NOTE BRÈVE

NO EFFECT OF UROPYGIAL GLAND SECRETIONS ON HATCHING SUCCESS IN GREAT TITS *PARUS MAJOR*

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RÉSUMÉ.— *Absence d'effet des sécrétions de la glande uropygienne sur le succès à l'éclosion de Mésanges charbonnières* Parus major.— Quelques travaux expérimentaux récents démontrent, chez les oiseaux, le rôle important de l'incubation dans la protection des œufs contre les contaminations au travers de la coquille par des bactéries et champignons pathogènes. Toutefois le mécanisme exact de cette protection demeure inconnu, bien qu'il ait été suggéré que le rôle protecteur puisse être lié aux sécrétions uropygiennes de la couveuse. Dans la présente note, cette hypothèse est testée chez la Mésange charbonnière *Parus major*, en prédisant que, si les sécrétions de la glande uropygienne protègent les œufs des bactéries et des champignons durant l'incubation, le succès à l'éclosion devrait être positivement lié à la taille de la glande uropygienne, qui reflète le volume des sécrétions. Contrairement à ce à quoi on s'attendait, la taille de la glande uropygienne de la couveuse n'est apparue associée ni au succès à l'éclosion ni à la durée de l'incubation, ni au succès à l'envol, ou au succès reproducteur ou encore à la masse corporelle des jeunes à l'envol. Il est donc conclu que, au moins chez la Mésange charbonnière, d'autres facteurs que les sécrétions de la glande uropygienne, comme l'inoculation d'espèces microbiennes protectrices ou la protection contre les précipitations et l'humidité par les couveurs, contrôleraient la réduction, facilitée par l'incubation, d'un développement d'agents pathogènes.

Avian incubation is an energy demanding activity (Thomson *et al.,* 1998), and thus bird condition and health during this period determine the success of the breeding performance. For example, birds infected with hemoparasites may have a decreased ability to thermoregulate, which in turn causes a reduction in hatching success (Sanz *et al.,* 2001). Environmental factors also affect the incubation output, mainly by changes in temperature and microbial growth (Stoleson & Beissinger, 1999; Cook *et al.,* 2003, 2005a). In the last few years, eggshell microbiota has appeared as a primary factor determining the breeding success of birds, since bacteria and fungi communities can multiply rapidly on the eggshell, from where they can penetrate into the egg content through the eggshell pores, thus decreasing the hatching success (Cook *et al.,* 2003, 2005a).

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Cook *et al.* (2005b) and Shawkey *et al.* (2009) have experimentally demonstrated that incubation decreases the probability of trans-shell infection by reducing harmful bacteria and fungi contents on eggshells in a hole-nesting passerine. However, these authors did not identify the underlying mechanism related to incubation causing a decrease in microbial growth, but proposed that: (1) incubation could reduce humidity and exposure to precipitation, factors that enhance microbial growth, and (2) the parents could inoculate shells with protective microbial species or with antimicrobial agents from the uropygial oil contained in their feathers or from epidermal secretions (Cook *et al.,* 2005b). The aim of the present study is to examine correlational evidence of these hypotheses by searching for a possible relationship between uropygial gland size and hatching success and other reproductive parameters in a population of Great Tits *Parus major* breeding in nest boxes. Uropygial gland size reflects the amount of oil secreted by birds, as the secretory capsules (which produce the lipid substances) occupy the greatest proportion of the gland, the size of the gland is positively correlated with the diameter of its artery, and uropygial gland weight is positively correlated with bird body weight within species, which is in turn correlated with the weight of gland secretions (Galván *et al.,* 2008). Indeed, it has been shown that uropygial gland size predicts the amount of oil secreted (Martín-Vivaldi *et al.,* 2009) and accounts for variation in the abundance of feather mites, oil-feeding ectosymbionts (Proctor, 2003; Galván & Sanz, 2006; Galván *et al.,* 2008). Since larger uropygial glands secrete greater volumes of oil (Martín-Vivaldi *et al.,* 2009) and present greater antimicrobial activity (Møller *et al.,* 2009), a positive relationship between uropygial gland size and different measures of breeding performance, in particular hatching success (Cook *et al.,* 2003, 2005a), was predicted. Such a relationship has been recently reported by Møller *et al.* (2010) at an interspecific level. Here, this relationship is tested intraspecifically in Great Tits.

MATERIALS AND METHODS

The study was carried out in May-June 2005 in a deciduous forest of Pyrenean Oak *Quercus pyrenaica* at 1200 m above sea level in Miraflores de la Sierra, Sierra de Guadarrama, central Spain (40º 49' N, 03º 46' W). Frequent checks of nest-boxes provided data on dates of clutch initiation and clutch size for all breeding pairs. On the fourth day after hatching, adults were captured and banded with numbered rings. The birds were weighed with a portable electronic balance to the nearest 0.1 g and classified as first year breeder or older according to plumage (Jenni & Winkler, 1994). A total of 23 female Great Tits (10 adults and 13 yearlings) belonging to 23 breeding pairs were captured. One female laid a second clutch, and therefore its first clutch was the only considered in the analyses. In Great Tits, incubation and brooding are carried out only by the female (Gosler, 1993; Sanz & Tinbergen, 1999), and only data on uropygial gland size of females were thus used.

Duration of incubation was defined as number of days between completion of clutch and first signs of hatching. Nestlings were ringed on day 14 after hatching (hatching date = day 0) and weighed in the same way as adults. Broods were visited again on day 15 to establish numbers of fledged and dead young, since a high number of nestlings have already left the nest on day 15 in the study area. Hatching success (proportion of eggs hatched), fledging success (proportion of hatchlings that resulted in fledged young), breeding success (proportion of eggs that resulted in fledged young) and fledgling mass were considered as partial measures of reproductive success.

The uropygial gland of Great Tits was visually inspected by soaking the surrounding feathers with water. This holocrine gland is embedded beneath the skin just dorsal to the levator muscle of the tail (Elder, 1954), and its relatively large size makes it easy to measure. Three measurements were taken to estimate the size of the uropygial gland: maximum width, maximum length and "height", which were then multiplied to obtain an estimate of the volume of the gland expressed in mm^3 (see Galván & Sanz, 2006; Galván *et al.,* 2008). This measure of gland size is also correlated with the size of excised glands (Møller *et al.,* 2010). Daily production of oil is regulated by the levels of certain hormones (e.g. androgenic steroids, glucocorticoids and secretions from the thyroid gland; Jacob & Ziswiler, 1982; Blanco & Frías, 2001), and thus daily variations in the gland size are likely to occur. Therefore, the hour of capture was also recorded and considered in the analyses.

General linear models (GLM) with each reproductive performance variable as a dependent variable, age of birds as a fixed factor and hour of capture and uropygial gland size as covariates were used. Non-significant terms were removed through a backward stepwise procedure. Hatching, fledging and breeding success were $\log_{10}$-transformed. Inspections of residuals from the models confirmed that the normality assumption was fulfilled.

RESULTS

Mean (± SE) values were 115.3 ± 6.0 mm^3 for uropygial gland size (n = 23, range: 75.2-180.2), 83.3 ± 3.6% for hatching success (n = 20, range: 44.4-100), 22.5 ± 0.4 days for incubation period (n = 23, range: 18-26), 94.1 ± 2.2% for fledging success (n = 16, range: 75-100), 80.1 ± 4.0% for breeding success (n = 16, range: 44-100), and 17.1 ± 0.2 g for fledgling mass (n = 16, range: 15.5-18.4).

Hatching success was not significantly related to uropygial gland size ($F_{1,18}$ = 0.37, p = 0.55; Fig. 1), and all terms were removed from the model (hour of capture: $F_{1,16}$ = 0.05, p = 0.83; age: $F_{1,17}$ = 0.04, p = 0.85). Similarly, uropygial gland size was not significantly related to incubation period ($F_{1,20}$ = 0.59, p = 0.45), fledging success ($F_{1,13}$ = 1.49, p = 0.24), breeding success ($F_{1,13}$ = 1.68, p = 0.217) nor fledgling mass ($F_{1,14}$ = 1.05, p = 0.32), and the rest of variables were also removed from the models (results not shown).

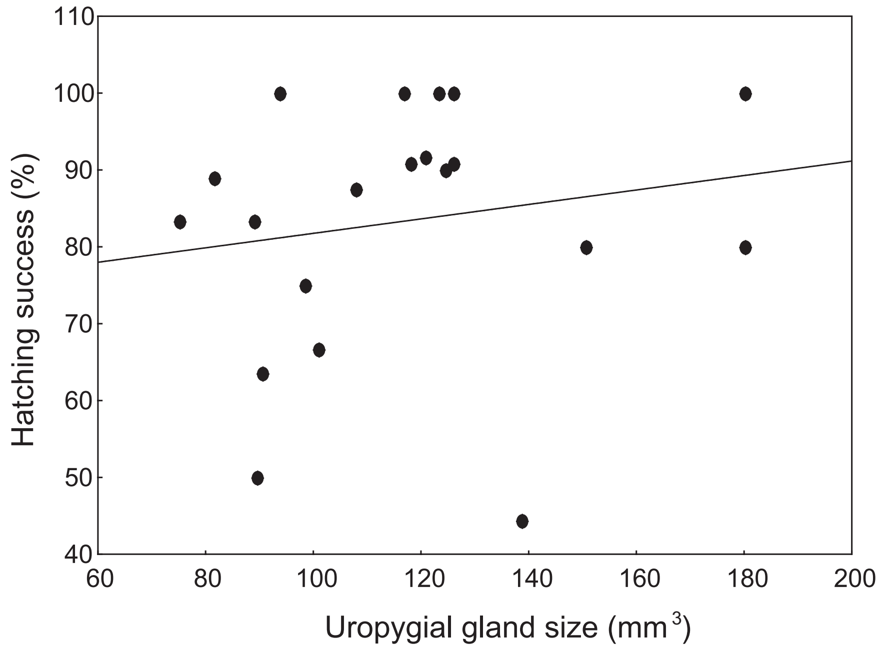

Figure 1. — Relationship between uropygial gland size and hatching success in female Great Tits. The line is the regression line.

DISCUSSION

Contrary to the prediction that uropygial secretions could be responsible for the observed reduction in microbial growth on eggshell during incubation (Cook *et al.,* 2005b), the size of the uropygial gland of female Great Tits was not significantly correlated with hatching success nor with any other reproductive parameter. Therefore, the antimicrobial agents of uropygial secretions (Shawkey *et al.,* 2003; Martín-Vivaldi *et al.,* 2009) are not likely to be the underlying mechanism causing a reduction in eggshell microbiota, as higher hatching success should be expected for greater amounts of a substance able to control these microorganisms (Cook *et al.,* 2003, 2005a). Although the size of the uropygial gland predicts the capacity to control feather-degrading bacteria (Møller *et al.,* 2009), this gland may not be responsible for protection against bacteria specific to eggshells in the Great Tit. This contradicts the results of Møller *et al.* (2010), who found a positive association between gland size and hatching success in 212 species of birds. Nevertheless, it is possible that the pattern of covariation between gland size and hatching success is differently expressed at intra- and interspecific levels. Thus, the relationship may be only apparent at a wide taxonomic level, but not between individual in certain species if the intensity of the association greatly varies across species. This would suggest that several ecological factors are affecting the magnitude of selective pressures acting on the size of uropygial gland in birds (Galván *et al.*, 2008).

However, the uropygial gland size was measured four days after the eggs hatched, so that the possibility that gland size has changed during the time interval between the start of incubation and gland measurements should not be discarded. Additional information on possible variations in uropygial gland size across the breeding cycle is needed. Furthermore, it is possible that intraspecific variation exists not only in the quantity of uropygial gland secretions, but also in the chemical composition of these secretions, as reported at an interspecific level between passerine bird species (Haribal *et al.*, 2005). These possibilities should be explored by future studies.

Since high rates of microbial growth are not restricted to specific habitats or climates but seem to be widespread among bird eggs in different climatic regimes (Cook *et al.,* 2005b), the results of the present study may be of general value. Indeed, moderate rates of trans-shell infection have been found in a Mediterranean climate (Cook *et al.,* 2005b and cited references), a similar climate to the one encountered in this study. However, the fact that all results in the present study were non-significant suggests that hypotheses alternative to that linking microbial growth to uropygial gland secretions could be more likely. In any case, this study is entirely correlational, so that causal relationships cannot be established at this stage of research. Additionally, the sample size here was small, so that the statistical power of the analyses is weak. It should be considered that this would preclude the possibility of finding any relationship unless the magnitude of the association was high. Therefore, the present study discards the possibility that the association between uropygial gland size and hatching success is intense in Great Tits.

Antimicrobial agents secreted by the epidermis was proposed as another mechanism reducing microbial growth, but the resemblance between the chemical nature of the uropygial secretions and those of the epidermis, which is also considered an independent holocrine secretory unit producing lipoid substances (Menon & Menon, 2000; Sandilands *et al.,* 2004 and cited references), makes this possibility unlikely. Therefore, the inoculation of protective microbial species or the protection from precipitation and high levels of humidity by the incubating parents (Cook *et al.,* 2005b) should be considered the most probable mechanisms linking incubation with the reduction of microbial growth on eggshells. Future studies using different species and larger sample sizes should corroborate the results found here.

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