BEHAVIOURAL ADAPTATIONS OF CAVITY NESTING BIRDS

ALEJANDRO CANTARERO CARMONA

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Memoria presentada por el Licenciado D. Alejandro Cantarero Carmona para optar al grado de Doctor en Biología, dentro del programa de doctorado "Comportamiento Animal" de la Universidad de Córdoba, dirigida por el Dr. Juan Moreno Klemming del Museo Nacional de Ciencias Naturales – CSIC y el Dr. Alberto José Redondo Villa de la Facultad de Ciencias de la Universidad Córdoba.

Córdoba, 2015

El Doctorando

Alejandro Cantarero Carmona

Vº Bº del Director

Juan Moreno Klemming

Vº Bº del Director

Albert

Alberto José Redondo Villa

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Memoria presentada para optar al grado de Doctor en Biología por la Universidad de Córdoba por el Licenciado

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TÍTULO DE LA TESIS: BEHAVIOURAL ADAPTATIONS OF CAVITY NESTING BIRDS

DOCTORANDO/A: ALEJANDRO CANTARERO CARMONA

INFORME RAZONADO DEL/DE LOS DIRECTOR/ES DE LA TESIS

La Tesis Doctoral denominada "Behavioural Adaptations of Cavity Nesting Birds", realizada bajo nuestra dirección por Alejandro Cantarero Carmona continúa la línea de investigación iniciada por el doctorando en su Trabajo Fin de Máster. El presente trabajo comenzó a raíz de la realización de un trabajo en vídeo de divulgación científica para la serie de televisión "Discovering Animal Behaviour" de la que Alberto J. Redondo es director. En el capítulo tercero trataba los trabajos sobre papamoscas cerrojillo (*Ficedula hypoleuca*) del Dr. Juan Moreno Klemming. Para la filmación de esta película desarrollamos una serie de técnicas que permitían grabar con una gran calidad en el interior de las cajas nido.

Esta vez la divulgación científica supuso un camino de ida y vuelta, ofreciendo una herramienta para avanzar en el descubrimiento científico de la biología reproductora de las aves que anidan en cavidades.

El doctorando Alejandro Cantarero ha realizado un excelente trabajo aprovechando al máximo esta técnica, complementándola con numerosas mejoras, de forma que ha podido ser testigo de cientos de horas de intimidad en la vida de las aves. Su capacidad, su dedicación y su excelente trabajo bajo la experta dirección del Dr. Juan Moreno han dado como resultado un magnífico trabajo que aporta numerosos avances en el conocimiento del comportamiento reproductor de estas aves y sus implicaciones evolutivas.

Los objetivos planteados durante el desarrollo de la Tesis se han cumplido con éxito. Los experimentos planteados han sido satisfactorios encontrándose resultados muy interesantes que abren nuevas fronteras en la investigación del comportamiento animal. Fruto de la gran calidad de este trabajo son las 5 publicaciones en las más destacadas revistas científicas indexadas en el Journal Citation Report que tratan sobre este campo:

- CANTARERO, A., LÓPEZ-ARRABÉ, J. y MORENO, J. 2015. Selection of nest-site and nesting material in the Eurasian Nuthatch *Sitta europaea*. Ardea (in press).

- CANTARERO, A., LÓPEZ-ARRABÉ, J., SAAVEDRA-GARCÉS, I., RODRÍGUEZ-GARCÍA, V., PALMA, A. y MORENO, J. 2014. The significance of nest structure and nesting material for hole-nesting passerines: an experimental study with Nuthatches *Sitta europaea*. Acta Ornithologica 49: 143-155.

- CANTARERO, A., LÓPEZ-ARRABÉ, J., PALMA, A., REDONDO, A. J. y MORENO, J. 2014. Males respond to female begging signals of need: a handicapping experiment in the Pied flycatcher *Ficedula hypoleuca*. Animal Behaviour 94: 167–173.

- CANTARERO, A., LÓPEZ-ARRABÉ, J., REDONDO, A. J. y MORENO, J. 2013. Behavioural responses to ectoparasites in Pied Flycatchers *Ficedula hypoleuca*: an experimental study. Journal of Avian Biology 44: 591-599.

- CANTARERO, A., LÓPEZ-ARRABÉ, J., RODRÍGUEZ-GARCÍA, v., GONZÁLEZ-BRAOJOS, S., RUIZ-DE-CASTAÑEDA, R., REDONDO, A. J. y MORENO, J. 2013. Factors affecting the presence and abundance of generalist ectoparasites in nests of three sympatric hole-nesting bird species. Acta Ornithologica 48: 39-54.

Por lo tanto, consideramos que la Tesis cumple todos los requisitos de calidad para su presentación.

Córdoba, 2 de Marzo de 2015

Firma del/de los director/es

Albert

Fdo.: Juan Moreno Klemming

Fdo.: Alberto José Redondo Villa

TÍTULO DE LA TESIS: BEHAVIOURAL ADAPTATIONS OF CAVITY NESTING BIRDS

DOCTORANDO/A: ALEJANDRO CANTARERO CARMONA

ESCRITO RAZONADO DEL RESPONSABLE DE LA LÍNEA DE INVESTIGACIÓN

El doctorando Alejandro Cantarero Carmona, ha demostrado una alta capacidad y dedicación en su trabajo investigación, habiendo publicado ya 5 artículos en revistas indexadas en el Journal Citation Report del tema de su Tesis doctoral, además de algunas otras publicaciones dentro de la línea de Ecología Evolutiva y de las Estrategias Vitales en Aves.

El grupo de investigación donde está desarrollando su labor de investigación es puntero a nivel internacional y de reconocido prestigio, lo que asegura una muy fructífera carrera investigadora del doctorando.

Por lo tanto, considero que la Tesis cumple todos los requisitos de calidad para su presentación.

Córdoba, 2 de Marzo de 2015

Firma del responsable de línea de investigación

Fdo.: Luis Arias de Reyna Martínez

This thesis has been presented as a compendium of publications. The following works have been published in journals included in the three first quartiles according to the Journal Citation Reports:

1. **Title:** Behavioural responses to ectoparasites in Pied Flycatchers *Ficedula hypoleuca*: an experimental study

   Authors: Cantarero, A., López-Arrabé, J., Redondo, A. J. & Moreno, J

   Journal: Journal of Avian Biology 44: 591-599

   Year of publication: 2013

   Subject category: Ornithology

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   Journal rank: 2/21

   Reference: Cantarero, A., López-Arrabé, J., Redondo, A. J. & Moreno, J. 2013. Behavioural responses to ectoparasites in Pied Flycatchers *Ficedula hypoleuca*: an experimental study. Journal of Avian Biology 44: 591-599

2. **Title:** Factors affecting the presence and abundance of generalist ectoparasites in nests of three sympatric hole-nesting bird species

   Authors: Cantarero, A., López-Arrabé, J., Rodríguez-García, V., González-Braojos, S., Ruiz-de-Castañeda, R., Redondo, A. J. & Moreno, J.

   Journal: Acta Ornithologica 48: 39-54

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3. **Title:** Males respond to female begging signals of need: a handicapping experiment in the Pied flycatcher *Ficedula hypoleuca*

   Authors: Cantarero, A., López-Arrabé, J., Palma, A., Redondo, A. J. & Moreno, J.

   Journal: Animal Behaviour 94: 167-173

   Year of publication: 2014

   Subject category: Zoology

   Impact factor: 3.068

   Journal rank: 7/153

   Reference: Cantarero, A., López-Arrabé, J., Palma, A., Redondo, A. J. & Moreno, J. 2014. Males respond to female begging signals of need: a handicapping experiment in the Pied flycatcher *Ficedula hypoleuca*. Animal Behaviour, 94, 167-173.

4. **Title:** The significance of nest structure and nesting material for hole-nesting passerines: an experimental study with Nuthatches *Sitta europaea*

   Authors: Cantarero, A., López-Arrabé, J., Saavedra-Garcés, I., Rodríguez-García, V., Palma, A. & Moreno, J.

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5. **Title:** Selection of nest-site and nesting material are affected by nest construction costs in the Eurasian Nuthatch *Sitta europaea*

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   Journal: Ardea

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   Impact factor: 0.989

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   Reference: Cantarero, A., López-Arrabé, J. & Moreno, J. (2015). Selection of nest-site and nesting material in the Eurasian Nuthatch *Sitta europaea*. Ardea (in press).

A Pepe

CHAPTERS

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ABSTRACT

Nesting cavities offer conditions of relatively constant temperature and humidity as well as protection from rain, solar radiation and. Availability of nesting cavities, thermal properties of the nests and ectoparasites loads are among the main factors implicated in the evolution of cavity nesting behaviour. The main goal of this thesis is increased understanding from an evolutionary perspective of the behavioural strategies arising during several stages of the reproductive cycle in small altricial cavity-nesting birds such as the Pied Flycatcher *Ficedula hypoleuca*, Eurasian Nuthatch *Sitta europaea* and Blue Tit *Cyanistes caeruleus*. This PhD thesis involves descriptive studies and field experiments aimed at understanding the mechanisms underlying adaptations for nest site selection and defense, for determining nest structure and composition, for defense against ectoparasites and for female nutrition during the incubation stage in their natural habitats.

Given that nesting cavities are a scarce resource, there may be strong competition over them. We found that levels of testosterone differ between populations of the same species, being higher in populations where the likelihood of nest-site usurpation by intruders is greater. We also found that the level of female aggressiveness against intruders decrease with higher T levels in high density areas. After obtaining a nest cavity or nest-box, nest building begins. Some species such as Nuthatches show clear preferences for certain nesting materials and cavities for breeding. The selection of nesting material and the amount of mud in Nuthatch nest-boxes can be explained as a trade-off between requirements for nest construction and availability as a function of transport distance.

Nesting cavities constitute micro-environments very likely to be colonized by ectoparasites and certain nesting materials may be used due to their insecticidal properties. Additionally, nests without any structure such as those built by Nuthatches may offer fewer opportunities for hiding to ectoparasites and nest composition may affect ectoparasite development through the effects of microclimatic conditions associated with different nest materials. Here we show that the replacement of unstructured nests by structured nests did not result in changes in ectoparasite loads for Nuthatches, which suggests that the preferences for nest materials in Nuthatches are unrelated to ectoparasitism. We suggest that Nuthatch nests contribute to reduce the thermal loss for nestlings and possibly eggs during female absences by remaining buried into loose and heat-producing bark flakes.

Generalist ectoparasites infest nests of avian cavity-nesting passerines as a response to different factors exhibited by host species. Differences in nest composition among host species are not the main factor explaining ectoparasite prevalences and abundances, while nest size, breeding phenology, brood size and nest-cavity micro-climate may all affect levels of infestation in different ways for each host-parasite association. Given the negative impact of ectoparasites on nestlings there will be selection on hosts to reduce parasite infestations through behavioural means. We found that frequency and intensity of female anti-parasite behaviours during the incubation and nestling periods decreased as a consequence of the experimental reduction of ectoparasite infestation and these behaviours were more intense in the host species with highest infestation levels.

As a response of higher nutritional needs arising from higher ectoparasite loads we showed that nestlings begged more intensely. While begging by nestlings has received a fair deal of attention as an honest system of communication, begging between mates has received scant attention. We tested if female begging during incubation is an honest signal of energetic need and found that experimentally handicapping female pied flycatchers intensified begging displays arising from condition impairment and that males were able to respond by increasing their feeding rates to females.

RESUMEN

Las cavidades de nidificación ofrecen unas condiciones de humedad y temperatura relativamente constantes así como protección de la lluvia, la radiación solar y los depredadores. La disponibilidad de dichas cavidades, las propiedades térmicas de los nidos y la carga de ectoparásitos son los principales factores implicados en la evolución del comportamiento de las aves que las utilizan para su reproducción. El objetivo principal de esta tesis es incrementar el conocimiento desde una perspectiva evolutiva de las estrategias de comportamiento que surgen durante diversas etapas del ciclo reproductivo en aves paseriformes que anidan en cavidades, tales como el Papamoscas Cerrojillo *Ficedula hypoleuca*, el Trepador Azul *Sitta europaea* y el Herrerillo Común *Cyanistes caeruleus*. Esta tesis incluye estudios descriptivos y experimentos de campo destinados a comprender los mecanismos subyacentes en la adaptación a la selección y defensa de sitios de nidificación, en la determinación de la estructura y composición del nido, en las respuestas a ectoparásitos y en la comunicación de señales de necesidad entre la pareja reproductora durante la incubación.

Dado que las cavidades de nidificación son un recurso escaso hay una fuerte competencia sobre ellas. Se encontró que los niveles de testosterona en hembras de papamoscas difieren entre poblaciones de la misma especie, siendo más elevados en poblaciones donde la probabilidad de usurpación de nidos por intrusos es mayor. Además, se encontró que el nivel de agresividad contra intrusos de hembras que criaron en zonas de alta densidad disminuye con altos niveles de testosterona. Tras la elección de un sitio de nidificación comienza la construcción del nido. Algunas especies como los trepadores mostraron patrones claros en selección de cajas nido y en la preferencia de ciertos materiales de nidificación. La selección del material de nidificación y la cantidad de barro que utilizan los trepadores puede explicarse como un compromiso entre la necesidad de su utilización y la disponibilidad de los mismos en función de la distancia de transporte.

Las cavidades de nidificación constituyen microambientes muy propensos a ser colonizados por ectoparásitos y ciertos materiales de nidificación pueden ser

utilizados por sus propiedades insecticidas. Además, los nidos desestructurados pueden ofrecer menos oportunidades de ocultación a los ectoparásitos y la composición del nido puede afectar al desarrollo de los ectoparásitos como consecuencia de las condiciones microclimáticas asociadas a diferentes materiales de nidificación. Se mostró que la sustitución de los nidos desestructurados por nidos estructurados no dio lugar a cambios en la carga de ectoparásitos, lo que sugiere que la preferencia de ciertos materiales no está relacionada con el ectoparasitismo. Sugerimos que los trepadores construyen nidos formados por material suelto para reducir la pérdida de calor de los pollos al poder permanecer enterrados en cortezas que podrían generar calor.

Los ectoparásitos generalistas infestan nidos de aves como respuesta a diferentes factores exhibidos por las especies huésped. Las diferencias en la composición del nido entre especies no son el principal factor que explica la prevalencia y abundancia de ectoparásitos, mientras que el tamaño del nido, la fenología reproductiva, el tamaño de la nidada y el microclima del nido pueden afectar a los niveles de infestación de diferentes formas. Dado el impacto negativo de ectoparásitos sobre los pollos, habrá una selección en los hospedadores para reducir dichos efectos a través de diversos mecanismos de comportamiento. Se encontró que la frecuencia y la intensidad de los comportamientos anti-parásitos de las hembras durante varias etapas del ciclo disminuyeron como consecuencia de la reducción experimental de la infestación de ectoparásitos y estos comportamientos fueron más intensos en las especies hospedadoras que presentaban los niveles de infestación más altos.

Como respuesta a necesidades nutricionales producidas por los parásitos, los pollos solicitaron comida más intensamente. Mientras que la petición de los pollos se ha considerado un sistema honesto de comunicación, la petición entre machos y hembras ha recibido escasa atención. Hemos probado si la petición femenina durante la incubación es una señal honesta de necesidad energética y encontramos que hembras de papamoscas dificultadas para el vuelo experimentalmente intensificaron sus señales de petición y que los machos fueron capaces de responder a dicha petición incrementando su tasa de cebas.

INTRODUCTION

Bird nests are structures constructed by reproducing adults for developing eggs and chicks (Collias and Collias 1984; Hansell 2000). Bird nests are considered extended phenotypes (Dawkins 1982) whose main function is to increase the probability of offspring survival. Nest building is an energetically costly and time demanding activity (Hansell 2000) that may increase detectability by potential predators of both builders and developing offspring inside nests (Collias & Collias 1984), so selection pressures exerted by predators must have influenced the evolution of nest characteristics. Nest predation is usually the greatest cause of nest loss for nesting birds (Lack 1954; Nice 1957; Ricklefs 1971). The properties that contribute to a predation-resistant structure may also contribute to maintaining an optimal microclimate (Rhodes et al. 2009). Thus, nest predation and thermal conditions may be the main factors implicated in the evolution of cavity nesting behaviour (Hansell 2000). Nesting cavities offer conditions of relatively constant temperature and humidity as well as protection from rain, solar radiation and predators (Hansell 2000; Nilsson 1984), and therefore the relative breeding success of cavity-nesting bird species is higher than that of open-nesting species (Lack 1954; Nice 1957). However, cavity nesting may require specific adaptations given the presumably limited availability of adequate nest holes and the sanitary and microclimatic implications of enclosed breeding.

The present PhD thesis is an approach to understanding from an evolutionary perspective the behavioural strategies arising at several stages of the reproductive cycle in cavity-nesting birds such as the Pied Flycatcher *Ficedula hypoleuca*, Eurasian Nuthatch *Sitta europaea* and Blue Tit *Cyanistes caeruleus.*

1. ADAPTATIONS FOR NEST DEFENSE

Natural selection favors individuals that choose resources that enhance breeding success, but limited availability of such resources can limit the number of individuals that breed (Li and Martin 1991). For obligate secondary cavity nesters, unable to excavate their own cavity, nest holes constitute a scarce resource that may limit the availability of breeding opportunities and there inducing a strong competition over them (Ingold 1994; Leffelaar and Robertson 1985; Li and Martin 1991; Nilsson 1984). Thus, Eurasian Nuthatches narrow the entrance of cavities by plastering mud on the outside (Matthysen 1998), thereby making the entrance narrower in order to avoid nest site competitors such as the European Starling *Sturnus vulgaris* (Collias and Collias 1984). Losing the nest site would be one of the worst scenarios for the breeding pair (Rätti 2000). Some cavity nesters have become accustomed to use artificial nest-boxes , and populations of some of these species may be increased dramatically in this way (Collias and Collias 1984). Competition for nest-boxes takes place between individuals of different or of the same species (Collias and Collias 1984) and may favour aggressive nest defense behaviours (Bentz et al. 2013). Males are typically considered the more aggressive sex (Kokko 1999; Lewis et al. 2001; Wiley and Poston 1996; Wittenberger 1981), although females may display aggressive behaviours towards conspecifics in some of the same contexts as males such as the defence of their nest site (Karlsen and Slagsvold 1997; Kral et al. 1996; Male et al. 2006; Sandell 2007; Slagsvold et al. 1992).

Aggressiveness is partly determined by hormonal status (Moss et al. 1994). Many aspects of male reproduction in vertebrates are influenced or controlled by the steroid hormone testosterone (Smith et al. 2005). Testosterone (T) levels are generally lower in females than in males (Moreno et al. 2014; Silverin and Wingfield 1982), and there is mixed evidence concerning the importance of T for female social aggressiveness. Some studies have shown that circulating T concentrations in females are associated with aggressive behaviour (Gill et al. 2007; Lahaye et al. 2012; Moss et al. 1994; Sandell 2007; Veiga and Polo 2008), while others have not (Hau et al. 2004; Jawor et al. 2006). Territorial exclusion of female intruders may be especially necessary during the initial stages of reproduction when nests-sites may be taken over (Gowaty and Wagner 1988; Rosvall 2011). Competition among females for breeding sites can be rough and even lethal, especially during the nest-building period (Morales et al. 2014), where it is known that T levels can be increased rapidly in response to territorial intrusions (Silverin 1993).

2. ADAPTATIONS IN NEST STRUCTURE AND NESTING MATERIAL

Hole-nesting bird species vary in the characteristics of their nest sites (Li and Martin 1991) and build their own characteristic nests (Hansell 2000). Birds typically make use of local materials but some birds are nest material specialists (Hansell 2000). However, for many species the functional properties of their nests remain unknown. Nest construction may be influenced by factors such as the availability of nest materials (Moreno et al. 2009) and may involve a large expenditure of time and energy (Moreno et al. 2008) mainly due to the costs of transporting material to the nest site (Putnam 1949). These costs may be reduced by using old nest material (Nores and Nores 1994) and/or if nesting material is located close to the nest site (Collias and Collias 1984). Availability may play an important role in the selection of nest materials.

The physical structure of the nest is determined by the degree of cohesion between the different materials used and may influence embryo development and chick growth, so that nest quality may have important consequences for the condition and reproductive success of parents (Álvarez and Barba 2011; Dawson et al. 2011; Lambrechts et al. 2012; Lombardo et al. 1995; Moreno et al. 2010b). Nest construction from a thermal aspect represents a compromise between heat conservation, heat dissipation, and protection from external heat sources (Heenan and Seymour 2011). It is reasonable that heat loss can be minimized by optimizing the physical structure of the nest (Heenan and Seymour 2011; Hilton et al. 2004; Reid et al. 2000) or by choosing a suitable nesting material (Álvarez et al. 2013). Consequently, we expect birds to adjust their nest characteristics in response to environmental conditions (Deeming 2011; Hansell 2000). Moreover, the regulation of thermal conditions within acceptable limits may be energetically costly for parents (Nord and Nilsson 2012; Williams 1996). The structure of nests may mitigate this energetic demand on parents (Hansell 2000). Thus, building a thermally favorable nest saves parental energy by reducing heat loss from attended and non-attended clutches (Heenan and Seymour 2011; Moreno et al. 2010b).

Nesting cavities are not always waterproof (Wesolowski et al. 2002). The insulation quality of nests is dependent on several factors, such as nest structure

(McGowan et al. 2004), thickness, height and volume (Alabrudzińska et al. 2003; Grubbauer and Hoi 1996), nest material quality (Mertens 1977) and moisture content (Deeming 2011; Pinowski et al. 2006). Large amounts of nest material, although of benefit to reduce incubation costs (Moreno et al. 2010a), may collect and retain humidity above optimal levels. The risk of the nests getting wet could be reduced by incorporating more hydrophobic material such as bark flakes (Wesolowski and Rowiński 2004). Furthermore, bark flakes could help to stabilize thermal fluctuations in the nesting cavity by conserving heat during the cooler hours of the day. Dense accumulations of bark flakes may produce heat due to microbiological activity as observed in composting (Collias and Collias 1984). Other birds like megapodes also use the heat of accumulated vegetal material to keep their eggs warm. Heat production may be especially noteworthy during the night when nest materials cool down after heating up during the day.

Incubation behaviour may be affected by nest microclimate and structure (Álvarez and Barba 2009). Nuthatches *Sitta* spp. use nests made of loose heaps of bark flakes without any structure or nest cup to contain eggs and nestlings (Matthysen 1998). Eggs and nestlings are found buried in the loose material. When the Nuthatch female returns to the nest, she lowers herself on the clutch and turns about in half-circles until the eggs are free from nest material (Matthysen 1998). This may reduce hatching success in unstructured nests by losing contact with some eggs within the nest material. The same may occur when nestlings are small. Moreover, the dispersion of the nestlings within the nest resulting from the lack of a structured nest cup may reduce contact among nestlings and thereby heat exchange, an important factor during periods of parental inattention (Webb 1993). Heat loss can be reduced by decreasing the area exposed through postural changes of nestlings or huddling (Webb 1993). Nuthatch nestlings may show no tendency for huddling due to the difficulty in keeping together during female absences in unstructured nests made of loose bark flakes. Moreover, nestlings may experience lower thermoregulatory costs while buried in the insulating nest material.

3. ADAPTATIONS TO ECTOPARASITES

Given relatively constant environmental conditions offered, nesting cavities constitute micro-environments very likely to be colonized by bacteria, decomposers and detritivores due to the presence of faeces and food remains of breeding birds, and by ectoparasites that feed on blood, skin and feathers of avian hosts (Collias and Collias 1984; Mazgajski 2007). Nest ectoparasites feeding on the blood of nestlings and adults constitute an important selective force affecting avian life history evolution as they remove nutritional and energy resources from hosts that could otherwise be used for growth, maintenance, or reproduction (Møller 1993). Accordingly, ectoparasite presence and abundance in nesting cavities may have constituted an additional important evolutionary factor modulating adaptations of hole-nesting birds (Heeb et al. 2000; Tripet et al. 2002).

Ectoparasites cause removal of nutritional and energy resources from hosts that could otherwise be used for growth, maintenance, or reproduction (Møller 1993). They may also induce costly immune and inflammatory responses (Møller et al. 2005). Moreover, the immature immune systems of altricial nestlings may result in stronger direct impacts from ectoparasitism due to the need to assign sufficient nutritional resources to growth (Saino et al. 1998; Szep and Møller 1999). Given the negative impact of ectoparasites on host fitness, there will be selection on hosts to avoid parasite infestations through behavioural, physiological and immunological responses (Hart 1992; Hart 1997; Heeb et al. 1998). All these responses are complementary and may be induced in adults, nestlings or both (Hart 1992; Keymer and Read 1991; Simon et al. 2005). Nesting adults may avoid nest sites with high ectoparasite loads (Moore 2002) due to the association between old nest material and higher abundance of certain types of ectoparasites (López-Arrabé et al. 2012; Mazgajski 2007) and bacteria (González-Braojos et al. 2012). Adults may also take measures to indirectly minimize the effects of nest parasites through incorporation of fresh plant material containing compounds that either directly affect the development of parasites (Clark and Mason 1988; Lafuma et al. 2001; Malan et al. 2002) or stimulate elements of the immune system of chicks that help them to cope better with the harmful activities of ectoparasites (Mennerat et al. 2009). Nevertheless, given counter-adaptations in ectoparasites, adult cavity-nesting birds are faced with their presence, and may have evolved a suite of behaviours directed a minimizing their impact (Hart 1992; Keymer and Read 1991; Loye and Zuk 1991).

Avian hosts may try to compensate for the deleterious effects of ectoparasitism through behavioural modifications (Hart 1992; Keymer and Read 1991; Loye and Zuk 1991; Simon et al. 2005). Hosts can increase their provisioning rates to the offspring (Tripet and Richner 1997b) which may affect their current and future reproduction (Richner and Tripet 1999). Given potential fitness costs, they can evolve behavioural responses to minimize ectoparasite loads (Christe et al. 1996; Tripet et al. 2002; Waite et al. 2012). The main behavioural defenses against ectoparasites are grooming and nest sanitation (Christe et al. 1996). Grooming behaviour may be operationally defined as manipulation of the plumage with the bill (Murray 1990; Nelson et al. 1977). One of its functions may be to dislodge ectoparasites hiding or residing among feathers (Cotgreave and Clayton 1994; Waite et al. 2012). Thus both adults and nestlings may groom themselves in the presence of ectoparasites (O'Connor et al. 2010). Nest sanitation (Welty 1982) refers to behaviours by parents in altricial species tending to remove ectoparasites on nestlings or nest material (Hurtrez-Boussès et al. 2000), removing from the nest both these as well as eggshells (Montevecchi 1974), fecal material (Blair 1941) or dead nestlings (Skutch 1976). Parents are expected to allocate time to nest sanitation in order to control the load of harmful ectoparasites in the nest materials and on the nestlings. Such anti-parasite behaviours may be time-consuming (Cotgreave and Clayton 1994) and therefore may reduce the time that a parent bird can devote to foraging and provisioning offspring. Behavioural adaptations to control and reduce ectoparasite impacts may mainly be detected in host populations where ectoparasites have important effects on reproductive success. That nest sanitation may be important is suggested by the fact that the condition and health of breeding females can determine the rates of ectoparasite infestation (López-Arrabé et al. 2012; Tomás et al. 2005; Tomás et al. 2007a; Tomás et al. 2007b).

For some hole-nesting passerines, fleas *Ceratophyllus gallinae*, blowflies *Protocalliphora azurea* and mites *Dermanyssus gallinoides* constitute the most important groups of nest-dwelling ectoparasites (Merino et al. 1998; Merino and

Potti 1995; Moreno et al. 2009; Rendell and Verbeek 1996). Species of these arthropod taxa are usually not host-specific (López-Arrabé et al. 2012; Moreno et al. 2009; Tripet and Richner 1997a). Their relative abundance differs according to host species even in conditions of strict sympatry (Bauchau 1998; Bennett and Whitworth 1991; Moreno et al. 2009). Nuthatches, Pied Flycatchers and Tits *Paridae* coexist frequently in European deciduous woodlands and present different prevalences and intensities of infestation by the different ectoparasite taxa (Bauchau 1998; Moreno et al. 2009). Matthyssen (1998) found that Nuthatch nests contained fewer fleas than Great Tit *Parus major* nests in similar nest-boxes and habitats, and that more fleas were found in nests built of leaves instead of pine bark. Nuthatches may prefer pine bark as nest material because it contains toxic secondary compounds that may have insecticidal properties, in particular the monoterpene limonene (Carroll 1994). Limonene (and other plant compounds such as hydrocyanic acid) repel northern fowl mites (*Ornithonyssus sylviarum*), an ectoparasitic mite (Carroll 1994). Bauchau (1998) found that Great Tit nests showed higher abundances of mites, fleas and blowflies than Pied Flycatcher nests in the Netherlands. One of the factors suggested to explain differences in ectoparasite loads between species is nest design and composition (Bauchau 1998; Moreno et al. 2009; Remeš and Krist 2005). Unstructured nests like those of Nuthatches offer fewer opportunities for hiding to ectoparasites, and nest composition may affect ectoparasite development through the effects of microclimatic conditions associated with different nest materials (Heeb et al. 2000). Large amounts of nest material, although of benefit to reduce incubation costs (Moreno et al. 2010a), may collect and retain humidity above optimal levels and attract parasitic arthropods and pathogenic bacteria (Moreno 2012). Thus, interspecific differences in ectoparasite abundances could be explained by interspecific differences in nest composition and structure.

4. ADAPTATIONS FOR INCUBATION

Males of many avian species in which only the female incubates provision their mates during the incubation period (Lifjeld and Slagsvold 1986; Lyon and Montgomerie 1985; Ricklefs 1974). In some species, for example hornbills, females are totally dependent on males for food during the incubation stage (Poonswad et al. 2004). In a variety of other species it is more common for incubating females to receive only some of their food from their mates, although they also leave the nest to forage in order to sustain their energy requirements (Poonswad et al. 2004). Mate feeding may have evolved as a behavioural strategy to compensate for energetically costly activities for the female during reproduction (Galván and Sanz 2011), which may include the posthatching stage.

Food provided by males during incubation has been proposed to be an important energy source for females, a proposal termed the ‘female nutrition hypothesis’ (Niebuhr 1981). In fact, several studies have demonstrated that higher rates of male incubation feeding to their mates can improve female body condition (Lifjeld and Slagsvold 1986) and increase nest attentiveness by reducing the amount of time the female spends foraging off the nest (Boulton et al. 2010; Halupka 1994; Leclaire et al. 2011; but see Lifjeld and Slagsvold 1989; Matysioková and Remeš 2010; Matysioková and Remeš 2011; Moreno and Carlson 1989; Pearse et al. 2004; Smith et al. 1989; Stein et al. 2010) and thereby help to advance hatching (Lyon and Montgomerie 1985; Nilsson and Smith 1988), improve hatching success (Galván and Sanz 2011; Lyon and Montgomerie 1985; Nilsson and Smith 1988) or improve fledgling condition (Lifjeld and Slagsvold 1986; Røskaft 1983). This suggests that incubation feeding has evolved as a behavioural strategy to partly compensate for the energetic limitations of females while incubating (Galván and Sanz 2011). Although there are probable fitness advantages for the breeding pair derived from male incubation feeding, there may also be costs for males induced by intensified foraging activity at an early stage of the season (Leclaire et al. 2011; Lifjeld and Slagsvold 1986; Smith et al. 1989). Thus males may experience a trade-off between provisioning their mate and feeding themselves (Lifjeld and Slagsvold 1986; Lifjeld et al. 1987; Lyon and Montgomerie 1985; Moreno et al. 2011). They may also allocate more or less effort to finding and copulating with extrapair mates (Hill et al. 2011; Wagner 1992). Male incubation feeding intensity could thus be more a product of differences in male age, condition and mating strategy than of female nutritional needs (Lifjeld and Slagsvold 1986; Lifjeld and Slagsvold 1989; Lifjeld et al. 1987).

To distinguish between the 'female nutrition' and alternative scenarios it is necessary to experimentally manipulate female condition and study male responses, as males may adjust their feeding activity to the optimal level of attendance at each nest in a non experimental situation (Moreno et al. 2011). Only according to the 'female nutrition' hypothesis would we expect a direct male response by either increasing (experimentally reduced female condition) or reducing (experimentally increased female condition) his provisioning rate. Both experimental approaches have provided support for the female nutrition hypothesis (reduced condition: Moreno et al. 2011; Paillisson et al. 2007; improved condition: Smith et al. 1989; Wright and Cuthill 1989; Wright and Cuthill 1990a; Wright and Cuthill 1990b).

To improve their condition during incubation, females should be able to communicate their needs to mates. As both sexes have at least partially overlapping reproductive interests (Moore and Rohwer 2012), communication between incubating females and their mates should be reliable (Searcy and Nowicki 2005). Begging by nestlings has received a fair amount of attention as an honest system of communication (Cotton et al. 1996; Mock et al. 2011; Wright and Leonard 2002), whereas begging between mates has received scant attention. Females beg to their mates in courtship contexts (Clancy 2005; East 1981; Ellis 2008; Otter et al. 2007; Tobias and Seddon 2002), while incubating (Ellis 2008; Moore and Rohwer 2012; Tobias and Seddon 2002) and also during the nestling feeding phase before apportioning food to the nestlings (Clancy 2005). Female begging displays include loud vocalizations, body postures and wing fluttering, which closely resemble the begging displays of older nestlings (Ellis et al. 2009; Godfray 1991; Harper 1986). The striking similarity of female and nestling begging displays suggests the retention into adulthood in females of typically juvenile behaviours (Moore and Rohwer 2012). Otter et al. (2007) manipulated the hunger levels during egg laying of black-capped chickadee, *Poecile atricapillus*, females and showed no effect on male provisioning, even finding a decrease in female food solicitation. Furthermore, Moore and Rohwer (2012) found a correlation between begging displays of incubating yellow warbler, *Setophaga petechia*, females and mate provisioning rate in relation to environmental conditions. However, to our knowledge, it has never been confirmed experimentally that males adjust incubation feeding effort to female begging intensity. To test this link, begging intensity could be manipulated directly although this is difficult. Several behavioural components (posture, vocalizations, wing fluttering) presumably contribute to begging behaviour but the information content expressed in each component is still unknown. Alternatively, begging behaviour may be manipulated through hunger. Hunger depends on energy balance which may be experimentally altered through either food supplementation or handicapping (see above).

AIMS AND HYPOTHESES

This thesis is framed within the scientific field of Evolutionary Ecology and the main goal is increase understanding from an evolutionary perspective of the behavioural strategies arising during several stages of the reproductive cycle in small altricial cavity-nesting birds such as the Pied Flycatcher, Eurasian Nuthatch and Blue Tit. This PhD thesis involves descriptive studies and field experiments aimed at understanding the mechanisms underlying adaptations for nest site selection and defense, for determining nest structure and composition, for defense against ectoparasites and for female nutrition during the incubation stage in their natural habitats.

- **Objective I.** Explore the implications of the steroid hormone testosterone in relation to female-female competition for breeding resources by studying natural levels of aggressiveness between three geographically separated populations of pied flycatchers (Chapter I).

- **Objective II.** Elucidate if selection of nesting material and the amount of mud in Nuthatch nest-boxes can be explained as a trade-off between requirements for nest construction and availability as a function of transport distance (Chapter II).

- **Objective III.** Understand through a field experiment the implications of unstructured bark flake nests in Nuthatches for microclimatic conditions in the nest, ectoparasite infestation, parental care and nestling begging (Chapter III).

- **Objective IV.** Explore experimentally whether the ectoparasite loads lead to change the frequency and duration of anti-parasite behaviours by adult hosts, as well as whether such anti-parasite behaviours are able to compensate for the deleterious effects that parasites may have on nestlings (Chapter IV).

- **Objective V.** Study the implications of ectoparasitism in three sympatric avian cavity-nesters, namely Pied Flycatchers, Blue Tits and Nuthatches, to explore if differences in prevalence and abundance of generalist ectoparasites (blowflies, fleas and mites) can be related to interspecific differences in their nest size, nest composition and cavity microclimate. Furthermore, we have aimed at detecting if interspecific variation in the incidence and intensity of anti-parasite behaviours is a consequence of the abundance of ectoparasites in these three species (Chapter V).

- **Objective VI.** Test experimentally whether female begging during incubation is an honest signal of energetic need and whether mates respond to it (Chapter VI).

To answer these aims, this thesis combines information obtained during intense fieldwork campaigns from observations and diverse experiments. The thesis is divided into six chapters, whose development has been linked to different aspects of the reproductive cycle of the cavity-nesting birds.

1. ADAPTATIONS FOR NEST DEFENSE

CHAPTER I. Nesting holes are a scarce resource for cavity nesting birds and the need to occupy them may be an important selective force for the evolution of aggressive female behaviours, which may be mediated by testosterone (T) levels. The aim of this study was to explore the variation in circulating T levels of females between three geographically separated populations of pied flycatchers. We exposed female pied flycatchers from two of these populations to simulated territorial intrusions using a stuffed female when nest construction was almost complete. We also collected female blood samples to measure T levels in all populations. The purpose of the study was to explore the implications of T levels for female-female competition by studying natural levels of aggressiveness towards intruders in two populations with a marked difference in female plumage phenotype, a southern population in Valsaín (central Spain) and a northern population on the island of Ruissalo (Finland). We also collected female blood samples from a high density population located near the village of Lozoya (central

Spain) to further explore density-related variation in T levels. Furthermore, we aimed to detect if variation of T levels may explain female incubation attendance. We predicted that if T level is a proximate mechanism regulating female behaviour in the early stages of breeding, then:

1. T levels should be related to female aggressiveness towards female decoys.
2. T levels should be higher in females from populations more exposed to aggressive territorial interactions.
3. T levels should be higher when the availability of nest-boxes for breeding is lower later in the season.
4. Incubation attendance should be lower when T levels are higher.

2. ADAPTATIONS IN NEST STRUCTURE AND NESTING MATERIAL

CHAPTER II. Selection of nest sites and nesting material may have important implications for avian reproductive behaviour and performance. Nest construction may involve costs arising of transporting material that may be reduced considerably if nest materials are located close to the nest-site. Nuthatch nests in our nest-box study area are mainly composed of pine bark flakes or alternatively of strips of bark of the widespread shrub *Cistus laurifolius*, with variable amounts of mud used for plastering the entrance. In the present study we have attempted to elucidate if selection of nesting material and the amount of mud in Nuthatch nest-boxes can be explained as a trade-off between requirements for nest construction and availability as a function of transport distance. We have explored whether:

1. The selection of nest-boxes by Nuthatches depends on the distance to streams where mud can be collected;
2. This relation is stronger for Nuthatches than for another sympatric cavity-nester, the Pied Flycatcher;
3. Selection of Nuthatch nesting material (pine or *Cistus* bark) depends on the availability of pine, in particular the distance to the nearest pine;

4. The amount of mud in Nuthatch nest-boxes is negatively associated with the distance to streams.

CHAPTER III. Nest structure and nesting material may have important consequences for avian reproductive behaviour and performance. Nuthatches *Sitta* spp. build nests made of loose bark flakes without any structure or nest cup to contain eggs and nestlings. We have aimed at understanding the implications of unstructured bark flake nests in Nuthatches for microclimatic conditions in the nest, ectoparasite infestation, parental care and nestling begging through a nest exchange experiment. To that end, we have experimentally replaced natural bark nests of Nuthatches by structured moss nests built at the same time by Great Tits for some pairs and compared their ectoparasite abundances, nest microclimate variables and the behaviour of nestlings and parents with those in natural Nuthatch nests. We have predicted that the experimental nest replacements would affect ectoparasite abundance and nest microclimate and possibly the behaviour of nestlings and parents depending on the magnitude and sign of effects on ectoparasites and cavity microclimate. This in turn could affect nestling growth and reproductive success.

3. ADAPTATIONS TO ECTOPARASITES

CHAPTER IV. Nests of cavity-nesting birds usually harbor some species of haematophagous ectoparasites that feed on the incubating adults and nestlings. Given the negative impact of ectoparasites on nestlings there will be selection on hosts to reduce parasite infestations through behavioural means. We have experimentally reduced the abundance of all ectoparasites in nests of pied flycatchers *Ficedula hypoleuca* to explore both whether there are changes in the frequency and duration of putative anti-parasite behaviours by tending adults, as well as whether such anti-parasite behaviours are able to compensate for the deleterious effects that parasites may have on nestlings. Our objectives were to explore changes in the frequency and duration of parental grooming and nest sanitation behaviours as a consequence of the abundance of ectoparasites, and to examine the impacts of these behaviours of adult birds. We have hypothesized that:

1. Behavioural responses to ectoparasites should be more frequent in control nests than in experimental nests. This pattern should occur during both the incubation and nestling periods;
2. There should be a trade-off between brooding nestlings and nest sanitation behaviours at the early nestling stage;
3. Nestlings should beg more intensely in control nests due to the increased food demand induced by ectoparasites;
4. Parents should respond to higher begging levels in control nests by increasing provisioning rates only if time consumed by anti-parasite behaviours does not compromise that available for foraging.

CHAPTER V. Nesting cavities constitute micro-environments very likely to be colonized by ectoparasites which feed on blood of the incubating female and the nestlings. Given the negative impact of ectoparasites on nestlings there will be selection on hosts to minimize ectoparasite loads through behavioural defenses. We have addressed the implications of ectoparasitism in three sympatric avian cavity-nesters, namely Pied Flycatchers, Blue Tits and Nuthatches, to explore if differences in prevalence and abundance of generalist ectoparasites (blowflies, fleas and mites) can be related to interspecific differences in their nest size, nest composition and cavity microclimate. Furthermore, we have aimed at detecting if interspecific variation in the incidence and intensity of anti-parasite behaviours is a consequence of the abundance of ectoparasites. We have explored if:

1. Variation in ectoparasite abundance between host species is associated with interspecific differences in nest size and composition;
2. Avian hosts using pine bark as nest building material (Nuthatches and some Pied Flycatchers) show lower prevalence and abundances of some ectoparasites;
3. Cavity microclimate affects ectoparasite abundance;

4. Behavioural responses to ectoparasites are more frequent in avian hosts with higher infestations. This pattern should occur during both the incubation and nestling periods;
5. There is a trade-off in time allocation between brooding nestlings and nest sanitation behaviours during the early nestling stage.

4. ADAPTATIONS FOR INCUBATION

CHAPTER VI. The ‘female nutrition’ hypothesis proposes that food provided by males during incubation is an important energy source for females in bird species in which females alone incubate. Females should be able to communicate their needs through begging signals to mates and males may compensate for the energetic limitations of females through their feeding visits, owing to their overlapping reproductive interests. To test whether female begging during incubation is an honest signal of energetic need and whether mates respond to it we experimentally handicapped female pied flycatchers at the beginning of incubation by clipping two primary flight feathers on each wing. We assumed that clipping should increase the female’s flight costs and therefore her energy requirements during incubation (Matysioková and Remeš 2011; Pennycuick 1982). We then compared control and experimental females by video filming their behaviour within the nest-box during incubation (before and after female manipulation) and at two stages of the nestling period (3 and 9 days of age). We predicted following the ‘female nutrition hypothesis’ that impaired flight ability caused by handicapping would (1) lead to increase female begging displays during incubation because of the manipulation of female condition and hunger and (2) induce more male incubation feeding in response to female needs. Depending on the effects of the experiment on male incubation feeding rate, we might or might not expect changes in female incubation behaviour and in body mass loss between incubation and the nestling phase.

STUDY SITE AND STUDY SPECIES

The general methods of this thesis are focused on the study site and study species. A more detailed description of the methods used in each experiment will be found in each chapter.

1. STUDY SITE

Picture of the study site (Valsaín, Segovia).

The studies presented in this thesis were conducted during the springs of 2011, 2012, 2013 and 2014. The study site is a montane forest of Pyrenean oak, *Quercus pyrenaica*, at 1200 m.a.s.l. in Valsaín, central Spain (40˚ 54’ N, 4˚ 01’ W) where passerines birds breeding in nest-boxes have been studied since 1991 (see Sanz et al. 2003 for general description). The mean annual temperature in this area is 10-11º C with a mean rainfall of 650-1000 mm. Scattered pines *Pinus sylvestris* are found among the oaks while the shrub layer consists mainly of *Cistus laurifolius* (Moreno et al. 2009, Cantarero et al. 2013). The study area is crossed by some seasonal watercourses.

There are 570 nest-boxes erected in the study area placed hanging from a branch attached to a metal hook (see Lambrechts et al. 2010 for dimensions, structure and placement of nest-boxes) and they are occupied mainly by Pied Flycatchers, Great tits, Nuthatches, Blue tits and Rock sparrows *Petronia petronia*. The use of such artificial cavities in avian research has greatly advanced our understanding of breeding behaviour in cavity-nesting species. To characterize the study area each nest-box location is identified with a Global Positioning System (GPS) waypoint. Breeding activities are followed routinely every year and laying and hatching dates and brood sizes at hatching and fledging are determined. All the nest-boxes are cleaned every year after the breeding season.

2. STUDY SPECIES

a. Pied Flycatcher *Ficedula hypoleuca*

The Pied Flycatcher is a small (12-13g g) passerine bird, which breeds in many forested areas of the Palaearctic region (Lundberg and Alatalo 1992). It only stays in the north for the spring and summer, spending the rest of the year on migration or in the wintering areas in tropical West Africa (Lundberg and Alatalo 1992). The Pied Flycatcher breeds naturally in hole in trees but it adapts readily to breeding in nest-boxes. This is one of the main reasons why it has become such a popular object of research. Pied Flycatchers are easily catchable in nest-boxes, which is an enormous advantage in behavioural studies.

In Central Spain, the first males usually start to arrive at the middle of April and, as in most migratory passerines, males arrive ahead of females. Nest building is performed mainly by the female although males also provide materials (Martínez-de la Puente et al. 2009). Egg laying in the population under study typically begins in late May, and clutch sizes range from 4 to 7 eggs. In our study area Pied Flycatchers incorporate strips of bark of *Cistus laurifolius*, pine *Pinus sylvestris* bark and dry grass as nest material (Moreno et al. 2009). The length of the incubation period is about 13-16 days (Lundberg and Alatalo 1992), wherein the female incubates alone and receives part of her food from her mate (Moreno et al. 2011). Both male and female contribute to feeding the nestlings. The mean nestling period varies between 13 and16 days (Järvinen 1990). From the first egg, the mean duration of the breeding cycle is about 36 days.

European Pied Flycatcher male (left image) and female (right image).

b. Eurasian Nuthatch *Sitta europaea*

The Nuthatch is a small (23 g) cavity-nesting bird which breeds mainly in mature deciduous or mixed woodland with large, old trees, preferably oaks (Matthysen 1998). Pairs hold permanent territories and they prefer to build their nests in existing cavities in trees but can also use nest-boxes for breeding.

The foundation of the nest consists of pieces or rotten wood or bark and on the top of this comes a layer of lining material, typically bark flakes of Scots pine or other trees (Matthysen 1998), in our study area mainly pine bark flakes and strips of bark of *Cistus laurifolius.* Besides bark flakes, Nuthatches use mud in nest building by narrowing the entrance of cavities (Matthysen 1998) in order to avoid nest site competitors (Collias and Collias 1984). The total amount of mud may weigh up to 1 - 2 kg and is collected by the female in small pellets and carried to the nest (Matthysen 1998). Egg laying in central and western Europe typically occurs during the second half of April, and clutch sizes range from 5 to 9 eggs (Matthysen 1998). The length of incubation period is about 13-18 days (Löhrl 1958), wherein the female incubates alone and receives part of her food from her mate (Matthysen 1998). In our population, females always cover the eggs with flakes of bark before leaving the nest during incubation. Young Nuthatches develop more slowly than other passerines of comparable size (Löhrl 1958) such as Great Tit *Parus major*. Both sexes feed the young (Matthysen 1998). The mean nestling period varies between 23-27 days (Järvinen 1990). From the first egg, the mean duration of the breeding cycle is 48 days.

Eurasian Nuthatch

c. Blue Tit *Cyanistes caeruleus*

The Blue Tit is a small (10 g) hole-nesting passerine of European woodlands, which breeds mainly in deciduous forests (Cramp and Perrins 1993). It is a resident bird, which adapts readily to breeding in nest-boxes. Blue Tits build their nests mainly of moss and hair. Egg laying in central Spain typically begins in the second half of April, and clutch sizes range from 4 to 14 eggs (Fargallo 2004), and the number of fledglings averages 7 (Fargallo and Johnston 1997). Females incubate and brood the chicks alone, receiving part of their food from their mates, and both sexes feed the young (Fargallo and Johnston 1997; Moreno et al. 1996). Both male and female provision the nestlings. Young fledge within 17–20 days of hatching (Cramp and Perrins 1993). From laying of the first egg, the mean duration of the breeding cycle is 42 days.

Blue Tit