

RESEARCH ARTICLE

Benefits associated with escalated begging behaviour of black-billed magpie nestlings overcompensate the associated energetic costs

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SUMMARY

Several experimental results support the existence of costs associated with exaggerated begging behaviour, which are assumed by some theoretical models of honest signalling in parent–offspring communication. However, to understand how honest begging behaviour is evolutionarily maintained in nature, the long-term cost–benefit output associated with exaggerated signals should also be estimated. As far as we know, the net cost–benefit balance of begging display has not previously been explored. Here, we used an appetite stimulant, cyproheptadine hydrochloride, to increase the feeling of hunger in some magpie nestlings. Supporting the use of cyproheptadine to manipulate hunger level and thereby begging behaviour, we found that experimental nestlings increased the frequency of begging and received more food than their control nestmates. Contrary to the expectation that physiological costs *per se* counteract the associated benefits of escalated begging signals, we found that near-fledging experimental magpies showed a better physical condition than control nestlings. These findings stress the interesting question of why magpie nestlings do not show to adults an escalated level of hunger if it implies an advantage. We discuss the responsibility of inclusive fitness costs and indirect genetic effects for the maintenance of honesty in parent–offspring communication.

Key words: begging behaviour, black-billed magpie, costs and benefits of begging, cyproheptadine, food allocation, honest signalling, parent–offspring conflict, *Pica pica*.

INTRODUCTION

Offspring are genetically different to their parents, and gaining more parental care than parents are selected to provide is of selective advantage (Trivers, 1974). Since this parent–offspring conflict was proposed, several models have tried to explain its resolution in general and the evolution of conspicuous and extravagant offspring signals during solicitation of care in particular (for reviews, see Mock and Parker, 1997; Godfray and Johnstone, 2000; Budden and Wright, 2001; Johnstone and Godfray, 2002). A group of these models suggests that the conflict can be solved by the existence of costs associated with offspring solicitation signals that are constraining their expression. The alluded costs include physiological costs of production of signals and costs related to risk of predation and loss of indirect fitness (e.g. depriving relatives – either nestmates or future unborn siblings – of food) (Godfray and Johnstone, 2000). In this situation, dishonest begging behaviour would not be compensated by the extra benefits gained (i.e. more food). Parents, thus, would be able to use these honest signals actively to properly adjust their effort as well as the food allocation among siblings (active-choice hypotheses) (*sensu* Mock et al., 2011), or passively by feeding the offspring that emerges victorious from scramble competition (Rodríguez-Gironés et al., 2001a; Parker et al., 2002) or negotiation (Roulin, 2002) among siblings. Between the active-choice models, the most widely recognized is that developed by Godfray (Godfray, 1991; Godfray, 1995a; Godfray, 1995b), which assumes that begging signals could be reflecting offspring need, defined as the

marginal fitness gain experienced by an offspring from receiving the next unit of parental investment [but see Mock et al. (Mock et al., 2011) for a review about other two active-choice alternatives]. According to Godfray's model, parents would preferentially feed those offspring showing the most intense signals because they would be the most needy.

Three predictions are generated from this hypothesis (see Kilner and Johnstone, 1997; Royle et al., 2002): (1) parental investment is regulated according to offspring signals; (2) the intensity of the begging signal varies according to offspring need; and (3) begging signals should be costly to produce. There exists a substantial experimental and empirical body of results supporting parent use of offspring begging behaviour to adjust feeding effort and to allocate food within the brood (e.g. Smith et al., 1988; Ottosson et al., 1997; Kilner and Johnstone, 1997; Burford et al., 1998) (but see Clark and Lee, 1998). However, although considerable evidence also exists about the relationship between begging intensity and levels of food deprivation and offspring condition (Redondo and Castro, 1992b; Price et al., 1996; Iacovides and Evans, 1998; Sacchi et al., 2002), it is still not clear how food deprivation (i.e. hunger) and offspring condition are related to offspring need as defined by Godfray (Godfray, 1991) [see Mock et al. (Mock et al., 2011) for a further discussion about this issue]. With regard to costs associated with offspring begging behaviour, its existence is not widely supported and sometimes is controversial (reviewed in Kilner and Johnstone, 1997; Budden

and Wright, 2001; Wright and Leonard, 2002; Moreno-Rueda, 2007).

Perhaps the clearest support for the costs of escalated begging behaviour comes from several articles showing that predation risk can constrain begging expression (especially offspring vocalizations) (e.g. Redondo and Castro, 1992a; Haskell, 1994; Haskell, 2002; Leech and Leonard, 1997; Briskie et al., 1999; Dearborn, 1999) and from four articles giving support to the existence of physiological costs associated with begging signals (Kilner, 2001; Rodríguez-Gironés et al., 2001b; Moreno-Rueda, 2010; Noguera et al., 2010). Predation costs of begging signals are based on the possibility that predators could more easily detect nests that contain nestlings that beg loudly. However, in broods with more than one nestling, predation costs would be shared by the entire brood because, once a nest is located, all nestlings will probably be eaten. For this reason, predation costs would hardly explain individual differences in intensity of begging signals among nestlings within a brood and, thus, this cost would not prevent an escalation of dishonest nestling behaviour [see Rodríguez-Gironés et al. (Rodríguez-Gironés et al., 2001b) for further discussion]. Regarding the physiological cost of escalated begging, the first two articles from the four referred to above have shown the existence of negative effects on nestling growth in canaries (*Serinus canaria*) (Kilner, 2001) and black-billed magpies (*Pica pica*) (Rodríguez-Gironés et al., 2001b); the third article gives support to the immunological cost of exaggerated begging signals in the house sparrow (*Passer domesticus*) (Moreno-Rueda, 2010); and the fourth provides evidence about the oxidative cost of begging in yellow-legged gulls (*Larus michahellis*) (Noguera et al. 2010). In the first three studies, experimental nestlings were forced to beg for food at a higher rate than control nestlings (different costs) and both groups received the same amount of food (same benefits) (Kilner, 2001; Rodríguez-Gironés et al., 2001b; Moreno-Rueda, 2010). The fourth study showed an increased begging display of nestlings experimentally supplemented with vitamin E, possibly because of the reduced oxidative cost of begging (Noguera et al., 2010). Nonetheless, these experimental approaches do not allow the estimation of net cost–benefit output of the exaggeration of begging behaviour, nor do they allow us to determine whether exaggerated begging behaviour is cost-effective in the sense of garnering more energy than the signal's transmission expends. Parents, following the exaggerated signal of 'selfish' offspring could even overcompensate energetic costs associated with the production of the signal. Thus, it is possible that, even assuming extra costs, a certain level of exaggeration could still be advantageous for offspring. Theoretical studies modelling the evolution of honest begging behaviours refer to the net balance between benefits and costs, rather than merely the costs of signals, as the keystone that predicts honesty in parent–offspring communication (e.g. Godfray and Johnstone, 2000). Therefore, the experimental increase in offspring solicitation signals in natural conditions and the estimation of the net physiological cost–benefit balance of this manipulation are important for the understanding of the evolution of honest begging behaviour.

In the present study, we performed such an experiment in black-billed magpies, one of the two bird species in which the cost of exaggerated begging behaviour on nestling growth has been reported (Rodríguez-Gironés et al., 2001b). Briefly, we increased the hunger level in some nestlings within broods by the administration of an appetite stimulant, cyproheptadine hydrochloride, which affected begging behaviour (see Results). This pharmaceutical is a type 2 serotonin receptor antagonist, which directly acts on the hunger centre at the hypothalamus (Stone et al., 1961; Chakrabarty et al.,

1967). Cyproheptadine is broadly used in humans, including children, as a safe and effective appetite stimulant (Chinuck et al., 2007; Couluris et al., 2008; Mahachoklertwattana et al., 2009). It is also often used in other animals, mainly in cats (see e.g. Plumb, 1999), and its property as appetite stimulant has also been manifested in two bird species, domestic fowl (*Gallus domesticus*) (Injidi and Forbes, 1987; Muralidharan et al., 1998) (but see Rao and Varadarajulu, 1979) and domestic pigeons (*Columba livia*) (Gunturkun et al., 1989). The use of this methodology allows nestlings to be raised in their natural nests being fed by their parents. Thus, and conversely to traditional methodologies based on food deprivation to increase begging signals, this experimental approach allows us to estimate variables related to the net balance between benefit and costs (e.g. fledging performance) associated with the exaggerated begging signals.

The aims of this experimental study were to test three different methodological and theoretical predictions: (1) cyproheptadine, by increasing the level of hunger of nestling, also increases the intensity of begging signals (frequency and/or intensity of begging); (2) the enhanced beggars receive more food from the parents than their control nestmates; and (3) if physiological costs associated to signals are at least partially responsible for maintenance of honesty in signalling, the energetic net balance of an exaggerated begging behaviour should be negative for enhanced beggars. We addressed these predictions by using four different methodological approximations. First, we video-recorded the behaviour of magpie nestlings and adults in their nests in order to determine whether nestlings treated with cyproheptadine beg more (frequency and/or intensity) and whether they are preferentially fed over their control nestmates. Second, in laboratory conditions, we estimated the effect of experimental treatment and food deprivation time on the begging behaviour of nestlings. Third, in natural conditions, we quantified the amount of food received and change in body mass (used as an index of begging effort) for control and experimental nestlings for a period of 2.5 h. Finally, we estimated the effect of our experimental treatment on variables related to nestling probability of recruitment, such as T-cell immune response (e.g. Møller and Saino, 2004; Cichon and Dubiec, 2005; Moreno et al., 2005), body size (lengths of tarsi, wings and tails) and body mass (e.g. Magrath, 1991; Gebhart-Henrich and Richner, 1998; Schwagmeyer and Mock, 2008).

MATERIALS AND METHODS

Study area and species

Fieldwork was performed in two close localities of southern Spain during the springs of 2002–2003 and 2008: in the Hoya de Guadix (37°14'N, 3°11'W) and in Iznalloz (37°25'N, 3°33'W), respectively. The Hoya de Guadix is a high-altitude plateau (~1000 m above sea level) with cereal crops (especially barley, *Hordeum vulgare*) that alternate with more or less dispersed plots of almond groves (*Prunus dulcis*) or holm-oak trees (*Quercus rotundifolia*), which are preferentially used by magpies to build their nests (for details, see Soler, 1990). Iznalloz has an undulated landscape mainly consisting of small patches of holm-oak forest coexisting with plantations of olive trees (*Olea europea*). In this population, magpies prefer to build their nests mainly in holm-oak trees and secondarily in olive trees.

The black-billed magpie (*Pica pica* Linnaeus 1758) is a territorial, sedentary and relatively long-lived passerine bird with a well-studied biology (reviewed in Birkhead, 1991). It occurs throughout large parts of the Holarctic region where, in some places, it becomes abundant. Magpies lay a single clutch between March and May with five to seven eggs (range=3–10). The female starts to incubate before

clutch completion (usually once the fourth egg is laid); this results in broods that hatch asynchronously where some of the smallest nestlings may starve soon after hatching (Birkhead, 1991).

General field procedures

At the beginning of the breeding season (end of March to early April), we intensively searched the study areas to find the location of new nests. Once a new nest was detected, we visited it regularly to determine the laying date. During the laying period, nests were checked at least twice per week to determine laying date, clutch size and the occurrence of brood parasitism by the great spotted cuckoo (*Clamator glandarius*), which is common in these areas (Soler and Soler, 2000). We used magpie nestlings from natural non-parasitized broods (natural broods) and from experimental parasitized broods, where two magpie and two great spotted cuckoo nestlings shared the nest during development (parasitized broods). Parasitized broods were also created to study the begging behaviour of great spotted cuckoos in comparison with that of nestling hosts; however, we only used data from magpie nestlings from these parasitized broods for the present study.

Magpie nestlings in natural broods were weighed 2–4 days after hatching (Pesola spring balance, accuracy 0.1 g; Pesola Ag, Baar, Switzerland) and marked by painting the tarsus with non-toxic waterproof pens of different colours (Mitsubishi Pencil, Rubi, Barcelona, Spain). Nestlings were ranked according to their mass and alternately assigned to the experimental and control treatments. Thus, we created pairs of experimental–control chicks of similar mass within broods (nestling dyads). In this way, we could use paired statistical tests to reduce the within-nest variance due to other variables such as body size and competitive abilities of nestlings. Further, we alternated the order of assignment of the experimental and control treatment between consecutive nests; thus, the heaviest nestling was assigned to the experimental treatment in some broods and to the control treatment in others. Parasitized broods were also created when nestlings were 2–4 days old both in magpie nests that were naturally parasitized by great spotted cuckoo (26 broods) and in unparasitized magpie nests (18 broods). The magpie nestlings used in each experiment came mainly from the same magpie brood (in 39 of 44 experimental broods); they were randomly selected from those that hatched within the first or second day of hatching and had masses similar to those of cuckoo nestlings. However, and because it is difficult to find two cuckoo nestlings of similar mass in the same magpie nest, the two cuckoo nestlings came mainly from two different nests (36 of 44 experimental broods). Similar to natural broods, nestlings were ranked within species and we alternated the order of assignment of experimental and control treatment between nests. In this case, we alternated the four possible combinations: the heaviest nestlings of each species received the same treatment or the heaviest nestlings of each species received different treatment.

Treatments consisted of oral administration (by a plastic 1 ml syringe) of 0.1 mg cyproheptadine hydrochloride (Acofarma, Inc., Barcelona, Spain) diluted in 0.25 ml mineral water to the experimental nestlings every 2 days (i.e. 0.05 mg day⁻¹). Control nestlings were administered with 0.25 ml mineral water. We calculated the dose of cyproheptadine based on that recommended for children (0.4 mg kg⁻¹ day⁻¹) (Peisker, 2000) and extrapolated to a nestling of 100 g. This dose was similar to that previously used in chickens (average dose was *ca.* 0.5 mg kg⁻¹ day⁻¹) (Rao and Varadarajulu, 1979; Injidi and Forbes, 1987; Muralidharan et al., 1998). Drug dilution was also the same as that used for children (0.4 mg ml⁻¹) (Peisker, 2000) and similar to that used in chicken

(0.32 mg ml⁻¹) (Injidi and Forbes, 1987). After the first dose (at first weighing, 2–4 days old), we revisited nests every 2 days to recolor the tarsi, weigh the nestlings and supply them with the experimental or control doses. Survivor nestlings received the treatment with cyproheptadine or water on alternative days and on six occasions, except those used for video recordings (see below), which received treatment on five occasions, i.e. until they were filmed.

Statistical analyses

When required for analysis, outcome variables were graphically checked for normal distribution of their frequencies (density and normal probability plots) and, if necessary, ln-transformed. Data from video recordings, laboratory and neck-collar trials were analysed mainly with linear mixed models (LMMs) and generalized linear mixed models (GLMMs) in R version 2.9.2 (R Development Core Team, 2009) using *lme4* (R package v.0.999375-31) (Bates and Maechler, 2009), and were fitted by the restricted maximum likelihood and Laplace approximations, respectively. For LMMs, we used Markov-chain Monte Carlo (MCMC) simulations performed by using the *pvals.fnc* command of *languageR* (Baayen, 2008) to compute the highest posterior density (HPD) 95% confidence intervals (CI) of the model estimates and *P*-values from MCMC simulations (P_{MCMC}). The chain length for MCMC sampling was fixed at 10,000. For GLMMs, we used Wald-*Z* tests for hypothesis testing. All first-order interactions were initially included in the model; the non-significant interactions were later removed to improve model parsimony (Zuur et al., 2009). To analyse the effect of cyproheptadine on the physical condition of magpie nestlings, we used general linear models (GLMs) with a repeated-measures design. GLMs were performed in Statistica version 8.0 (StatSoft Ibérica, Lisbon, Portugal). All tests were two-tailed and values in text and tables are reported as means ± s.e.m.

Video recordings in the field

Between May and July 2008 and when nestlings were 10–12 days old, we filmed nestling begging behaviour and food delivery by parents in 16 non-parasitized magpie nests (natural broods). We used a wireless microcamera (KPC-S500, black and white CCD camera, eSentia Systems Inc., Baton Rouge, LA, USA), a hard disk device media recorder (EMTEC, Gennevilliers, France) and a 3 inch portable monitor. Before filming, nestlings were administered with their fifth doses (cyproheptadine or water) and weighed. Nestlings were individually marked with a unique combination of white points drawn on the crown using correction fluid (Tipp-Ex). These marks have a similar appearance to excremental remains, which sometimes can be found on nestlings. The mean duration of the video recordings was 3:27 h (range=2:23–4:39 h), resulting in a mean of 5.5 adult visits per hour (range=2.9–9.3 visits h⁻¹).

We watched the video recordings using TMPGEnc DVD Author 3 software (Pegasys Europe, London, UK), which allows forward and backward frame stepping. During the visits by the adults, we noted whether each nestling begged (begging) (i.e. if the nestling responded to the adult visit by at least opening its mouth towards the adult), received food or defecated (faeces). Magpies have a throat pouch used to carry food items to the nest, and thus we could not directly quantify the amount of food received by each nestling from video recordings; however, this was estimated using neck collars (see below). We also ranked: (1) nestling body position in the nest with respect to distance to adult location (nest position), (2) height of nestling head whilst begging (height), and (3) the order in which each nestling begged in relation to nestmates (order). Two or more nestlings had the same rank value when differences

between them were not easily discernible. Because both brood sizes vary among nests (as does the number of begging nestlings within nests), we standardized ranking values of nest position, height and order by the formula: $(\text{rank}-1)/(N-1)$ (modified from Smith and Montgomerie, 1991), where N equals brood size for the variable nest position, or the number of nestlings that begged during the adult visit for the variables height and order. Standardized values therefore vary between 0 and 1, so those values closest to 0 correspond to nestlings relatively close to the adult, the highest nestlings or the first to beg.

In order to reduce within-nest variance, we analysed the effect of cyproheptadine treatment within nestling dyads (i.e. paired tests); further, to homogenize nestling mass within dyads, we only used those dyads with nestling mass differences <15 g (18 nestling dyads belong to 11 different nests). Differences in mass between nestlings within dyads were on average $6.37 \pm 0.98\%$ of nestling mass. Thereby, we compared nestlings with similar competitive abilities. In such a situation, begging behaviour within a nestling dyad is assumed to reflect individual differences in interest in receiving food, independently of whether parent or offspring has total control over food distribution (see Royle et al., 2002). We have no data about whether parents or offspring control food distribution during video recordings. However, nestling behaviours associated with sibling negotiation or sibling competition should also be constrained by their associated costs [as is suggested by Royle et al. (Royle et al., 2002) and Johnstone and Roulin (Johnstone and Roulin, 2003)]. Therefore, the net balance of costs and benefits associated with escalated begging behaviour could be estimated using our methodology independently of whether parent or offspring has control over food distribution. The effect of our treatment on begging and faeces was analysed using binomial GLMMs, and the effect of the experimental treatment on the nest position, height and order was estimated using LMMs. Nest identity and nestling dyad (nested in nest identity) were used as random factors, whereas experimental treatment, standardized time $[(x-\text{mean})/s.d.]$ in frames, 1 frame=0.04 s) from the last feeding received (time without food) and nestling weight (g) were included as covariates. For GLMMs, we also included the number of nestmates competing for food in a given adult visit (nestmates begging) as a further covariate. The success of nestlings begging for food was analysed using a GLM with the percentage of feedings each nestling received relative to the number of adult visits in which it begged as a dependent variable, experimental treatment as a within factor, the mass differences within nestling dyad as a covariable and nest identity as a fixed factor.

Estimations of begging behaviour in the laboratory

During May and June 2003 and late in the evening (between 20:00 and 21:30 h), 50 magpie nestlings (8–10 days old) from 22 experimental nests (nine natural broods and 13 parasitized broods) were moved to our laboratory (~30 min by car). We used only one magpie nestling dyad from the same nest, except for three natural broods where we used two nestling dyads per nest. When necessary, nestlings from non-experimental nests were placed in the experimental nests in place of those removed in order to prevent adults from abandoning the nests. Once in the laboratory, the nestlings were administered with their fourth dose of cyproheptadine or water and weighed on a portable digital balance (Sartorius Portable PT600, precision ± 0.01 g; Data Weighing Systems Inc., Elk Grove, IL, USA). Nestlings were kept separately overnight in artificial nests in a soundless room and under a heating lamp (30 to 35°C). The artificial nests consisted of metal nest-shaped moulds lined with raw cotton. Each artificial nest was covered with a small

Table 1. Results from the analyses of video-recordings in magpie nests to assess the effect of treatment with cyproheptadine on begging behaviour and adult food allocation

Variable	Estimate	s.e.m.	z	Pr(> z)
Begging				
(Intercept)	-4.17	1.71	-2.45	0.014
Treatment	3.09	0.95	3.25	0.001
Mass	0.02	0.02	1.12	0.26
Time without food	-2.65	0.61	-4.38	<0.001
Nestmates begging	1.38	0.13	10.51	<0.001
Treatment \times mass	-0.03	0.01	-3.44	0.001
Variable	Estimate	(HPD) 95% CI	P_{MCMC}	
Order				
(Intercept)	0.39	0.22 to 0.60	<0.001	
Treatment	0.04	-0.02 to 0.10	0.17	
Mass	<0.001	-0.002 to 0.001	0.71	
Time without food	0.15	0.03 to 0.256	0.011	
Mass \times time without food	0.002	-0.003 to -0.001	0.003	
Height				
(Intercept)	0.81	0.61 to 1.05	<0.001	
Treatment	0.04	-0.02 to 0.09	0.21	
Mass	-0.004	-0.007 to -0.002	<0.001	
Time without food	0.18	0.07 to 0.29	0.001	
Mass \times time without food	-0.003	-0.004 to -0.001	<0.001	
Nest position				
(Intercept)	0.45	0.16 to 0.69	0.007	
Treatment	-0.04	-0.10 to 0.01	0.13	
Mass	<0.001	-0.002 to 0.003	0.68	
Time without food	0.004	-0.03 to 0.03	0.80	

Analyses are generalized mixed linear models (GLMMs) for begging (788 observations in 229 adult visits) and linear mixed models (LMMs) for order (580 observations in 217 adult visits), height (573 observations in 217 adult visits) and nest position (579 observations in 217 adult visits). Nest identity (11 different nests) and nestling dyad* (nested in nest identity, 18 different nestling dyads) were used as two random factors, whereas experimental treatment, time from the last feeding received (time without food) and nestling mass were included as covariates. For GLMMs, we also included the number of nestmates competing for food (nestmates begging) as a further covariate. We used Wald z-tests [z-values and $\text{Pr}(>|z|)$] for GLMMs, and for LMMs we estimated the highest posterior density 95% confidence intervals (HPD 95% CI) and P -values (P_{MCMC}) from Markov-chain Monte Carlo (MCMC) simulations.

*Nestling dyad, one experimental and one control nestling from the same nest that were of similar mass.

piece of fabric to avoid any visual stimulation that could cause the nestlings to beg. Early in the morning, each nestling was satiated with a mixture of commercial nestling food with water and raw cow heart. After 1 h, each nestling was separately stimulated to beg for food, but was not fed. We encouraged nestlings to beg by gently touching the border of the nest, whistling three times (the stimulus that we previously used to feed the nestlings) and moving our fingers above their heads for 35 s. Nestlings were encouraged to beg every 20 min eight times (begging trials), except for three nests where nestlings were only stimulated seven (one nest) and five times (two nests). After each begging trial, we again covered the experimental nestlings to prevent any further begging efforts by the experimental nestlings. During these begging trials, we were blind to the treatment group of the nestlings.

Nestling behaviour during begging trials was video-recorded. Afterwards, video recordings were watched to collect information on the following variables: begging (nestling responded to the stimulus at least by opening its mouth towards our fingers); begging calls (nestling emitted a sound when begging); body posture (nestling had its tarsi extended for begging); and begging duration

[time (s) that the nestling spent begging in each trial]. After the begging trials, nestlings were again fed until they were satiated. Differences in begging behaviour between experimental and control nestlings were tested using binomial GLMMs (response variables: begging, begging calls and body posture) and LMMs (response variable: begging duration) with nestling treatment (cyproheptadine or water) and brood type (i.e. natural or parasitized broods) as fixed factors, and time (min) from the beginning of the experiment and nestling mass as covariates. Nestling dyad was included in the model as a random factor.

During all trials, faecal sacs were removed and weighed on a portable digital balance (precision ± 0.01 g). Although nestlings defecated mostly during the hand-feeding performed after begging trials, we used data from all faeces successfully weighed.

Neck-collar trials

During May and June 2002 and 2003 and when nestlings were ~10–12 days old (i.e. after their fifth dose), we placed neck collars on all the chicks in a group of parasitized and natural broods. The ligature wire around the neck was tight enough to hinder the swallowing of food and loose enough to avoid strangling the chicks [see Soler et al. (Soler et al., 1995) for a further description of the neck-collar method in magpies]. Nestlings wore the neck collar for a period of 2 to 2.5 h. This period is ~1 h shorter than that previously used in magpies (Soler et al., 1995). Although this shorter experimental period may reduce the hypothetical differences between nestlings of different treatments, it may also reduce the possibility of some nestlings regurgitating boluses of food. The food that was delivered by parents to each nestling during neck-collar trials was kept in absolute ethanol until it was analysed in the laboratory. Biomass of food received by each nestling (mg) was estimated by dry mass of food samples. Food samples were placed in an oven at 60°C for a period of 24 h (i.e. until a constant mass), after which they were weighed (Adam equipment 120/0.0001 g; Adam Equipment Co. Ltd, Bletchley, UK). Nestlings were weighed at the beginning and end of the neck-collar trial and change in body mass was calculated accordingly. Differences in body mass (before–after the experiment) and biomass of food received were standardized by dividing by the duration of the experiment (h). Because a gain in nestling body mass during neck-collar application would mean that the neck collar was not correctly placed, we only used data from those nestlings that lost weight during neck-collar trials. In only 16 of 226 cases did nestlings maintain or gain mass during neck-collar trials. Moreover, we only used in the analyses those nests with data from at least one control and one experimental magpie nestling. Differences in body mass were also used as an estimate of begging effort of nestlings (e.g. Kilner, 2001) during neck-collar trials.

Nests in which regurgitated boluses of food were detected in the nest cup (i.e. they could not be assigned to nestlings) were not included in the analyses and, similar to body mass loss, we included in the analyses only those nests with data from at least one control and one experimental magpie nestlings (i.e. excluding zeros). Consequently, sample sizes for biomass received (33 nests; 17 natural broods and 16 parasitized broods) and body mass loss (59 nests; 31 natural broods and 28 parasitized broods) differed. We used LMMs to explain the effect of the experiment on $\ln(\text{biomass of food received})$ (mg) and $\ln(\text{change in body mass})$ (g) per hour and per nestling. Nest identity was included in the models as a random factor, brood type (i.e. natural or parasitized broods) as a second fixed factor and nestling mass (g) before the neck-collar trial began as a covariable.

Neck-collar trials were performed in 2002 (18 and 41 experimental nests with data for biomass received and body mass loss, respectively) and 2003 (15 and 29 experimental nests with data for biomass received and body mass loss, respectively). Nonetheless, we decided to pool data from these two years because no significant differences between years appeared with respect to biomass received per nestling (LMM, year effect: HPD 95% CI of \ln -transformed values = -0.84 to -0.37 mg h^{-1} , $P_{\text{MCMC}}=0.44$) or body mass loss during the experiment (LMM, year effect: HPD 95% CI of \ln -transformed values = -0.38 to 0.03 g h^{-1} , $P_{\text{MCMC}}=0.09$). Furthermore, the effect of the experiment did not differ significantly between years for biomass received (LMM, interaction between year and treatment factors: HPD 95% CI = -1.10 to 0.54 mg h^{-1} , $P_{\text{MCMC}}=0.46$) or body mass loss (LMM, interaction between year and treatment factors: HPD 95% CI = -0.16 to 0.40 g h^{-1} , $P_{\text{MCMC}}=0.44$). Besides treatment, year and its interaction, nestling mass (g) before the neck-collar trial began was also included as a covariate in these analyses.

The food deprivation during begging trials performed in the laboratory (see above) reproduces that suffered by nestlings with neck collars in the field (i.e. begging but not getting food, see below). In field conditions, adult magpies removed faecal sacs from the nest; thus, it was not possible to know the mass of faecal sacs produced during the neck-collar trials. By weighing faecal production of experimental and control nestlings during the laboratory trials, we were able to test whether any change in body mass of experimental and control nestlings in natural nests with neck collars could be due to previous experience (i.e. food processing in the digestive tract), and whether faecal production was related to nestling body mass (see below). We analysed the relationship between the mass of faecal sacs produced during laboratory trials and nestling mass by estimating the correlation coefficient; moreover, we tested the possible effect of experimental treatment on faeces production (see above) by performing a GLM analysis with treatment as a fixed factor, nestling mass as a covariable and mass of faecal sacs as the outcome variable. Sample sizes were, in this case, reduced to 41 nestlings because in nine nestlings some of the faecal sacs leaked before they were weighed. Nestling mass was positively correlated with the mass of the faecal sacs produced during the laboratory trials ($r=0.33$, $N=41$, $t=2.19$, $P=0.035$), whereas the experimental treatment did not explain a significant proportion of the variance in mass of faeces ($F_{1,38}=0.06$, $P=0.81$). Similarly, during the video recordings of the magpie nests in natural conditions, we found that probability of nestling defecation in a given adult visit was significantly and positively related to nestling mass [binomial GLMM, nestling mass effect (estimate \pm s.e.m.): 0.026 ± 0.005 , $z=2.97$, $\text{Pr}(>|z|)=0.003$, 705 observations (i.e. whether a nestling defecated during a visit), 209 adult visits, 18 nestling dyads in 11 nests], whereas experimental treatment did not affect the probability of nestling defecation (binomial GLMM, treatment effect (estimate \pm s.e.m.): -0.014 ± 0.202 , $z=-0.069$, $\text{Pr}(>|z|)=0.95$, 705 observations, 209 adult visits, 18 nestling dyads in 11 nests). Therefore, the inclusion in the models of the nestling masses before the neck-collar experiment allowed us to statistically control the variation in faecal-sac production among nestlings during the neck-collar trials.

Physical condition of magpie nestlings

During May and June 2002, we estimated the physical condition of control and experimental nestlings from natural broods close to fledging time (ca. 18 days old). We measured nestling mass (spring balance, 300 g ± 1 g), lengths of tarsi (with a digital calliper, ± 0.01 mm), wings and tail (using a ruler, ± 0.1 cm), and cell-mediated

immune response estimated from values of skin swelling elicited by injection of the mitogen phytohemagglutinin (PHA) (reference no. L8754, Sigma Chemical Co., St Louis, MO, USA) (see Tella et al., 2008). Briefly, we injected fledglings subcutaneously in the right wing web with 0.5 mg of PHA dissolved in 0.1 ml of physiological saline solution (Bausch and Lomb). As a control, the left wing web was injected with 0.1 ml of physiological saline solution. Before and 24 h after injection, we measured the thickness of each wing web at the injection site with a pressure-sensitive digital micrometer (model 547-301, ± 0.01 mm; Mitutoyo, Andover, Hants, UK). The immune response variable was then estimated as the change in swelling of the right wing web minus that of the left wing web (Lochmiller et al., 1993). We repeated measurements of each wing web three times and used the mean values in our analyses.

For these analyses, we used a repeated-measures analytical approach to check the possibility that the treatment effects were influenced by nestling hierarchy in body mass. We only used natural broods where at least the two heavier nestling dyads had survived to this age ($N=37$ nests). In order to obtain the balanced statistical design needed for repeated-measures analyses, only data from these two nestling dyads were used. We included the experimental treatment as the first within factor, and the assigned rank in the body mass hierarchies of nestling (i.e. first or second heaviest nestlings within each treatment) as the second within factor. This design allowed us to determine whether the treatment effect was different for different nestling hierarchies (interaction factor). We used a Sigma-restricted model to code categorical factors and the type VI [the effective hypothesis method (Hocking, 1996)] to get the sums of squares. Moreover, we performed a repeated-measures multivariate ANOVA (RM-MANOVA) by using the same design but including all dependent variables describing nestling condition.

RESULTS

Effect of cyproheptadine on begging behaviour and food allocation during video recordings in magpie nests

We found a statistically significant association between experimental treatment and probability of begging during adult visits (begging; Table 1). Nonetheless, we did not find a significant effect of cyproheptadine on further variables related to nestling begging intensity (nest position, height and order; Table 1). In relation to food allocation by magpie adults, we found that the proportion of parent visits where nestlings were fed relative to the total of visits where they begged was greater for the experimental nestlings ($46.81 \pm 2.62\%$) than for their control nestmates ($40.47 \pm 2.33\%$; GLM, treatment effect: $F_{1,6}=7.27$; $P=0.036$). This suggests that experimental nestlings were more efficient when begging for food than control nestmates.

Effect of cyproheptadine on begging behaviour during laboratory trials

Results from the laboratory were similar to those obtained from video recordings in the field. Nestlings experimentally provided with cyproheptadine begged more frequently than their control nestmates (begging, treatment effect; Table 2). The probability of emission of sound (begging calls), body posture during begging and time of begging during a certain trial (begging duration) were not correlated with the experimental treatment (Table 2).

Neck-collar trial

Heavier magpie nestlings received more food [LMM, with $\ln(\text{food received})$ as the dependent variable, nestling mass effect, HPD 95%

Table 2. Results of comparisons between begging behaviour of nestlings with an experimentally increased level of hunger and control nestlings with no treatment during the laboratory trials

Variable	Estimate	s.e.m.	z	Pr(> z)
Begging				
(Intercept)	6.70	3.13	2.14	0.033
Treatment	1.55	0.63	2.47	0.014
Brood type	1.19	0.88	1.35	0.18
Mass	0.08	0.04	-2.12	0.034
Time	0.03	0.01	5.39	<0.001
Treatment \times brood type	2.83	0.88	-3.22	0.001
Begging calls				
(Intercept)	-0.44	2.42	-0.18	0.86
Treatment	-0.41	0.86	-0.47	0.64
Brood type	1.18	0.77	1.54	0.12
Mass	-0.03	0.03	-0.94	0.35
Time	0.00	0.01	-0.59	0.56
Treatment \times brood type	-1.45	0.68	-2.13	0.033
Treatment \times time	0.02	0.01	2.07	0.038
Body posture				
(Intercept)	-0.10	1.72	-0.06	0.95
Treatment	-0.37	0.25	-1.47	0.14
Brood type	-0.02	0.46	-0.05	0.96
Mass	-0.02	0.02	-0.91	0.36
Time (min)	0.02	0.00	6.57	<0.001
Variable	Estimate	(HPD) 95% CI	P_{MCMC}	
Begging duration				
(Intercept)	15.41	9.06 to 20.69	<0.001	
Treatment	0.07	-0.73 to 0.82	0.86	
Brood type	-0.46	-2.13 to 1.17	0.62	
Mass	-0.10	-0.16 to -0.02	0.014	
Time	0.02	0.01 to 0.03	<0.0001	

Analyses are GLMMs for begging (374 observations in 25 groups), begging calls (332 observations in 25 groups) and body posture (327 observations in 25 groups); and LMMs for begging duration (327 observations in 25 groups). Nestling treatment and brood type (i.e. natural or mixed broods) were included in the model as fixed factors, and time from the beginning of the experiment and nestling mass were included as covariates. Nestling dyad was considered as a random factor. For GLMMs, we used Wald z-tests for hypothesis testing [z and $\text{Pr}(>|z|)$]. For LMMs, we estimated the HPD 95% CI and P_{MCMC} .

CI=0.02–0.04 mg h^{-1} , $P_{MCMC}<0.001$] but lost more body mass [LMM, with $\ln(\text{body mass lost})$ as the dependent variable, nestling mass effect: HPD 95% CI=0.003–0.01 g h^{-1} , $P_{MCMC}<0.001$] during the standardized time of the experiment. After controlling for the allometric effects of nestling mass before trials and the possible effect of brood type (see Materials and methods), experimental nestlings received more food [LMM with $\ln(\text{food received})$ as the dependent variable, treatment effect: HPD 95% CI=1.82–5.93 mg h^{-1} , $P_{MCMC}=0.0004$; Fig. 1] but lost more body mass [LMM with $\ln(\text{body mass lost})$ as the dependent variable, treatment effect: HPD 95% CI=0.03–0.31 g h^{-1} , $P_{MCMC}=0.015$; Fig. 1] than control nestlings.

Effect of cyproheptadine on the physical condition of magpie nestlings

Magpie nestlings experimentally treated with cyproheptadine showed a better physical condition than control nestlings when they were ~18 days old (RM-MANOVA, Wilks' $\lambda=0.66$, $F_{5,27}=2.79$, $P=0.037$). Experimental nestlings exhibited a higher immune response than their control nestmates (experimental vs control, 1.25 ± 0.06 vs 1.12 ± 0.07 mm, GLM: $F_{1,34}=4.14$, $P=0.0497$), had a longer tarsus (48.0 ± 0.4 vs 47.2 ± 0.4 mm, GLM: $F_{1,36}=7.31$, $P=0.010$),

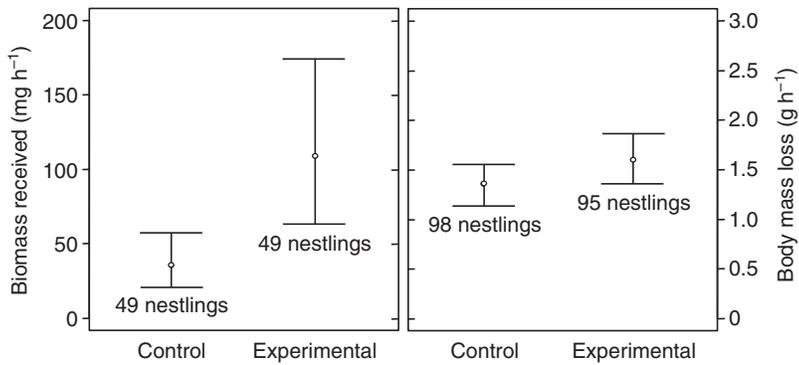


Fig. 1. Partial effect of treatments with cyproheptadine (experimental) or water (control) on biomass received [mg h^{-1} , $N=33$ nests, effect size (i.e. range)=73.88 mg] and body mass loss (g h^{-1} , $N=59$ nests, effect size=0.25 g) during neck-collar trials in magpie nestlings. Values were calculated by back-transformation of estimates obtained from linear mixed models with $\ln(\text{biomass received})$ and $\ln(\text{body mass loss})$ as dependent variables. Values are means \pm highest posterior density (HPD) 95% confidence intervals.

wing (81.9 ± 1.5 vs 79.8 ± 1.4 mm, GLM: $F_{1,36}=5.24$, $P=0.028$) and tail (24.6 ± 0.9 vs 23.3 ± 1.0 mm, GLM: $F_{1,34}=4.35$, $P=0.045$) and a higher body mass (138.3 ± 2.7 vs 132.3 ± 2.9 g, GLM: $F_{1,35}=7.19$, $P=0.011$). Further, the effect of cyproheptadine was similar in the first and second nestling hierarchical dyads (interaction between treatment and rank of nestling dyad: in all cases $P>0.35$).

DISCUSSION

In the present study, by using an appetite stimulant (cyproheptadine hydrochloride), we have for the first time explored the net cost–benefit balance of escalated begging behaviour in natural conditions, where the extra costs of begging might be compensated by the acquisition of extra resources from parents. In accordance with the widely manifested effects of the level of hunger on begging behaviour, we found that experimental nestlings increased the frequency of begging (but not intensity) and received more food than their control nestmates. Our main result was that the net benefit of escalated begging behaviour was positive, i.e. experimental nestlings showed a better physical condition than control nestlings close to the age of nest abandonment. This result suggests that the costs associated with exaggerated begging behaviour do not solely counteract associated benefits as suggested and/or assumed by previous studies (e.g. Kilner, 2001; Rodríguez-Gironés et al., 2001b; Moreno-Rueda, 2010; Noguera et al., 2010). Below we discuss the methodological approach used for manipulating nestling begging behaviour in natural conditions, as well as the resulting experimental effects in a scenario of parent–offspring communication and honest signalling.

The use of cyproheptadine to increase begging behaviour

Cyproheptadine is a type 2 serotonin receptor antagonist. It is assumed that cyproheptadine acts directly on the hypothalamus – cats administered with this drug demonstrated an increased neuronal activity at this site (Chakrabarty et al., 1967) – by modifying the activity of serotonin on the feeding centre (Delitala et al., 1975). Its action as appetite stimulant appears not to be due to a hypoglycaemic-induced hyperphagia or an increase in endogenous growth hormones (Bergen, 1964; Stiel et al., 1970). Cyproheptadine is broadly used in human patients, including children, that suffer from diseases such as cancer, HIV, cystic fibrosis and eating disorders (see Chinuck et al., 2007; Couluris et al., 2008; Mahachoklertwattana et al., 2009), where an increase in body mass is desirable. Cyproheptadine is also used as an antihistaminic because it blocks H_1 histamine receptors (Stone et al., 1961) and, similar to other antihistaminic substances, cyproheptadine has minimal side effects, consisting mainly of transient drowsiness [see Homnick et al. (Homnick et al., 2004) and references therein]. In birds, the effect of cyproheptadine has been tested in two species, chicken (Injidi

and Forbes, 1987; Muralidharan et al., 1998) and domestic pigeons (Gunturkun et al., 1989), where it resulted in a significant increase of food ingestion. Thus, the use of this drug as appetitive stimulant was well founded and we tested its effects on begging behaviour of magpie nestlings.

The use of cyproheptadine as appetite stimulant has several advantages. It is administered orally diluted in water, and thus the treatment can be easily done in the field and during short nest visits. Importantly, experimental nestlings can be administered during development while they are being fed by their parents, who are the receptors of the begging signals. This approach makes possible the estimation of variables related to the net cost–benefit balance associated with the experimentally escalated begging behaviour. In addition, experimental nestlings may also suffer from other possible sources of energetic costs not directly related to the production of the begging signals and difficult to take into account in food deprivation laboratory experiments. For example, the effect of sibling competition (e.g. Johnstone, 1999; Rodríguez-Gironés, 1999; Royle et al., 2002; Neuenschwander et al., 2003), the possible costs related to the nestling vigilance for parent arrival (Roulin, 2001) or those costs suggested for behaviours related to sibling negotiation during the absence of parents (Johnstone and Roulin, 2003). Moreover, because cyproheptadine would exclusively affect hunger level, this experimental approach allow us to disentangle the effects of body condition and nestling hunger level on begging behaviors (e.g. Clark, 2002) to address what offspring are really communicating to their parents *via* their begging signals (need, quality or just hunger) (see Mock et al., 2011).

In accordance with previous results of the effects of hunger level on begging behaviour (e.g. Redondo and Castro, 1992b; Price et al., 1996; Iacovides and Evans, 1998; Sacchi et al., 2002), we found that nestlings provided with cyproheptadine begged more frequently than their control nestmates. In addition, we found that begging signals of experimental nestlings were more effective in attracting parental feeding than those of their control nestmates. This suggests the existence of a treatment effect on non-measured variables of begging intensity. The most important described side effect of cyproheptadine is the reduction of physical activity because of transient drowsiness, which would predict a reduced rather than an increased begging activity. Thus, the detected effects on begging behaviour are not explained by the drug side effects but by the increased feeling of hunger of the experimental nestlings.

Evidence of short-term benefits and costs of escalated begging behaviour

Experimental nestlings received more food than their control siblings did during the neck-collar trials. Therefore, based on our

results on the influence of hunger level on begging behaviour and the extensive literature concerning this issue (e.g. Smith and Montgomerie, 1991; Cotton et al., 1996; Kacelnik et al., 1995; Kilner, 1995; Kölliker et al., 1998), relative to the use of begging by parents to determine which chick to feed (e.g. Smith et al., 1988; Ottosson et al., 1997; Kilner and Johnstone, 1997; Burford et al., 1998) (but see Clark and Lee, 1998), the effect of our experimental treatment on biomass received during neck-collar trials by each nestling was probably mediated by a change in begging behaviour associated with the experimentally increased level of hunger.

The neck-collar approach may, however, have methodological problems that could affect to the interpretation of the results. For instance, feeding decisions of parents might depend on detecting food in the nestling's mouth, or adults might remove non-swallowed food from one nestling and place it in another chick's mouth. However, rather than predicting more food in the gullet of cyproheptadine-treated nestlings, the above scenario predicts more equally distributed food among nestlings. We compared experimental and control nestlings from the same nest, and thus our result of experimental nestlings receiving more food than their control siblings likely does arise from our experimental approach (increased level of hunger). In accordance with this interpretation, results from video recordings indicate that parents selected experimental nestlings among nestlings that beg for food. Parents should preferentially feed the hungriest nestlings in the nests (i.e. those with more exaggerated begging behaviour); thus, because the hunger level of experimental nestlings was increased, a more efficient begging behaviour of cyproheptadine-treated nestlings can be predicted. Moreover, during neck-collar trials, nestlings cannot be satiated by parents, and nestlings would therefore beg for food during more feeding visits than in natural conditions (i.e. video recordings). This scenario is therefore the most plausible explanation of the resulting larger biomass received by experimental nestlings during the neck-collar trials.

Rodríguez-Gironés et al. reported a delayed growth of magpie nestling chicks in relation to experimentally increased begging activity (Rodríguez-Gironés et al., 2001b), which is likely to affect probability of survival of magpie nestlings (Husby and Slagsvold, 1992; Ponz Miranda et al., 2007) (M. Molina-Morales and J. G. Martínez, unpublished data). This suggests that escalated begging would be a costly behaviour for magpie nestlings. In our case, experimental nestlings lost more mass than their control nestmates during the two and a half hours that nestlings wore neck collars (Fig. 1). This result cannot be explained by drowsiness, which is the main side effect of cyproheptadine (see above) and from which the expected influence on mass loss is just the opposite to that detected (i.e. weight gain) (see Stiel et al., 1970). Another possibility is that, because experimental nestlings may have ingested more food before the experiment, they may also have produced more and/or larger faecal sacs during the time that nestlings wore neck collars. However, this is unlikely for two reasons. First, our analyses were statistically controlled by body mass, a variable that is positively related to the production of faecal sacs of nestlings in the laboratory and in video recordings in the field. Second, experimental treatment did not explain either faecal weight or probability of defecation. An alternative explanation would be that because faecal sac production often occurs simultaneously with feeding, the greater mass loss of experimental nestlings might be the direct consequence of being preferentially fed by parents. We have no data to discount this possibility; therefore, although differences in begging effort between experimental and control nestlings is a tentative explanation of the

resulting differences in mass loss during collar trials, this conclusion should be cautiously interpreted.

Net cost–benefit balance of exaggerated begging behaviour during the nestling period

If the costs associated with escalated begging behaviour surpass the extra benefits, as is assumed by some theoretical models (for reviews, see Mock and Parker, 1997; Godfray and Johnstone, 2000; Budden and Wright, 2001; Johnstone and Godfray, 2002; Royle et al., 2002), and the direct costs are physiological and/or energetic, we should find a negative effect of our experiment on nestling phenotypic quality. Contrary to this expectation, experimental nestlings showed a better physical condition than their control siblings when abandoning the nest. Briefly, we found that after controlling for the effect of body-size hierarchy, experimental nestlings showed a higher immune response and larger body mass and tarsus, tail and wing lengths than their control siblings. Because these variables are related to the probability of nestling survival (see Introduction and above), these results suggest that magpie nestlings could escalate some aspect of their solicitation signals and gain more resources that are plausibly connected to enhanced personal fitness. Therefore, these results give rise to an interesting evolutionary question, namely, why has natural selection not shaped magpie begging behaviour to this higher level?

Apart from non-functional explanations including physiological or phylogenetic constraints, responses to this question could be related to the existence of costs other than the energetic costs associated with the escalation of begging behaviour and suffered during development. One possibility is the existence of costs related to risk of predation, because predators would more easily detect nests with nestlings that beg loudly (e.g. Redondo and Castro, 1992a; Haskell, 1994; Leech and Leonard, 1997; Briskie et al., 1999; Dearborn, 1999; Haskell, 2002). However, and as mentioned previously, these costs are mainly suffered by the whole brood, and different costs for nestlings that beg and do not beg are necessary for explaining the evolution of begging behaviour as an honest signal [see Rodríguez-Gironés et al. (Rodríguez-Gironés et al., 2001b) for further discussion], and thus this cost would not prevent an escalation of dishonest nestling behaviour. Further, predation cost would only constrain those signals whose exaggeration implies an increased nest detectability for predators, as is the case for vocalizations, but not for other kinds of signals such as visual ones. Another cost that might prevent escalation of begging behaviour is that related to the loss of indirect fitness (e.g. Briskie et al., 1994; Johnstone, 1998; Lotem, 1998). Bergstrom and Lachmann (Bergstrom and Lachmann, 1998) argued that a cost-free signalling equilibrium is possible between parent and offspring if parents and/or siblings pay a disproportionately extra cost for dishonest signalling and these indirect fitness costs for escalated signallers outweigh the direct benefit that they gain. Other non-explored costs associated with escalated begging behaviour are those related to the costs of rearing dishonest offspring. If begging behaviour has a genetic basis (see Kölliker et al., 2000; Kölliker and Richner, 2001; Dor and Lotem, 2009), nestlings with an escalated begging display would rear offspring with an escalated begging display. This possibility was proposed by Alexander, who stated that a certain allele causing the dishonest trait in an offspring that, because of its selfish behaviour, receives more than the optimum parental inversion could not spread because the advantage of this allele to the young offspring would be counteracted by the disadvantage of producing dishonest offspring when adult (Alexander, 1974). Although this idea was controversial (e.g. Dawkins, 1976; Blick, 1977), costs of rearing

offspring with inherited begging behaviour should be added as a further cost in the equation explaining the maintenance of signal honesty (Dawkins, 1976). In agreement with the importance of such costs, Kölliker et al. recently modelled the evolution of traits that confer benefits to individual offspring and are positively related to parental investment, and found that under antagonistic selection a zone for equivalent co-adaptation outcomes exists in which stable levels of parental inversion can evolve and be maintained (Kölliker et al., 2010). They also concluded that the modelled antagonistic co-adaptation between parents and offspring to begging-related traits would explain the evolutionary stability of these traits independently of the existence of begging costs (Kölliker et al., 2010).

A long-term study in which parents and offspring are monitored for several generations, however, is necessary to test the role of indirect fitness and genetic effects in the maintenance of honesty in parent-offspring communication. Here, our results strongly suggest that energetic costs associated with exaggerated signalling are not sufficient to explain the begging behaviour of magpies. These results, therefore, urge us to explore costs, other than energetic, associated with begging display that could prevent the evolution of escalated signalling. We hope the experimental approach described and tested in the present study will facilitate the discovery of such costs in future experimental studies.

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