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Feathers, suspicions, and infidelities: an experimental study on parental care and certainty of paternity in the blue tit

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Theoretical models on parental care predict that males should decrease their parental effort when paternity is in doubt. Males may use some cues to assess their certainty of paternity, and try to avoid rearing offspring sired by extra-pair males. We have previously reported in a socially monogamous passerine, the blue tit (*Cyanistes caeruleus*), that males decorate their nests with feathers, and that when this ornament is manipulated, males appear to have suspicions about the presence of an intruder male. Here, we decrease the male's certainty of paternity through experimental feather supplementation to analyse whether the outcome of our experiment supports the assumptions of the parental care theory. Male *C. caeruleus* responded to the feather supplementation experiment by reducing their parental investment (feeding frequency and nest defence) in comparison with control males. The occurrence of extra-pair offspring in experimental nests was double than that in controls. This suggests that the manipulation was successful not only in altering males' perceived paternity, but also, indirectly, the actual paternity. Furthermore, males that gained extra-pair young also had a higher than average probability to lose paternity in their nest, which may imply that male *C. caeruleus* faced a trade-off between obtaining extra-pair fertilizations and maintaining paternity in their own nest. Overall, this study supports the idea that males are prone to decrease their parental effort when they perceive that the risk of losing paternity is high. © 2013 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2013, **109**, 552–561.

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INTRODUCTION

A fundamental prediction of the parental care theory is that parents invest in their offspring according to the reproductive value (i.e. genetic relatedness) of their young (Trivers, 1972). How males respond in terms of parental care when faced with a real or apparent loss of paternity has been the subject of a large body of theoretical and empirical research (Alonzo & Klug, 2012). According to optimality models, the optimal parental investment of the male should be influenced by his share of paternity (Whittingham, Dunn & Robertson, 1992; Westneat & Stewart, 2003; Houston, 1995). As extra-pair paternity is a widespread phenomenon in socially monogamous birds (Griffith, Owens & Thuman, 2002; Kempenaers & Schlicht, 2010), the relationship between parental care and paternity has been explored in a wide range of species (reviewed in Whittingham & Dunn, 2001; Sheldon, 2002). The results of these studies are inconsistent, in part because of the diverse methods employed to relate these two variables. Some of these methods are regarded as unsuitable to address the key question,

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i.e. if male parental care decreases when the likelihood of being cuckolded increases. Kempenaers & Sheldon (1997) argued that a causal relationship between paternity and paternal care cannot be established in most studies relying on correlations across individuals, because of the potential influence of several other variables (e.g. condition; for a thoughtful discussion on this topic see Kempenaers & Sheldon, 1996, 1998; Sheldon, 2002). Experimental studies seem to be the most appropriate approach to establish a causal link between paternity and paternal care, thereby avoiding the problems associated with confounding factors (e.g. quality of the territory or of the individual); however, a difficulty that must be taken into account when designing these kinds of experiments is that it is unlikely that males can directly estimate their own paternity. Thus, the manipulated variable should be a third one (a surrogate variable) that correlates with the parameters of interest, i.e. certainty of paternity (see more below).

In this study, we use the blue tit *Cyanistes caeruleus*, a socially monogamous hole-nesting passerine, as a study species. Extra-pair fertilizations are common in *C. caeruleus*, typically ocurring in 40–60% of broods (for a review, see Vedder, 2011). Behavioural observations suggest that fertile females conduct forays into the territories of other males in search of extra-pair copulations (Kempenaers *et al*., 1992). On the other hand, male *C. caeruleus* often intrude into neighbouring territories, but in the presence of the male partner, intruding males are usually detected early and immediately chased out of the territory (Kempenaers, Verheyen & Dhondt, 1995). Such behaviour (male harassment) is likely to be especially frequent at high densities (Charmantier & Perret, 2004).

We have reported elsewhere (Sanz & García-Navas, 2011) that *C. caeruleus* breeding at Montes de Toledo (central Spain) are characterized by behaviour not previously described in this species: males regularly place feathers of a variety of species in their nests, with an ornamental purpose (feathers are deposited outside the nest cup in a conspicuous manner). This post-mating display may be used by females to ascertain the phenotypic quality of the partner (parental quality) and modulate their investment in reproduction. We tested this hypothesis experimentally, and found that in the nests to which we added feathers, females laid larger clutches (Sanz & García-Navas, 2011). However, a side effect of our feather-addition experiment was that introduced feathers increased the male desertion rate in comparison with the control group (Sanz & García-Navas, 2011). A possible explanation is that male *C. caeruleus* may infer the existence of a second male from the presence of foreign feathers, and thus invest less in the care of the brood because of a reduced confidence in paternity. In this sense, indeed, it has been suggested that males may use some cues that provide them with information about their certainty of paternity in the current breeding attempt (Kempenaers & Sheldon, 1997: fig. 1). Most studies performed with the aim of manipulating certainty of paternity have employed temporary removals of one of the pair members (female detention, e.g. Wright & Cotton, 1994; Sheldon, Rãsãnen & Dias, 1997; Kempenaers, Lanctot & Robertson, 1998; Valera, Hoi & Kristin, 2003; male detention or removal, e.g. Lifjeld, Slagsvold & Ellegren, 1998; Møller, 1998; Sheldon & Ellegren, 1998). These studies assume that males use the absence of their mate as a cue to assess their paternity. The major flaw of this method is that the manipulation involves the individual itself, which may influence its physiological condition and/or provisioning behaviour. More recent studies have employed other methods without involving the capture of adults. In birds, Mougeot, Arroyo & Bretagnolle (2001) used decoy presentations as a means to manipulate the perception of risk of extra-pair copulation by male Montagu’s harriers *Circus pygargus*. In fishes, Neff (2003) manipulated a visual cue (the presence of parasitic cuckolder males) used by male bluegill sunfishes *Lepomis macrochirus* to assess their paternity. In either case, the main goal of these kinds of experiments is not necessarily to change paternity in the brood, but to change the male’s perception about the female partner’s fidelity (Kempenaers *et al*., 1998). The rationale of this approach is that any male response must be based upon a male’s certainty of paternity, and not on actual genetic parentage, as there is evidence that male birds are not able to discriminate between their own and extra-pair offspring (Kempenaers & Sheldon, 1996; Penn & Frommen, 2010).

Here, we test indirectly whether the addition of feathers to the nest is perceived by male *C. caeruleus* as a reduction in their certainty of paternity. According to optimality models on paternal investment, males should expend less parental effort when their certainty of paternity within a brood is low (e.g. Whittingham *et al*., 1992; Houston, 1995). We first examine how *C. caeruleus* respond to the manipulation of this male ornament, which is also used to convey information in an intrasexual context (i.e. male–male interaction). Second, we study male and female parental investment (in terms of feeding effort and defence against potential predators) in control and experimental nests. Third, we test whether the addition of feathers has an effect on the rate of extra-pair paternity. Finally, we analyse the consequences of the experimental manipulation on breeding performance (fledgling success and offspring body condition).

MATERIAL AND METHODS

Study area and general field procedures

The study was conducted over two consecutive breeding seasons (2010–2011) on a *C. caeruleus* nesting box population at Quintos de Mora (39°25′N, 4°04′W, Toledo, central Spain). The area consists of deciduous forest dominated by Pyrennean oak, *Quercus pyrenaica*, with 100 wooden nesting boxes erected at 30–40-m intervals. Nesting boxes were inspected daily throughout the breeding season until every fledgling left the nest. The breeding traits were: laying date (defined as the date the first egg is laid); clutch size; number of hatchlings; and number of fledged young. Parents were captured by means of spring traps while feeding nestlings, at 8 days after hatching. Upon capture, adults were tagged with metal sex-specific coloured bands, sexed (based on the presence/absence of a brood patch), and aged as first-year breeders or older (based on plumage characteristics; Svensson, 1992). On day 13 after hatching, nestlings were banded, weighed using a portable electronic balance (accuracy ± 0.1 g), and their tarsus length was measured to the nearest 0.01 mm using a digital calliper (for more details on field routines, see García-Navas, Ferrer & Sanz, 2012). In 2010, adults and nestlings were bled for parentage analyses by brachial venipuncture. Blood samples (about 20 µl) were stored in 96% ethanol (in the case of adults) or on Whatman FTA cards (in the case of nestlings; Whatman Ltd, Kent, UK).

Experimental feather supplementation

In the field, the average number of feathers found per nest in these populations is six (mean ± SE, 6.41 ± 0.65; range, 0–30; $N = 110$). We experimentally manipulated the number of ornamental feathers in 66 *C. caeruleus* nests (2010, $N = 16$ control nests, $N = 16$ experimental nests; 2011, $N = 17$ control nests, $N = 17$ experimental nests) to induce the males of manipulated nests to infer the presence of a second male. We used commercial feathers (Almacenes Cobian SA, Madrid, Spain) of similar size (80–90 mm in length), and dyed orange and blue. We chose these colours because they seem to be preferred and easily detectable by *C. caeruleus* (Sanz & García-Navas, 2011; V. García-Navas, pers. observ.). We assigned each nest to either the experimental or the control groups at the beginning of the egg-laying period, once the nest-building period was complete (see Fig. 1). We took

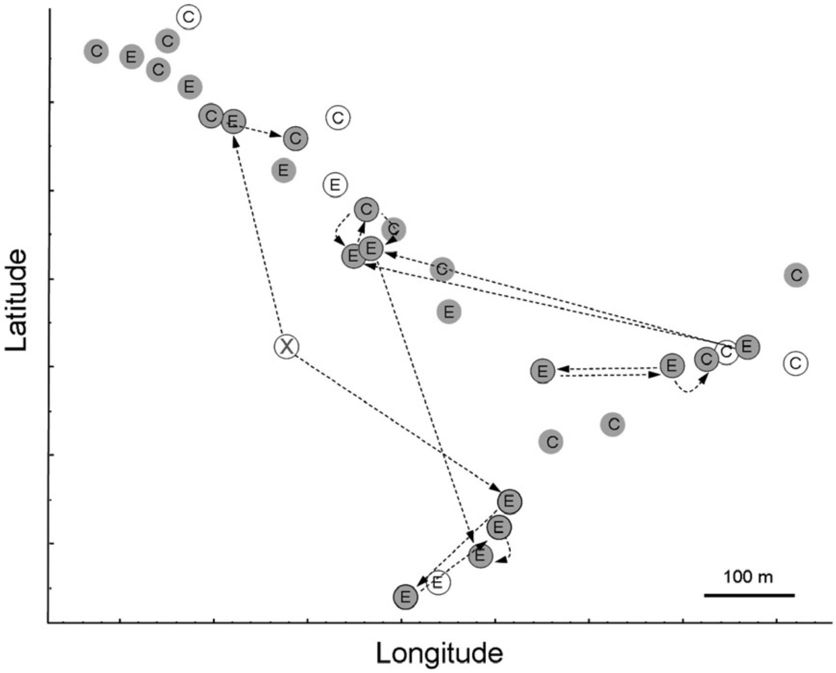

Figure 1. Map showing the spatial distribution of experimental (E; $N = 16$) and control (C; $N = 16$) nests in the study area in 2010, and the extra-pair mating behaviour of blue tits (*Cyanistes caeruleus*) from a male perspective (i.e. assuming that the male is responsible for moving into other territories). Arrows originate in the territory of the 'cuckolding male' and point at the territory of the extra-pair female ('cuckolded male'). Grey circles indicate active nests of *C. caeruleus*; white circles were not included in paternity analyses (in most cases because one of the parents was not captured). The cross indicates a male whose nest was not identified (i.e. a floating individual or a breeding individual from a natural cavity).

into account whether the nests were originally decorated. Nests in which we noted that *C. caeruleus* were beginning to add feathers were assigned to the experimental group, and those in which we perceived that this behaviour (feather carrying) was absent or rare were assigned to the control group. In control nests we did not add or remove feathers, so that the number of feathers added by *C. caeruleus* naturally (if any) was unaltered. In experimental nests we added five feathers: three orange and two blue. Feathers were placed outside the nest cup, simulating the natural pattern observed in these populations (for additional details, see Sanz & García-Navas, 2011). Experimental and control nests were checked every day until the end of the egg-laying period. Control nests were exposed to a similar level of disturbance as the experimental nests (for less than 3 min). At each visit, we counted the number of supplemented feathers and uncovered feathers that were buried with nesting material by *C. caeruleus*. We replaced any feathers that had been removed by *C. caeruleus* (see below). In this way, we tried to ensure that experimental nests always contained supplemented 'foreign' or 'non-family' feathers. Experimental nests were classified into two types: nests with and without feather-removal behaviour. There were no differences in laying date or brood size on day 10 after hatching between treatments (both *P* values > 0.2).

In a subsample of experimental nests ($N = 10$) we filmed the feather-removal behaviour of *C. caeruleus* in order to identify the sex responsible for this activity. We used a handycam (Sony DCR-SR290E, Tokyo, Japan) placed on a tripod at a distance of > 5 m from the nest, and set in such a way that the entrance to the nest was visible in frame. Video recordings (mean duration, 120 min) took place a few minutes after we introduced feathers to the nest, and occurred opportunistically throughout the study period.

Cyanistes caeruleus is an unsuspecting species, whose less elusive character makes it a good model for experimental studies. Several authors have manipulated some nest characteristics (e.g. the presence of aromatic plants) or exchanged nests without causing significant changes in the provisioning behaviour of *C. caeruleus* parents (e.g. see Tomás *et al*., 2007; Mennerat *et al*., 2008, 2009). For instance, the insertion of model eggs (Vedder *et al*., 2007), small trays (Grieco, 2002), or Petri dishes (Tomás *et al*., 2008, V. García-Navas, pers. observ.) into the nesting box did not lead to an increase in the desertion rate of males or a decrease in the frequency with which these males fed their young. *Cyanistes caeruleus* accept these kind of manipulations well, and rapidly resume their feeding activity after a disturbance (V. García-Navas, pers. observ.). Thus, there is no *a priori* reason to think that the result of our experiment is linked to the disturbances caused by manipulating the nest (irrespective of the manipulated trait). If so, both sexes should respond to the addition of feathers in a more or less similar way (by decreasing their feeding rates in comparison with that of the pairs in control nests).

PATERNITY ANALYSES

We obtained samples corresponding to 26 families (12 control broods and 14 experimental broods). DNA was isolated from blood samples using NucleoSpin Tissue Kits (Macherey-Nagel, Duren, Germany). *Cyanistes caeruleus* were genotyped using 14 polymorphic microsatellite markers developed for *C. caeruleus* and other passerine species. The primer details, PCR amplification conditions, and other details are described in García-Navas, Ortego & Sanz (2009). Genetic parentage was determined by using a likelihood-based approach in CERVUS 3.0 (Kalinowski, Taper & Marshall, 2007). We inspected progeny arrays for allelic mismatches with the putative (social) mother. Nestlings that mismatched those of the social mother at more than one locus were considered cases of intraspecific brood parasitism, and were excluded from analyses. We then compared nestling genotypes with those of the social parents. If a nestling mismatched their social father at more than two loci, the social father was excluded as a sire, and this was considered to be a case of extra-pair paternity (EPP). For all cases of EPP, we searched for the extra-pair sire among all breeding males for complete matches (for details of a similar procedure, see Bouwman & Komdeur 2005). The array of loci used had a total exclusionary power of 0.99. To analyse levels of EPPs, we recorded both the proportion of extra-pair offspring (EPO) and the proportion of broods containing EPO (extra-pair broods, EPBs).

PARENTAL INVESTMENT

In the 2011 breeding season we monitored the provisioning effort of *C. caeruleus* parents by means of video recordings. One day prior to filming we replaced the original nesting box with another adapted to house a handycam, such that the birds could get habituated to this device. Taping sessions were carried out on the day that the chicks reached 10 days of age. Each nest was recorded for 90 min and the first half hour was discarded because birds took time (5–15 min) to resume feeding activity after the disturbances from setting the equipment. We scored the number of male and female feeding visits during the last 60 minutes of the recording for a total of 26 pairs ($N = 14$ control nests; $N = 12$ experimental nests).

In 2011, we also recorded whether parents were present while the nestlings were manipulated, and if they exhibited anti-predator behaviour when the integrity of their offspring was threatened by our presence. We noted whether adults performed anti-predatory displays (gliding flights, hissing display) while we ringed and measured the nestlings on day 13 after hatching.

The cases in which one of the parents was not observed feeding the young, and was not seen again later (e.g. exhibiting defence behaviour to protect the nestlings while they were being handled), were excluded from the analyses to avoid false zeros, as such individuals were assumed to have deserted the nest.

Data analyses

During two breeding seasons, a total of 66 *C. caeruleus* nests were assigned to one of the two treatments ($N = 33$ control nests; $N = 33$ experimental nests). In 2010, 26 complete families ($N = 12$ control; $N = 14$ experimental) were sampled to obtain parentage data. Six families were not genotyped (mostly because we failed to capture both parents), and were then excluded from paternity analyses. In 2011, we gathered information on parental care (feeding effort, defence behaviour) for a total of 26 nests ($N = 14$ control; $N = 12$ experimental). The effect of the treatment on parental feeding rates was assessed by comparing the two experimental groups by means of Student's *t*-tests. Proportions (number of broods containing EPO with respect to the total number of broods analysed, frequency of occurrence of nest-defence behaviour) or percentages (% EPO per brood) were compared using non-parametric tests (2 × 2 contingency tables, chi-square test). We used general linear mixed models to test for the effect of the experimental treatment on breeding success and nestling condition in both years. Models included study year and treatment as categorical variables, female ID as a random factor, and two explanatory terms that may affect offspring development: laying date and brood size. Body mass was corrected by regression for body size (tarsus length). We used a backward stepwise procedure to remove non-significant variables and interactions from the models. Statistics are only reported for the variable of interest (i.e. treatment), and those that had a significant effect on the dependent variable. Percentages were arcsine square root transformed. Sample sizes varied slightly among analyses because of missing data for some variables in some nests. All tests are two-tailed and means ± SEs are presented. All statistics were carried out using STATISTICA 7 (Statsoft Inc. Tulsa, OK, USA) and SAS 9.1 (SAS Institute, Cary, NC, USA).

RESULTS

Males' response to the treatment

In more than 75% of nests in which we added artificial feathers we found evidence that *C. caeruleus* removed or hid the feathers (2010, 75%, 12/16; 2011, 88%, 15/17). In most of the cases we observed that some or all feathers were expelled from the nest (92%, 25/27; Fig. 2). Sometimes, the time elapsed between the addition of the feathers and their removal by the bird was less than an hour. On a few occasions (7% 2/27), feathers were not removed but were hidden with nesting material. Both strategies (feather removal and feather concealment) were recorded in some nests (33%, 9/27; Fig. 2). In all cases in which we gathered observational data on the removal of feathers from the nest, we found that only the social male was involved in this behaviour ($N = 8$). The frequency with which males visited the nest was 1.4 visits per hour (range, 0–3.5, $N = 10$).

Of the 33 experimental nests decorated with feathers, two (6%) were deserted during the egg-laying phase; during the brood-rearing period, two (6%) were deserted by both parents (7–9 days after hatching), two (6%) were deserted by the male, and one (3%) was deserted by the female. In the control group, two nests (5%) were deserted prior to egg hatching and no nest was deserted during the chick-rearing period.

Paternity

A total of 52 adults and 171 chicks from 26 broods were genotyped for paternity analyses. Almost half of the sampled broods (46%, 12/26) contained at least one offspring whose genotype mismatched those of their observed fathers. Twenty of 171 chicks (14%) were the result of extra-pair fertilizations. The proportion of EPBs differed significantly between treatments. More than half of the experimental broods contained nestlings that were sired by an extra-pair male (experimental, 64%, 9/14; control, 25%, 3/12; $\chi^2 = 4.01$, $P = 0.04$). There was no difference in the percentage of EPO per brood between experimental and control nests (experimental, 13%, range 0–33; control, 10%, range 0–50; $t = 0.36$, $P = 0.72$). Males that obtained extra-pair copulations were more likely to lose paternity in their own nests (Fig. 1). Seven of the eight males (88%) that sired extra-pair young also lost paternity with their social mate, whereas more than half (72%, 13/18) of males that did not engage in extra-pair copulations avoided being cuckolded ($\chi^2 = 7.95$, $P = 0.005$). Cuckolding males did not obtain any overall advantage in terms of gained paternity because the number of extra-pair chicks ('gains') was not significantly higher than the number of 'paternity

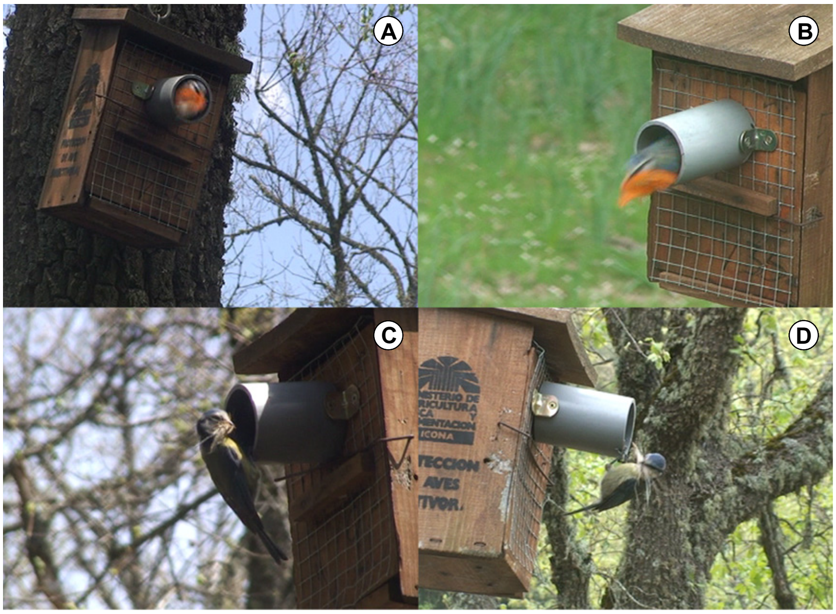

Figure 2. Examples of the behaviour of the males observed in response to the feather-addition treatment: male blue tits (*Cyanistes caeruleus*) removing feathers from their nest (A, B); a male introducing a 'natural' feather (pigeon down) to the nest (C); and a male introducing new nesting material (straw, dry grass) to cover the feathers added experimentally by researchers (D). All pictures were taken once the nest construction period was finished. The nesting boxes are protected with wire mesh, and a plastic pipe is fixed to the entrance to deter predators.

losses' (number of extra-pair gains, 2.25 ± 1.48, range 1–5; number of within-pair losses, 1.43 ± 0.53, range 1–2; $t = 1.37$, $P = 0.19$).

EFFECTS OF THE TREATMENT ON PARENTAL CARE

We found an effect of treatment on the frequency with which male *C. caeruleus* provisioned their young. Chicks from experimental nests were fed less frequently than those from control nests (Fig. 3; experimental, 5.83 ± 6.46, $N = 12$; control, 11.77 ± 7.21, $N = 14$; $t = 2.09$, $P = 0.04$). There was no effect of treatment on female provisioning rates (Fig. 3; experimental, 10.36 ± 4.16, $N = 12$; control, 10.91 ± 6.00, $N = 14$; $t = 0.15$, $P = 0.78$). When considering both sexes jointly, we did not find an effect of adding feathers on the total number of provisioning events (feeds per h: experimental, 17.17 ± 6.95, $N = 12$; control, 21.29 ± 8.92, $N = 14$; $t = 1.29$, $P = 0.20$).

The frequency with which male *C. caeruleus* defended their nestlings while they were manipulated was significantly lower for experimental nests in comparison with control nests (experimental, 17%, 2/12; control, 79%, 11/14; $\chi^2 = 9.90$, $P < 0.01$). In the case of females, the difference between treatments was not significant (experimental, 50%, 6/12; control, 79%, 11/14; $\chi^2 = 2.33$, $P = 0.12$).

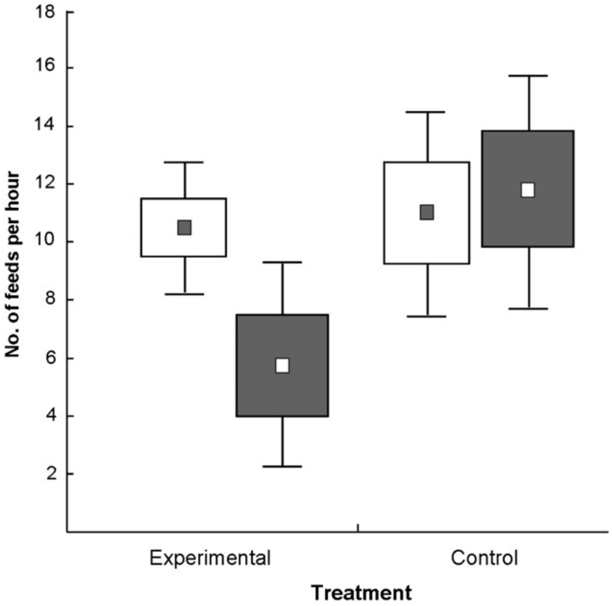

Figure 3. Effect of the treatment (experimental versus control) on the provisioning effort (feeds per h, means ± SEs) of males (filled boxes) and females (empty boxes) on day 10 after hatching.

BREEDING SUCCESS AND NESTLING CONDITION

There was no effect of treatment on the proportions of fledged young (experimental, 80.07 ± 4.79; control, 83.55 ± 4.72; $F_{1,51} = 0.73$, $P = 0.39$). Nestling mass did not differ significantly between experimental and control nests after controlling for other influencing variables (experimental, 9.91 ± 0.20; control, 10.19 ± 0.14; treatment, $F_{1,45} = 0.05$, $P = 0.82$; laying date, $F_{1,45} = 6.26$, $P = 0.01$; brood size, $F_{1,45} = 6.52$, $P = 0.01$; nestling tarsus length, $F_{1,45} = 21.3$, $P < 0.001$). Likewise, neither was there a significant effect of treatment on nestling tarsus length (experimental, 15.49 ± 0.09; control, 15.62 ± 0.12; treatment, $F_{1,48} = 0.07$, $P = 0.79$; laying date, $F_{1,48} = 12.29$, $P < 0.001$).

DISCUSSION

We found that male *C. caeruleus* responded to the feather supplementation experiment by reducing their parental investment in comparison with males whose nests were not manipulated. Our experiment suggests that male *C. caeruleus* may infer the existence of a second male and perceive an increased risk of cuckoldry by the presence of 'foreign' feathers in the nest. Our findings are thus in agreement with theoretical models predicting that when the genetic contribution to progeny is decreased by cuckoldry, males will reduce their care of the offspring in their nest (Winkler, 1987; Westneat & Stewart, 2003). Some previous studies have found correlational evidence for this prediction (e.g. Burke *et al.*, 1989), whereas others found no adjustment of paternal effort with decreased paternity (e.g. Westneat, 1995; Bouwman, Lessells & Komdeur, 2005; García-Vigón, Veiga & Cordero, 2009). Here, we did not directly relate parentage and parental effort, but we used a third variable (certainty of paternity) to establish a relationship between paternity and the extent of paternal care (Kempenaers & Sheldon, 1997; Sheldon, 2002). In this regard, it should be stressed that it is practically impossible to measure such a thing as 'certainty of paternity' (Kempenaers & Sheldon, 1997). Nonetheless, we obtained evidence that the perceived paternity of males was decreased by the experimental treatment, and that it was successful in affecting the male's parental care. Male *C. caeruleus* decreased their provisioning effort and tended to reduce their invesment in the defence of offspring against potential predators when they found 'foreign' feathers introduced to their own nest. As far as we know this study is the first to provide experimental evidence that *C. caeruleus* can alter their paternal investment in response to their confidence in paternity. Our findings are consistent with those found by Neff (2003) in fishes. He found that when the perceived paternity of male bluegill sunfishes was experimentally reduced, they tended to abandon their nests and that their willingness to defend the brood from a potential predator was lower in comparison with the control males (Neff, 2003).

From the females' point of view, one can argue that it would be reasonable to expect a greater share of maternal effort at experimental nests because the presence of supplemented feathers (an indicator of male quality) may induce them to increase their investment in reproduction ('differential allocation', reviewed in Sheldon, 2000; Horváthová, Nakagawa & Uller, 2012). As reported in a previous study, we found that the presence of feathers induced females to lay slightly larger clutches (Sanz & García-Navas, 2011, see also García-López de Hierro, Moleón & Ryan, 2013). However, we did not find differences in provisioning rates between females from experimental and control nests. This means that females from experimental nests did not appear to compensate for the decrease in male investment. This result is striking, considering that we have previously reported that the relative contribution of females to offspring feeding was lower in nests decorated with feathers, compared with undecorated nests (Sanz & García-Navas, 2011). That is, females from nests decorated (naturally or experimentally) with feathers tended to decrease their parental effort when the partner contribution was high, but were unwilling to increase their share of provisioning in the opposite situation. In other words, females were sensitive to changes in their partner's contribution only when it suited them. In previous breeding seasons, we observed that chicks from nests naturally decorated with feathers were significantly heavier than those from nests in which such behaviour was not detected, and that the absolute number of fledged young was also significantly higher in the former (Sanz & García-Navas, 2011). In the present study, the lower contribution of males to the care of young observed in experimental nests could explain the absence of an effect of the treatment on fledgling success or nestling condition.

A remarkable finding of this study was that males tended to remove or hide the feathers that we added (Fig. 2). In these populations, *C. caeruleus* often use feathers to decorate their nests, as has been previously reported in other species (e.g. the spotless starling, *Sturnus unicolor*, Polo & Veiga, 2006; Veiga & Polo, 2011; firewood-gatherer, *Anumbius annumbi*, Zyskowski & Prum, 1999; K. Delhey, unpubl. data; rock sparrow, *Petronia petronia*, V. García-Navas, unpubl. data). In a previous study, in which we supplied *C. caeruleus* with feathers piled in the nest surroundings, we observed that males made use of this resource as soon as they discovered it

(Sanz & García-Navas, 2011). In the present study, we observed that some of the feathers that were expelled from the nest by its owners were later reused by other individuals (V. García-Navas, pers. observ.). Therefore, it is reasonable to think that *C. caeruleus* just perceived the feathers that we added as being introduced by male intruders. Such a perception resulted in the resident males hiding or removing them. Nest ornamentation may also play an informative role against potential rivals, and have implications on male–male interactions. In a recent study with *C. caeruleus*, Holveck *et al*. (2010) found that males visited their nest multiple times during the egg-laying period. Moreover, they reported that a large proportion of males visited the nests of neighbouring males. The frequency of nest visits that we reported for experimental nests was higher than that observed by Holveck *et al*. (2010) in unmanipulated conditions, a fact that could be explained as a male response to the supplementation of 'foreign' feathers. Males of several bird species frequently add bizarre materials to the nest (fur, cocoons, bones, and plastic; e.g. Eckerle & Thompson, 2005; Sergio *et al*., 2011), and their function, in some cases, remains unknown. The use of nest decorations as a means to avoid provisioning of unrelated offspring is a topic that deserves further research.

We found that some males from the experimental group deserted their nest after hatching, which may be related to their increased perception of risk of cuckoldry. In addition, in two deserted experimental nests we found some eggs (the quality, or at least appearance, of which was good) had been destroyed by pecking. Such circumstances had not been observed previously in the study population (V. García-Navas, pers. observ.), and can be considered as unusual in this species, whose rate of conspecific brood parasitism is thought to be low (Vedder *et al*., 2007; Griffith *et al*., 2009). Egg destruction could be interpreted as an infanticidal behaviour, as has been reported in some mate-switching studies in which a male was replaced by another male (Robertson & Stutchbury, 1988; Veiga, 1990). In this vein, Osorio-Beristain & Drummond (2001) reported that male blue-footed boobies, *Sula nebouxii*, expelled the eggs from their nest when the risk of being cuckolded was increased experimentally.

In natural circumstances, one would expect that the presence of feathers (as an indicator of high-quality males) would incite female *C. caeruleus* to be less likely to engage in extra-pair copulations. The result of our experiment contradicted this prediction. The rate of extra-pair paternity in experimental nests was significantly higher compared with that of control nests. This suggests that engagement in extra-pair copulations is not only driven by females, and that males may also play an important role in this behaviour (Griffith *et al*., 2002; Westneat & Stewart, 2003; Akçay *et al*., 2012). In this sense, a possible explanation for our finding is that males responded to the feather-supplementation experiment by reducing their mate-guarding behaviour. This is in agreement with previous studies that showed a negative relationship between apparent loss of paternity and mate-guarding intensity (see Møller & Birkhead, 1993, and references therein). If the addition of feathers caused the certainty of paternity of males to reach a critically low level, then males may be reluctant to devote any effort to paternity guarding because the amount of time and energy devoted to these behaviours may not be compensated by a substantial gain in terms of reproductive success. Thus, the high rate of extra-pair paternity found in experimental nests may be linked to a decrease in mate-guarding effort (i.e. 'distrustful' males drop their guard). In this regard, we found that males that gained extra-pair young via extra-pair copulation were also more likely to have reduced paternity in their nest in comparison with non-cuckolding males. Accordingly, it seems that male *C. caeruleus* experienced a trade-off between ensuring paternity at home and pursuing copulations elsewhere. This result can be easily explained by taking into account that a male faces a dilemma while pursuing extra-pair copulations outside their territory, as their absence can be exploited by other neighbouring males to gain paternity (Chuang-Dobbs, Webster & Holmes, 2001; Hill *et al*., 2011; Canal, Jovani & Potti, 2012).

Overall, our results suggest that feather supplementation was perceived by social males as indicating a reduced certainty of paternity, which seems to have negatively affected their mate-guarding behaviour and resulted in a higher rate of extra-pair paternity in comparison with control nests. According to optimality models of paternal care, male investment in the brood was lower in experimental nests, an effect that can be attributed to the lower certainty of paternity after feather supplementation. This study reinforces the view that experimental approaches are necessary to control for potentially confounding variables and disentagle the nature of the association between paternal investment and the male's certainty of paternity. Future observational studies could also help to determine the association between the presence of ornamental feathers and extra-paternity rates in a natural context, and whether the fitness benefits of this behaviour (e.g. increased female fecundity) exceed other potential costs.

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REFERENCES

Akçay Ç, Searcy WA, Campbell SE, Reed VA, Templeton CN. 2012. Who initiates extrapair mating in song sparrows? *Behavioral Ecology* **23:** 44–50.

Alonzo SH, Klug H. 2012. Maternity, paternity and parental care. In: Royle NJ, Smiseth PT, Kölliker M, eds. *The evolution of parental care*. 189–205. Oxford: Oxford University Press.

Bouwman KM, Komdeur J. 2005. Old female reed buntings (*Emberiza schoeniclus*) increase extra-pair paternity in their broods when mated to young males. *Behaviour* **142:** 1449–1463.

Bouwman KM, Lessells M, Komdeur J. 2005. Male reed buntings do not adjust parental effort in relation to extrapair paternity. *Behavioral Ecology* **16:** 499–506.

Burke T, Davies NB, Bruford MW, Hatchwell BJ. 1989. Parental care and mating-behavior of polyandrous dunnocks prunella-modularis related to paternity by DNA fingerprinting. *Nature* **338:** 249–251.

Canal D, Jovani R, Potti J. 2012. Male decisions or female accessibility? Spatiotemporal patterns of extra pair paternity in a songbird. *Behavioral Ecology* **23:** 1146–1153.

Charmantier A, Perret P. 2004. Manipulation of nest-box density affects extra-pair paternity in a population of blue tits (*Parus caeruleus*). *Behavioral Ecology and Sociobiology* **56:** 360–365.

Chuang-Dobbs HC, Webster MS, Holmes RT. 2001. Paternity and parental care in the black-throated blue warbler *Dendroica caerulescens*. *Animal Behaviour* **62:** 83–92.

Eckerle KP, Thompson CF. 2005. Addition of arthropod cocoons to house wren nests is correlated with delayed pairing. *Behavioral Ecology* **16:** 1–7.

García-López de Hierro L, Moleón M, Ryan PG. 2013. Is carrying feathers a sexually selected trait in House Sparrows? *Ethology* **119:** 199–211.

García-Navas V, Ferrer ES, Sanz JJ. 2012. Plumage yellowness predicts foraging ability in the blue tit *Cyanistes caeruleus*. *Biological Journal of the Linnean Society* **106:** 418–429.

García-Navas V, Ortego J, Sanz JJ. 2009. Heterozygosity-based assortative mating in blue tits (*Cyanistes caeruleus*): implications for the evolution of mate choice. *Proceedings of the Royal Society of London B* **276:** 2931–2940.

García-Vigón E, Veiga JP, Cordero PJ. 2009. Male feeding rate and extrapair paternity in the facultatively polygynous spotless starling. *Animal Behaviour* **78:** 1335–1341.

Grieco F. 2002. Time constraint on food choice in provisioning blue tits, *Parus caeruleus*: the relationship between feeding rate and prey size. *Animal Behaviour* **64:** 517–526.

Griffith SC, Barr I, Sheldon BC, Rowe LV, Burke T. 2009. Egg patterning is not a reliable indicator of intraspecific brood parasitism in the blue tit *Cyanistes caeruleus*. *Journal of Avian Biology* **40:** 337–341.

Griffith SC, Owens IPF, Thuman KA. 2002. Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Molecular Ecology* **11:** 2195–2212.

Hill CE, Akçay Ç, Campbell SE, Beecher MD. 2011. Extrapair paternity, song and genetic quality in song sparrows. *Behavioral Ecology* **22:** 73–81.

Holveck M-J, Doutrelant C, Guerreiro R, Perret P, Gomez D, Grégorie A. 2010. Can eggs in a cavity be a female secondary sexual signal? Male nest visits and modelling of egg visual discrimination in blue tits. *Biology Letters* **6:** 453–457.

Horváthová T, Nakagawa S, Uller T. 2012. Strategic female reproductive investment in response to male attractiveness in birds. *Proceedings of the Royal Society of London B* **279:** 163–170.

Houston AI. 1995. Parental effort and paternity. *Animal Behaviour* **50:** 1635–1644.

Kalinowski ST, Taper ML, Marshall TC. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology* **16:** 1099–1106.

Kempenaers B, Lanctot RB, Robertson RJ. 1998. Certainty of paternity and paternal investment in eastern bluebirds and tree swallows. *Animal Behaviour* **55:** 845–860.

Kempenaers B, Schlicht E. 2010. Extra-pair behaviour. In: Kappeler P, ed. *Animal behavior: evolution and mechanisms*. 359–411. Heidelberg: Springer.

Kempenaers B, Sheldon BC. 1996. Why do male birds not discriminate between their own and extra-pair offspring? *Animal Behaviour* **51:** 1165–1173.

Kempenaers B, Sheldon BC. 1997. Studying paternity and paternal care: pitfalls and problems. *Animal Behaviour* **53:** 423–427.

Kempenaers B, Sheldon BC. 1998. Confounded correlations: a reply to Lifjeld *et al*. and Wagner *et al*. *Animal Behaviour* **55:** 241–244.

Kempenaers B, Verheyen GR, Dhondt AA. 1995. Mate guarding and copulation behavior in monogamous and polygynous blue tits: do males follow a best-of-a-bad-job strategy. *Behavioral Ecology and Sociobiology* **36:** 33–42.

Kempenaers B, Verheyen GR, Vandenbroeck M, Burke T, Vanbroeckhoven C, Dhondt AA. 1992. Extra-pair paternity results from female preference for high-quality males in the blue tit. *Nature* **357:** 494–496.

Lifjeld JT, Slagsvold T, Ellegren H. 1998. Experimentally reduced paternity affects paternal effort and reproductive success in pied flycatchers. *Animal Behaviour* **55:** 319–329.

Mennerat A, Perret P, Bourgault P, Blondel J, Gimenez O, Thomas DW, Heeb P, Lambrechts MM. 2009. Aromatic plants in nests of blue tits: positive effects on nestlings. *Animal Behaviour* **77:** 569–574.

Mennerat A, Perret P, Caro SP, Heeb P, Lambrechts MM. 2008. Aromatic plants in blue tit *Cyanistes caeruleus* nests: no negative effect on blood-sucking *Protocalliphora* blow fly larvae. *Journal of Avian Biology* **39:** 127–132.

Møller AP. 1998. Paternity and parental care in the swallow. *Animal Behaviour* **36:** 996–1005.

Møller AP, Birkhead TR. 1993. Certainty of paternity covaries with paternal care in birds. *Behavioral Ecology and Sociobiology* **33:** 261–268.

Mougeot F, Arroyo BE, Bretagnolle V. 2001. Decoy presentations as a means to manipulate the risk of extra-pair copulation: an experimental study in a semi-colonial raptor, the Montagu's harrier *Circus pygargus*. *Behavioral Ecology* **12:** 1–7.

Neff BD. 2003. Decisions about parental care in response to perceived paternity. *Nature* **422:** 716–719.

Osorio-Beristain M, Drummond H. 2001. Male boobies expel eggs when paternity is in doubt. *Behavioral Ecology* **12:** 16–21.

Penn D, Frommen JG. 2010. Kin recognition: an overview of conceptual issues, mechanisms and evolutionary theory. In: Kappeler P, ed. *Animal behavior: evolution and mechanisms*. 55–85. Heidelberg: Springer.

Polo V, Veiga JP. 2006. Nest ornamentation by female spotless starlings in response to a male display: an experimental study. *Journal of Animal Ecology* **75:** 942–947.

Robertson RJ, Stutchbury BJ. 1988. Experimental evidence for sexually selected infanticide in tree swallows. *Animal Behaviour* **36:** 749–753.

Sanz JJ, García-Navas V. 2011. Nest ornamentation in blue tits: is feather carrying ability a male status signal? *Behavioral Ecology* **22:** 240–247.

Sergio F, Blas J, Blanco G, Tanferna A, López L, Lemus JA, Hiraldo F. 2011. Raptor nest decorations are a reliable threat against conspecifics. *Science* **331:** 327–330.

Sheldon BC. 2000. Differential allocation: tests, mechanisms and implications. *Trends in Ecology and Evolution* **15:** 397–402.

Sheldon BC. 2002. Relating paternity to paternal care. *Philosophical Transactions of the Royal Society of London B* **357:** 341–350.

Sheldon BC, Ellegren H. 1998. Paternal effort related to experimentally manipulated paternity of male collared flycatchers. *Proceedings of the Royal Society of London B* **265:** 1737–1742.

Sheldon BC, Rãsãnen K, Dias PC. 1997. Certainty of paternity and paternal effort in the collared flycatcher. *Behavioral Ecology* **8:** 421–428.

Svensson L. 1992. *Identification guide to European Passerines*. Stockholm: Lars Svensson.

Tomás G, Merino S, Martínez-De la Puente J, Moreno J, Morales J, Lobato E. 2008. A simple trapping method to estimate abundances of blood-sucking flying insects in avian nests. *Animal Behaviour* **75:** 723–729.

Tomás G, Merino S, Moreno J, Morales J. 2007. Consequences of nest reuse for parasite burden and female health and condition in blue tits, *Cyanistes caeruleus*. *Animal Behaviour* **73:** 805–814.

Trivers RL. 1972. Parental investment and sexual selection. In: Campbell BG, ed. *Sexual selection and the descent of man*. 139–179. Chicago, IL: Aldine.

Valera F, Hoi H, Kristin A. 2003. Male shrikes punish unfaithful females. *Behavioral Ecology* **14:** 403–408.

Vedder O. 2011. *All's fair in love and war – Reproductive strategies, intra-specific competition and sexual selection in blue tits*. PhD dissertation. Groningen: University of Groningen.

Vedder O, Kingma SA, von Engelhardt N, Korsten P, Groothuis TGG, Komdeur J. 2007. Conspecific brood parasitism and egg quality in blue tits *Cyanistes caeruleus*. *Journal of Avian Biology* **38:** 625–629.

Veiga JP. 1990. Infanticide by male and female house sparrow. *Animal Behaviour* **39:** 496–502.

Veiga JP, Polo V. 2011. Feathers in the spotless starling nests: a sexually selected trait? *Behaviour* **148:** 1359–1375.

Westneat DF. 1995. Paternity and paternal behaviour in the red-winged blackbird *Agelaius phoeniceus*. *Animal Behaviour* **49:** 21–35.

Westneat DF, Stewart IRK. 2003. Extra-Pair paternity in birds: causes, correlates, and conflict. *Annual Review of Ecology, Evolution and Systematics* **34:** 365–396.

Whittingham LA, Dunn PO. 2001. Male parental care and paternity in birds. *Current Ornithology* **16:** 257–298.

Whittingham LA, Dunn PO, Robertson RJ. 1992. Confidence of paternity and male paternal care. *The American Naturalist* **139:** 1115–1125.

Winkler DW. 1987. A general model for parental care. *The American Naturalist* **130:** 526–543.

Wright J, Cotton PA. 1994. Experimentally induced differences in parental care: an effect of certainty of paternity? *Animal Behaviour* **47:** 1311–1322.

Zyskowski K, Prum RO. 1999. Phylogenetic analysis of the nest architecture of neotropical ovenbirds (Furnariidae). *The Auk* **116:** 891–911.