Male spotless starlings adjust feeding effort based on egg spots revealing ectoparasite load

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Parents may vary their parental behaviour and investment in reproduction in response to parasite-induced changes in the fitness prospects of their offspring. Thus, parents may use the physical condition of their offspring, or any other trait related to parasite load, to adjust parental effort. The immaculate eggs of the spotless starling, *Sturnus unicolor*, often become densely spotted owing to the activity of the ectoparasite carnid fly *Carnus hemapterus*. Spot density anticipates the intensity of fly infestation suffered by nestlings and, therefore, may serve as a cue for parents to adjust reproductive investment. By cleaning spots produced by *C. hemapterus* on eggs of spotless starlings, we manipulated the parasite's traces revealing its presence in broods of starlings, without modifying the level of infestation, to test whether parents use these signals to adjust reproductive effort. We found support for the hypothetical negative effect of *Carnus* flies since nestlings raised in nests with a higher fly load had lower body mass. The experimental egg cleaning during incubation did not change the intensity of carnid fly infestation during nestling development. However, it had a significant positive influence on paternal but not maternal effort. Our experimental results support the idea that spotless starling males adjust their effort in response to their perception of the fitness prospects of their nestlings as indirectly estimated by traces of parasites on the eggshells. As far as we know, this is the first evidence of the use of parasite traces to infer risk of parasitic infestation by animal hosts.

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Parasites can impose fitness costs by reducing their host's fecundity, survival and reproductive success when they affect developing offspring. Selection therefore favours the evolution of physiological and behavioural defensive mechanisms in the hosts to reduce the harmful effects of parasitism (e.g. Loye & Zuk 1991; Toft et al. 1991). In birds, it has been experimentally demonstrated that ectoparasites negatively affect both offspring body mass and fledging success (Richner et al. 1993). Nestling body mass is a reliable predictor of nestling survival (e.g. Perrins 1965; Smith et al. 1988; Tinbergen & Boerlijst 1990; Moreno et al. 2005a) and, therefore, ectoparasites may considerably lower the value of the current brood to the parent. Parents may, therefore, vary their parental behaviour and the investment in reproduction in response to parasite-induced changes in the fitness prospects of their offspring. For instance, parents could reduce their investment in the current parasitized brood in order to invest more in future

broods (see Linden & Møller 1989; Richner & Tripet 1999). Alternatively, parents could increase their investment to compensate for the negative effects of parasites on the current brood (e.g. Christe et al. 1996; Tripet & Richner 1997). The optimal reproductive strategy of parents in terms of reproductive effort is likely to depend on the fitness costs of parasite infestation for parents and their offspring, as well as on the possibility of future reproduction (e.g. Stearns 1992; Møller 1997).

Studying behavioural responses of parents to ectoparasite-induced changes in the reproductive value of the offspring is difficult because parental responses may, for instance, be driven by the effects of ectoparasites on offspring begging behaviour. Parasites may affect the begging behaviour of nestlings that parents evaluate to adjust provisioning rates either directly for their own benefit or indirectly through an increase in nestling needs (e.g. Wright & Leonard 2002). Parents, thereby, by simply following the rule of adjusting parental effort to the honest begging behaviour of their offspring, might adaptively compensate for the negative effects of parasitism (Christe et al. 1996; Tripet & Richner 1997). On the other hand, a reduction in parental effort may reflect the negative effects of ectoparasite infestation on parents rather than an adaptive parental response to a parasite-induced change in the reproductive value of the offspring (e.g. Råberg et al. 2000; Gallizzi

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et al. 2008). Consequently, such negative or positive relationships between experimental parasitism loads and parental effort might not be the direct adaptive responses to parasitism suffered by offspring, but the consequence of parasites affecting nestling needs and/or the energy budget of parents.

One way to disentangle parasite effects on parents' perception of offspring value from a parasite-induced effect on offspring begging or parental behaviour would be to explore the possibility that parents could detect the parasite before hatching and examine parental feeding behaviour after removing parasites from the nest (see Gallizzi et al. 2008). However, even in this case, a reduction in parental effort could be caused by parasites affecting the parents (Gallizzi et al. 2008). Another unexplored way of testing the hypothesis of parasitism 'per se' influencing the reproductive investment of parents is related to the idea that parents could infer intensity of infestation from parasite traces in the nest that could be experimentally manipulated without modifying the actual level of infestation.

The immaculate blue-greenish eggs of some members of the genus *Sturnus* often became finely spotted with red-brownish spots that increase in density as incubation progresses (Feare & Constantine 1980; López-Rull et al. 2007; Fig. 1a). Recent observations suggest that these spots are the droppings of the imagos of an ectoparasite of nestlings: the carnid fly *Carnus hemapterus* (López-Rull et al. 2007). In general, the imagines of the carnid fly acquire food resources by feeding on carcasses, faeces or umbelliferous flowers. However, imagines of the genus *Carnus* are blood-sucking ectoparasites of nestling birds (Grimaldi 1997). Carnid flies are highly prevalent and abundant in *Sturnus* colonies, and are present in nests in their adult form a few days before incubation starts (Liker et al. 2001). The abundance of flies in a nest at the nestling phase can be anticipated some days after the beginning of egg incubation by the density of spots on the eggshell (López-Rull et al. 2007). Indeed, the experimental reduction of carnid fly load with an insecticide resulted in a reduction in the number of spots 4 days after the treatment, which suggests that spot density on the eggshell is an indicator of carnid fly density in spotless starling broods (López-Rull et al. 2007).

We tested the hypothesis that male spotless starlings, *Sturnus unicolor*, use egg spottiness, revealing parasite load, to adjust parental investment because spottiness would predict the fitness prospects of their offspring. The hypothesis is based on a visual mechanism of assessing fitness prospects based on egg spottiness that requires covariation between the parasite load of the brood and the current value of reproduction. A number of correlative studies have found a negative correlation between carnid fly load in the nests and different estimators of nestling health that suggest that carnid fly parasitism generates costs (e.g. Soler et al.

1999; Martín-Vivaldi et al. 2006; Václav et al. 2008), although a number of studies have failed to find such a link (e.g. Dawson & Bortolotti 1997; Liker et al. 2001). More convincingly, Wiebe (2009) experimentally fumigated half of the nests of a northern flicker, *Colaptes auratus*, population parasitized by carnid flies. Nestlings from control nests had lower body mass than those from fumigated nests after 15 days and fledged at a lower weight, suggesting that carnid fly parasitism has a negative effect on nestling development (Wiebe 2009). None the less, as a prerequisite for testing the hypothesis that male starlings use carnid fly costs related to egg spottiness, we first evaluated whether fly load relates to the fitness prospects of nestlings in spotless starling nests in terms of their body mass. Second, we manipulated egg spottiness during incubation to explore the behavioural responses of the targeted males. Our prediction was that the experimental cleaning of eggs would affect parental feeding investment of males.

METHODS

Study Site and System

We carried out the study in a spotless starling colony in Guadix, southeastern Spain (37°18′N, 3°11′W), during the breeding seasons in 2006–2007 with the authorization of the Dirección General de Gestión del Medio Natural, Consejería de Media Ambiente, Junta de Andalucia. Starlings in the colony breed in nestboxes established since the early spring of 2005. Adults were captured inside nestboxes during the nest-building phase, then sexed (Hiraldo & Herrera 1974) and marked with a metallic numbered ring and a unique combination of coloured plastic rings.

Females lay one egg per day, mainly during the morning, and start incubation before clutch completion which leads to asynchronous hatching (Cramp 1998). In our population, females mostly lay one clutch of four or five eggs and, with a few exceptions, incubation is a female task (Soler et al. 2008; unpublished data). Nestlings are fed mainly with insects (Motis et al. 1997) by both male and female (Cramp 1998; Veiga et al. 2002) and paternal feeding effort is mainly concentrated in the first third of the nestling period (Soler et al. 2008).

The carnid fly is a 2 mm blood-sucking ectoparasite of nestling birds of a wide diversity of species excluding ground-nesting species (e.g. Dawson & Bortolotti 1997; Grimaldi 1997; Roulin et al. 2003; Martín-Vivaldi et al. 2006; Chakarov et al. 2008; Václav et al. 2008). The parasite overwinters as pupae inside the nests. After emergence the adult dispersive form is winged, but it loses its wings soon after finding a suitable host (Roulin 1998, 1999). Fly populations increase from host hatching through nestling
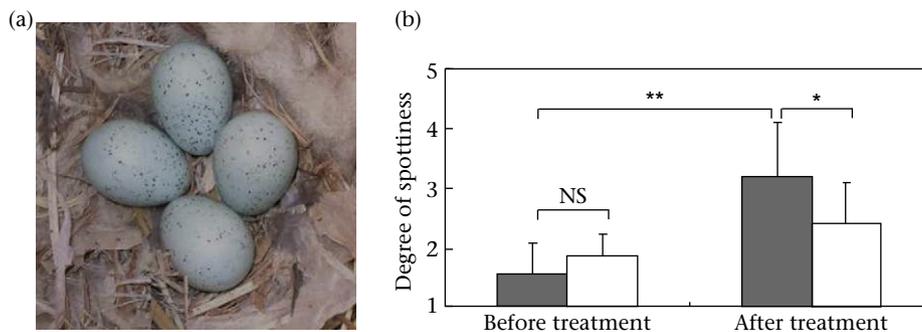
Figure 1. (a) Representative spottiness coverage of eggs in control nests 2 days before hatching. (b) Average spottiness coverage ± SD of the eggshell in nests where spots were experimentally removed (□; N = 11) and in control nests (■; N = 13) before (the day of clutch completion) and after (2 days before egg hatching) treatment application. **P < 0.01; *P < 0.05.

development, and disappear once host nestlings become feathered (Roulin 1998).

Parasite Load and Nestling Growth

We studied the association between parasite load and body weight of nestlings as a variable related to nestling survival (Tinbergen & Boerlijst 1990) in 18 starling broods (12 broods in 2006 and six in 2007). For this particular analysis we disregarded experimental nests in 2006 in which we cleaned the eggs (11 nests, see below) because we expected that our manipulation would affect parental effort and therefore nestling growth. Furthermore, we ignored one control nest (see below) in which, because of logistic problems, we could not estimate parasite load. Nests were visited every day during egg laying and, afterwards, every second day until hatching. Once hatching occurred starling broods were carefully taken from the nest inside a white cloth bag every second day thus minimizing the possibility that flies jumped into the nest material. We counted the *C. hemapterus* on the body surface of each chick as well as the remaining flies in the bag to estimate parasite load for every brood. We did not attempt to catch flies that were returned together with nestlings to the nests. Previous studies have shown that this method reliably assesses parasite load (Roulin et al. 2003; Václav et al. 2008).

When the first-hatched nestling was 8 days old, all nestlings in the nest were weighed (with a Pesola spring balance, ± 0.5 g) and the average body mass per brood was calculated. We relied on weight at day 8 because flies disappear once nestlings became feathered and weight at day 8 is a good correlate of fledging weight in our population ($r_{15} = 0.72$, $P = 0.001$).

Experimental Design

We did the experiment in 2006. Nests were visited daily during egg laying and were randomly assigned to a clutch-cleaning ($N = 11$ nests) or a control ($N = 13$ nests) treatment the day of clutch completion. The cleaning treatment consisted of the careful removal of the eggshell spots of all eggs in the clutch with a tap water-moistened piece of cotton. In fly-infested nests intensity of spots increases as incubation progresses (López-Rull et al. 2007; Fig. 1b), so we cleaned the eggs on the day of clutch completion, on day 6 of incubation and 2 days before the estimated hatching date. Control nests were visited with the same frequency and their eggs were also smoothly touched with dry cotton but no attempt was made to remove spots.

To examine the effect of the cleaning treatment on the visual perception of spottiness, we estimated the degree of spottiness on the eggshell in clean and control nests on a scale of 5 before (i.e. the day of clutch completion) and after (i.e. 2 days before hatching) the treatment application. Clutches were classified as: (1) all eggs unspotted; (2) small isolated speckles on the eggshell; (3) small speckles widely distributed on the eggshell; (4) large speckles covering entire portions of the eggshell; (5) total coverage of spots obscuring the original blue colour of the eggshell. Confirming previous anecdotal evidence, the spottiness in control nests increased throughout incubation (Wilcoxon test: $Z = 3.059$, $N = 13$, $P = 0.002$; Fig. 1b). Clean ($N = 11$) and control ($N = 13$) nests did not differ in spot coverage before treatment application (Mann–Whitney U test: $U = 51.5$, $P = 0.24$; Fig. 1b). However, cleaned nests that were infested by flies ($N = 11$) displayed globally lower spot coverage than control nests ($N = 13$) before the last cleaning visit (i.e. 2 days before hatching; Mann–Whitney U test: $U = 31.0$, $P = 0.04$; Fig. 1b), which suggests that our treatment affected the visual perception of spot coverage on the eggshell.

The date of the first day of hatching (hatching date hereafter; $1 = 1$ April) of experimental nests (mean hatching date $\pm$ SE = 23.55 $\pm$ 5.84) did not differ significantly from that recorded in control nests (28.66 ± 5.81, one-way ANOVA: $F_{1,19} = 0.36$, $P = 0.55$). In addition, the brood size of nests where eggs were cleaned (mean brood size $\pm$ SE = 3.77 $\pm$ 0.54) did not differ significantly from that in control nests (3.00 ± 0.30; one-way ANOVA: $F_{1,19} = 1.76$, $P = 0.20$). In a previous study we have shown that the intensity of the blue-green coloration reflects female individual condition and affects parental provisioning in spotless starlings (Soler et al. 2008). Therefore, the results of our experiment could be biased if the clean and control treatments were not randomly assigned in regard to egg blueness. To explore this possibility we estimated the intensity of blue-green egg coloration in 16 randomly selected nests from the 24 nests in which we performed the experiment. We could not measure coloration in the full sample of experimental nests because of logistic problems. Coloration information was collected at every nest 2 days after the end of laying, following the protocol of Soler et al. (2008). Briefly, reflectance spectra (300–700 nm) of all the eggs in a clutch were recorded with an Ocean Optics S2000 spectrometer, connected to a deuterium–halogen light (D2-W, mini) by a coaxial reflectance probe (QR-400-7-UV-vis), and the OOIBase32 operating software (Ocean Optics, Inc., Dunedin, FL, U.S.A.). Reflectance was always measured with the probe placed at a constant distance and reaching the egg at $45°$ (e.g. Avilés et al. 2006a, b). Measurements were relative and referred to standard white (WS-2) and dark references, which we calibrated before measurement of each clutch. As a variable indicating intensity of blue-green coloration we used the proportion of reflectance in the blue-green region of the spectrum (R400–575/ R300–700), the region where biliverdin shows the lowest absorbance (Falchuk et al. 2002) and where reflectance of starling eggs reaches its maximum (see López-Rull et al. 2008; Soler et al. 2008). Intensity of blue-green egg coloration of nests where eggs were cleaned (mean blueness $\pm$ SE = 0.54 $\pm$ 0.008) did not differ significantly from that in control nests (0.53 ± 0.008; one-way ANOVA: $F_{1,14} = 0.10$, $P = 0.74$). Globally these results provide evidence that our experiment was properly randomized in regard to the main known factors associated with individual quality and/or parental investment in spotless starlings.

Male feeding visits occur mainly during the first half of the nestling period, when feeding rates of males are usually higher than those of females (Soler et al. 2008). Therefore we measured provisioning behaviour of adults when nestlings were approximately 4 days old (mean $\pm$ SD = 3.7 $\pm$ 1.0 days, $N = 24$ experimental nests). All observations were made during the morning (i.e. 0700–1100 hours). Parental visits were recorded by direct observation of the nests with a telescope (from a hide or a car) during periods of approximately 1 h (mean $\pm$ SD = 59.8 $\pm$ 3.37 min, $N = 24$ observations). Visit rates were corrected to 1 h periods by dividing the number of visits by the number of minutes recorded and multiplying by 60. For each visit we identified the sex, and also whether adults carried detectable food for the offspring (i.e. feeding visits). We decided to estimate parental attendance based on total number of visits instead of only using feeding visits because small prey could have been undetected. This is reasonable considering that the number of visits by males was strongly and positively correlated with the number of visits in which we identified the male carrying food ($r^2 = 0.46$, $F_{1,19} = 16.57$, $P = 0.0007$).

Statistical Methods

For analyses we used SAS statistical software (SAS 2001 Institute Inc., Cary, NC, U.S.A.). Nestling body mass and natural log-transformed number of flies were approximately normally

distributed (Kolmogorov–Smirnov test for continuous variables: $P > 0.2$).

We studied the relationship between fly infestation and nestling body mass with Pearson correlations and a general linear model (GLM) in which nestling body mass was the dependent variable and log-transformed number of flies was the independent variable. We used average number of flies per brood because flies moved from one nestling to another during counts and cannot, therefore, be considered a nestling characteristic. To account for between- and within-season variation in fly numbers we entered study year as a fixed term (2006 versus 2007) and hatching date as a covariate. In addition, we entered brood size as a further covariate to account for possible nonrandom distribution of files among sampled nests.

To test for the effect of experimental removal of spottiness on the evolution of parasite load we performed a repeated measures ANOVA with log-transformed number of flies at different dates as the within-nest effect, and experimental treatment (clean versus control) as the between-nest effect. Hatching date and brood size were entered as covariates in the model. Finally, to test the effect of our experimental removal of spottiness on male provisioning rates, we used a GLM with experimental treatment (clean versus control) as the fixed effect. Because male investment may depend on that of females, but also on brood size and hatching date, these variables were included in a first model as covariates. After testing the model including all mentioned factors and covariables, we systematically removed the variable associated with the largest P value and ran the model again until we reached a model with all factors and covariables associated with two-tailed P values smaller than 0.1 (hereafter, final model). All statistical tests were two tailed.

RESULTS

Fly Infestation and Nestling Growth

Carnus hemapterus files were detected in all nests in which we checked ($N = 29$ nests, i.e. 12 nonmanipulated broods in 2006, 11 egg-cleaned broods in 2006 and six nonmanipulated broods in 2007). The average $\pm$SE number of flies per brood at age day 8 of nestlings was 16.85 ± 3.37. Among the nonmanipulated nests, the intensity of *C. hemapterus* infestation did not change seasonally (Pearson correlation: $r_{16} = -0.10$, $P = 0.66$), and was unrelated to brood size ($r_{16} = -0.03$, $P = 0.89$). The number of flies was negatively related to nestling body mass at 8 days old ($r_{16} = -0.61$, $P = 0.007$; Fig. 2). A GLM in which we controlled for the effect of year, brood size and hatching time to assess the relationship between infestation intensity and nestling body mass yielded qualitatively identical results (number of flies per nest versus nestling body mass: full model: $\beta \pm SE = -0.62 \pm 0.29$, $F_{1,13} = 4.61$, $P = 0.05$; final model: $\beta \pm SE = -0.61 \pm 0.19$, $F_{1,16} = 9.54$, $P = 0.007$).

### Egg Spottiness Manipulation and Parasite Load

The experimental cleaning of eggs had no effect on parasite load (repeated measures ANOVA, log-transformed number of flies as the dependent variable, measures at different nestling ages as the within-nest effect, and experimental treatment as the between-nest effect; interaction term: $F_{5,70} = 0.30$, $P = 0.91$; age effect term: $F_{5,70} = 0.20$, $P = 0.96$; experimental treatment term: $F_{1,14} = 3.07$, $P = 0.10$; Fig. 3), once hatching date (hatching date term: $F_{1,14} = 1.08$, $P = 0.31$) and brood size (brood size term: $F_{1,14} = 3.71$, $P = 0.07$) were considered as covariates.
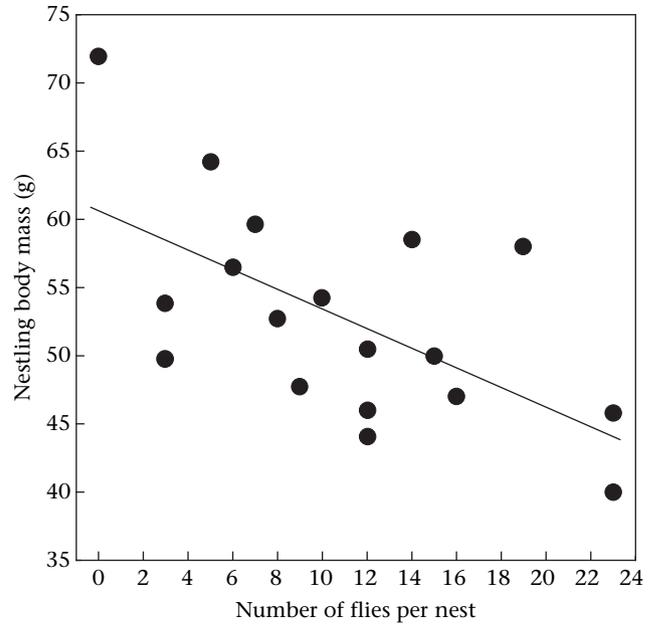

Figure 2. Relationship between intensity of *Carnus hemapterus* infestation at nesting day 8 and brood mean body mass (g; $N = 18$ nests).

Egg Spottiness Manipulation and Parental Care

Egg spottiness was not significantly related to female visit effort (experimental treatment term: $F_{1,16} = 0.13$, $P = 0.72$). Neither hatching date (hatching date effect: $F_{1,16} = 4.12$, $P = 0.06$), brood size (brood size effect: $F_{1,16} = 0.00$, $P = 0.98$) nor male visit effort (male visit effect: $F_{1,16} = 0.63$, $P = 0.43$) explained a significant proportion of female visit effort. However, males from nests with experimentally cleaned eggs visited the nestlings more frequently than those from nests with control eggs (experimental treatment term: $F_{1,22} = 12.03$, $P = 0.02$; Fig. 4). This result was qualitatively confirmed (experimental treatment term: $F_{1,16} = 4.65$, $P = 0.04$) when we controlled for number of nestlings in the nest (brood size
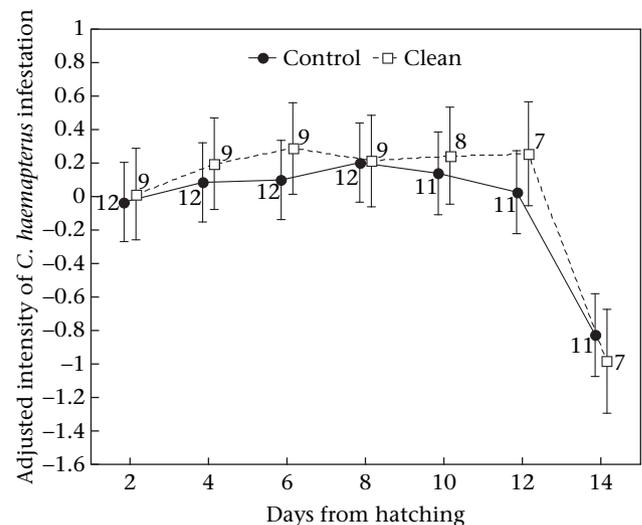
Figure 3. Mean intensity $\pm$ SD of *Carnus hemapterus* infestation in relation to nestling development in control and experimental spotless starling nests. Number of sampled nests at every nestling period is shown above average values. The *Y* axis represents the residuals from the general linear model performed after controlling for the effects of hatching date and brood size on log-transformed total number of adult flies in the nest.
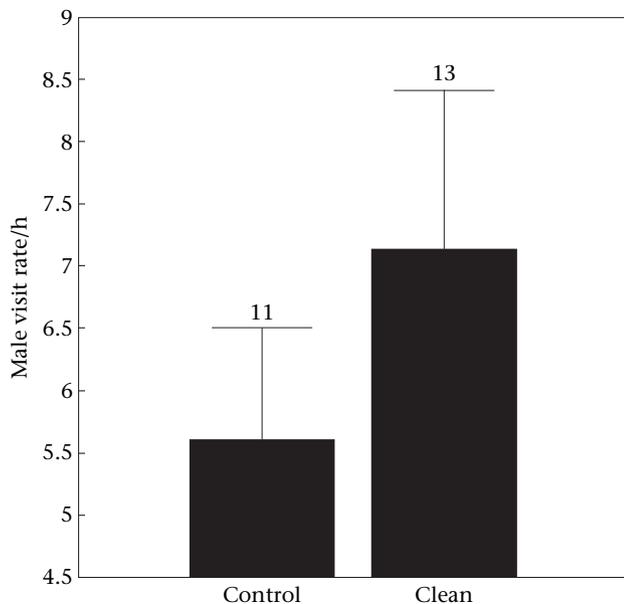
Figure 4. Mean number $\pm$ SD of visits/h by males in nests with experimentally cleaned or naturally spotted (control) eggs.

effect: $F_{1,16} = 1.12$, $P = 0.30$), female visit effort (female visit effect: $F_{1,16} = 0.63$, $P = 0.43$) and hatching date (hatching date effect: $F_{1,16} = 0.05$, $P = 0.82$).

DISCUSSION

Our study reveals that male spotless starlings can adjust their feeding rate during nestling growth in response to a change in the density of spots covering the eggshell during incubation, a trait that reliably reflects the level of parasite load by carnid flies in spotless starling broods (López-Rull et al. 2007). In addition, we have shown in this study that the level of infestation by carnid flies was associated with nestling body weight among broods. Altogether these results are in agreement with the proposed hypothesis that male spotless starlings use spottiness as a cue of parasite infestation to adjust parental investment because spottiness predicts the fitness prospects of their offspring.

We found that the number of carnid flies in the brood was negatively related to nestling body mass at age 8 days, which is a good correlate of weight at fledging in our population (see Methods). A number of correlative studies have previously shown the existence of a negative correlation between parasite infestation by *C. hemapterus* and different estimators of nestling body condition (e.g. Soler et al. 1999; Martín-Vivaldi et al. 2006; Václav et al. 2008). In addition, carnid fly infestation increases with darkness of the plumage in the common buzzard, *Buteo buteo*, which suggests that fly parasitism may have partly shaped the evolution of eumelanin polymorphism in this species (Chakarov et al. 2008). More recently, Wiebe (2009) has found experimental support for a negative effect of carnid fly infestation on nestling growth in northern flickers. Some other studies with the American kestrel *Falco sparverius* (Dawson & Bortolotti 1997) and the European starling, *Sturnus vulgaris* (Liker et al. 2001) have failed to find a link between carnid fly parasitism and different estimators of nestling phenotypic quality. The existence of a negative relationship between parasitism and nestling body mass suggests that *C. hemapterus* parasitism has detrimental effects on nestling health (see Wiebe 2009). However, given this variety of results and the absence of an experiment, we cannot preclude a nonrandom

distribution of parasites in relation to brood qualities as the mechanism leading to this association. The mechanisms of host selection by carnid flies remain to be studied although it is well established that carnid flies can actively colonize hosts' nests during the winged phase of their cycle (Grimaldi 1997). Irrespective of the mechanism promoting the parasite–nestling condition link, our results confirm the existence of such a relationship which is a prerequisite for the use of spots as a cue revealing parasite load.

Spotless starling males increased their feeding effort in those nests in which we experimentally reduced parasite traces on the host eggshell. Parental responses to parasitism vary among species and/or populations and are likely to be influenced by lifehistory trade-offs. If parasites lower the value of the current brood, as could be the case in the spotless starling–carnid fly system (present study), parental response to parasitism may depend on the trade-off between current and future reproduction. Previous studies have found that nestling infestation leads to increased parental feeding effort, which in turn compensates for the parasite-induced costs in nestlings of great tits, *Parus major* (Christe et al. 1996; Tripet & Richner 1997) and barn swallows, *Hirundo rustica* (Møller 1994). Parents might also decrease breeding effort in current parasitized reproduction and increase the energy budget for future, possibly nonparasitized broods. In accordance with this last possibility, Møller et al. (1994) found that house martin, *Delichon urbicum*, adults in experimentally parasitized nests reduced their energetic investment in these nests, and Gallizzi et al. (2008) found that experimental parasitism during the prehatching phase of reproduction reduced the frequency of brooding and nestling care in great tits. However, parental responses to parasitism may be driven by the effect of ectoparasites on nestling begging (Christe et al. 1996), or on adult health-related behaviours (e.g. Råberg et al. 2000; Gallizzi et al. 2008), rather than being a pure response of parents to the current value of the parasitized brood (see above). Importantly, our experiment did not affect fly load at experimental and control nests throughout nestling growth (Fig. 3), but did affect parent perception of parasite traces that we hypothesized males might use as information revealing future ectoparasite loads. Therefore, changes in level of need, and therefore in begging behaviour, of nestlings or physical condition of parents as a result of parasitism cannot explain the reported experimental effect on the feeding behaviour of male parents in nests with experimentally removed parasitic traces on the eggshell.

The removal of spots from the eggshell modified the feeding rate of males but not that of females. Females and males often differ in the costs and benefits of providing care to offspring versus using this time in other activities such as obtaining more mates, setting the conditions for a sexual conflict over parental care (Trivers 1972; Houston et al. 2005). Sexual conflict frequently leads to a resolution in which one sex provides most of the parental care (Bennett & Owens 2002), whereas the sex providing less care has more chance of pursuing extrapair mating opportunities (Trivers 1972; Royle et al. 2002). The spotless starling is a facultative polygynous species (Veiga et al. 2002), and males may trade between investment in different breeding attempts and modify their investment in the different broods based on their perception of the fitness benefits arising at each nest. In this context, polygynous males might, for instance, concentrate their feeding effort in nonparasitized nests by reducing paternal investment in nests with traces of parasitism.

In the last few years a growing body of studies has provided support for a possible signalling role of egg coloration in birds based on a thorough inspection of the egg colour pattern by males (Moreno et al. 2005b, 2006a, b; Siefferman et al. 2006; Soler et al. 2008). Here we have shown that spots covering the eggshell and produced by the activity of a blood-sucking ectoparasitic fly (López-Rull et al. 2007) are used as a cue revealing parasite load by male

spotless starlings. This finding provides further support for the hypothetical role of egg pattern as a trait from which males acquire important information after mating that they may use to adjust paternal effort. The presence of spots on the eggshell would reflect a reduction in the fitness prospects of nestlings and, therefore, would offer the opportunity to males of differentially investing in broods or females without parasite traces on the eggshell.

Alternatively, it could be argued that spots caused by fly activity may hinder the male's capacity to ascertain female quality from the blue egg coloration. In a previous study we have shown that male spotless starlings can adjust their feeding effort in relation to the intensity of the blue coloration of their eggs (Soler et al. 2008). Perhaps our removal of spottiness triggered the use of the blue signal by males which would be difficult to perceive in naturally spotted eggs. Although we cannot definitively discount this possibility, it is unlikely because control and experimental nests did not differ significantly in the intensity of blue coloration before the experiment (see Methods). In addition, at least for humans, blueness seems easily perceivable from spotted eggs even in the final stages of incubation since a large portion of the eggshell remains unspotted (Fig. 1a).

In species with biparental care the amount of parental investment that each partner should provide is a major source of conflict between provisioning parents (Trivers 1972). Most theoretical and experimental research to date has focused on understanding how parents adjust their work rate in relation to that of their, partner as well as to the hunger levels of their offspring (reviewed in Hinde 2006). Comparatively, far less attention has attracted the study of parental allocation in relation to offspring quality. Here we have studied how parents adjust provisioning in relation to a change in their perception of the fitness prospects of the offspring without altering true nestling health. We found that male but not female spotless starlings modulate the intensity of parental effort in relation to their perception of the intensity of parasitism as estimated by the density of spots covering the eggs. This suggests that males and females vary in how readily they obtain information on offspring quality at the different stages of offspring development. Alternatively, optimal parental investment for males and females may differ and, therefore, open the possibility for selection of female behaviours directed to control the male's perception of offspring quality as a form of sustaining paternal care in the spotless starling. As far as we know, this is the first evidence of the use of parasite traces for inferring risk of parasitic infestation by animal hosts. *Carnus* traces are, however, frequent on the eggs of several bird species and, therefore, their use as signals to adjust reproductive effort might be widespread in nature.

Acknowledgments

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