Corticosterone levels in host and parasite nestlings:
Is brood parasitism a hormonal stressor?

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ABSTRACT

Parasite chicks from non-evictor species usually try to monopolize host parental care, thereby increasing considerably the level of food competition in the nest. Here, we propose that brood parasitism is an important stressor for host and parasite nestlings and explore this hypothesis in the non-evictor great spotted cuckoo (*Clamator glandarius*) and its main hosts, the same-sized black-billed magpie (*Pica pica*) and the larger carrion crow (*Corvus corone*). We experimentally created 3-nestling broods of different brood compositions (only cuckoo chicks, only host chicks, or cuckoo and host chicks together) and measured baseline corticosterone levels of nestlings along their developmental period (early, middle and late). We found that brood parasitism increased corticosterone levels in magpie nestlings in the mid and late nestling period compared to those raised in unparasitized nests. Interestingly, carrion crow nestlings from parasitized nests only increased their corticosterone levels in the mid nestling period, when the competition for food with the cuckoo nestling was highest. Our results suggest that brood parasitism could be a potential physiological stressor for host nestlings, especially during the developmental stages where food requirements are highest. Conversely, cuckoo nestlings could be physiologically adapted to high competition levels since they did not show significant differences in corticosterone levels in relation to brood composition.

Introduction

Avian brood parasitism is a type of parental care parasitism (Roldán and Soler, 2011) in which some individuals (the parasites) place their eggs in the nests of other species or conspecifics, the hosts (Davies, 2000; Johnsgard, 1997). Hosts usually incubate these eggs and rear the chicks hatched from them as their own (Davies, 2000; Johnsgard, 1997). In general, avian parasite–host systems are considered one of the best models to study coevolution due to the important selective pressures that both parasite and host impose on each other (Avilés et al., 2006; Langmore et al., 2003; Rothstein, 1990; Rothstein and Robinson, 1998; Soler and Soler, 2000). Indeed, numerous studies from many points of view provide us with excellent examples of ongoing coevolution in birds with behavioral (Briskie et al., 1992; Kilner et al., 1999; Madden and Davies, 2006;

Soler et al., 1995a, 1995c, 2011), morphological (Brooke and Davies, 1988; Krüger and Davies, 2004; Krüger et al., 2007; Langmore et al., 2011; Lemons and Sedinger, 2011; Spottiswoode, 2010; Welbergen and Davies, 2011), genetic (Fossoy et al., 2011; Gibbs et al., 2000; Martín-Gálvez et al., 2006, 20707) and ecological consequences of parasitism (Krüger et al., 2009; Møller et al., 2011; Smith et al., 2002; Ward and Smith, 2000). However, studies about physiological adjustments due to brood parasitism are still scarce and those that exist focus on maternal effects in eggs (Cariello et al., 2006; Hahn et al., 2005; Hargitai et al., 2010; Hauber and Pilz, 2003; Pilz et al., 2005; Schmaltz et al., 2008) or the immune system of the nestlings (Soler et al., 1999a). To the best of our knowledge nothing is known about other physiological indicators like hormones in developing nestlings in response to brood parasitism.

Hormones are good candidates to study physiological changes in animals as they are mediators of many behavioral responses (Landys et al., 2006; McEwen and Wingfield, 2003). Furthermore, the study of hormonal changes in developing organisms, like chicks, is very important because even short-term changes in these substances may alter the proper development and have important fitness consequences (Gil, 2003; Lindström, 1999; Schoech et al., 2011). For

example, baseline corticosterone (cort) levels during early development have been shown to affect growth, immunity and even personality in birds (Butler et al., 2010; Müller et al., 2009; Schoech et al., 2011). Cort levels in nestlings are also related with stressful situations (Kitaysky et al., 1999, 2001b; Love et al., 2003; Saino et al., 2003) and are known to increase with energetically costly processes, such as competition for food (Kitaysky et al., 2003; Loiseau et al., 2008b; Love et al., 2003).

In general, competition for food in host nests parasitized by non-evictor brood parasites is enormous (Davies, 2000; Rothstein, 1990) and frequently gives rise to the starvation of some or all host nestlings (Lorenzana and Sealy, 1999; Robinson et al., 1995; Soler et al., 1996).However, sometimes host nestlings can also outcompete parasitic chicks, especially if the latter starts the competition in disadvantage, i.e. in case host nestlings are larger than parasitic chicks or host nestlings hatch before parasitic chicks (Dearborn, 1998; Dearborn and Lichtenstein, 2002; Lichtenstein, 2001; Lichtenstein and Dearborn, 2004; Payne et al., 2001; Rivers et al., 2010b; Soler et al., 1998, 2001). This, strongly suggests that a parasitized nest is a stressful environment for both parasite and host nestlings and hence it is expected that chicks from a parasitized nest would have elevated levels of cort.

Furthermore, the level of competition among parasite and host chicks could vary along the nestling period due to a boost in food requirements. This may lead to increased food restrictions and higher levels of competition within the nest (Moskat and Hauber, 2010). However, cort responses also vary with the developmental stage of the individual and usually increase with nestling age (Sims and Holberton, 2000; Suorsa et al., 2003; Tilgar et al., 2009; Wada et al., 2007). Therefore, it is also important to take into account the developmental stage of nestlings when studying a potential effect of brood parasitism on nestling cort levels.

Brood parasites are also known to potentially parasitize different host species (Davies, 2000; Rothstein and Robinson, 1998). Each host species may have different begging ability and parental feeding capacity, which in addition to the size-difference between host and parasite may crucially influence the level of competition experienced by the nestlings in parasitized nests (Lichtenstein, 2001; Lichtenstein and Sealy, 1998; Madden and Davies, 2006; Rivers, 2007). Thus, different hosts may impose different physiological costs to the same parasite species and vice versa.

In this study we investigate the effect of brood parasitism on hormonal (cort) nestling responses using the great spotted cuckoo (Clamator glandarius), a non-evictor brood parasite, and its two main host species in the Palearctic, the black-billed magpie (Pica pica) and the carrion crow (Corvus corone) as model species (Cramp, 1998; Soler, 1990b). These two host species are considerably different in size, the magpie being only slightly larger, while the crow almost triples the size of the cuckoo (Cramp, 1998). We experimentally created different brood compositions of host and parasite nestlings and measured baseline cort levels of chicks along the nestling period (early, middle and late). We predict that: (i) the presence of a cuckoo chick should lead to an increase in baseline cort levels in magpie nestlings given the known negative impact of this parasite on magpie nestlings in natural conditions (Soler, 1990b; Soler and Soler, 1991); (ii) the competition with a cuckoo chick should not lead to an increase in baseline cort levels in carrion crow nestlings because this host is far larger than the parasite and is little influenced by cuckoo chicks (Soler, 1990b); (iii) plasma cort levels in cuckoo nestlings should not be affected by brood composition since the elevation of this hormone impairs growth (Butler et al., 2010; Müller et al., 2009; Schoech et al., 2011). Rapid growth is an important adaptation in non-evictor brood parasites to outcompete host nestlings (Rivers, 2007; Rivers et al., 2010b; Rodriguez-Gironés et al., 2002; Zanette et al., 2005). Therefore we reason that a low cort production should have been selected for in cuckoos to favor their fast growth.

Materials and methods

Research was conducted according to national (Real Decreto 1201/2005, de 10 de Octubre) and regional guidelines (permission granted by the Consejería de Medio Ambiente of the Junta de Andalucía, Spain).

Study area and species

This study was conducted from April to June 2008 in the Hoya de Guadix in southern Spain (37° 18′ N, 3° 11′ W; 1000 m a.s.l.). This is an area where magpies and carrion crows breed sympatric and where both species are frequently parasitized by the great spotted cuckoo (Soler, 1990a; Soler and Møller, 1990), a medium-sized brood parasite, member of the order Cuculiformes, that winters in Africa and breeds in southern Europe during spring (Cramp, 1998). During the breeding seasons 2006–2009 a mean of 44.7% of the magpie nests was parasitized ($N = 153$ out of 342 magpie nests) of which 60.1% ($N = 92$) contained more than one cuckoo egg and 88.9% ($N = 136$) reached hatching (De Neve and Soler, unpublished data). The most common natural situation in parasitized magpie nests is that cuckoo chicks hatch several days before the magpie chicks, which gives them an important size advantage to outcompete magpie chicks for food (Soler et al., 2001). In fact, one cuckoo chick was raised alone in the nest in 33.8% ($N = 46$ out of 136) of the hatched nests, and in 47.1% ($N = 64$) of the nests more than one cuckoo chick hatched and several cuckoo chicks were raised together in the same nest (Guadix 2006–2009; De Neve and Soler, unpublished data). When the cuckoo egg was laid when magpies already started incubation, cuckoo nestlings can also grow up together with one or several host nestlings (11.8%, $N = 16$ out of 136 hatched parasitized nests) or together with other cuckoo and host nestlings in the same nest (7.3%, 10 out of 136 hatched parasitized nests; De Neve and Soler, unpublished data). In the case of carrion crows as host species, 24% of the nests was parasitized (Guadix 2006–2008, $N = 123$ nests; Roldán, 2011) of which 60% contained more than one cuckoo egg (Guadix 2006–2008; Roldán and Soler, unpublished data). However, we never observed cuckoo nestlings to outcompete carrion crow nestlings and they always grew up together with host nestlings (Soler, 1990b; Roldán, personal observation).

Field procedures

At the beginning of the breeding season, we actively searched the area for magpie and carrion crow nests. Once a nest was located we visited it regularly (every 2 days) to assess laying date, clutch size and brood parasitism. Close to hatching, the nests were visited daily in order to detect newly hatched chicks. Experimental manipulation consisted in swapping two-day old nestlings (± 1 day) between nests with the same hatching date in order to generate nests with different brood compositions. The methodological procedure to handle chicks has previously been successfully utilized by our group to carry out other cross-fostering experiments (Soler and Soler, 2000; Soler et al., 1999a). Briefly, during cross-fostering, the nestling was placed in an artificial cotton nest maintaining the temperature between 25 and 30 °C while transporting it immediately to the foster nest. No nestling died during the process and none of the nests from which chicks were taken or introduced was deserted.

Using this methodology, we created five different types of three-nestling nests: (i) parasitized magpie broods (1 cuckoo + 2 magpie chicks), (ii) parasitized crow broods (1 cuckoo + 2 crow chicks), (iii) non parasitized magpie broods (3 magpie chicks), (iv) non parasitized crow broods (3 crow chicks), and (v) multiparasitized magpie broods (3 cuckoo chicks). In addition we also created (vi) single parasitized magpie broods (1 cuckoo chick). We did not

create multiparasitized and single parasitized crow broods because of the lower density of crow nests in the area, the lower parasitism rate and the unusual natural situation of cuckoo chicks growing up alone in crow nests (see above). Experimental broods were randomly composed upon the availability of same-aged chicks in different nests and 48% of the experimental nestlings were cross-fostered while the rest of the experimental nestlings remained in their nest of origin. Therefore, we believe that possible confounding effects of maternally deposited egg yolk hormones in relation to brood parasitism have been randomized across species and experimental nest compositions.

Nests were visited regularly after the experimental manipulation in order to obtain blood samples for each chick of the brood representing the early, mid or late nestling period. Given that the three species show different developmental times and nestling periods (cuckoos: 15–16 days, magpies 19–20 days, carrion crows 24–25 days), the early, middle and late nestling stage were selected proportionally to each particular developmental period. Specifically, cuckoos were sampled at the age of 4, 9 and 13 days (± 1 day), magpies were sampled at the age of 5, 11 and 17 days (± 1 day), and carrion crows were sampled at the age of 5, 13 and 22 days (± 1 day). We collected one blood sample (100–180 µl) from the brachial vein using sterile needles and heparinized microcapillary tubes. All blood samples included in the analyses were taken within 3:16 minutes starting from the moment the nest was reached with the aim of measuring baseline cort levels in plasma (Romero and Reed, 2005; Wada et al., 2007).

Chicks were always sampled in the morning (8:30 to 11:30 a.m.) avoiding the potential problem of circadian cort changes (Breuner et al., 1999). We also recorded ambient temperature in the shade (range: 10–24.5 °C; mean $\pm$ SE: 17.0 $\pm$ 0.2 °C) and estimated wind speed (scale 0 (no wind) to 4 (stormy wind)) during each visit, as weather circumstances may affect the stress that chicks experience when parents leave the nest for feeding (Bize et al., 2010; Lobato et al., 2008; Romero et al., 2000; Wingfield, 2000). In addition, we also measured wing length with a ruler (accuracy 1 mm) and body mass with a digital balance (accuracy 0.1 g) of all chicks on each visit.

Blood samples were kept in a portable fridge at 4 °C for up to 3 hours. Then, blood samples were centrifuged at 9279g for 5 minutes. Plasma samples were stored at −20 °C until further analyses.

Corticosterone assays

Plasma baseline cort levels were determined by radio immunoassay at the Centre d'Etudes Biologiques de Chizé (CEBC) following the procedure of Lormée et al. (2003). Plasma cort was measured in samples (70–80 µl) after diethyl-ether extraction by radioimmunoassays using antiserum. Corticosterone antiserum was developed in rabbit against corticosterone-21-thyroglobulin (C8784; Sigma, USA). Cross-reaction was 20% with 11-deoxycorticosterone, 15.7% with progesterone, 8.8% with 20a-hydroxyprogesterone, 7.9% with testosterone and less than 5% with other plasma steroids. Duplicate aliquots of the extracts (100 µl) were incubated overnight at 4 °C with antiserum and ^{3}H-corticosterone (3000 cpm). The bound and free steroid was separated by adding dextran-coated charcoal. The bound fraction was counted in a Packard liquid scintillation counter β (Model 1600 TR, Canberra) after adding scintillant to samples. Intra-assay coefficient of variation was 2.31% ($n = 3$ duplicates). The lowest detectable concentration was 470 pg ml^{-1}.

Statistical procedures

We evaluated the effect of different brood composition on baseline cort levels (log transformed) for each species separately by fitting linear mixed models (LMM) using SAS 9.2 (SAS Institute Inc. 2002–2003, Cary, NC, USA).

In all initial models, we included brood composition, nestling stage and the interaction term as fixed factors. All models were initially corrected for the hour of the day (08:30–11:30), sampling time (77–196 seconds), ambient temperature, wind speed and nestling wing length and body mass. Individual nestlings nested within nest identity and nest identity were included as random factors to account for the non-independence of samples taken from the same nestling along the nestling period and for similarities between nestlings reared in the same nest. Non-significant fixed effects were sequentially dropped from the initial model until the model with lowest AIC value was obtained. Degrees of freedom were estimated following the method described by Kenward and Roger (1997). We ran this model for each species separately comparing first cuckoo chicks raised in different nest situations in magpie nests (1 cuckoo, 1 cuckoo + 2magpies and 3 cuckoos) and also cuckoo chicks raised in different host species (1 cuckoo + 2 magpies and 1 cuckoo + 2 crows). Second, baseline cort levels for each host were compared between host nestlings that were raised together with other host chicks and those that were raised with a cuckoo chick.

Due to predation (7 nests) and because we did not always succeed in taking the blood sample from each chick before the time limit of 3 and a half minutes, the sample sizes that could be used in the analyses were as follows: at least 1 blood sample from 39 cuckoo chicks proceeding from 26 different nests (97 blood samples), 16 magpie chicks proceeding from 8 different nests (39 blood samples) and 23 crow chicks proceeding from 11 different nests (66 blood samples).
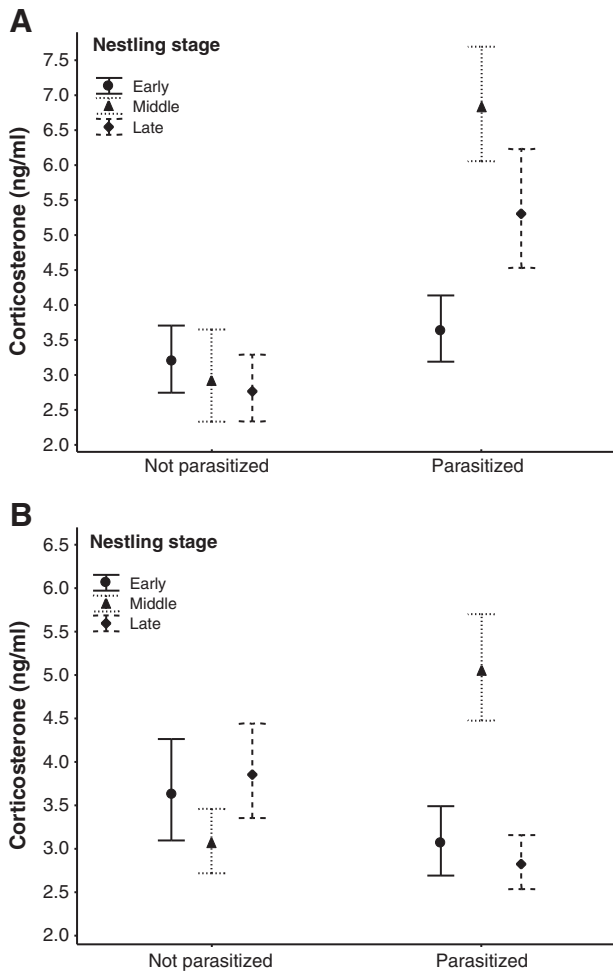
Results

Magpie nestlings

Overall, baseline cort levels in magpie chicks were highly influenced by the type of nestmate (Table 1; Fig. 1A), with magpie nestlings bearing significantly higher plasma cort in the presence of a cuckoo nestling compared to the presence of conspecifics. In addition, the final model with lowest AIC values for magpie nestlings also retained the interaction between brood composition and nestling stage (Table 1). Variation in cort levels along the nestling period was almost null in magpie nestlings sharing the nest with conspecifics, while it increased significantly from the beginning to the mid-nestling period in magpies from parasitized nests (post-hoc $t = 3.27$; $P = 0.0046$; Fig. 1A). Cort levels in mid and late-nestling period did not differ in parasitized nests (post-hoc $t = 1.18$; $P = 0.25$; Fig. 1A). With respect to brood composition, magpie nestlings from parasitized nests tended to show higher baseline cort levels during the mid and late-nestling period compared to those from unparasitized nests (post-hoc $t = 2.71$; $P = 0.011$ and $t = 2.83$; 0.0089 respectively; Fig.1A). With respect to covariates, when it was warmer outside and with stronger winds, baseline cort levels of magpie nestlings significantly increased (Table 1).

Table 1
LMM models that best explained (lowest AIC) the variation in baseline corticosterone levels in magpie and carrion crow nestlings.

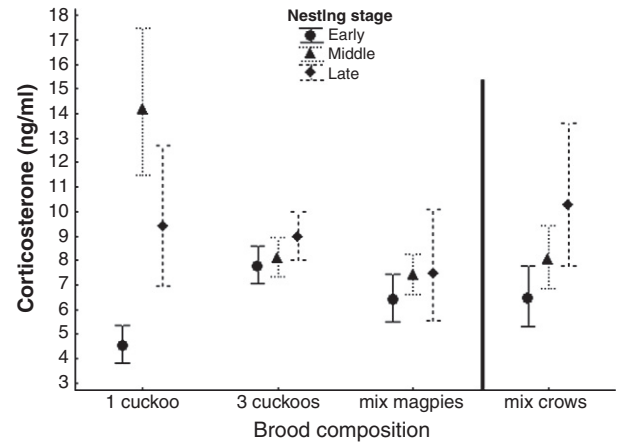| Variable	Estimate	F (Z)	df	P
Magpie nestlings				
Brood composition		9.67	1.24	0.0048
Nestling stage		2.13	2.24	0.14
Brood composition × nestling stage		1.97	2.24	0.16
Outside temperature	0.09	5.47	1.24	0.028
Wind speed	0.41	21.38	1.24	0.0001
Crow nestlings				
Brood composition		0.00	1.59	0.96
Nestling stage		1.24	2.59	0.29
Brood composition × nestling stage		5.93	2.59	0.0045
Wing length	−0.01	7.95	1.59	0.0065

Fig. 2. The combined effect of nestling stage and brood composition (results from LMM) on baseline corticosterone levels (least square means ± SE, log back-transformed values) in great spotted cuckoo nestlings. Results in magpie and crow nests (separated by vertical line) are obtained from different mixed models (see results). SE bars are asymmetrical due to log back-transformation.

composition in magpie nests (LMM brood composition × nestling stage $F_{4,43.3} = 3.47$; $P = 0.015$; Fig. 2). Nestlings alone in the nest showed a huge variation in baseline cort levels along the nestling period, with the highest values measured during the mid nestling stage (Fig. 2). In contrast, cuckoo nestlings sharing the nest with other nestmates showed only small variations in baseline cort levels along the nestling period (post-hoc all $t < 0.96$; $P > 0.34$; Fig. 2). During the mid-nestling period, post-hoc tests revealed higher baseline cort levels in cuckoos being alone in the nest compared to those sharing the nest with magpies or other cuckoo nestlings (post-hoc $t = 2.34$; $P = 0.023$ and $t = 2.87$; $P = 0.0055$ respectively). From the covariates, only ambient temperature remained in the final model having a significant negative effect on baseline cort levels in cuckoo nestlings ($F_{1,58.3} = 12.13$, estimate = 0.058, $P = 0.0009$). Furthermore, when comparing cuckoo nestlings raised in different host species nests, cort levels were similar in cuckoo nestlings being raised with magpie or crow nestlings (LMM, effect before being dropped from the model $F_{1,26.4} = 0.26$; $P = 0.61$; Fig. 2).

Discussion

To the best of our knowledge, this is the first study looking at endocrine consequences of brood parasitism in nestlings. Our main result shows that magpie, but not crow, nestlings had elevated plasma baseline cort levels when they shared the nest with a cuckoo instead of a conspecific, suggesting variability in the effect of brood parasites on different hosts. However, we found that both host species modified their baseline cort levels according to their developmental stage differently in non-parasitized compared to parasitized nests, i.e. they did not modify their baseline cort levels during development in the first situation, while in the latter they increased their baseline cort levels when the competition for food was highest (mid nestling stage). In contrast, cuckoo nestlings did not modify their cort levels according to brood composition; they had similar levels sharing the nest with magpies, crows or other cuckoo nestlings.

Hormonal response of hosts to brood parasitism

Our results showed that magpie nestlings had elevated baseline cort levels when sharing the nest with a cuckoo confirming the prediction that a parasitized nest could be a more stressful environment for hosts than an unparasitized nest. The elevated levels of cort in magpie nestlings from parasitized nests could reflect either a higher level of hunger, because food deprivation is related with

Fig. 1. The combined effect of nestling stage and brood composition (results from LMM) on baseline corticosterone levels (least square means ± SE, log back-transformed values) in magpie (A) and carrion crow (B) nestlings. SE bars are asymmetrical due to log back-transformation.

Crow nestlings

We did not find an overall effect of brood composition on baseline cort levels for crow nestlings (Table 1; Fig. 1B). However, cort levels in crow nestlings were differently affected by the nestling stage in unparasitized and parasitized nests (significant interaction term, Table 1). While crow nestlings showed similar baseline cort levels along the nestling period in unparasitized nests (post-hoc, all $t < 1.20$; all $P > 0.23$; Fig. 1B), in parasitized nests baseline cort levels increased from the beginning towards the middle of the nestling period (post-hoc $t = 2.69$; $P = 0.0091$; Fig. 1B) and steeply decreased again near the end of the nestling period (post-hoc $t = 3.61$; $P = 0.0063$; Fig. 1B). When comparing crow nestlings between parasitized and unparasitized nests, in the middle of the nestling period nestlings from parasitized nests showed higher cort levels compared to those from unparasitized nests (post-hoc $t = 2.90$; $P = 0.0052$; Fig. 1B). The only covariate that remained in the final model was wing length having a significant negative effect on crow baseline cort levels (Table 1).

Cuckoo nestlings

There was no overall effect of brood composition on baseline cort levels for parasite chicks (LMM $F_{2,29} = 1.15$; $P = 0.33$; Fig. 2). However, changes in baseline cort levels according to the developmental stage of the nestling differed significantly depending on the brood

increased cort levels in nestlings (Kitaysky et al., 1999; Nuñez de la Mora et al., 1996). Alternatively, magpie nestlings could also elevate their cort levels in order to increase their begging activity. Several studies related high cort levels to an increase in begging behavior (Kitaysky et al., 2001b, 2003; Loiseau et al., 2008a; Quillfeldt et al., 2006), and there is numerous empirical evidence that nestlings beg in accordance to their level of hunger and food deprivation (Leonard and Horn, 2001; Price et al., 1996; Sacchi et al., 2002; Smiseth et al., 2003; Smith and Montgomerie, 1991). In addition, nestlings may also increase their begging levels in response to the behavior of their nestmates (Forbes, 2002; Leonard et al., 2000; Smith and Montgomerie, 1991). Given that brood parasite nestlings are known to produce exaggerated begging displays compared to the individual host nestlings (Dearborn, 1998; Dearborn and Lichtenstein, 2002; Lichtenstein and Sealy, 1998; Rivers, 2007; see Rodríguez-Gironés et al., 2002; Soler et al., 1999b for the magpie-cuckoo system), the continuous high begging levels of cuckoo nestlings may also increase begging activity in host nestlings in parasitized nests as has already been observed in cowbirds (Rivers et al., 2010a, 2010b). Hence, the increased cort levels in magpie nestlings sharing the nest with a parasite nestling might result from a combination of both an increased level of need and begging activity. The causal relationships between nest environment, begging and hunger level with baseline cort levels in host nestlings from parasitized nests, need however further experimental study.

Interestingly, and in accordance with our prediction, we did not find such general effect for the other main host species of the great spotted cuckoo, the carrion crow. This result indicates that the effect of brood parasites may vary between different host species. Nestling body size and condition are very important traits for sibling competition in many bird species (Fargallo et al., 2003; Jeon, 2008; Lack, 1954; Oddie, 2000; Price and Ydenberg, 1995; Smiseth et al., 2003), also in non-evictor brood parasites (Rivers, 2007; Rivers et al., 2010b; Rodríguez-Gironés et al., 2002; Zanette et al., 2005). In fact, non-evictor brood parasite nestlings have several adaptations to obtain a size advantage with respect to their host nestmates, like shorter incubation periods or higher growth rates (Hauber, 2003; Krüger, 2007). Magpie fledglings are only slightly larger than cuckoo fledglings, while crow fledglings nearly triple the size of the parasite (Cramp, 1998). Thus, the competition level imposed by the presence of a cuckoo nestling in a magpie nest should be much higher than that imposed in a crow nest (Rivers et al., 2010b; Soler, 1990a; Soler et al., 2002). In fact, this prediction has been already confirmed in non-evictor American cowbirds (*Molothrus* spp.) where the competition outcome between parasite and host nestlings depended on size differences between host species: a parasite nestling usually outcompeted smaller sized host nestlings (Lichtenstein and Sealy, 1998; Lorenzana and Sealy, 1999; Robinson et al., 1995) while it was not able to do the same with larger sized host species (Rivers et al., 2010b). Therefore, the difference in competition level due to host size probably caused the different impact of brood parasitism on host baseline cort levels, though we cannot discard that other, non-measured, differences between these two species had an additional impact.

Taking the age of the nestlings into account, we found that host nestlings modified their baseline cort levels along their developmental period differently in parasitized than in non-parasitized nests. Both magpie and crow nestlings growing up with a parasite significantly increased their cort levels from the beginning to the middle of the nestling stage. Moreover, in both species, baseline cort levels were higher in parasitized nests compared to unparasitized nests at the mid-nestling period. By the late nestling period, especially crow nestlings down-regulated this hormone again (Fig. 2). Soon after hatching, host parents most likely can provide easily all the food required by their nestlings, independently of their nature. However, at the mid nestling period, host nestlings are still in full development and competition for food in parasitized nests might increase

considerably given the really fast development of the cuckoo chick that is already close to fledging (Soler and Soler, 1991). This situation could explain the increase in baseline cort levels for both host species in parasitized nests compared to the stable levels in non-parasitized nests in the mid-nestling period. In contrast, food competition during the final stage of host development is reduced again in the case of the carrion crow, but not in the magpie. On the one hand, this is probably because adult carrion crows preferentially feed their own nestlings, which enjoy a larger size, instead of parasitic chicks (Roldán, 2011). On the other hand, great spotted cuckoo nestlings start to leave the nest around day 15 of development. Thus, cuckoo nestlings parasitizing magpie nests only left very recently the nest when magpie nestlings reached their fledging state. Soon after fledging cuckoos still visit frequently the nest and depend completely on their foster parents (Soler et al., 1994), so magpie nestlings might still perceive the competition with the cuckoo nestling at the end of the nestling period. In contrast, when crow nestlings reached their fledgling state, the cuckoo already left the nest for more than 1 week and at this stage cuckoos do not visit the nest anymore and decreasingly depend on host parents (Soler, De Neve and Roldán, unpublished data). Carrion crows that have their own chicks at the nest decrease attendance of fledging cuckoos and these, as is also the case for cuckoos reared by magpie hosts, start to join communal groups (Soler et al., 1995b). Therefore, crow nestlings did probably not compete for food with the cuckoo during their last days in the nest. This difference between host developmental periods might explain the steep drop in cort levels at the end of the nestling period in crow nestlings, but not in magpies. These results, particularly those referring to the elevation of cort levels at the moment of maximum food competition (mid nestling stage), could indicate that, regardless of the size difference between hosts, both host species were affected by the presence of the parasite. In addition, our results also highlight the importance of the age of sampling in this kind of physiological studies: no effects can be found at some developmental stages while strong effects can be found at others.

In evolutionary terms, the elevation of cort levels may bear important costs in developing organisms, in terms of growth, immune response and eventually survival and fitness (Butler et al., 2010; Müller et al., 2009; Schoech et al., 2011). Therefore, host nestlings of parasitized nests should cope with other additional costs than those reared in non-parasitized nests. In fact, these additional costs associated to the elevation of cort could help to explain the observed negative effects of brood parasitism on host long-term fitness (Hoover and Reetz, 2006).

Hormonal response of parasites to different brood composition

Overall and in accordance with our third prediction, great spotted cuckoo nestlings did not modify their baseline cort levels according to brood composition. This finding is very interesting, especially given that we did find an effect of brood composition on baseline cort levels in host nestlings. It is possible that cuckoo chicks maintain their cort at the same levels independently of brood composition given the negative effects on growth that entails a high level of this steroid hormone (Müller et al., 2009). A slower growth rate would involve an important handicap for parasite nestlings, especially due to the relevance of the size in host–parasite nestling competition and the urgent need of cuckoos to grow as fast as possible (Rivers et al., 2010b; Robinson et al., 1995; Rodríguez-Gironés et al., 2002). This is also supported by the fact that we did not find significant differences in cort levels in cuckoos depending on the host species or in multiparasitized nests (competing with other cuckoos) and cuckoos alone in the nest (Fig. 2).

Alternatively, cuckoo chicks could always follow the same high food-demanding behavior independently of the competition level within the nest, and consequently have always high baseline cort levels (Fig. 2). However, this possibility is less likely because constant

high levels of cort may entail important fitness costs (see references above), and hence, cuckoo nestlings should be physiologically adapted to minimize these costs associated to a situation of constant high baseline cort levels during their development.

Apart from these adaptive explanations, hunger level is also an important determinant of cort levels (e.g. Kitaysky et al., 2001a; Quillfeldt et al., 2004). Therefore, the lack of differences in cuckoo cort levels could also be explained if cuckoos did not experience food restraints in any of the experimental brood compositions. However, further research is needed to disentangle whether cuckoos maintain low cort levels because they are better adapted to competition (due to selection over evolutionary time), or because they are always well fed (a proximate factor).

Nevertheless, we found significant differences between brood compositions in the pattern of cuckoo chicks' hormonal responses along the nestling period (Fig. 2). Parasitic nestlings being reared alone in a magpie nest significantly increased their cort levels from the early to mid nestling period, in contrast with those cuckoos sharing the nest with other chicks of any kind (magpie, crow or cuckoos). Avian parents usually adjust their feeding effort according to their brood size (Chamberlain et al., 1999; Hinde and Kilner, 2007; Saino et al., 2000), and, it has been shown that nestlings of the non-evicting parasite brown-headed cowbird (*Molothrus ater*), grow faster and survive better when they share the nest with some host chicks than when raised alone (Kilner, 2003; Kilner et al., 2004). Single-cuckoo nestlings could therefore show increased baseline cort levels in the mid nestling period because they become hungrier and have to increase their begging activity, encouraging the food delivery by their foster parents to meet up their needs.

Conclusions

Our study, testing for the first time that brood parasitism could be a physiological stressor for host nestlings, finds support for this hypothesis, especially during periods of high food requirements. In contrast, great spotted cuckoo nestlings, that shared the nest with conspecifics or host nestlings, could be either physiologically adapted to levels of high competition or could have received sufficient food in all experimental nests.

Our results also highlight the importance of developmental stage in sampling baseline cort levels in relation to ecological factors, as environmental factors may affect stress levels in a different way along development.

Acknowledgments

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