Efecto del medioambiente sobre la biología reproductiva de dos especies de aves insectívoras forestales en los Montes de Toledo

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A mis padres y mis hermanos Pedro y María.

A mi abuela.

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RESUMEN

Resumen

El fenotipo de las poblaciones animales que observamos está altamente influenciado por las condiciones del medio ambiente donde dichas poblaciones viven. Por lo tanto, para entender en profundidad las características de una población es necesario saber las presiones del medio ambiente que dicha población está soportando. Conocer el papel que el medio ambiente tiene sobre el fenotipo de los individuos que componen una población no es sólo importante a nivel de investigación básica, si no que reviste de gran importancia para su conservación, porque sólo de este modo sabremos de qué manera el medio ambiente afecta a la eficacia biológica de las individuos de las poblaciones y podremos predecir y adelantarnos a cambios que se produzcan en el medio ambiente, tales como el cambio global que actualmente acontece en el planeta. Con este objetivo, durante la primavera de tres años consecutivos (2011-2013), estudiamos el efecto de cambios medio ambientales sobre la biología reproductiva de dos poblaciones simpátricas de Carbonero común (*Parus major*) y Herrerillo común (*Cyanistes caeruleus*) en el municipio de San Pablo de los Montes (Toledo, España). En concreto estudiamos el efecto del tamaño del parche forestal (debido a la fragmentación del hábitat) y el tipo de cavidad (caja nido) sobre la biología reproductiva de ambas poblaciones. También estudiamos el efecto de las propiedades acústicas del hábitat, concretamente el nivel de ruido ambiental y la estructura del hábitat, sobre las características del canto del Carbonero común, debido a que éste es un rasgo fundamental en su biología por su papel en la selección sexual. Además, estudiamos el efecto de las condiciones climáticas propias de nuestra latitud sobre el comportamiento de incubación de ambas especies. Por último estudiamos el marco ecológico en el que se producen ausencias prolongadas en el cuidado de los huevos durante la incubación, pues probablemente indican condiciones extremadamente limitantes para las hembras incubadoras, lo que puede tener consecuencias sobre su propia eficacia biológica y la de los pollos. Los resultados de nuestro estudio confirmaron que, efectivamente, el fenotipo de los individuos en nuestras poblaciones de Carbonero y Herrerillo comín estaba condicionado por el medio ambiente y que ambas poblaciones fueron capaces de responder plásticamente a dichos cambios ambientales para amortiguar sus efectos. Así, la calidad de los pollos a los 13 días de edad en ambas especies estuvo condicionada por el tamaño del parche forestal donde fueron criados, siendo peor en los parches forestales pequeños (**capítulo I**). El tipo

de caja nido utilizado para anidar también influyó en la biología reproductiva de ambas especies. En las cajas nido de cemento los nidos fueron más altos, los huevos más pequeños y los pollos tenían un ala más corta a los 13 días de edad. En dichas cajas nido la fecha de puesta se adelantó alrededor de una semana, la atención al nido durante la incubación disminuyó así como el éxito reproductor de las parejas criando en ellas, debido a una mayor tasa de depredación en ese tipo de cajas y posiblemente a unas mayores condiciones de hipertermia (**capítulo II**). Ciertas características del canto de los Carboneros comunes se relacionaron con las propiedades acústicas en cada hábitat. Así, en el **capítulo III** comparamos los cantos entre Toledo y dos poblaciones forestales cercanas. Dicha comparación reveló que los Carboneros comunes en Toledo cantaban más rápido y con una frecuencia pico y máxima mayor que sus conspecíficos en zonas forestales, además aumentaron el uso de notas con una frecuencia pico mayor. Los Carboneros comunes también ajustaron las características de su canto dentro de la misma población según la estructura del hábitat en cada territorio. En los territorios con una mayor cobertura de matorral cantaron más despacio y con una frecuencia mínima menor, mientras que en los territorios con una mayor proporción de suelo desnudo incrementaron la longitud de sus estrofas (**capítulo IV**). La estrategia de incubación de nuestra población de Carboneros comunes difirió de la estrategia mostrada en poblaciones más norteñas, posiblemente debido a la variación climática latitudinal, y también difirió de la estrategia de incubación de los Herrerillos comunes, pese a que ambas especies proporcionaron la misma atención a sus huevos (**capítulo V**). De entre todos los patrones de incubación registrados en las dos especies, aproximadamente el 12 % de ellos presentaban al menos una ausencia del nido extremadamente larga, en donde la temperatura del termómetro situado entre los huevos descendía muy por debajo del límite fisiológico cero de ambas especies. En este tipo de patrones de incubación, la atención proporcionada por las madres era inferior que en los patrones donde dichas ausencias prolongadas no aparecían. Este comportamiento negligente se produjo con mayor frecuencia en el año y la especie que las hembras presentaban peor condición corporal (2012 y Herrerillos comunes respectivamente) y en las cajas nido de cemento más que en las de madera. Además normalmente se producía después de noches largas y frías, lo que hace pensar que las ausencias prolongadas del nido durante la incubación suceden debido a limitaciones energéticas de las madres incubadoras.

El periodo de incubación en dichas puestas donde se produjo al menos una ausencia prolongada se alargó en promedio cuatro días (**capítulo VI**). En rasgos generales, ambas poblaciones de páridos mostraron que son susceptibles a los cambios en su medio ambiente y pese a que son capaces de responder plásticamente a dichos cambios ambientales, su eficacia biológica se vio comprometida en algunas ocasiones.

Abstract

*The phenotype of the populations of animals that we observe is highly influenced by the environmental conditions in which those animals live. In order to understand the characteristics of a population in great depth, it is therefore necessary to know the environmental pressures being exerted on that population. Knowing the role that the environment plays in the phenotype of the individual of the population is not only important at a basic research level, but is also of great importance for its conservation, because it is the only way in which we can know how the environment affects the fitness of populations and be able to predict and get ahead of changes that occur in the environment, such as the global change that is currently affecting the planet. With this objective in mind, during the spring of three consecutive years (2011-2013), we studied the effect of environmental changes upon the reproductive biology of two sympatric populations of Great tits (*Parus major*) and Blue tits (*Cyanistes caeruleus*) in the municipality of San Pablo de los Montes (Toledo, Spain). More specifically, we studied the effect of the size of the forest patch (owing to the fragmentation of the habitat) and the type of cavity (nestbox) on the reproductive biology of both populations. We also studied the effect of the acoustic properties of the habitat, and more specifically the level of environmental noise and the structure of the habitat, on the characteristics of the Great tit's song, since this is a fundamental characteristic of its biology owing to the role that it plays in sexual selection. We also studied the effect of the climatic conditions of the latitude in question on the incubation behaviour of both species. Finally, we studied in an ecological context the prolonged parental absences during incubation, since this will probably indicate extremely*

limiting conditions for incubating females, which may have consequences in both their own biology and that of their nestlings. The results of our study confirmed that the phenotype of our populations of Great and Blue tits was effectively conditioned by the environment and that both populations were capable of plastically respond to these environmental changes in order to cushion their effects. The quality of 13-day-old nestlings of both species was therefore conditioned by the size of the forest patch in which they were bred, the worst being in the small forest patches (Chapter 1). The type of nestbox used for nesting also influenced the breeding biology of both species. The nests in the woodcrete nestboxes were taller, the eggs smaller and the 13-day-old nestlings had a smaller wing span. The laying date in these nest boxes was a week earlier, and the attention paid to the nest during incubation decreased, as did the reproductive success of the pairs breeding in them, owing to a greater rate of predation and possibly more hypothermia (Chapter II). Certain characteristics of the Great tit's song were related to the acoustic properties of each habitat. In Chapter III we compared the song of the Great tits in Toledo with that of two populations in nearby forests. This comparison revealed that that the Great tits in Toledo sing faster and with a greater peak and maximum frequency than their counterparts in forest areas, in addition to increasing the use of notes with a greater peak frequency. Great tits within the same population also adjusted the characteristics of their song according to the habitat in each territory. They sang more slowly and with less minimum frequency in those territories with greater shrub cover, while the length of their strophes increased in those territories with a greater proportion of bare ground (Chapter IV). Our Great tit population's incubation strategy was also different from that of more northern populations, possibly owing to the variation in latitudinal climate. Their incubation strategy was additionally different to that of the Blue tits, in spite of the fact that both species paid the same amount of attention to their eggs (Chapter V). Of all the incubation patterns registered for the two species, approximately 12% of them spent at least one extremely long period away from the nest, during which the thermometer located among the eggs showed that the temperature descended well below the Physiological Zero Temperature of both species. In the case of this type of incubation patterns, the attention paid by the mothers is less than in the case of those patterns in which prolonged periods of absence do not occur. This negligent behaviour occurred with greater frequency during the year and in the *species in which females had a worse body condition (2012 and Blue tits, respectively), and in woodcrete nestboxes more than in wooden nestboxes. Moreover, this behaviour usually occurred after long cold nights, which leads us to believe that prolonged absences from the nest occur owing to the incubating mothers' energy limitations. The incubation periods in those clutches where at least one prolonged absence occurred were longer on average four days (Chapter VI). With regard to general features, both breeding populations were susceptible to changes in their environment and, in spite of their capacity to adapt to the aforementioned environmental changes, their fitness was on occasions compromised.*

INTRODUCCIÓN

Introducción

Importancia del medio ambiente como modelador de la biodiversidad

El medio ambiente, constituido por factores abióticos (ej. clima, recursos, estructura del hábitat) y bióticos (ej. competencia por recursos, depredación, mutualismo, parasitismo y otras formas de interacciones intra e inter-específicas), actúa como un gran molde regulador de la *evolución* (ver glosario) de las especies (Darwin 1859, Grant y Grant 2008, Price 2008). Así pues, la diversidad biológica que encontramos en nuestro planeta se puede explicar en gran medida por la gran variedad de ambientes en el mismo (Levin *et al.* 2001, Nevo 2001, Whittaker *et al.* 2001). La forma en que el medio ambiente regula la evolución de las especies es mediante la selección natural (y *selección sexual*). Esto es, aquellos *genotipos* que confieren una mejora en la *eficacia biológica* de los individuos portadores bajo unas condiciones medioambientales determinadas, son transmitidos a la siguiente generación en mayor proporción. Este proceso hace que las *frecuencias alélicas* de una población varíen con el transcurso de las generaciones, acumulándose las que son beneficiosas y produciéndose así la evolución de dicha población (Fisher 1930, Rundle y Nosil 2005, Schuster 2009). Sin embargo para que la selección natural pueda actuar, las poblaciones deben contener una *diversidad genética* suficiente que confiera a cada individuo un grado de eficacia biológica distinto, y de este modo puedan ser seleccionados los genotipos más aptos (Frankham 1996). El principal mecanismo generador de diversidad genética es la *mutación*, pero también es importante el *flujo genético*. En contraposición, la *deriva genética* disminuye la diversidad genética de las poblaciones por procesos azarosos (Muller 1964, Felsenstein 1976, Slatkin 1987). Estos tres últimos mecanismos, pese a ser independientes del medio ambiente, constituyen junto con la selección natural (y sexual) los cuatro motores de la evolución (Kutschera y Niklas 2004).

Todos los organismos del planeta viven en un medio ambiente y por tanto son susceptibles a la presión de la selección natural. El blanco último sobre el que actúa la selección natural, como se ha explicado anteriormente, son los genes del individuo. Sin embargo, la selección de dichos genes se hace a través del fenotipo que codifican (Lewontin 1970, Doolittle y Sapienza 1980, Schluter 2001). El fenotipo es cualquier característica o rasgo observable de un individuo, como su morfología, desarrollo, propiedades bioquímicas, fisiológicas o su comportamiento; pero también los productos de dicho comportamiento, como el nido de un ave o la madriguera de un conejo (Dawkins 1982). El fenotipo, además de tener una base genética (que permite su herencia) está también influenciado por el medio ambiente, y suele representarse mediante la siguiente ecuación:

Genotipo + Medio Ambiente + Genotipo x Medio Ambiente = Fenotipo

Donde la interacción ‘Genotipo x Medio Ambiente’ hace referencia a la variación del fenotipo medio de una población a lo largo de un gradiente ambiental sin que se produzcan cambios en el genotipo, es decir, a la plasticidad fenotípica (Agrawal 2001, Langerhans *et al.* 2007). Esta plasticidad fenotípica confiere a las especies la posibilidad de adaptarse a cambios bruscos en el medio ambiente sin necesidad de evolucionar genéticamente (Charmantier *et al.* 2008). Un ejemplo de plasticidad fenotípica puede ser el cambio de altura del nido de un ave dependiendo del nivel de depredación (Kaliński *et al.* 2014), el cambio en la frecuencia del canto de un ave dependiendo de la intensidad de ruido ambiental (Salaberria y Gil 2010, Slabbekoorn 2013) o el aumento de la masa corporal de las especies a medida que aumenta la latitud, conocido como la Ley de Bergmann (Bergmann 1847). Es por esto que durante muchos años el efecto del medio ambiente sobre el fenotipo se haya considerado como algo ‘embarazoso’ en los estudios evolutivos, incluso como un error del ‘sofisticado’ mecanismo de la *herencia genética*. Como consecuencia, clásicamente, los biólogos no han considerado a la plasticidad fenotípica como un factor clave en el proceso de evolución de las especies (West-Eberhard 1989). Sin embargo, recientes estudios están poniendo de manifiesto la importancia de la plasticidad fenotípica en la evolución, concretamente en el proceso de *especiación*; por lo que actualmente se discute su consideración como un quinto ‘motor’ de la evolución (Hendry *et al.* 2000, Price *et al.* 2003, Palacios *et al.* 2011, Fitzpatrick 2012). La plasticidad fenotípica se puede describir mediante su ‘norma de reacción’, que es la forma en

que un rasgo fenotípico (representado en el eje Y) varía a lo largo de un gradiente medioambiental (representado en el eje X; Healy y Schulte 2015). Las 'normas de reacción' sí que tienen una base genética y por lo tanto cambios en dichas normas de reacción puede resultar adaptativos y promover la evolución mediante la selección natural en *alopatría* (Dayan *et al.* 2015, Dowle *et al.* 2015).

Importancia del medio ambiente para el mantenimiento de la biodiversidad

Como se ha expuesto anteriormente, el medio ambiente resulta crucial para los organismos pues condiciona su eficacia biológica, es decir su supervivencia y éxito reproductor. Esto se debe a que a través de la selección natural, los organismos han evolucionado para maximizar su eficacia biológica en el medio ambiente donde viven, lo que les permite perdurar en el tiempo. Así pues, en un escenario de estabilidad ambiental y durante un periodo suficientemente largo de tiempo, la selección natural hará que el fenotipo medio observable en las poblaciones de seres vivos sea aquel que maximice la eficacia biológica de dichas poblaciones, llamado fenotipo óptimo (Levins 1968, Endler 1986, Michel *et al.* 2014). El problema aparece cuando se produce una alteración inesperada en el medio ambiente a la que las poblaciones no están adaptadas, como puede ser un incendio, una inundación, una sequía, la erupción de un volcán, la colisión de un cometa o la introducción de una especie nueva en el *ecosistema*, por ejemplo. Estos cambios ambientales imprevistos comprometen la supervivencia de las poblaciones, pues se desajustan los mecanismos de eficacia biológica de los individuos. Ante dichos cambios medioambientales imprevistos las poblaciones tienen tres posibles respuestas:

1. Dispersarse hacia otras zonas que reúnan las condiciones idóneas para su supervivencia.
2. Responder mediante plasticidad fenotípica ante el cambio medioambiental producido.
3. Evolucionar genéticamente para adaptarse a las nuevas condiciones medioambientales reinantes.

Mientras que la primera respuesta entraña la extinción local de la especie, las dos restantes previenen dicha extinción. Conviene destacar que estas tres respuestas no son excluyentes y a menudo más de una opera simultáneamente, incluso las tres (Giennap *et al.* 2008).

Actualmente, la práctica totalidad de ecosistemas del planeta Tierra se encuentran bajo la presión de cambios imprevistos, producidos en un periodo muy corto de tiempo y en su mayor parte por acción del hombre. Este escenario se ha denominado 'cambio global' y es consecuencia de la acción conjunta del cambio climático global y los efectos directos de la actividad humana en el medio ambiente. El cambio climático global, producido por la acumulación de gases de efecto invernadero en la atmósfera, hace que las especies estén modificando sus rangos de distribución y su *fenología*. Estos cambios están alterando la composición de las comunidades y la naturaleza de las interacciones entre especies (Walther *et al.* 2002, Parmesan y Yohe 2003, Parmesan 2006, Hoffmann y Sgró 2011). A esta presión sobre las especies hay que sumarle los drásticos y extensos cambios ambientales derivados de la actividad humana, que son principalmente la sobreexplotación, la transformación del hábitat y la introducción de especies invasoras (Hoffmann *et al.* 2010). Debido a la extensión y celeridad de estos dos procesos, las especies no encuentran zonas adecuadas de refugio, se desborda su nivel de respuesta fenotípica y no tienen tiempo de adaptarse genéticamente, lo que está provocando la llamada 'sexta extinción global' (Bellard *et al.* 2012, Dirzo *et al.* 2014); en donde la tasa de extinción de las especies es de entre tres y cuatro órdenes de magnitud superior a la tasa de extinción de las anteriores cinco extinciones globales que han acontecido en el planeta (Barnosky *et al.* 2011). Es por esto que es crucial entender cuáles son los requerimientos básicos de las poblaciones que evitan la disminución de su eficacia biológica y por tanto su extinción en el escenario de cambio global en que nos encontramos. Para ello también es importante comprender cómo afecta el medio ambiente a las poblaciones y cómo éstas responden. Sólo de este modo se podrá prevenir el efecto del cambio global y tomar medidas eficaces de conservación de la biodiversidad.

Importancia del medio ambiente en los Herrerillos y Carboneros comunes

En esta tesis doctoral se ha estudiado alguno de los efectos del medio ambiente sobre diversos aspectos cruciales de la biología de dos especies de aves paseriformes insectívoras forestales, el Carbonero común (*Parus major*) y el Herrerillo común (*Cyanistes caeruleus*). En concreto hemos estudiado el efecto de la **fragmentación del hábitat** sobre la biología reproductiva de estas dos especies. La fragmentación del hábitat es una de las principales causas de extinción de especies en ecosistemas forestales (Laurance 2010, Bregman *et al.* 2014), donde el paisaje forestal original queda fragmentado en un conjunto de 'parches' de bosque de distinto tamaño y separados entre sí por una matriz de hábitat diferente del original (Bender *et al.* 1998). El tamaño del parche forestal donde crían las aves se ha visto que es una variable importante que predice su éxito reproductor (Hinsley 1999, Donovan y Lamberson 2001), y se ha sugerido que la reducción de recursos clave en parches forestales pequeños puede ser una causa responsable del citado fracaso reproductor (Møller 1991, Burke y Nol 1998, Zannette *et al.* 2000). Además del efecto de la fragmentación del hábitat, la naturaleza de la oquedad donde anidan estas aves también condiciona su eficacia biológica pues determina el microclima que habrá en el nido (Maziarz y Wesolowski 2013), su aislamiento frente a las condiciones ambientales externas (Amat-Valero *et al.* 2014) y la seguridad frente a los depredadores (Mazgajski y Rykowska 2008). En esta tesis hemos estudiado el efecto del tipo de oquedad seleccionada para reproducirse, en nuestro caso, **el tipo de caja nido**, una fabricada con una mezcla de cemento y serrín y otra fabricada con madera, sobre la biología reproductiva de nuestras poblaciones de Carbonero y Herrerillo comunes. Cada tipo de caja nido utilizado en estudios de biología condicionará de forma distinta el microclima que las aves tendrán en el nido durante la reproducción, debido a las diferencias en las propiedades térmicas y grado de aislamiento de cada tipo de caja nido. Además del material, la forma y el volumen interno de cada tipo de caja nido puede variar, lo que determina el tamaño del nido en su interior y puede tener consecuencias sobre algunos parámetros de la reproducción de las aves como el tamaño de puesta (Møller *et al.* 2014). Determinar el efecto del tipo de caja nido en las poblaciones de aves tiene mucho interés, porque cada tipo de caja puede afectar a cada especie en un bosque de forma distinta, lo que a largo plazo puede estar beneficiando a unas especies sobre otras y produciendo cambios en las dinámicas poblaciones de la comunidad de aves (Browne *et al.* 2006). Esto es especialmente relevante en ciertos países como España o Polonia, donde las políticas agrarias han suministrado miles de cajas nido a lo largo de más 50 años en los bosques sin ningún tipo de control o seguimiento, afectando sin ninguna duda a las comunidades de aves de dichos bosques (Lambrechts *et al.* 2010). Pero además porque distintos tipos de caja nido se utilizan de manera no aleatoria en distintos grupos de investigación y localidades. Por lo tanto, es posible que se estén produciendo sesgos en los resultados obtenidos en estudios dependiendo del tipo de caja nido utilizado; además de introducir errores cuando se comparan los resultados de dichos estudios entre sí, o en los resultados obtenidos en estudios donde más de un tipo de caja nido se utiliza indistintamente (Møller *et al.* 2014). Además del éxito reproductor general de las aves, aspectos concretos de su biología pero cruciales, pueden verse afectados por el medio ambiente. Tal es el caso del canto por su papel en la comunicación y selección sexual (Catchpole y Slater 2008). Debido a que el canto de las aves, para cumplir su función biológica (comunicación), debe llegar nítidamente desde un emisor hasta un receptor atravesando un hábitat, la transmisión de dicho canto queda condicionada por las características del hábitat. Dichas características se denominan propiedades acústicas del hábitat y las aves ajustan las características de su canto a dichas propiedades para optimizar la transmisión de su sonido, bien evolutivamente según la Hipótesis de la Adaptación Acústica (HAA, Morton 1975) o fenotípicamente (Brumm y Naguib 2009). Atendiendo a las propiedades que más influyen en la transmisión del canto de las aves se encuentra el **ruido ambiental**, porque enmascara las señales acústicas y disminuye su alcance funcional; dificultando la comunicación (Brumm y Slabbekoorn 2005). En la actualidad, debido al crecimiento continuado de los núcleos urbanos, cada vez más especies de aves se ven forzadas a vivir en el interior de las ciudades o en sus aledaños, y por tanto a hacer frente a las radicalmente nuevas condiciones ambientales que la urbanización supone (Warren *et al.* 2006). Entre estas nuevas condiciones ambientales urbanas, el elevado ruido ambiente como consecuencia principal de los medios de transporte supone un serio obstáculo para el establecimiento de las aves cantoras. Esto se debe a que sólo aquellas especies que son capaces de modificar su canto para conseguir distinguir su señal acústica del ruido ambiente prosperan, mientras que las especies que no son capaces de modificar su canto eficazmente desaparecen (Francis *et al.* 2011, Proppe *et al.* 2013). Además del ruido ambiente, la **estructura del hábitat** también afecta al canto de las aves, ya que su transmisión a través de un hábitat se ve comprometida por dos procesos fundamentales (Catchpole y Slater 2008): atenuación (disminución de la intensidad de la señal acústica con la distancia debido al choque de las ondas sonoras con el aire, pero también con el ambiente físico de cada hábitat: vegetación, rocas, suelo, etc.) y reverberación (degradación de la señal acústica debido a las interferencias causadas por los ecos y reflejos de las ondas sonoras con el ambiente físico de cada hábitat). En paisajes fragmentados, además de producirse una pérdida de hábitat original, normalmente se produce una degradación del hábitat remanente (Fahrig 2003); lo que puede alterar la estructura del hábitat de manera distinta en cada parche forestal. Esta situación hace que las aves tengan que ajustar las características de su canto a las propiedades acústicas en cada parche. Los ajustes del canto que las aves hacen frente a cambios en las propiedades acústicas de un hábitat, pese a que en un principio parezcan positivos pues mejoran la transmisión del canto, pueden entrañar costes en su eficacia biológica si el mensaje queda comprometido (Warren *et al.* 2006, Halfwerk *et al.* 2011, Read *et al.* 2014). Además de al canto, el medio ambiente también puede afectar al periodo de incubación de las aves. Este periodo es crucial para ellas porque mediante la incubación los padres proporcionan las condiciones óptimas para el desarrollo de sus embriones, lo que tiene efectos directos sobre el fenotipo de los pollos (Pérez *et al.* 2008; Nord y Nilsson 2011). Este efecto sobre los pollos tiene consecuencias indirectas en la eficacia biológica de los padres, pero también directas, ya que debido al elevado gasto energético que la incubación supone se compromete su supervivencia y se altera su nivel de involucración en etapas posteriores de la cría como la ceba; así como en la probabilidad de realizar segundas puestas (Reid *et al.* 2000, DuRant *et al.* 2013). Variables ambientales asociadas a la **latitud** como la temperatura o la cantidad de horas de luz influyen en el gasto energético de la incubación, ya que afectan inversamente en el coste energético de mantener los huevos calientes (Haftorn y Reinertsen 1985, Conway y Martin 2000a) y directamente al tiempo disponible para alimentarse (Ricklefs y Brawn 2013, Shaw y Cresswell 2014) respectivamente. Mientras que, según nuestros conocimientos, ningún estudio ha descrito el comportamiento de incubación del Herrerillo común, casi todos los estudios de incubación con el Carbonero común se han realizado en poblaciones del norte de Europa, con la única excepción del realizado por Álvarez y Barba (2014) en Valencia (España). En el norte de Europa las condiciones ambientales difieren sustancialmente de las de nuestras poblaciones de páridos, situadas cerca del límite meridional de su rango de distribución (Cramp y Perrins 1993). Es por esto que estudiar el comportamiento de incubación de estas dos especies ecológicamente similares, en la misma zona de estudio y criando en las mismas cajas nido, puede ayudar a entender los factores próximos que están detrás de la gran variación que existe en las estrategias de incubación entre las especies (Conway y Martin 2000b). No sólo eso, conocer el comportamiento de incubación de nuestras poblaciones de páridos y comparar dichos resultados con el de poblaciones más norteñas es interesante para ver el efecto que tiene la latitud sobre la incubación de dichas especies. Estos estudios ayudarán a entender qué variables ambientales son las más limitantes en la incubación de las especies de aves, lo que reviste de gran importancia en el escenario de cambio global en que nos encontramos para poder predecir sus efectos.

ESPECIES DE ESTUDIO

Especies de estudio

Carbonero común

El Carbonero común (*Parus major*, Linneo 1758) es un ave forestal insectívora de pequeño tamaño perteneciente al Orden *Passeriformes* y a la familia *Paridae*. Su longitud oscila entre 12 y 14 cm y tiene una envergadura de 22,5 y 25,5 cm; siendo su peso aproximado de 17-19 g. Es una especie fácilmente reconocible por el color amarillo de su vientre que contrasta con el azul grisáceo de las alas y la cola, y por la presencia de una característica banda de color negro en el centro del pecho. El dorso es verde-oliváceo, algo más amarillento hacia la nuca y el obispillo es grisáceo; siendo la cabeza de color negro con manchas blancas en las mejillas. El pico es de color negro también (Figura 1a). Los sexos son similares en plumaje salvo que las hembras además de ser sutilmente más pequeñas presentan tonos más apagados y una banda estrecha en el pecho que se desdibuja hacia la mitad (Figura 1b). En España se distribuye por casi toda la península e Islas Baleares, aunque falta en algunas zonas deforestadas de su interior; así como en las Islas Columbretes y las Canarias. En general se le puede encontrar en todo tipo de hábitats siempre que exista arbolado y presencia de agujeros para anidar.

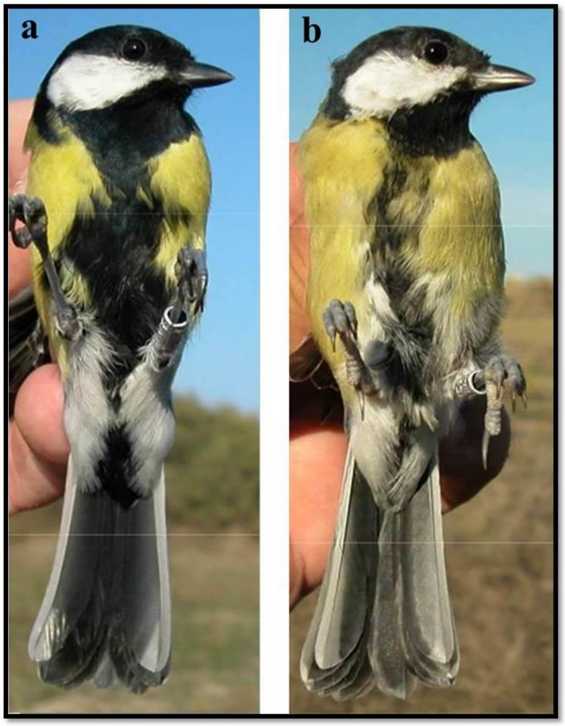

Figura 1. Diseño de la coloración del plumaje del pecho del macho (a) y hembra (b) del Carbonero común. Fotos tomadas Javier Blasco-Zumeta, Obra Social y Cultural de Ibercaja.

Es una especie sedentaria que forma bandos inter-específicos en invierno. Sin embargo durante la época de cría, entre marzo y junio, los machos se vuelven territoriales. Se trata de una especie monógama aunque a veces realizan puestas extra-parentales y los juveniles se pueden reproducir en el primer año de edad. Las hembras son las encargadas de construir el nido, incubar y empollar, necesitando oquedades ya

existentes para anidar, como agujeros en árboles, grietas en rocas etc. aunque aceptan fácilmente la utilización de cajas nido. En general tardan una semana en la construcción del nido y ponen un huevo diario hasta alcanzar un tamaño de puesta que oscila entre 8-10 huevos dependiendo de la población. Normalmente sólo realizan una puesta por temporada, aunque en todas las poblaciones hay parejas que ponen dos puestas. El macho ceba a la hembra mientras esta incuba, periodo que suele durar 13 días y los dos miembros de la pareja ayudan en la ceba de los pollos. Éstos vuelan a los 18-20 días de haber nacido y su dispersión natal es de corta distancia. Se alimentan principalmente de insectos en la estación reproductora, siendo las larvas de lepidópteros (principalmente de las familias *Noctuidae* y *Tortricidae*) su principal fuente de alimento, aunque otros grupos de presas pueden ser importantes también dependiendo de las poblaciones como arácnidos o imagos de lepidópteros. Los principales depredadores son mamíferos como la garduña (*Martes foina*) o la comadreja (*Mustela nivalis*), reptiles como la culebra de escalera (*Rhinechis scalaris*) o aves como el pico picapinos (*Dendrocopos major*) y el gavilán (*Accipiter nissus*) (Atiénzar *et al.* 2012).

Herrerillo común

El Herrerillo común (*Cyanistes caeruleus*, Linneo 1758) es un ave forestal insectívora de pequeño tamaño perteneciente al Orden *Passeriformes* y a la familia *Paridae*. Presenta una longitud aproximada de 11,5 cm y una envergadura de 17,5 a 20 cm, con un peso aproximado de 10 g. Al igual que el Carbonero común presenta un dorso de color verde-oliváceo y un vientre amarillo, pero en vez de una banda ancha central negra presenta una raya grisácea poco patente. El píleo, alas y cola son de color azul, pero las mejillas y parte superior de la cabeza son blancas. El pico es de color negro, con una banda oscura que le llega hasta la nuca pasando por el ojo. Esta especie apenas presenta dimorfismo sexual (Figura 2). Su distribución abarca la práctica totalidad de la península ibérica aunque está ausente de algunas regiones deforestadas del interior. En las Islas Baleares sólo está presente al norte de la isla de Mallorca, faltando también en las islas Canarias. Se la puede encontrar en todo tipo de hábitats siempre que haya una densidad suficiente de arbolado y huecos para anidar, aunque evita los boques de coníferas.

Figura 2. Diseño de la coloración del plumaje del pecho y cara del Herrerillo común. Foto JBE.

Las poblaciones en España son sedentarias, aunque se han capturado individuos provenientes de poblaciones centroeuropeas. Al igual que los Carboneros comunes, en invierno forman bandos mixtos con otras especies pero durante la época de cría, que dura aproximadamente tres meses entre marzo y junio, los machos se vuelven territoriales. Es una especie monógama con cierto grado de poliginia que necesita de oquedades ya existentes para anidar, aceptando con facilidad las cajas nido. Las hembras se encargan de la construcción del nido, de incubar y de empollar, pero ambos miembros de la pareja ayudan en la ceba de los pollos. La incubación dura unos 13 días y el tamaño medio de puesta es de 9 huevos, cada uno de ellos puesto en un día diferente. Los pollos vuelan entre los 15-20 días después de la eclosión. Durante la época de reproducción su alimentación es insectívora, depredando fundamentalmente sobre larvas de lepidópteros de las familias *Noctuidae* y *Tortricidae*; aunque presentan una dieta ligeramente más variada que los Carboneros comunes incorporando elevadas proporciones de otros tipos de orugas, arañas, coleópteros u ortópteros. En ecosistemas mediterráneos comparte los mismos depredadores que los Carboneros comunes (Salvador 2005).

ZONA DE ESTUDIO

Zona de estudio

El trabajo de campo para la realización de esta tesis doctoral se ha llevado a cabo en el municipio de San Pablo de los Montes (Toledo, España), situado a 50 km al sur de la ciudad de Toledo y lindando con la provincia de Ciudad Real, en plena cordillera de los Montes de Toledo (39°32′44″N, 4°19′41″O). El municipio presenta una altura media de 907 msnm y un clima mediterráneo-continental, con una marcada amplitud térmica a lo largo del año de unos 15ºC, ya que la temperatura media los meses de invierno es de 5-6ºC con frecuentes heladas y nevadas y la temperatura media de los meses de verano es de 25ºC siendo bastante secos. Los valores medios de temperatura y precipitación anual son 15ºC y entre 700 y 800 mm respectivamente.

La orientación de los Montes de Toledo en dirección este-oeste determina la presencia de laderas con orientación norte o ‘umbrías’ pertenecientes a la cuenca hidrográfica del río Tajo y de laderas con orientación sur o ‘solanas’ pertenecientes a la cuenca hidrográfica del río Guadiana. Las condiciones ambientales en ambos tipos de laderas son muy diferentes condicionando la vegetación en cada una de ellas. Así en la solana el bosque es el típico bajo mediterráneo de porte arbustivo, constituido por especies perennes donde predomina la encina (*Quercus ilex*) y la jara pringosa (*Cistus ladanifer*), acompañadas de una cohorte de arbustos en las que destacan el madroño (*Arbutus unedo*), la cornicabra (*Pistacea terebinthus*), brezo español (*Erica australis*) o la lavanda (*Lavandula Stoechas*). En la umbría la vegetación está constituida en su mayoría por especies de hoja caduca, principalmente el melojo (*Quercus pyrenaica*), acompañado de algunos ejemplares de arce (*Acer monspessulanum*) y mostajo (*Sorbus aria*). Entre la cohorte arbustiva de estos bosques destaca el majuelo (*Crataegus monogyna*), el rusco (*Ruscus aculeatus*), el rosal silvestre (*Rosa spp*) o la retama negra (*Cytisus scoparius*).

La densidad de parejas reproductoras en el matorral mediterráneo es muy escasa, debido al bajo porte de las encinas y a la ausencia de cajas nido en dichas manchas forestales (García-Navas *et al.* 2014). Por tanto los bosques caducifolios, considerados el hábitat óptimo de los páridos en esta región (Atiénzar *et al.* 2012), se encuentran

fragmentados formando ‘parches’ de mayor o menor tamaño separados entre sí por una matriz de matorral mediterráneo, cultivos y pastos para el ganado; tal y como sucede en otras regiones de la cuenca mediterránea (Blondel y Aronson 1999). En este paisaje fragmentado de melojares colgamos, durante el otoño de 2010, cajas nido en ramas de melojos a una altura aproximada de 2-3 m y separadas entre sí por una distancia media de 40 m. Para ello seleccionamos 20 parches de melojo y los clasificamos según su tamaño en tres categorías: 3 parches grandes, 3 parches medianos y 14 parches pequeños; colgando en cada uno de ellos un número acorde a su superficie de cajas nido, tanto de cemento como de madera (Tabla 1 y Figura 3). Ambos tipos de cajas las protegimos frente a los depredadores cubriendo a las cajas nido de madera con una malla de metal para evitar que el pico picapinos las agujerease y a ambos tipos de cajas nido les acoplamos un tubo de unos 5-7 cm de largo y 4 cm de grosor de policloruro de vinilo (PVC) (Figura 4).

Tabla 1. Relación, descripción y localización del número de ‘parches’ forestales utilizados en esta tesis doctoral situados en el municipio de San Pablo de los Montes (Toledo, España) en la cordillera de los Montes de Toledo.

Nombre	Tipo de parche	Superficie (Ha)	Nidales de madera	Nidales de cemento
El Marchés	Grande	26,01	80	20
La Morra	Grande	17, 85	80	20
La Fuenlabrada	Grande	21,72	80	20
Las Majadillas	Mediano	8,41	20	20
Las Casillas	Mediano	10,03	20	20
El Avellanar	Mediano	9,74	20	20
Navalahuerta	Pequeño	1,72	5	-
Tumba del Peregrino	Pequeño	1,33	5	-
Fuente Cantarranas	Pequeño	1,79	5	-
El Robledillo	Pequeño	2,06	5	-
Los Baños	Pequeño	1,40	5	-
El Tobarejo	Pequeño	1,12	5	-
La Ermita	Pequeño	1,63	5	-
Fuente Fría	Pequeño	2,02	5	-
Puerto de el Lanchar	Pequeño	1,91	5	-
Fuente Cerecera	Pequeño	1,42	5	-
El Canchorral	Pequeño	1,75	5	-
Arroyo	Pequeño	1,99	5	-
Campo de tiro	Pequeño	1,93	5	-
Puerto del Robledillo	Pequeño	1,69	5	-

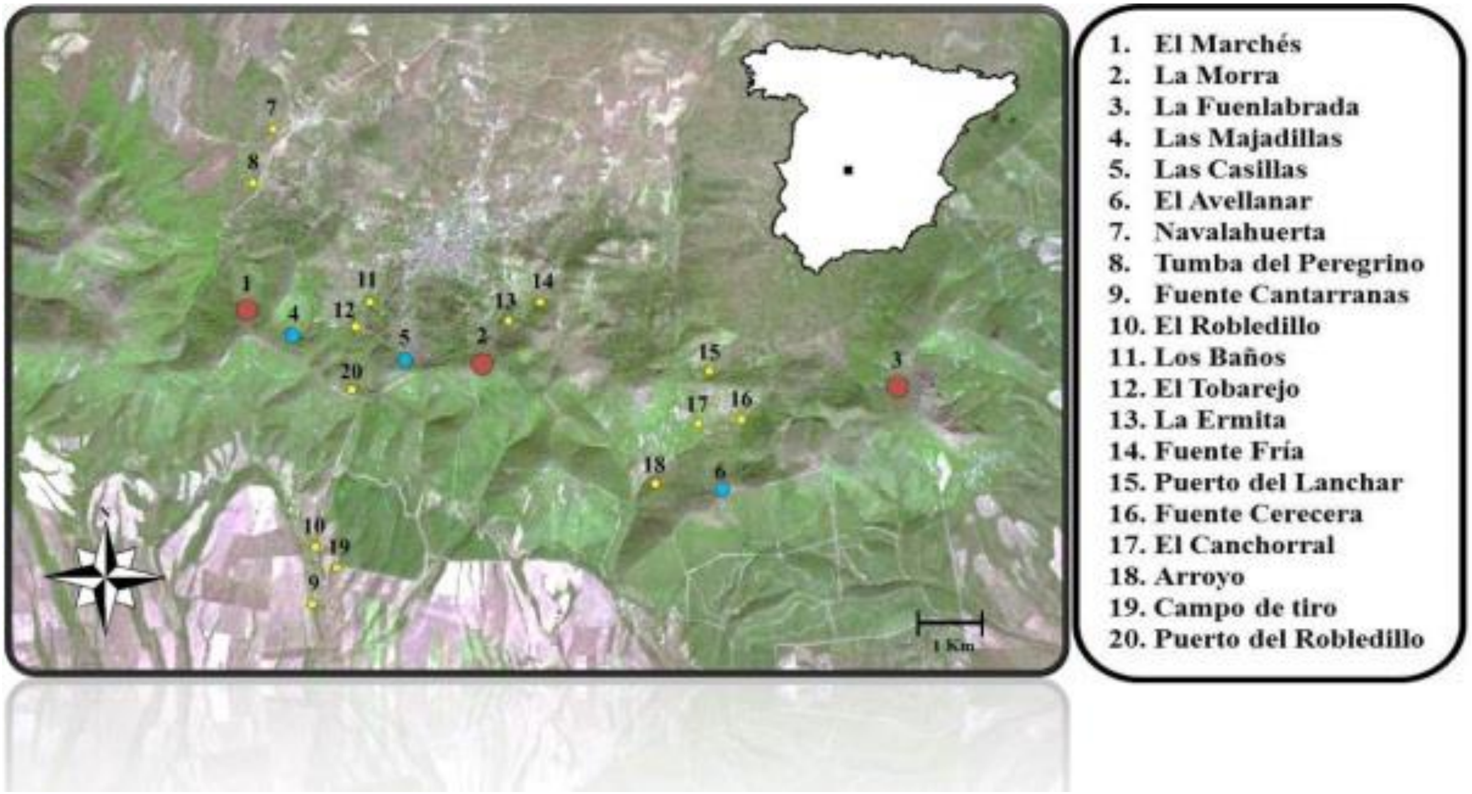

Figura 3. Mapa de la situación de los parches forestales en la zona de estudio en el municipio de San Pablo de los Montes. Colores, rojo: parche forestal grande, azul: parche forestal mediano y amarillo: parche forestal pequeño. Véase Tabla 1.

PROCEDIMIENTO GENERAL DE CAMPO

Procedimiento general de campo

Durante las primaveras del año 2011 al año 2013 y desde finales de marzo, comenzamos a revisar periódicamente cada tres o cuatro días todas las cajas nido colocadas en la zona de estudio con el fin de monitorizar la biología reproductiva de los Carboneros y Herrerillos comunes de nuestra población. De este modo obtuvimos los parámetros reproductivos básicos de nuestra población de páridos, tales como la fecha de puesta (1 = 1 de abril), el tamaño de puesta, el número de pollos eclosionados y el número de pollos volados. Con estos parámetros calculamos una serie de variables para cada caja nido ocupada, como el éxito de eclosión (número de pollos eclosionados entre el tamaño de puesta), éxito reproductor (número de pollos volados entre el tamaño de puesta) y éxito de vuelo (número de pollos volados entre el número de pollos eclosionados). La altura de cada nido (mm) se midió con una regla una vez encontrado el primer huevo. Además, en las dos primeras temporadas de campo hicimos una foto de cada huevo con una regla de referencia de manera que a posteriori pudiésemos calcular la longitud (mm) y la anchura máxima (mm) de cada huevo y así, siguiendo la ecuación de Hoyt (1982), calcular su volumen (mm^3). Los padres fueron capturados mientras cebaban a sus pollos cuando éstos contaban con 8 días de edad, mediante la colocación de trampas en las cajas nido. Cada individuo adulto se anilló en el tarso derecho si se trataba de una hembra o en el tarso izquierdo en el caso de que fuese un macho, se pesó con una balanza electrónica (0,01 g) y se le midió la longitud del ala con una regla (mm) y la longitud del tarso con un calibre electrónico (0,01 mm). Los pollos fueron anillados a los 13 días de edad y se les tomaron las mismas medidas que a los adultos. Previamente, cuando los pollos contaban con 10 días de edad se grabó su dieta acoplando una cámara de video a la parte trasera de una caja nido especialmente modificada para ello, de manera que apuntase a la entrada de la caja nido desde el interior. De esta manera durante una hora se grabó las cebas que los padres traían a los pollos. Para dar tiempo a los padres a habituarse a la caja nido modificada, el día anterior a la grabación de la dieta se intercambiaba la caja nido original por la caja nido especialmente modificada.

OBJETIVOS

Objetivos

El objetivo principal de esta tesis ha sido estudiar el efecto del medio ambiente sobre la biología reproductiva de dos especies de aves insectívoras forestales, el Carbonero común y el Herrerillo común, y evaluar si su eficacia biológica se ve comprometida; haciendo especial énfasis en las presiones ambientales que el cambio global inducido por el hombre ejerce sobre aspectos vitales de la biología reproductiva de estas aves. Para ello, en cada capítulo hemos abordado el efecto de una variable ambiental sobre la biología reproductiva o un rasgo de ésta en dichas especies

Capítulo I

Profundizar en los mecanismos que empeoran el éxito reproductor de las poblaciones de aves forestales en paisajes fragmentados. Concretamente estudiar la posibilidad de que una disminución de recursos cruciales, tales como el alimento, en los parches forestales pequeños pueda estar detrás del fracaso reproductor de las aves en dichos parches.

Capítulo II

Estudiar el efecto de dos tipos diferentes de caja nido, tanto en material como en forma, sobre la biología reproductiva de nuestra población de Carboneros y Herrerillos comunes; prestando especial atención a la arquitectura del nido y a la fase de incubación.

Capítulo III

Ahondar en los mecanismos que permiten que las aves modifiquen su canto en las ciudades presuntamente para evitar solaparse con el elevado ruido ambiente de origen antropogénico. Concretamente se ha estudiado el efecto de la transmisión cultural en la divergencia acústica del Carbonero común entre poblaciones forestales y urbanas.

Capítulo IV

Estudiar el efecto que la estructura del hábitat en cada territorio tiene sobre las características del canto de los machos de Carboneros comunes propietarios de dichos territorios dentro de la misma población, controlando por las características de cada macho.

Capítulo V

Describir el comportamiento de incubación de nuestras poblaciones de Carboneros y Herrerillos comunes situada en el límite meridional de su rango de distribución y compararla con los estudios de incubación realizados en poblaciones más norteñas, para así ver el efecto que la latitud tiene en la incubación. Además, comparar el comportamiento de incubación entre Carboneros y Herrerillos comunes para ahondar en los factores próximos que están detrás de la gran variabilidad en el comportamiento de incubación entre especies.

Capítulo VI

Estudiar en un contexto ecológico la realización de recesos extensos durante la incubación de Carboneros y Herrerillos comunes y si su realización conlleva costes en la eficacia biológica.

GLOSARIO

Glosario

- Alopátrico: Referente al aislamiento geográfico. En evolución se conoce como especiación alopátrica al mecanismo por el cual una especie da lugar a otras en áreas diferentes.
- Deriva genética: Es un cambio en la frecuencia alélica de una población debido a un muestreo aleatorio de individuos en la reproducción.
- Diversidad genética: Es el número total de variaciones alélicas en el acervo génico de una población.
- Ecosistema: Es el conjunto de la comunidad de organismos vivos y el medio físico donde viven y con el que se relacionan formando un sistema natural.
- Eficacia biológica: Es la proporción de los genes de un individuo en los genes totales de la siguiente generación.
- Especiación: Proceso evolutivo por el que una nueva especie se origina.
- Evolución: Es el cambio de los rasgos hereditarios o frecuencias alélicas de las poblaciones biológicas a lo largo de sucesivas generaciones.
- Fenología: Es la relación entre el medio ambiente y los ciclos de vida de los seres vivos, por ej. el comienzo de la migración de algunas especies de aves en relación a cambios ambientales.
- Flujo genético: También conocido como migración, es la transferencia de genes de una población a otra.
- Frecuencia alélica: Es la frecuencia relativa de un alelo (variante de un gen) en una población, expresada en fracción o porcentaje. Específicamente, es la fracción de todos los cromosomas de una población que portan dicho alelo.
- Genotipo: Es la información genética que posee un organismo en particular, en forma de ADN.
- Herencia genética: Es el proceso por el cual las características de un individuo (fisiológicas, bioquímicas, morfológicas o comportamentales) se transmiten a su descendencia, ya sea por reproducción sexual o asexual.

- Mutación: Es un cambio azaroso en la información genética de un individuo que puede transmitirse a su descendencia.

- Selección sexual: Es una forma de selección natural en donde típicamente los miembros de un género seleccionan miembros del género opuesto para reproducirse.

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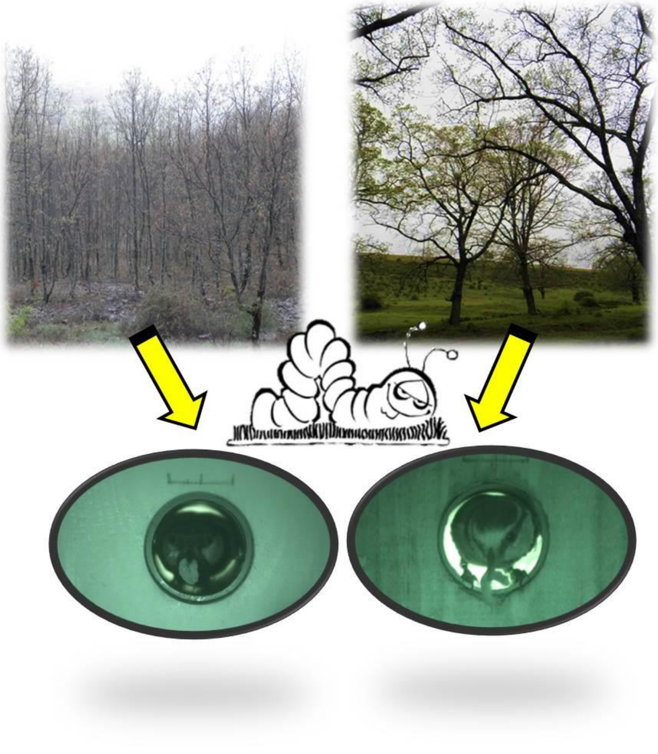
CAPÍTULO I

LA FRAGMENTACIÓN DEL HÁBITAT INFLUYE EN EL CRECIMIENTO DE LOS POLLOS DE CARBONERO Y HERRERILLO COMÚN MEDITERRÁNEOS



Bueno-Enciso J, Ferrer ES, Barrientos R, Serrano-Davies E y Sanz JJ. Habitat fragmentation influences nestling growth in Mediterranean Blue and Great tits.

Under review

Abstract

In patchy forest areas, the size of the forest patch where birds breed is a main issue that influences their breeding success. However, the proximate effects worsening the breeding success in small forest patches remains unclear; and a shortage of crucial resources in those forest patches has been suggested to account in some degree for this failure. With the aim to further investigate this topic, we have monitored the breeding cycle of two populations of Blue and Great tits in three ‘large’ forest patches (ranging between 26.5-29.6 ha) and twelve ‘small’ forest patches (ranging between 1.1-2.1 ha) in a Mediterranean area in central Spain, during three years (2011-2013). We also recorded the nestling diet inside the nests with the aid of handy-cams specially placed. Only males significantly differed between forest patch size categories. Reproductive traits did not vary between forest patch size categories, but the body condition of Blue tit nestlings and the size of Great tit nestlings significantly did, being better and higher in large forest patches respectively. Their recruitment rate was also higher in large patches, although this pattern was only significant in Blue tits. Regarding nestling diet, Blue tits did not differ but Great tits did, delivering a higher amount of caterpillars in large forest patches. Most variation in the reproductive traits was given among years, probably due to the different environmental conditions in each one. This study suggests that food supply in small forest patches could be limiting the breeding success of birds in those patches.

Introduction

Habitat fragmentation is one of the major threats that forest biodiversity faces (Laurance 2010; Amos *et al.* 2014; Bregman *et al.* 2014), and its effects have been widely studied in forest birds (Fahrig 2003). The ultimate effect of habitat fragmentation is the decline of bird species richness and population abundances (Moller 1987; Debinski and Holt 2000; Boulinier *et al.* 2001). These numerical responses may stem, at least in part, in demographic changes, i.e. proximate effects given at a regional-scale (Lampila *et al.* 2005). However, the mechanisms underlying these proximate effects remain unclear, and it is of vital importance to light them in the sake of conservation biology (Boulinier *et al.* 2001; Le Tortorec *et al.* 2013). One feature that affects the breeding success of birds, is the size of the forest patch where they breed (Paton 1995; Hinsley *et al.* 1999, 2009; Shochat *et al.* 2001; Loman 2003; Zitsque *et al.* 2011, but see Nour *et al.* 1998), and a reduction of crucial resources in small forest patches has been suggested to be a responsible cause (Kuitunen and Makinen 1993; Tremblay *et al.* 2005; Hinam and Clair 2008).

In this regard, food supply could be a crucial resource limiting the breeding success of birds in small forest patches, as it is one of the most important limiting factors affecting life-history in birds (Lack 1968; Martin 1987; but see Martin 1995). Food supply could be compromised in small forest patches just because their small surface area (Moller 1991); but also because in small forest patches the proportion of forest edges increases (Helzer and Jelinski 1999), which harsh the environmental conditions (Zanette *et al.* 2000) and may drive to a decrease in the amount of invertebrates (Didham *et al.* 1996; Burke and Nol 1998). Furthermore, the process of habitat fragmentation typically implies degradation, which changes the structure of the remaining habitat (Hinsley *et al.* 1999; Fahrig 2003). These changes in the vegetation structure usually involve microclimate alterations too; which apart from its direct effects on the abundance of invertebrates, it could promote a change in the composition of the vegetation which could also alter the composition of invertebrates (Cramp and Perrins 1993; Laurance *et al.* 2002). In addition, for altricial bird species, breed in a small forest patch could be challenging due to

they are ‘central-place foragers’, as they are attached to a fixed point when they breed, their nests (Tremblay *et al.* 2005). If they are not able to cope with the food demand of their broods within the patch, they will be forced to travel longer distances to reach other foraging patches, crossing unsuitable foraging habitats, which will limit their feeding rate (Bruun and Smith 2003). In other cases, in landscapes containing little habitat, the distance between forest patches may exceed a species gap-crossing tolerance, constraining the size of the home ranges and limiting the availability of resources (Desrochers and Hannon 1997). The ultimate effect of both scenarios is a reduction of the breeding success (Frey-Roos *et al.* 1995; Hinsley 2000).

The aim of the present study was to test whether there is an effect of the forest patch size on the breeding performance of two populations of Blue (*Cyanistes caeruleus*) and Great tits (*Parus major*). To do this, we studied the breeding performance of these two species of tits in a fragmented landscape in central Spain during three years. Both species result idoneous to study this topic as they need an enormous supply of food when they breed. For example Great tits while feeding their chicks made up to 700 feeding visits per day, and Blue tits even more (Perrins 1991). Because of this, it is crucial for tits to match the maximum food demand period of their chicks with the food peak in the forest (Naef-Daenzer and Keller 1999; Matthysen *et al.* 2011); when they do not achieve this match, their reproductive success decrease (Svensson and Nilsson 1995; Naef-Daenzer *et al.* 2001; Tremblay *et al.* 2003). We hypothesized that in small patches the breeding performance will be worse (Moller 1991; Riddington and Gosler 1995), and predict that the lack of resources in small patches will be the most responsible, concretely foods supply (Buke and Nol 1998; Zanette *et al.* 2000; Razeng and Watson 2014).

Material and Methods

Study area

The present study was conducted in the locality of San Pablo de los Montes situated in Montes de Toledo (39°32′44″N, 4°19′41″W; Toledo, central Spain). This region presents continental Mediterranean climate, characterized by pronounced summer droughts and a high

daily thermal oscillation, with mean annual rainfall of 700-800 mm. The landscape of this area has suffered an intense fragmentation due to human activities, mainly agriculture and deforestation for raising cattle, as occurs in other regions from the Mediterranean basin (Blondel and Aronson 1999). As a consequence, deciduous woodlands, considered the most suitable breeding habitat for tits in this region (Atiénzar *et al.* 2012), are scattered and patched in a matrix of less suitable habitat, mainly Mediterranean scrubland with low tree cover and pastureland. Our study area consisted of fifteen oak (*Quercus pyrenaica*) forest patches: three '*large*' patches ranging between 26.46 and 29.60 ha, and twelve '*small*' patches ranging between 1.12 and 2.06 ha, separated each other for a mean distance of 4.23 km (range 0.53 - 9.84 km). Large patches were provided with 80 wood nest-boxes (internal dimensions: 12 x 11.5 x 16.5 cm.) and small ones with 5 wood nest-boxes; separated each other by at least 30 m.

Field work

During the 2011-2013 breeding seasons (day 1= April 1), nest-boxes were frequently inspected to obtain the basic reproductive parameters of our tit population, such as laying date, clutch size, hatching date and brood size. Body condition, size and age of parents were compared between patch size categories because these variables are indicators of status and thus, of dominance among resources (Gosler 1997; Stahl *et al.* 2001). To do this, parents were trapped and ringed while feeding their nestlings (8-9 days old). Females were ringed in the right tarsus while males in the left one to facilitate their differentiation in the video recordings. Tarsus length (to the nearest 0.01 mm), body weight (0.01 g) and age of parents (yearling or older, according to plumage characteristics) were noted. Due to technical difficulties, adult Great tits in 2011 were not trapped. Nestlings were ringed, measured and weighted when they were 13 years old. Tarsus length was employed as a surrogate of body size, and the weight analyses were corrected by the tarsus length (added as a covariate) to consider the body condition of birds. Nest-boxes were also visited the on day 22 to assess the number of chicks fledged. Hatching

success was calculated as the rate between the number of nestlings hatched and the clutch size; and fledgling success was calculated as the rate between nestlings fledged and hatched. To assess the breeding performance of each tit pair, seven variables were used (laying date, clutch size, hatching success, fledgling success, nestling body condition, nestling size and fledgling recruitment).

Feeding rate and Nestling diet

To evaluate potential differences in the breeding performance between patch size categories, nestling diet was filmed by placing an infrared handy-cam (Sony DCR-SR290E-like) in an adapted nest box when nestlings were 11 days old (see García-Navas and Sanz 2011, for details); that is, when nestling food requirements are maximum (Naef-Daenzer and Keller 1999). All filming sessions were carried out in similar weather conditions. First hour of video of each recording was discarded to allow parents habituate to the disturbance that the video camera installation entails. The second hour recording was analysed frame by frame using the software package Adobe Premiere Elements 7.0. In a previous study performed in a nearly area, it was confirmed that an hour is representative of the feeding behaviour in these species (García-Navas and Sanz 2012). A total of 319 film recordings were analysed, 240 belonged to Blue tits and 80 from Great tits. For each recording, the number of feeding events per hour was counted and was considered as a surrogate of parental provisioning effort (hereafter ‘feeding rate’). Diet of nestlings was estimated from identified preys of 6,054 feeding trips: 4,924 belonging to Blue tits and 1,130 to Great tits. Food items were firstly classified in two main trophic categories: Lepidoptera larvae (caterpillars) and “others”. We further distinguish between caterpillars of the three mayors Lepidoptera families presented in the study area: Tortricidae, Noctuidae and Geometridae. The second group “others” include: ‘spiders’ (Aranea), ‘imago’ (belonging from the orders Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera and Orthoptera), ‘Chrysalides’ and ‘Miscellaneous’ (heterogeneous group including vegetable tissues, fungus, eggshell, snails and lichen). Size of preys was assessed using a scale bar attached above the entrance of the nest box as a

reference. Within each taxonomic category, items of prey were classified to three ordinal categories of body size [1 = small (length <1.5 cm for caterpillars; <1.5 cm for imagos and abdomen diameter <0.2 cm for spiders); 2 = middle (length 1.5–2.5 cm, 1.5–2 cm and diameter 0.2–0.6 cm, respectively); 3 = large (length >2.5 cm for both caterpillars and imagos and diameter >0.6 cm for spiders)] to make individual prey volumes comparable among taxa (see García-Navas and Sanz 2010). Shannon index H' was calculated for each nest.

Statistical analysis

Only first clutches were used. A set of General Linear Mixed Model (GLMM) were performed in all cases with year and patch size category as fixed factors and laying date as a covariate. As mentioned above, body condition of birds was estimated by adding the tarsus length as another covariate, as advised by Darlington and Smulders (2001) and García-Berthou (2001). In all models, nest ID nested in forest patch ID was included as the random term. Normal distribution was assumed in all models, with the exceptions of proportions (hatching success, fledgling success and proportions of the main prey). For these analyses, GLMMs with a binomial distribution and a binary response variable were employed. In hatching and fledgling success models, the numerator of the binary response variable were the number of hatchling and fledglings respectively, and the denominator were the clutch size and the number of hatchlings respectively. For proportions of the main prey types, the numerator was the number of preys belonging to a specific prey type and the denominator was the total number of prey. Age of parents and probability to recruit next year were also analysed fitting the GLMMs with a binomial distribution. In age models the response variable was 0 for yearlings and 1 for adults; and in recruitment models the response variable was 0 for nestlings non-recruited and 1 for nestlings recruited. Nestling mass and nestling tarsus length were included as explanatory variables in the models of recruitment. When the interaction between the factor 'Year' and 'Patch size' was significant, the main effects of both factors on the response variable were not considered (even when $P<$ 005), in a similar way as MacDonald *et al.* (2014).

Assumptions of homogeneity, normality and independence were visually verified plotting the residuals against fixed values, doing a histogram of the residuals and plotting the residuals against each explanatory variable respectively (Zuur *et al.* 2009). All models were firstly constructed with all explanatory terms fitted including interactions, and final models were selected following a backward procedure, by progressively eliminating non-significant terms starting with the interactions. Thus, final models were those that explained the maximum variance with the less number of explanatory variables. All analyses were performed in R (R Core Team 2014) with the package lme4 (Bates *et al.* 2004). For non-parametric distributions Wald chi-square statistic (χ^2) is given. Mean ± SE (n) is given in the results.

Results

Breeding performance

In both species, the main difference found between forest patch size categories was at nestling stage. Body condition of Blue tit nestlings in large patches was better than conspecifics in small patches in all years, while nestlings of Great tits had longer tarsi than those from small patches (Table 1 and Fig.1). Regarding recruitment, the probability of a Blue tit yearling that was born in the study area to breed the next year, was higher for those that were born in large patches; whereas in Great tits, it was positively related to their body mass (Table 1). Almost none other trait of breeding performance differed between patch size categories, although there were some significant interactions between year and patch size category in Blue tits (Table1). Concretely, the clutch size and fledgling success differed in 2012, being smaller in small patches [clutch size (2012): large patches= 8.50 ± 0.20 (109) and small patches= 7.42 ± 0.51 (21); fledgling success (2012): large patches= 83% ± 0.04 (74) and small patches= 70% ± 0.11 (14)]; rest of years these variables did not differ between patch size categories.

The greatest differences in the breeding performance of tits were given among years (Table 1). Blue tit laying date was delayed year after year (Tukey HSD test< 0.05) (2011= 17.36 ± 1.09 (130), 2012= 25.00 ± 1.05 (128), 2013= 29.27 ± 0.78 (158)); and their hatching success, nestling condition, nestling size and recruitment significantly decreased in 2012 (Tukey HSD test< 0.05); [hatching success: 2011= 80.07% ± 2.00 (129), 2012= 71.47% ± 2.58 (120), 2013= 80.44% ± 1.99 (149); nestling condition: 2011= 10.02 ± 0.05 (108), 2012= 9.37 ± 0.10 (81), 2013= 10.17 ± 0.06 (126); nestling size: 2011= 15.88 ± 0.04 (108), 2012= 15.74 ± 0.05 (81), 2013= 15.93 ± 0.04 (126) and recruitment: 2011= 7.85% (815), 2012= 1.91% (478)]. Great tits showed similar phenology, and their laying date was also delayed in 2012 and 2013, although there were not differences between the latter years (Tukey HSD test> 0.05) [year 2011= 13.75 ± 1.80 (32), year 2012= 26.42 ± 1.47 (105), year 2013= 23.19 ± 0.75 (68)]. However, the clutch size in this species did not differ among years (Table 1). Rest of the reproductive variables varied among years in a similar way as Blue tits, significantly decreasing in 2012 (Tukey HSD test< 0.05); with the exception of hatching success (Table 1). [fledgling success: 2011= 91.63% ± 0.04 (29), 2012= 63.32% ± 0.05 (52), 2013= 81.40 ± 0.04 (46); nestling condition: 2011= 17.02 ± 0.17 (29), 2012= 16.32 ± 0.15 (50), 2013= 17.33 ± 0.12 (45); nestling size: 2011= 19.46 ± 0.09 (29), 2012= 19.28 ± 0.78 (50), 2013= 19.40 ± 0.06 (45) and recruitment: 2011= 4.65% (409), 2012= 0.52% (387)].

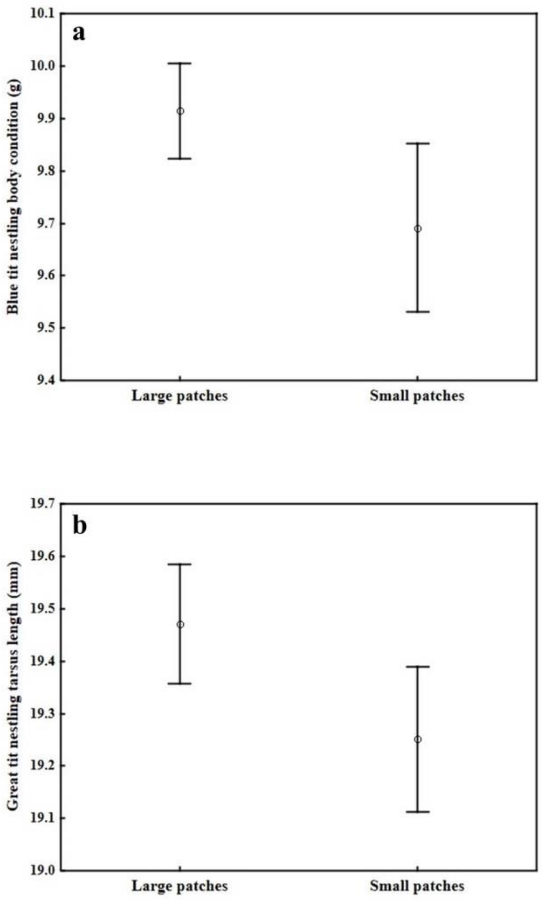


Figure 1. Differences in Blue tit (*Cyanistes caeruleus*) nestling body condition (a) and Great tit (*Parus major*) nestling tarsus length (b) between large and small forest patch sizes in a population of Montes de Toledo, central Spain. Body condition has been corrected by year, laying date and tarsus length, and tarsus length has been corrected by year and laying date. Vertical bars indicate ± SE.

Table 1. Results of the GLMMs showing the effects of year, patch size and laying date on the breeding performance of Blue (*Cyanistes caeruleus*) and Great tits (*Parus major*) in Montes de Toledo, central Spain. The ID nest nested in ID forest patch is included in all models as the random effect. *F* statistic is used for Laying date, Clutch size and Nestling condition and size; while χ^2 statistic is used for Hatching and Fledgling success and Recruitment. Statistics of non-significant terms are shown in Table S1.

		Blue tit					Great tit		
Parameter	Effect	d.f.	test	*P*	Parameter	Effect	d.f.	test	*P*
Laying day	Year	320	32.2	<0.001	**Laying day**	Year	150	8.1	<0.001
Rejected effects: Patch size and Year * Patch size.					Rejected effects: Patch size and Year * Patch size.				
Clutch size	Laying day	316	164.5	<0.001	**Clutch size**	Laying day	149	13.3	<0.001
	Year*Patch size	316	3.3	0.02					
Rejected effects: Patch size.					Rejected effects: Year, Patch size and Year * Patch size.				
Hatch. success	Year	296	6.9	0.03	**Hatch. success**				
Rejected effects: Patch size, Laying date and Year * Patch size.					Rejected effects: All.				
Fledg. success	Laying day	251	35.2	<0.001	**Fledg. success**	Year	100	11.8	0.003
	Year*Patch size	251	13.7	0.003					
Rejected effects: Patch size.					Rejected effects: Patch size, Laying date and Year * Patch size.				
Nest. condition	Year	224	26.4	<0.001	**Nest. condition**	Year	94	11.5	<0.001
	Patch size	224	5.5	0.02		Tarsus length	95	36.1	<0.001
	Laying date	224	5.5	<0.001					
	Tarsus length	224	28.6	<0.001					
Rejected effects: Year * Patch size.					Rejected effects: Patch size, Laying date and Year * Patch size.				
Nestling size	Year	225	6.9	0.001	**Nestling size**	Year	95	3.5	0.03
	Laying date	225	5.3	0.02		Patch size	95	4.9	0.03
Rejected effects: Patch size and Year * Patch size.					Rejected effects: Laying date and Year * Patch size.				
Recruitment	Year	1274	20.9	<0.001	**Recruitment**	Year	787	6.1	0.01
	Patch size	1274	6.3	0.01		Nestling mass	787	5.9	0.02
Rejected effects: Nestling mass, Tarsus length, Laying date and Year * Patch size.					Rejected effects: Patch size, Tarsus length, Laying date and Year * Patch size.				

Parents

Only males differed between patch size categories, although in a different way in each species. Blue tit males had a better body condition in small patches (GLMM: Estimate ± SE= 0.29 ± 0.08, $F_{1,\ 211}$= 11.91, $P<$ 0.001), while Great tit males were on average older in large patches (GLMM: Estimate ± SE= -1.19 ± 0.52, $\chi^2_{1,\ 69}$= 5.16, P= 0.02). Regarding yearly variation, body condition of Blue and Great tits significantly decreased in 2012 (Tukey HSD test< 0.05; GLMM: $F_{2,\ 255}$= 4.37, P= 0.01 and GLMM: $F_{2,\ 211}$= 8.46, $P<$ 0.001 for Blue tit females and males respectively, and GLMM: Estimate ± SE= 0.49 ± 0.20, $F_{1,\ 74}$= 6.12, P= 0.02 and GLMM: Estimate ± SE= 0.51 ± 0.21, $F_{1,\ 68}$= 5.97, P= 0.02 for Great tit females and males respectively). This year, the average age of Blue tit males was significantly lower (Tukey HSD test< 0.05; GLMM: $\chi^2_{1,\ 227}$= 8.21, P= 0.02), the same as the average age of female Great tits (Tukey HSD test< 0.05; GLMM: Estimate ± SE= 1.09 ± 0.49, $\chi^2_{1,\ 78}$= 5.09, P= 0.02).

Nestling diet

Blue tit nestling diet did not differ between patch size categories (Table 2). Proportions of total caterpillars, noctuids, tortricids and prey size were negatively affected by the date in this species, while proportion of spiders significantly increased with the date; both in a linear way (Table 2). Feeding rate was only positively affected by the brood size. The proportion of noctuids was negatively affected by brood size, whereas the proportion of tortricids was positively affected (Table 2). The proportion of caterpillars in this species increased in 2012 and 2013 regarding 2011 (Tukey HSD test< 0.05, Table 2), but these increases were sustained by different caterpillar families in each year. In 2012 the increase in the proportion of caterpillars delivered by Blue tits was above all, due to an increase in the intake of noctuids, while proportion of tortricids this year decreased (Tukey HSD test< 0.05); the increase in the consumption of noctuids was probably the cause of the significant increase in the prey size this year (Tukey HSD test <0.05). In 2013 the proportion of noctuids diminished, but the

proportion of tortricids increased with respect to the former years (Tukey HSD test <0.05, Table 2), resulting in the highest proportion of caterpillar in nestling diet this year. Finally the proportion of spiders decreased in 2013 (Tukey HSD test <0.05). Diet diversity (H') did not vary between patch sizes, but was positively affected by both laying date and brood size (GLMM: Estimate ± SE: 0.009 ± 0.03, $F_{1,\,177}$= 9.12, P= 0.003 and GLMM: Estimate ± SE: 0.04 ± 0.01, $F_{1,\,177}$= 6.26, P= 0.01).

Contrary to Blue tits, Great tit diet did differ between patch size categories (Table 3). The proportion of caterpillars and noctuids were higher in large patches (Table 3). These two prey types were also negatively affected by the date. As in Blue tits, feeding rate was positively affected by the brood size, but no prey type was (Table 3). Yearly comparisons showed a similar pattern than Bluet tit diet. The proportion of caterpillars increased in 2012 and 2013, although it did not differ between the latter years (Tukey HSD test> 0.05); in the same way as the proportion of noctuids (Table 3). The proportion of spiders also diminished in 2013 in this species (Tukey HSD test< 0.05). Opposite to Blue tits, proportion of tortricids and prey size were not affected by any explanatory variable (Table 3). Diet diversity (H') in this species was only affected by the patch size, being higher in large patches (GLMM: Estimate ± SE: 0.32 ± 0.15, $F_{1,\,56}$= 4.37, P= 0.04).

Table 2. Results of the GLMMs analyzing the effects of the year, patch size, laying date and brood size on Blue tit nestlings diet (*Cyanistes caeruleus*) in Montes de Toledo, central Spain. The ID nest nested in ID forest patch is included in all models as the random term. Wald chi-square (χ^2) statistic is given with non-parametric distributions. Significant results are highlighted in bold

Response variable	Explanatory term	Estimate ± SE	test	*P*	Response variable	Explanatory term	Estimate ± SE	test	*P*
Feeding rate	Intercept	8.44 ± 3.04			Tortricidae	Intercept	-2.00 ± 0.32		
	Year		$F_{2, 175} = 1.84$	0.16		Year		$\chi^2_{2, 176} = 37.57$	**<0.001**
	Patch size	-4.02 ± 2.47	$F_{1, 176} = 2.64$	0.13		Patch size	0.08 ± 0.20	$\chi^2_{1, 175} = 0.17$	0.68
	Laying day	-0.08 ± 0.09	$F_{1, 174} = 0.74$	0.40		laying day	-0.01 ± 0.005	$\chi^2_{1, 176} = 5.13$	**0.02**
	Brood size	2.50 ± 0.39	$F_{1, 177} = 40.31$	**<0.001**		Brood size	0.08 ± 0.03	$\chi^2_{1, 176} = 5.52$	**0.02**
	Year*Patch size		$F_{2, 172} = 0.09$	0.92		Year*Patch size		$\chi^2_{2, 173} = 4.24$	0.12
Caterpillars	Intercept	1.48 ± 0.13			Aranea	Intercept	-1.93 ± 0.10		
	Year		$\chi^2_{2, 177} = 11.32$	**0.003**		Year		$\chi^2_{2, 177} = 24.33$	**<0.001**
	Patch size	-0.11 ± 0.16	$\chi^2_{1, 176} = 0.50$	0.48		Patch size	-0.07 ± 0.10	$\chi^2_{1, 175} = 0.40$	0.52
	laying day	-0.03 ± 0.004	$\chi^2_{1, 177} = 38.87$	**<0.001**		laying day	0.009 ± 0.004	$\chi^2_{1, 177} = 5.21$	**0.02**
	Brood size	-0.007 ± 0.03	$\chi^2_{1, 175} = 0.08$	0.78		Brood size	-0.03 ± 0.004	$\chi^2_{1, 176} = 1.67$	0.20
	Year*Patch size		$\chi^2_{2, 173} = 3.02$	0.22		Year*Patch size		$\chi^2_{2, 173} = 4.22$	0.12
Noctuidae	Intercept	0.51 ± 0.23			Prey size	Intercept	1.99 ± 0.08		
	Year		$\chi^2_{2, 176} = 16.38$	**<0.001**		Year		$F_{2, 176} = 18.30$	**<0.001**
	Patch size	-0.09 ± 0.17	$\chi^2_{1, 175} = 0.32$	0.57		Patch size	-0.008 ± 0.08	$F_{1, 175} = 0.01$	0.92
	laying day	-0.02 ± 0.004	$\chi^2_{1, 176} = 12.48$	**<0.001**		laying day	-0.007 ± 0.003	$F_{1, 176} = 4.52$	**0.04**
	Brood size	-0.05 ± 0.02	$\chi^2_{1, 176} = 4.35$	**0.03**		Brood size	-0.002 ± 0.02	$F_{1, 172} = 0.02$	0.89
	Year*Patch size		$\chi^2_{2, 173} = 0.36$	0.84		Year*Patch size		$F_{2, 173} = 2.56$	0.08

Table 3. Results of the GLMMs analyzing the effects of the year, patch size, laying date and brood size on Great tit nestlings diet (*Parus major*) in Montes de Toledo, central Spain. The ID nest nested in ID forest patch is included in all models as the random term. Wald chi-square (χ^2) statistic is given with non-parametric distributions. Significant results are highlighted in bold.

Response variable	Explanatory term	Estimate ± SE	test	*P*	Response variable	Explanatory term	Estimate ± SE	test	*P*
Feeding rate	Intercept	8.40 ± 3.68			Tortricidae	Intercept	-2.45 ± 0.19		
	Year		$F_{2, 49}= 0.09$	0.91		Year		$\chi^2_{2, 53}= 0.97$	0.61
	Patch size	-0.83 ± 5.78	$F_{1, 49}= 0.03$	0.87		Patch size	-0.32 ± 0.33	$\chi^2_{1, 55}= 0.96$	0.32
	laying day	-0.09 ± 0.09	$F_{1, 54}= 1.07$	0.30		laying day	-0.02 ± 0.01	$\chi^2_{1, 54}= 1.63$	0.20
	Brood size	1.02 ± 0.45	$F_{1, 55}= 5.15$	**0.03**		Brood size	-0.01 ± 0.07	$\chi^2_{1, 52}= 0.03$	0.87
	Year*Patch size		$F_{5, 49}= 2.71$	0.06		Year*Patch size		$\chi^2_{2, 50}= 2.00$	0.37
Caterpillars	Intercept	2.28 ± 0.52			Aranea	Intercept	-2.34 ± 0.34		
	Year		$\chi^2_{2, 53}= 20.80$	**<0.001**		Year		$\chi^2_{2, 55}= 13.10$	**0.001**
	Patch size	-1.01 ± 0.41	$\chi^2_{1, 53}= 5.93$	**0.01**		Patch size	0.14 ± 0.43	$\chi^2_{1, 53}= 0.10$	0.75
	laying day	-0.05 ± 0.001	$\chi^2_{1, 53}= 10.17$	**0.001**		laying day	0.001 ± 0.02	$\chi^2_{1, 52}= 0.005$	0.95
	Brood size	-0.01 ± 0.08	$\chi^2_{1, 52}= 0.02$	0.89		Brood size	-0.03 ± 0.10	$\chi^2_{1, 54}= 0.13$	0.72
	Year*Patch size		$\chi^2_{2, 50}= 0.95$	0.62		Year*Patch size		$\chi^2_{2, 50}= 0.51$	0.77
Noctuidae	Intercept	0.93 ± 0.41			Prey size	Intercept	2.15 ± 0.08		
	Year		$\chi^2_{2, 51}= 21.93$	**<0.001**		Year		$F_{2, 54}= 1.65$	0.21
	Patch size	-0.60 ± 0.31	$\chi^2_{1, 51}= 4.34$	**0.03**		Patch size	0.05 ± 0.15	$F_{1, 51}= 0.11$	0.75
	laying day	-0.04 ± 0.01	$\chi^2_{1, 51}= 8.82$	**0.002**		laying day	-0.007 ± 0.01	$F_{1, 53}= 1.71$	0.20
	Brood size	0.02 ± 0.06	$\chi^2_{1, 50}= 0.09$	0.76		Brood size	-0.02 ± 0.03	$F_{1, 52}= 0.83$	0.37
	Year*Patch size		$\chi^2_{2, 48}= 2.17$	0.34		Year*Patch size		$F_{2, 49}= 0.85$	0.44

.Discussion

Patch size affected nestling growth in both species (Table 1, Fig. 1). Great tit nestlings in large patches were bigger than those from small patches, which suggest that nestling growing conditions were better in large patches. Worse feeding conditions in small patches may be behind the former result, as food is the main limiting factor of nestling growth (Van Noordwijk *et al.* 1988). In fact, the analysis of Great tit nestling diet revealed key differences between patch size categories (Table 3). Great tits delivered more caterpillars to theirs broods in large patches, concretely noctuids, which are the preferred prey of this species in the study area (García-Navas *et al.* 2013). The possibility to feed with a higher proportion of their preferred prey in large patches may not go unnoticed for Great tits, and males may compete to stablish there, as the age pattern suggests. This difference in the average age of Great tits between large and small patches has been also shown in other studies with this species (Moller, 1991, Riddington and Gosler 1995) and other species (Burke and Nol 1998); and is related to the acquisition of a territory (Riddington and Gosler 1995). In this species, the patch size did not affect their recruitment; although it was positively affected by the nestling mass; as other previous studies have shown (Tinbergen and Boerlijst 1990, Verhulst *et al.* 1997). Nonetheless, from all Great tit fledglings that achieved to breed in the next year, 82% were born in large patches (data not shown for brevity); and the 78% of them bred again in large patches, versus the 50% of the nestlings that were born in small patches.

Blue tit nestlings did not differ in body size between patch size categories (Table 1); which suggests that Blue tit nestlings did not have the limitations in their development that the Great tit nestlings seem to suffer in small patches. The reason of this dissimilarity in the results could rely in the different feeding habits of both species. Blue and Great tits, although they share a similar trophic ecology predating above all on Lepidoptera caterpillars (Perrins 1991), differ in the global amount of caterpillars in their diet and the composition of them. Great tits are more specialists on caterpillars, being highly selective for larger ones (Naef-Daenzer *et al.* 2001); which in our study area are chiefly noctuids. Blue tits additionally, feed

abundantly on tortricids and also incorporate a great amount of spiders in their diet (García-Navas *et al.* 2013). The particular feeding behaviour of each species was also patent when an increase in the parental effort was given. Both species increased their feeding rate with large broods, but in a different way. Blue tits faced this burden on parental effort through a change in the composition of their diet. This is partly shown with the reduction in the proportion of caterpillar as broods become larger and also with the increase in diet diversity (Table 2). An example of this switch in Blue tit diet accordingly to the degree of parental effort, is what happens with the two most abundant caterpillar families in our study area, noctuids and tortricids. The first ones, despite they are a high quality prey, are scarce and more difficult to find. On the contrary, tortricids are smaller but their higher abundance, gregarious behaviour and leaf-roller habits make them easy to find (Naef-Daenzer and Keller 1999; García-Navas *et al.* 2013). With small broods, Blue tits feed their nestlings with a high proportion of noctuids but, as the broods become larger, they start incorporating a higher proportion of tortricids at the expense of noctuids. This change in the composition of the diet allows them to increase their feeding rate (Table 2). This switch in Blue tit diet accordingly to the degree of parental effort was experimentally demonstrated by García-Navas and Sanz (2010). Great tits with larger broods did feed more frequently but they did not change their nestling's diet composition (Table 3).

This wider trophic niche of Blue tits (Matthysen *et al.* 2011), may allow them to face more efficiently the food limitation conditions in small forest patches, and to better satisfy the energetic demands of their broods in such patches. Indeed, their diet diversity did not change between patch size categories; which means that they in general find their food requisites in both patch size categories. This was not the case of the Great tits, which significantly increased their diet diversity in small patches, probably because they are not able to achieve their preferred diet in those patches and are forced to shift to secondary low-quality preys. Besides that the elementary feeding requisites of Blue tits may be satisfied in small patches, this does not mean that little differences in composition, even at species level

(García-Navas *et al.* 2013 and references there-in), influence the body condition of their nestlings due to differences in food quality, as food is the main limiting factor in nestling body condition (Naef-Daenzer and Keller 1999; Kaliński *et al.* 2014). In fact, for Blue tit nestlings was better to be born in large patches, because the probability to breed next year was higher in those patches (Table 1). In the same way as Great tits, the 81% of the Blue tit yearlings that achieved to breed were born in large patches, and the 97% of them did it in large patches. Surprisingly, Blue tit males were heavier in small patches; which a priori goes against the expected. However, Riddington and Gosler (1995) found a similar result with Great tit males breeding in good and poor habitats. The former authors discussed that in poor habitats, due to the scarcity of food, birds increase their fat reserves to face this uncertainty (McNamara and Houston 1990; Higginson *et al.* 2012). This could be happening in our study area, although we could not confirm this, as we did not measured the fat score levels (Gosler 1997). The forest patch size effect on nestling characteristics found in this study contrasts with the results obtained by Nour *et al.* (1998), where they did not find differences in the breeding success, diet or caterpillar abundances among a gradient of forest patch size categories. In our study area, small patches have suffered some kind of degradation due to the passage of cattle; which could have accounted in some degree for the worse breeding performance of tits in those forest patches.

Other feature that strongly marked this study was the yearly variation, probably because of the different environmental conditions in each year; which lustily influence the abundance of caterpillars as temperature is the primary factor influencing their phenology (Visser and Holleman 2001). In year 2012, the budding of the oaks was extremely delayed (some patches did not bud until mid-May, J. Bueno-Enciso per. Obs.). This probably made that all those caterpillars that hatched before this budding starved, as they only can survive few days without food (Durant *et al.* 2007). As a consequence, the abundance of caterpillars this year likely decreased in comparison with the others; which probably account for the decrease in the body condition and breeding performance of birds this year, due to the ‘reproductive

stress' hypothesis (Nagy *et al.* 2007; Neto and Gosler 2010). This variation in the breeding performance among years is well documented in bird population studies (Perrins, 1965, Lack, 1966). Tits also delayed their laying date in each year, probably in an attempt to synchronize the period of maximum food demand of their chicks with the peak of food (Van Noordwijk *et al.* 1995), a feature that enhances their reproductive success (Visser *et al.* 2006; Cresswell and McCleery 2003; Matthysen *et al.* 2011). Blue tits, but no Great tits, had a smaller clutch size and a worse breeding success in small patches in 2012. This result may suggest that for some species, the effects of habitat fragmentation only arise determinant years under particular conditions, such as a food shortage; as other authors have pointed out (Riddington and Gosler 1995; Nour *et al.* 1998). In 2012, the average age of birds decreased, although it was only significant for Blue tit males and Great tit females. This decrease in the average age of birds in 2012 was probably due to the 'call effect' of the box placement the former year, joined with the good environmental conditions prevailing in 2011; which allowed a higher recruitment rate in 2012 (Newton 1994; Robles *et al.* 2012). Blue tit nestling diet varied among years probably in consonance with the prey availability in each year, because of their more flexible feeding behaviour (Matthysen *et al.* 2011). Not the case of Great tits, which actively selected nocuids and its consumption increased each year (Table 3). Consumption of spiders decreased in 2013 in both species, probably due to a sudden decline of this type of prey in the forest this year (Tables 2 and 3).

Conclusions

This study suggests that food supply in small forest patches could be hampering the growing conditions of tits in such forest patches, and consequently, their recruitment rate. However, the observational nature of this study do not let to draw firm conclusions, and experiments of cross-fostering between large and small forest patches would be necessaries to confirm the factors involved in the worse breeding performance of tits in small forest patches.

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SUPPORTING INFORMATION

Table S1. Results of the GLMMs analyzing the effects of the year, patch size and laying date on the breeding performance of Blue (*Cyanistes caeruleus*) and Great tits (*Parus major*) in Montes de Toledo, central Spain. The ID nest nested in ID forest patch is included in all models as the random term. Wald chi-square (χ^2) statistic is given with non-parametric distributions. Significant results are highlighted in bold.

Blue tit					Great tit				
Response variable	**Explanatory term**	**Estimate ± SE**	**test**	***P***	**Response variable**	**Explanatory term**	**Estimate ± SE**	**test**	***P***
Laying day	Intercept	16.78 ± 1.36			Laying day	Intercept	13.43 ± 2.83		
	Year		$F_{1, 320}$= 32.24	**<0.001**		Year		$F_{2, 150}$= 8.12	**<0.001**
	Patch size	-2.61 ± 2.25	$F_{1, 319}$= 1.34	0.27		Patch size	-2.25 ± 3.91	$F_{1, 149}$= 0.33	0.58
	Year*Patch		$F_{2, 317}$= 0.40	0.67		Year*Patch		$F_{2, 147}$= 0.67	0.52
Clutch size	Intercept	10.10 ± 0.24			Clutch size	Intercept	9.82 ± 0.32		
	Year		$F_{2, 316}$= 11.14	<0.001		Year		$F_{2, 148}$= 3.00	0.06
	Patch size	0.46 ± 0.34	$F_{1, 316}$= 0.06	0.81		Patch size	-0.48 ± 0.45	$F_{1, 147}$= 1.17	0.34
	Laying day	-0.10 ± 0.008	$F_{1, 316}$= 164.54	**<0.001**		Laying day	-0.04 ± 0.01	$F_{1, 149}$= 13.25	**<0.001**
	Year*Patch		$F_{3, 316}$= 3.27	**0.02**		Year*Patch		$F_{2, 146}$= 0.41	0.66
Hatching success	Intercept	1.69 ± 0.13			Hatching success	Intercept	1.44 ± 0.15		
	Year		$\chi^2_{2. 296}$= 6.98	**0.03**		Year		$\chi^2_{2. 128}$= 3.81	0.15
	Patch size	-0.05 ± 0.22	$\chi^2_{1. 294}$= 0.05	0.82		Patch size	0.48 ± 0.27	$\chi^2_{1. 129}$= 3.05	0.08
	Laying day	0.007 ± 0.006	$\chi^2_{1. 295}$= 1.19	0.28		Laying day	0.004 ± 0.009	$\chi^2_{1. 127}$= 0.18	0.68
	Year*Patch		$\chi^2_{2. 292}$= 0.96	0.62		Year*Patch		$\chi^2_{2. 125}$= 4.12	0.13
Fledgling success	Intercept	7.03 ± 0.67			Fledgling success	Intercept	9.59 ± 1.41		
	Year		$\chi^2_{2. 251}$= 8.31	0.02		Year		$\chi^2_{2. 100}$= 11.77	**0.003**
	Patch size	-0.95 ± 0.79	$\chi^2_{1. 251}$= 0.13	0.72		Patch size	-1.75 ± 1.47	$\chi^2_{1. 99}$= 1.42	0.23
	Laying day	-0.09 ± 0.02	$\chi^2_{1. 251}$= 35.21	**<0.001**		Laying day	0.04 ± 0.04	$\chi^2_{1. 98}$= 0.87	0.35
	Year*Patch		$\chi^2_{3. 251}$= 13.67	**0.003**		Year*Patch		$\chi^2_{2. 96}$= 3.35	0.18
Nestling condition	Intercept	-2.30 ± 1.51			Nestling condition	Intercept			n.s.
	Year		$F_{2, 224}$= 26.36	**<0.001**		Year		$F_{2, 94}$= 11.45	**<0.001**
	Patch size	-0.24 ± 0.10	$F_{1, 224}$= 5.51	**0.02**		Patch size	-0.09 ± 0.18	$F_{1, 93}$= 0.24	0.63
	Laying date	-0.02 ± 0.004	$F_{1, 224}$= 5.51	**<0.001**		Laying date	-0.01 ± 0.007	$F_{1, 94}$= 1.97	0.16
	Tarsus length	0.51 ± 0.09	$F_{1, 224}$= 28.55	**<0.001**		Tarsus length	1.01 ± 0.17	$F_{1, 95}$= 36.06	**<0.001**
	Year*Patch		$F_{2, 222}$= 1.25	0.29		Year*Patch		$F_{2, 91}$= 0.29	0.75
Nestling size	Intercept	16.01 ± 0.07			Nestling size	Intercept	19.63 ± 0.12		
	Year		$F_{2, 225}$= 6.85	**0.001**		Year		$F_{2, 95}$= 3.50	**0.03**
	Patch size	0.004 ± 0.11	$F_{1, 225}$= 0.002	0.97		Patch size	-0.23 ± 0.12	$F_{1, 95}$= 4.86	**0.03**
	Laying date	-0.006 ± 0.003	$F_{1, 225}$= 5.29	**0.02**		Laying date	-0.002 ± 0.004	$F_{1, 94}$= 0.34	0.56
	Year*Patch		$F_{2, 223}$= 2.09	0.13		Year*Patch		$F_{2, 92}$= 0.33	0.72
Recruitment	Intercept	-2.85 ± 0.29			Recruitment	Intercept	-13.52 ± 4.18		
	Year	-1.88 ± 0.29	$\chi^2_{1. 1276}$=	**<0.001**		Year	-1.98 ± 0.8	$\chi^2_{1. 787}$= 6.13	**0.01**
	Patch size	-1.30 ± 0.52	$\chi^2_{1. 1276}$= 6.30	**0.01**		Patch size	-0.35 ± 0.69	$\chi^2_{1. 786}$= 0.26	0.61
	Nestling mass	0.09 ± 0.21	$\chi^2_{1. 1272}$= 0.20	0.66		Nestling mass	0.58 ± 0.24	$\chi^2_{1. 787}$= 5.98	**0.01**
	Tarsus length	-0.04 ± 0.22	$\chi^2_{1. 1275}$= 0.03	0.87		Tarsus length	0.18 ± 0.44	$\chi^2_{1. 784}$= 0.16	0.68
	Laying date	0.004 ± 0.02	$\chi^2_{1. 1271}$= 0.05	0.83		Laying date	-0.02 ± 0.04	$\chi^2_{1. 785}$= 0.19	0.67
	Year*Patch		$\chi^2_{1, 1270}$= 1.94	0.16		Year*Patch		$\chi^2_{1, 783}$= 0.001	0.98

CAPÍTULO II

EFECTOS DEL TIPO DE CAJA SOBRE LA BIOLOGÍA REPRODUCTIVA DE DOS ESPECIES PASERIFORMES QUE ANIDAN EN AGUJEROS

Bueno-Enciso J, Ferrer ES, Barrientos R y Sanz JJ. Effect of the nestbox type on the breeding performance of two secondary hole-nesting passerines

Under review

Abstract

The use of nestboxes to study secondary cavity nesters avian species has greatly improved the knowledge related to many fields of environmental sciences. The aim of this study has been to compare the breeding performance of Great and Blue tits in two types of nestboxes that differ as regards their shape and thermal properties. Both nestbox types were placed in the same area to avoid confounding factors. There were significant differences between the extreme values of temperature and relative humidity obtained for the two nestbox types, and this was also the case of their daily patterns. Secondary hole-nesting birds appear to prefer breeding in woodcrete nestboxes. Nest predation was, however, significantly greater in woodcrete nestboxes. The nest height was significantly higher in woodcrete nestboxes, as was the danger distance between the nest cup and the entrance hole, which could account for the fact that Great tits prefer this type of nestbox. The laying date was earlier in woodcrete boxes, but nestbox type did not influence clutch size. However, the eggs of both species were significantly smaller in woodcrete boxes. Breeding success was worse in woodcrete nestboxes. Nestbox type also affected the incubation pattern in both species, and attentiveness was significantly diminished in woodcrete nestboxes. This study reinforces the idea that the type of nestbox used in avian studies is not a trivial issue and may have strong biological effects on avian populations. It is important to take this into account when nestboxes are used as management measures for bird conservation.

Introduction

The use of man-made artificial nestboxes in avian research carried out with secondary hole-nesting species has greatly improved the knowledge related to the disciplines of ecology and evolution (Lambrechts *et al.* 2010 and references there in). However, nestboxes differ from natural cavities in several respects, and it has been suggested that this may bias the results obtained through the use of nestboxes and thus the validity of general conclusions and extrapolations to ‘natural’ populations (Møller 1989). Furthermore, different research groups use different nestbox designs in a non-random manner (Lambrechts *et al.* 2010; Møller *et al.* 2014a). Nestbox type use is not trivial and can alter phenotypical or reproductive traits over time (Lambrechts *et al.* 2010; Møller *et al.* 2014a). Furthermore, different hole-nesting birds inhabiting the same area may perceive each particular nestbox type in a different way, and the preference for one type or another may consequently vary among species (Browne 2006). Likewise, a particular nestbox type may affect each species in a different way (Møller *et al.* 2014b), and the placement of one particular type of nestbox may therefore benefit one species over the others (Lambrechts *et al.* 2010; Møller *et al.* 2014a). It is thus important to document the effects of the nestbox type used at the species level in order to better understand the results of avian population studies and the potential confounding factors associated with the use of nestboxes.

Unlike most previous studies, we have placed two different types of nestboxes in several forests in central Spain in order to avoid confounding factors such as geography, climatology or food supply (Møller *et al.* 2014b). The two types of nestboxes differ as regards their design and the material from which they are constructed, which may affect their thermal properties: woodcrete nestboxes made from a mixture of cement and sawdust, and wooden nestboxes made from pine wood. The size of each of these nestboxes is also different: the woodcrete nestboxes have a smaller surface area and internal volume than the wooden ones (García-Navas *et al.* 2008). Both parameters (thermal properties and size) probably influence the breeding performance of bird species. The nestbox size dictates the size of the nest, and we know that large nests are advantageous because they allow birds to increase their

clutch size (Møller *et al.* 2014b). Furthermore, temperature influences birds' energy expenditure as regards their self-maintenance, and could have effects on their phenology and breeding performance (Ardia *et al.* 2009). Before hatching, breeding in warmer nestboxes may be advantageous since the energy saved on thermoregulation can be allocated to egg production (Nager and van Noordwijk 1992; Yom-Tov and Wright 1993) or better embryo developmental conditions during incubation (Haftorn and Reinertsen 1990; Nilsson, Stjernman and Nilsson 2008; Nord and Nilsson 2011). However, once the chicks have hatched, some problems related to an excess of temperature may occur in terms of hyperthermia (Møller *et al.* 2014b), and fledgling success may be reduced (Thomas, Blondel and Perret 2001).

The aim of the present study has been to assess the effects of nestbox type on occupation and predation rates, and on breeding biology, with a particular emphasis on nest architecture and on the incubation phase of two hole-nesting birds, the Great tit (*Parus major* L.) and the Blue tit (*Cyanistes caeruleus* L.). In order to obtain robust conclusions, we placed both nestbox types in three nearby forests over three consecutive years so as to assess the impact of nestbox used in avian studies. Our study design avoids possible biases that may occur when comparing data obtained in different localities and years.

Methods

Study area

The study was conducted over three consecutive years (2011-2013) in three nearby forests situated in San Pablo de los Montes (39°32′44″N, 4°19′41″W; central Spain). A total of 180 wooden and 60 woodcrete nestboxes were erected in a grid of 30-50 m between adjacent nestboxes at the beginning of 2011 in three Pyrenean oak (*Quercus pyrenaica*) forests: Morra, Fuenlabrada and Avellanar). In the two first forests, 20 woodcrete and 80 wooden nestboxes were made available for hole-nesting passerines. In the third forest (Avellanar), 20 woodcrete and 20 wooden nestboxes were also made available for birds. Woodcrete or wooden nestboxes were placed in two parallel blocks of the forests separated by a 30-50 m wide-

corridor. The nestboxes were hung on the branches of oak trees at a height of 2.5-3 metres and oriented towards the south. They were protected from predators (mustelids, woodpeckers) with wire mesh and a polyvinyl chloride (PVC) pipe (length: 50–70 mm, diameter: 40 mm) fixed to the hole-entrance. We have analysed the effect of nestbox type on the predation by the ladder snake (*Rhinechis scalaris*), the most common predator in our study area (Salvador and Pleguezuelos, 2002). During the study period, nestboxes were occupied by Blue tits (*Cyanistes caeruleus*, n = 233), Great tits (*Parus major*, n = 151) and Nuthatches (*Sitta europaea*, n = 31).

Woodcrete nestboxes are cylindrical (model 1B, Schwegler, Germany) with a hole entrance of 32 mm and an internal chamber size of 17-18 x 12 cm in diameter (floor surface = 113.1 cm^2), thus signifying an internal volume of 1869 cm^3. The distance between the lower edge of the entrance hole and the base is 13.5 cm, and the removable frontal lid covers the whole front. Wooden nestboxes, which are provided by the Spanish forestry commission, are quadrangular and made of pine wood, with an entrance hole of 32 mm and an internal chamber of 17.5-19.5 x 11.5 x 13 cm (floor surface = 149.5 cm^2), thus signifying an internal volume of 2057 cm^3. The distance between the lower edge of the entrance hole and the base is 10 cm. The removable frontal lid leaves a slit of 1-1.5 cm between the roof and the frontal lid, which increases light conditions inside the box chamber.

General field procedures

Nestboxes were checked for occupation by birds, and the dates of clutch initiation (1 = 1 of April), clutch sizes and number of hatched or fledged young were determined. Once we had found the first eggs laid, we used a rule to measure the height of the fresh nest situated under the entrance hole to the nearest 0.5 mm, and then multiplied the measurement obtained by the inner surface of the nestbox floor in order to obtain a surrogate of the nest volume. We also calculated the danger distance from the nest cup to the entrance hole (Mazgajski and Rykowska 2008). In the first two study years (2011-2012), when clutch size was complete, a photograph of each egg was taken (side view) with a rule next to it (Sanz and García-Navas 2009). From each photograph the length

and breadth of each egg was measured (JBE) to calculate the egg volume (Hoyt 1979). The length of incubation was defined as the number of days between completion of the clutch and the first signs of hatching. Adults birds were captured when the nestlings were 8 days old (day of hatching = 0), aged (yearling vs. old) according to plumage characteristics, and their tarsus length was measured. In 2011, adult Great tits were not captured owing to technical difficulties. Nestlings were ringed, measured (tarsus and wing length) and weighed when they were 13 days old. All birds were weighed (± 0.01 g) with an electronic balance, and their tarsus and wing length were measured with a digital calliper (± 0.01 mm) and ruler (± 0.5 mm), respectively. Only non-predated nests and first clutches were considered. Hatching success was calculated as the proportion of eggs that had hatched and breeding success as the proportion of eggs that resulted in fledglings.

In the three study years, ambient temperature and relative humidity inside empty nestboxes were recorded every 10 min for 75 days (16 April to 31 May) in each forest by 24 data loggers (Gemini Tiny Tag Ultra 2, UK) placed inside twelve pairs of woodcrete and wooden nestboxes located in the same tree and with the same orientation. One data logger did not record relative humidity properly. Daily temperature and relative humidity (maximum, minimum and mean) for these 75 days were averaged to obtain a single value per nestbox type, forest patch and study year.

In 2012 and 2013, female incubation activity was recorded over 24 hours by using two DS1922L Thermochron i-Buttons (accuracy ±0.5 ºC in the range +20 ºC to +75 ºC) for temperature recordings inside the nest cup and nestbox (see below). Recordings took place between days 5 and 13 after the onset of full incubation (the day on which the female was found to be incubating). This date was called the incubation date. Data loggers were placed the day before we began to take the measurements and retrieved the day after. One i-Button was covered with brown medical tape (to tie a transparent line to it), and was introduced through the bottom of the nest up into the nest cup so as to place it among the eggs (hereafter, nest data logger). We tied the free end of the line to the front lid of the nestbox. The second i-Button was stuck to one of the internal sides of the nestbox

(hereafter, control data logger) with the aid of a piece of medical tape. The data loggers were programmed to take one measurement every 11 seconds. The Rhythm 1.0 programme (Cooper and Mills, 2005) was used to determine off-bouts after a visual rechecking of the output in the RavenPro 1.5 programme (Charif, Strickman and Waack 2010), with a minimum off-bout duration of 2 min and a minimum off-bout change in temperature of 2 ºC. The variables measured were: 1) Onset: minutes after sunrise until female left the nest for first time; 2) Offset: minutes before sunset since the last female arrived at the nest; 3) On-bout duration: average duration of the periods during which the females actively warmed the eggs; 4) Off-bout duration: average duration of the periods during which the females were off the nest;, 5) Number of off-bouts per day, 6) Attentiveness: percentage of time during the daily activity (from onset to offset) in which the female was actively incubating, and 7) Egg cooling: daily average egg temperature lost during off-bouts.

Statistical procedures

The daily temperature and relative humidity of the empty nestboxes were compared for each pair of nestboxes by means of two-tailed paired Student's t-test. Occupation rate was analysed with a GLZ (binomial distribution; occupied nestbox = 1, non-occupied = 0). Predation was also analysed with a GLZ (binomial distribution: nest predated by snakes = 1, non-predated nest = 0). General Linear Models (GLM) were used to test for the effect of nestbox type on the breeding performance of Great and Blue tits. The models included forest, study year, tit species and nestbox type as categorical variables, and laying date and/or clutch size as covariates. The body condition of birds was estimated by adding tarsus length as a further covariate. Hatching and breeding success were also analysed with a GLZ fitted with a binomial distribution, in which the numerator of the response variable was the number of hatchling or fledglings, and in both cases the denominator was clutch size.

In those GLM performed to test for the effect of nestbox type on the incubation performance of females, several continuous variables were included as covariates: 1) Incubation day, because the thermal properties of the eggs change with the development of the

embryo (Cooper and Voss 2013); 2) Mean temperature during the daylight hours or the previous night, as ambient temperature is the main factor to affect incubation (Conway and Martin 2000); 3) Daylight hours, because longer days increase the potential time devoted to incubation and decrease the night energy expenditure (Chalfoun and Martin 2007), and 4) Clutch size, as female energy expenditure is positively related to clutch size (Haftorn and Reinertsen 1985; Moreno and Sanz 1994). Attentiveness was analysed with a GLZ (binomial distribution), in which the numerator of the response variable was the total amount of time during daily activity (from onset to offset) in which the female was actively incubating, and the denominator was the duration of day.

Homoscedasticity, proper distribution employed and dependence were graphically verified with the residuals. Models were initially constructed with all the explanatory terms fitted, including interactions. The final models were then selected by means of a backward procedure during which non-significant terms were progressively eliminated, starting with the interactions. The final models were therefore those that explained the most variance with the fewest number of explanatory variables. All analyses were performed in STATISTICA 10.0 and SAS 9.4. Mean ± SE is shown in the results.

Results

Nestbox type effect on microclimate

The mean daily temperatures did not differ between nestbox types (Paired Student t-test. t = 0.56, *P* = 0.58). However, woodcrete nestboxes had significantly higher maximum temperatures and lower minimum temperatures than the wooden ones (Maximum temperature. t = 3.02, *P* = 0.011; Woodcrete = 21.22 ± 2.16 ºC, n =12; Wooden = 20.60 ± 2.40 ºC, n = 12. Minimum temperature. t = 2.53, *P* = 0.030; Woodcrete = 7.97 ± 1.45 ºC, n = 12; Wooden = 8.16 ± 1.49 ºC, n = 12). The daily temperature pattern proved to be significantly different between nestbox types during several periods of the day (Fig. 1a). From dawn until approximately midday the temperature inside woodcrete nestboxes was significantly lower (Fig. 1a). However, the temperature inside the woodcrete nestboxes during the afternoon

increased more sharply than in the wooden nestboxes, and started to become significantly higher at around 16:00 h. From that moment on until midnight, the temperatures inside the woodcrete nestboxes were significantly higher. Overnight temperatures were the same for both nestbox type (Fig. 1a).

With regard to relative humidity conditions, only the minimum values obtained for the two nestbox types were significantly different, since they were higher for the woodcrete nestboxes (Paired Student t-test: t = 3.50, *P* = 0.006; Woodcrete = 52.76 ± 9.27 %, n = 11; Wooden = 48.85 ± 10.90 %, n = 11). The mean and maximum daily values of relative humidity obtained for the two nestbox types did not differ (Mean relative humidity: t = 2.17, *P* = 0.06; Maximum relative humidity: t = 0.01, *P* = 0.99). Despite the fact that mean daily relative humidity was the same for both nestbox types, the daily pattern was different (Fig. 1b) and it was significantly more humid inside woodcrete boxes during more than half the day than it was in the wooden ones (Fig. 1b). This significant difference in humidity started at around 10:00 h and continued until late evening.

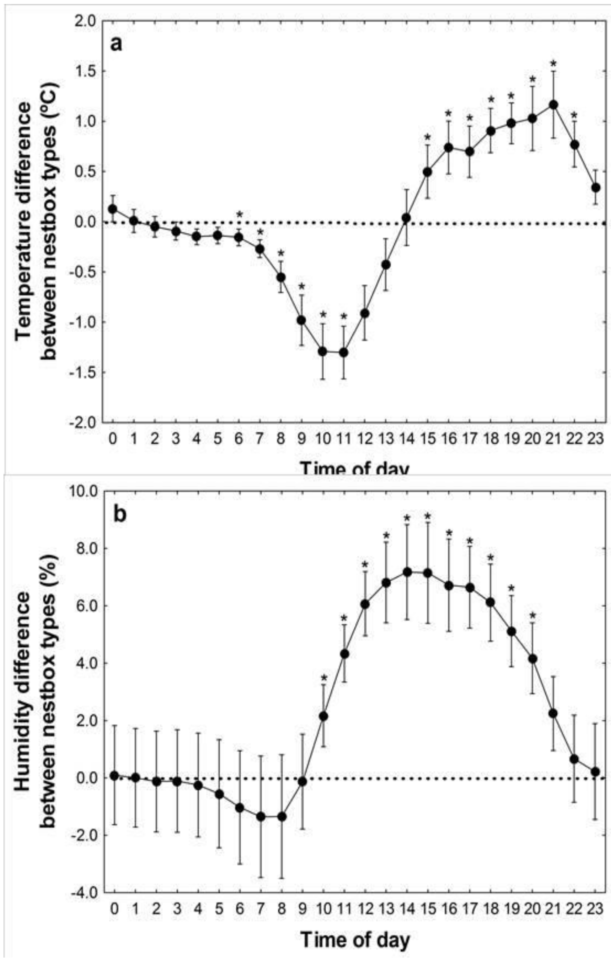

Figure 1. Differences in the daily pattern of mean temperature (a) and mean relative humidity (b) between woodcrete and wooden nestboxes. Positive values signify that the value in woodcrete nestboxes is higher than that in the wooden ones. Asterisks indicate signification. Vertical bars indicate ± SE.

Nestbox type effect on the occupation and predation rate

The occupancy rate of the two nestbox types was significantly different in the case of all three hole nesting species (GLZ. $\chi^2_1 = 14.03$, P < 0.001) and years (GLZ. $\chi^2_2 = 31.86$, P < 0.001), with

higher occupation occurring in woodcrete nestboxes (75 %) than in wooden ones (51 %). Upon taking into account the availability of both nestbox types, we found that Great tits proved to have a significant preference for woodcrete nestboxes (GLZ. $\chi^2_1 = 15.95$, $P < 0.001$. Wooden nestboxes: 17.59 %, n = 540. Woodcrete nestboxes: 31.11 %, n = 180). The occupancy rate of Great tits was significantly different as regards the years studied ($\chi^2_2 = 12.88$, $P = 0.002$), but was not so as regards forests (GLZ. $\chi^2_2 = 5.35$, $P = 0.07$). The occupancy rate of Blue tits was, however, the same for both nestbox types (GLZ. $\chi^2_1 = 0.40$, $P = 0.53$). Neither study year nor forest affected the occupancy rate of Blue tits (GLZ. Year: $\chi^2_2 = 3.15$, $P = 0.21$. Forest: $\chi^2_2 = 5.20$, $P = 0.07$).

The probability that a tit nest would be predated by snakes was significantly higher in the case of woodcrete nestboxes (GLZ. $\chi^2_1 = 6.42$, $P = 0.011$; Fig. 2) and significantly differed as regards the year studied (GLZ. $\chi^2_2 = 42.47$, $P < 0.001$). However, the snake predation rate was the same for Blue and Great tit nests (GLZ. $\chi^2_1 = 2.79$, $P = 0.10$).

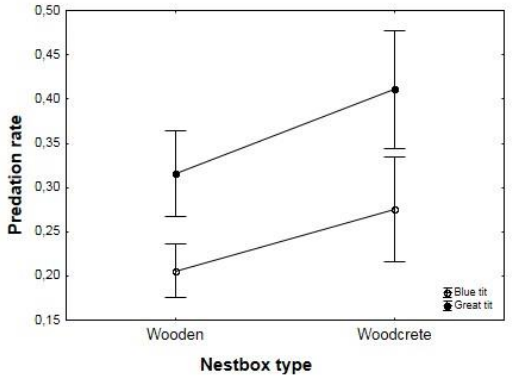

Figure 2. Predation rates by ladder snake (*Rhinechis scalaris*) on Blue and Great tit nests in relation to the nestbox types in the Montes de Toledo. Vertical bars indicate ± SE.

Table 1. Number of woodcrete and wooden nestboxes occupied by Blue and Great tits during the three study years in the Montes de Toledo

	2011		2012		2013		
	woodcrete	wooden	woodcrete	wooden	woodcrete	wooden	**Total**
Blue tit	18	53	15	59	25	63	**233**
Great tit	20	17	21	47	15	31	**151**

Nestbox type effect on the breeding performance

Nest height was not statistically influenced by forests or study year (Table 2), but it was significantly different for both nestbox type and species ($R^2 = 0.08$; Table 2). Great tits built shallower nests (53.34 ± 13.57 mm, n = 142) than Blue tits (59.96 ± 13.47 mm, n = 220). The nest height in wooden nestboxes (56.07 ± 12.81, n = 256) was shallower than that in woodcrete nestboxes (60.47 ± 15.80, n = 106). However, Great tits built significantly shallower nest than Blue tits only in wooden nestboxes (Wooden nestboxes: $t_{254} = 5.57$, $P < 0.001$. Woodcrete nestboxes: $t_{104} = 1.25$ $P = 0.21$. Fig. 3a).

On the other hand, nest volume did not differ among forests or study years ($R^2 = 0.19$; Table 2), although it was significantly larger in wooden nestboxes (838.31 ± 191.49 cm^3, n = 256) than in woodcrete ones (683.94 ± 178.70 cm^3, n = 106). The volume of Blue tits' nests was larger than that of Great tits (Blue tit: 840.68 ± 199.51 cm^3, n = 220. Great tit: 719.40 ± 178.71 cm^3, n = 142). The magnitude of the effect of nestbox type on nest volume was significantly different for the two species (interaction term), and was greater in the case of Blue tits (Fig. 3b). With regard to the danger distance between the nest cup and the entrance hole, it was not statistically affected by the forest and the study year (Table 2). However, it was significantly affected by nestbox type and species ($R^2 = 0.53$; Table 2). Great tits kept a longer danger distance (59.48 ± 1.54 mm, n= 142) than did Blue tits (48.63 ± 1.29 mm, n= 220), and this distance was significantly longer in woodcrete nestboxes (74.53 ± 1.53 mm, n= 106) than in wooden ones (43.92 ± 0.80 mm, n= 256. Fig. 3c). The differences between nestbox types were held within tit species (Table 2). The laying date was significantly different in the case of both forest and study years, but was the same in that of the two species (Table 2). The laying date of both species was significantly earlier in woodcrete nestboxes (Table 2. Fig. 3d). The differences between nestbox types was held within tit species ($R^2 = 0.22$; Table 2). The clutch size was statistically influenced by study year, forest and tit species ($R^2 = 0.37$; Table 2). Clutch size was larger for Great tits (9.23 ± 0.16, n = 151) than for Blue tits (8.18 ± 0.13, n =

233), and declined with the laying date (Table 2). Finally, the clutch size was not different as regards nestbox types (Table 2).

The egg volume was statistically influenced by study year, forest, tit species and nestbox types ($R^2 = 0.65$; Table 2), was larger in 2011 than in 2012, and was greater for the Great tit (2.19 ± 0.05 mm^3, n = 151) than for the Blue tit (1.47 ± 0.02 mm^3, n = 233). The egg volume proved to have smaller mean values in the woodcrete nestboxes (Table 2. Woodcrete: Blue tit, 1.45 ± 0.05 mm^3, n = 31; Great tit, 2.12 ± 0.07 mm^3, n = 39. Wooden: Blue tit, 1.47 ± 0.03 mm^3, n = 107; Great tit, 2.24 ± 0.06 mm^3, n = 57). Neither the laying date nor the clutch size affected the average egg volume of either tit species (Table 2).

Hatching success was not influenced by forest, tit species or nestbox types, and was not related to the laying date (Table 2). Hatching success was statistically different in the three years studied (Table 2), while breeding success was significantly different depending on the forests, study years and nestbox types (Table 2). Breeding success was significantly worse in the woodcrete nestboxes (Table 2. Fig. 3e). Finally, breeding success was the same for both tit species and was not related to the laying date (Table 2).

Table 2. Models (GLM, GLZ) showing the effects of forest, year, species and nestbox type on the breeding parameters of Blue and Great tits in the Montes de Toledo (non-significant terms are shown in Table S1).

Parameter	Effect	Estimate ± SE	Test	*P*
Nest height (mm)	Species		$F_{1, 359} = 25.36$	< 0.001
	Nestbox type		$F_{1, 359} = 12.18$	< 0.001
Rejected effects: Forest, Year and Nestbox type x Species.				
Nest volume (mm^3)	Species		$F_{1, 358} = 17.26$	< 0.001
	Nestbox type		$F_{1, 358} = 40.97$	< 0.001
	Nestbox type x Species		$F_{1, 358} = 4.42$	0.036
Rejected effects: Forest and Year.				
Danger distance (mm)	Species		$F_{1, 359} = 25.36$	< 0.001
	Nestbox type		$F_{1, 359} = 364.52$	< 0.001
Rejected effects: Forest, Year and Nestbox type x Species.				
Laying date	Forest		$F_{2, 378} = 13.33$	< 0.001
	Year		$F_{2, 378} = 36.12$	< 0.001
	Nestbox type		$F_{1, 378} = 9.64$	0.002
Rejected effects: Species and Nestbox type x Species.				
Clutch size	Forest		$F_{2, 377} = 7.04$	< 0.001
	Year		$F_{2, 377} = 10.36$	< 0.001
	Species		$F_{1, 377} = 30.49$	< 0.001
	Laying date	-0.087 ± 0.007	$F_{1, 377} = 191.7$	< 0.001
Rejected effects: Nestbox type and Nestbox type x Species.				
Egg volume (mm^3)	Forest		$F_{2, 228} = 12.63$	< 0.001
	Year		$F_{1, 228} = 67.79$	< 0.001
	Species		$F_{1, 228} = 354.4$	< 0.001
	Nestbox type		$F_{1, 228} = 5.80$	0.017
Rejected effects: Nestbox type x Species, Laying date and Clutch size.				
Hatching success (%)	Year		$\chi^2_2 = 25.39$	< 0.001
Rejected effects: Forest, Species, Nestbox type, Nestbox type x Species and Laying date.				
Breeding success (%)	Forest		$\chi^2_2 = 10.26$	0.006
	Year		$\chi^2_2 = 128.07$	< 0.001
	Nestbox type		$\chi^2_1 = 13.08$	< 0.001
Rejected effects: Species, Nestbox type x Species and Laying date.				

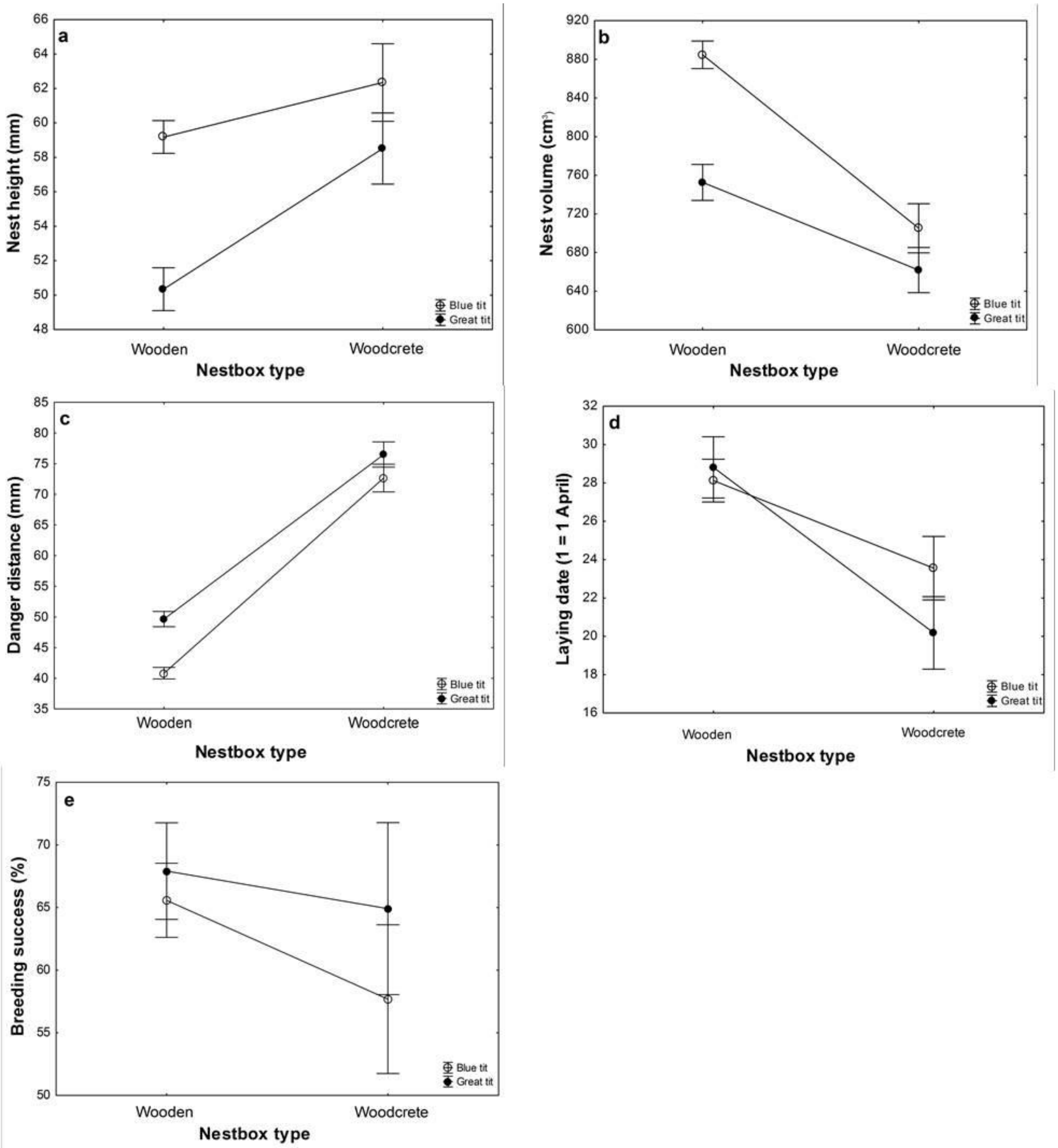

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Figure 3. Nest height (a), nest volume (b), danger distance (c), laying date (d) and breeding success (e) of Blue and Great tis breeding in wooden and woodcrete nestboxes in the Montes de Toledo. Vertical bars indicate ± SE.

Nestbox type effect on the incubation performance

Neither forest nor nestbox types had an effect on the length of the incubation period (GLM. Forest: $F_{2,\ 330}$ = 1.03, *P* = 0.36. Nestbox type: $F_{1,\ 333}$ = 1.73, *P* = 0.19). However, the length of the incubation period was significantly different in the years studied and for the two tit species (GLM. R^2 = 0.49. Year: $F_{2,\ 334}$ = 100.33, *P* < 0.001. Species: $F_{1,\ 334}$ = 8.39, *P* = 0.004) and was negatively related to the laying date and clutch size (GLM. Laying date: Estimate ± SE= -0.126 ± 0.009, $F_{1,\ 334}$ = 198.37, *P* < 0.001. Clutch size: -0.282 ± 0.063, $F_{1,\ 334}$ = 19.70, *P* < 0.001). The incubation period was shorter for the Great tit (13.81 ± 0.26 d, n = 135) than for the Blue tit (14.28 ± 0.15 d, n = 205). Nonetheless, the incubation performance of females was affected by nestbox type.

Incubating females left the nest half an hour after sunrise. The onset of daily activity was not influenced by forest and tit species, and was not related to incubation day or clutch size (Table 3). The onset of daily activity was significantly different in the years studied, and was negative- and positively related to the mean night temperature and the duration of daylight, respectively (Table 3). Females left the nest earlier when there were higher overnight temperatures and shorter daylight hours. The onset of daily activity was not different for the two nestbox types (Table 3), but the effect was not held within tit species (R^2 = 0.15. Interaction term on Table 3. Fig. 4a). The onset of daily activity in woodcrete nestboxes was the same for the Blue and Great tits (Tukey HSD test, P > 0.05. Fig. 4a), but it was significantly later in the case of Great tits than that of in Blue tits in wooden nestboxes (Tukey HSD test, P < 0.05. Fig. 4a). In the morning, the incubating female Great tit left the nest ten minutes later than the Blue tit females in wooden nestboxes (Fig. 4a). With regard to the end of daily incubation activity, the females of both species entered the nestboxes 50 minutes before sunset. The end of daily activity was the same for all forests, tit species, study years and nestbox types, and was not related to daily temperature or clutch size (Table 3). The end of the activity was only positive- and negatively related to the incubation day and daylight hours, respectively (R^2 = 0.07. Table 3). With regard to sunset, the

females entered earlier at the beginning of the incubation period and on days with more daylight hours.

The duration of on-bouts was not affected by forests and study years, and was not related to incubation day, mean daily temperature or clutch size (Table 3). The duration of on-bouts was significantly different for both tit species (Table 3), with higher values for the Great tit (Blue tit: 22.69 ± 5.52 min, n = 79. Great tit: 31.83 ± 10.12 min, n = 63). On the other hand, the duration of on-bouts was positively related to the duration of daylight (Table 3). Finally, the duration of on-bouts was the same for both nestbox types (Table 3), but the effect was not held within tit species (R^2 = 0.31. Interaction term on Table 3. Fig. 4b). The duration of the on-bouts of the females incubating in woodcrete nestboxes was the same for both tit species (Tukey HSD test, $P > 0.05$. Fig. 4b), but it was significantly longer in the case of Great tits than that of Blue tits in wooden nestboxes (Tukey HSD test, $P < 0.001$. Fig. 4b). However, the duration of the two tit species' off-bouts was significantly different (Table 3), with higher values being obtained for the Great tit (Blue tit: 8.75 ± 2.04 min, n = 79. Great tit: 12.40 ± 3.27 min, n = 63), and was positively related to the mean ambient temperature during daylight (Table 3). The duration of off-bouts was the same for both nestbox types (R^2 = 0.43. Table 3). The number of off-bouts was not affected by forests and study years, and was not related to incubation day, mean daily temperature or clutch size (Table 3). The number of the two tit species' off-bouts was significantly different (Table 3), with higher values for Blue tits (Blue tit: 27.18 ± 6.03, n = 79. Great tit: 19.84 ± 5.10, n = 63). Moreover, the number of off-bouts had a negative relationship with the duration of daylight (Table 3). The number of off-bouts was the same for both nestbox types (Table 3), but the effect was not held within tit species (R2 = 0.33. Interaction term on Table 3. Fig. 4c). The number of off-bouts of females incubating in woodcrete nestboxes was the same for both tit species (Tukey HSD test, $P > 0.05$. Fig. 4c), but it was significantly greater in Blue tits than in Great tits in wooden nestboxes (Tukey HSD test, $P < 0.001$. Fig. 4c).

The attentiveness of Blue and Great tits was significantly affected by forest, study year and tit species, and was

negatively and positively related to mean ambient temperature and clutch size, respectively (Table 3). Attentiveness did not change with incubation day (Table 3). Although the two tit species' attentiveness was significantly different (Table 3), this was owing to the different values obtained for both nestbox types. The attentiveness in the two nestbox types was also significantly different (Table 3), with lower values being obtained for the woodcrete nestboxes than for the wooden ones (Fig. 4d). However, the effect of nestbox type was not held within tit species (Interaction term. Fig. 4d). Attentiveness was the same for both tit species in the wooden nestboxes (Tukey HSD test, $P > 0.05$. Fig. 4c), but it was significantly lower in the case of the Great tits than in that of the Blue tits in woodcrete nestboxes (Tukey HSD test, $P < 0.001$. Fig. 4d). Moreover, female Great tit attentiveness proved to have significantly lower values in woodcrete nestboxes (Tukey HSD test, $P < 0.001$. Fig. 4d). Finally, attentiveness had a significant quadratic relationship with the duration of daylight (Table 3). This relationship was the same for both tit species (analysis not shown).

Egg cooling when females were not incubating was not affected by forests, tit species and study years, and was not related to incubation day or the mean daily temperature (Table 3). Egg cooling had a positive relationship with the duration of the on-bouts and clutch size (Table 3). The eggs lost less temperature when the females had longer on-bouts and larger clutch sizes (Table 3). However, the relationship with on-bout duration was not held within the tit species (Interaction term), and was significant only for Blue tits (analysis not shown). The egg cooling in the two nestbox types was significantly different ($R^2 = 0.24$. Table 3), with lower values being obtained for the wooden nestboxes than for the woodcrete ones (Fig. 4e). This effect was held within tit species (Interaction term. Fig. 4e).

The attentiveness of Blue and Great tits was significantly affected by forest, study year and tit species, and was negatively and positively related to mean ambient temperature and clutch size, respectively (Table 3). Attentiveness did not change with incubation day (Table 3). Although the two tit species' attentiveness was significantly different (Table 3), this was owing to the different

values obtained for both nestbox types. The attentiveness in the two nestbox types was also significantly different (Table 3), with lower values being obtained for the woodcrete nestboxes than for the wooden ones (Fig. 4d). However, the effect of nestbox type was not held within tit species (Interaction term. Fig. 4d). Attentiveness was the same for both tit species in the wooden nestboxes (Tukey HSD test, $P > 0.05$. Fig. 4c), but it was significantly lower in the case of the Great tits than in that of the Blue tits in woodcrete nestboxes (Tukey HSD test, $P < 0.001$. Fig. 4d). Moreover, female Great tit attentiveness proved to have significantly lower values in woodcrete nestboxes (Tukey HSD test, $P < 0.001$. Fig. 4d). Finally, attentiveness had a significant quadratic relationship with the duration of daylight (Table 3). This relationship was the same for both tit species (analysis not shown).

Egg cooling when females were not incubating was not affected by forests, tit species and study years, and was not related to incubation day or the mean daily temperature (Table 3). Egg cooling had a positive relationship with the duration of the on-bouts and clutch size (Table 3). The eggs lost less temperature when the females had longer on-bouts and larger clutch sizes (Table 3). However, the relationship with on-bout duration was not held within the tit species (Interaction term), and was significant only for Blue tits (analysis not shown). The egg cooling in the two nestbox types was significantly different ($R^2 = 0.24$. Table 3), with lower values being obtained for the wooden nestboxes than for the woodcrete ones (Fig. 4e). This effect was held within tit species (Interaction term. Fig. 4e).

Table 3. Models (GLM, GLZ) showing the effect of certain independent variables on the incubation performance of Blue and Great tits in the Montes de Toledo (non-significant terms are shown in Table S2).

Parameter	Effect	Estimate ± SE	Test	*P*
Onset of activity (min)	Year		$F_{1, 137} = 4.61$	0.033
	Nestbox type x Species		$F_{1, 137} = 10.33$	0.002
	Mean temperature (night)	-1.014 ± 0.366	$F_{1, 137} = 7.69$	0.006
	Daylight	18.85 ± 5.68	$F_{1, 137} = 11.03$	0.001
Rejected effects: Forest, Species, Nestbox type, Incubation day and Clutch size.				
End of activity (min)	Incubation day	3.359 ± 1.696	$F_{1, 139} = 3.92$	0.049
	Daylight	-26.92 ± 8.67	$F_{1, 139} = 9.63$	0.002
Rejected effects: Forest, Year, Species, Nestbox type, Nestbox type x Species, Mean temperature (day) and Clutch size.				
On-bout duration (min)	Species		$F_{1, 138} = 29.52$	< 0.001
	Nestbox type x Species		$F_{1, 138} = 4.71$	0.032
	Daylight	6.686 ± 2.321	$F_{1, 138} = 8.30$	0.005
Rejected effects: Forest, Year, Nestbox type, Incubation day, Mean temperature (day) and Clutch size.				
Off-bout duration (min)	Species		$F_{1, 139} = 79.23$	< 0.001
	Mean temperature (day)	0.196 ± 0.037	$F_{1, 139} = 28.38$	< 0.001
Rejected effects: Forest, Year, Nestbox type, Nestbox type x Species, Incubation day, Daylight and Clutch size.				
Number of off-bouts	Species		$F_{1, 138} = 38.18$	< 0.001
	Nestbox type x Species		$F_{1, 138} = 3.92$	0.049
	Daylight	-3.714 ± 1.685	$F_{1, 138} = 4.86$	0.029
Rejected effects: Forest, Year, Nestbox type, Incubation day, Mean temperature (day) and Clutch size.				
Attentiveness (%)	Forest		$\chi^2_2 = 6.61$	0.037
	Year		$\chi^2_1 = 5.32$	0.021
	Species		$\chi^2_1 = 8.23$	0.004
	Nestbox type		$\chi^2_1 = 28.88$	< 0.001
	Nestbox type x Species		$\chi^2_2 = 16.13$	< 0.001
	Mean temperature (day)	-0.006 ± 0.0004	$\chi^2_1 = 247.78$	< 0.001
	Daylight	1.584 ± 0.528	$\chi^2_1 = 9.03$	0.003
	Squared daylight	-0.052 ± 0.018	$\chi^2_1 = 8.31$	0.004
	Clutch size	0.003 ± 0.001	$\chi^2_1 = 6.99$	0.008
Rejected effects: Incubation day.				
Egg Cooling (ºC)	Nestbox type		$F_{1, 137} = 4.10$	0.045
	On-bout duration	0.057 ± 0.016	$F_{1, 137} = 12.98$	< 0.001
	Species x On-bout duration		$F_{1, 137} = 23.71$	< 0.001
	Clutch size	0.289 ± 0.062	$F_{1, 137} = 21.35$	< 0.001
Rejected effects: Forest, Year, Species, Nestbox type x Species, Incubation day and Mean				

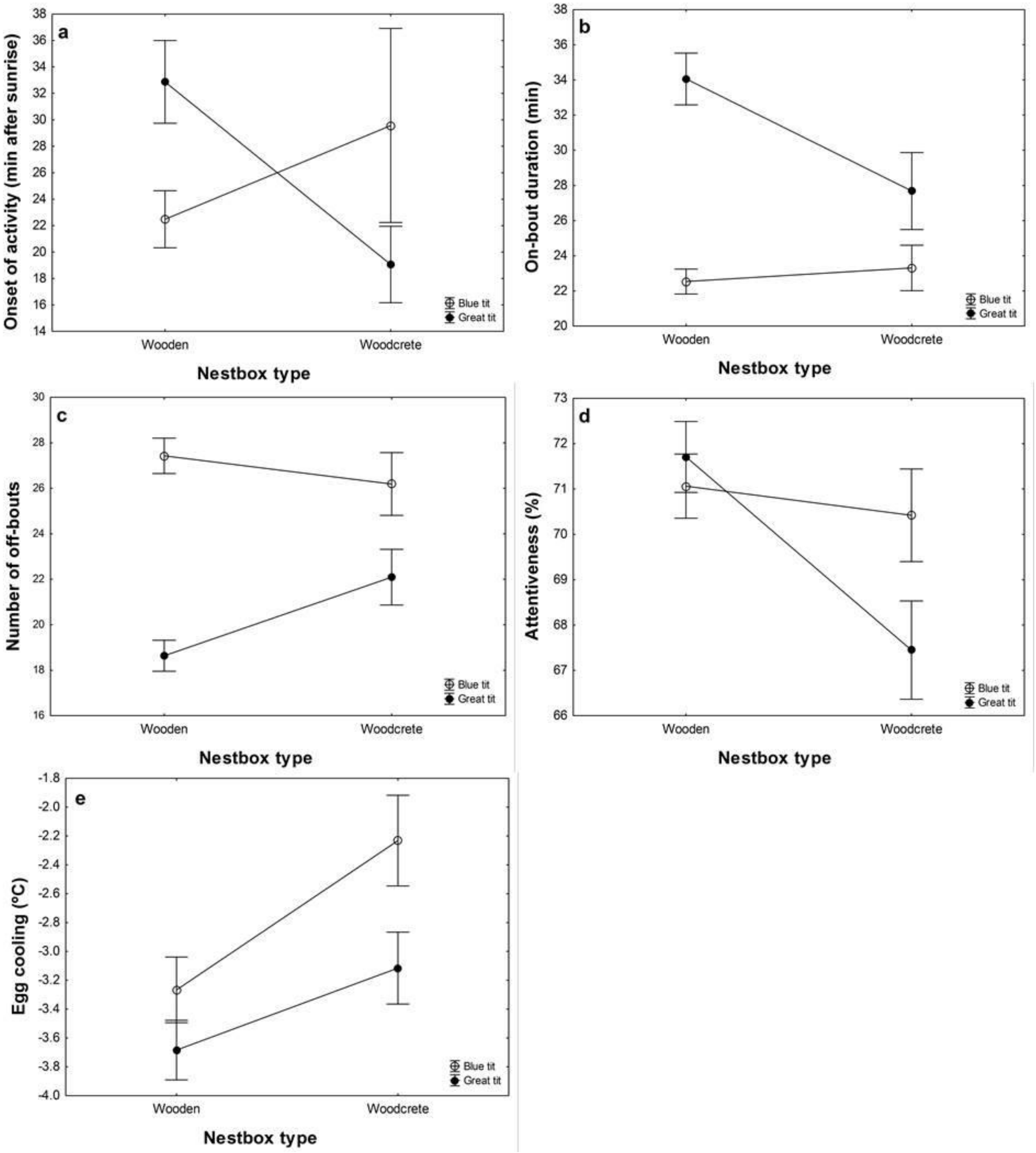

Figure 4. Mean onset of activity after sunrise (a), on-bout duration (b), number of off-bouts (c), attentiveness (d) and egg cooling (e) of Blue tits (open dots) and Great tits (close dots) breeding in wooden and woodcrete nestboxes in the Montes de Toledo. Vertical bars indicate ± SE.

Nestbox type effect on parental and nestling characteristics

There was no difference as regards either female age or male age in the two nestbox types (GLZ. Female: Species, χ^2_1 = 0.33, P = 0.56. Nestbox type, χ^2_1 = 0.10, P = 0.76. Species x Nestbox type, χ^2_1 = 0.69, P = 0.40. Male: Species, χ^2_1 = 0.03, P = 0.86. Nestbox type, χ^2_1 = 0.34, P = 0.56. Species x Nestbox type, χ^2_1 = 0.85, P = 0.36). The mean tarsus length of females or males feeding 8 day-old nestlings was the same for both nestbox types (Table S3), and parental body condition was also the same in both nestbox types (Table S3). There was no difference in either tarsus length or the body condition of 13 day-old nestlings differ in the two nestbox types (Table 4). However, nestling wing length at day 13 after hatching was significantly different depending on the nestbox type (Table 4. Fig. 5). This effect was held within tit species (Interaction term on Table 4).

Table 4. Models (GLM) containing nestling morphological measurements (tarsus length, body condition and wing length) at day 13 after hatching as response variables in relation to several explanatory variables of Blue and Great tits breeding in the Montes de Toledo (non-significant terms are shown in Table S4).

Parameter	Effect	Estimate ± SE	Test	*P*
Nestling tarsus length (mm)	Species		$F_{1,227} = 3132.0$	< 0.001
	Laying date	-0.006 ± 0.002	$F_{1,227} = 5.80$	0.017
	Brood size	0.034 ± 0.013	$F_{1,227} = 6.80$	0.01
Rejected effects: Forest, Year, Nestbox type and Nestbox type x Species.				
Nestling body condition (g)	Year		$F_{2,225} = 5.35$	0.005
	Species		$F_{1,225} = 149.95$	< 0.001
	Laying date	-0.011 ± 0.003	$F_{1,225} = 11.90$	< 0.001
	Nestling tarsus length	0.817 ± 0.090	$F_{1,225} = 83.18$	< 0.001
Rejected effects: Forest, Nestbox type, Nestbox type x Species and Brood size.				
Nestling wing length (mm)	Forest		$F_{2,225} = 11.72$	< 0.001
	Species		$F_{1,225} = 467.01$	< 0.001
	Nestbox type		$F_{1,225} = 4.11$	0.044
	Laying date	-0.032 ± 0.014	$F_{1,225} = 4.87$	0.028
	Brood size	0.184 ± 0.078	$F_{1,225} = 5.46$	0.02
Rejected effects: Year and Nestbox type x Species.				

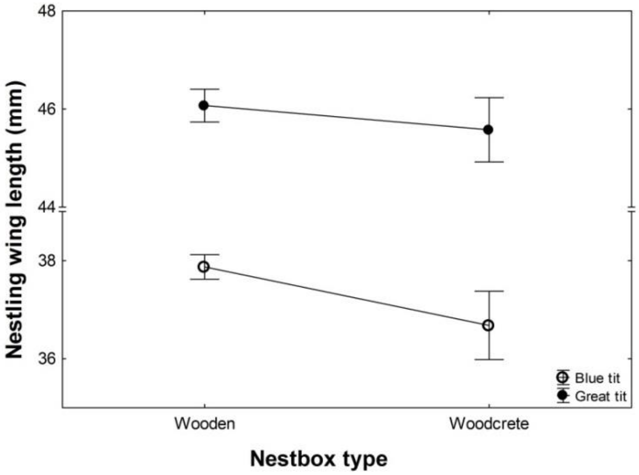

Figure 5. Mean nestling wing length at day 13 after hatching of Blue and Great tis breeding in wooden and woodcrete nestboxes in the Montes de Toledo. Vertical bars indicate ± SE.

Discussion

Nestbox type effect on microclimate

The daily temperature pattern is different in both nestbox types. Thermal conductivity might be higher in woodcrete nestboxes, thus causing faster temperature losses and gains. Birds breeding in woodcrete nestboxes therefore experienced a more variable microclimate with rapid temperature fluctuations. The nest humidity of both nestbox types was also different, and significantly higher values were obtained for woodcrete nestboxes during the majority of the day. The higher porous nature of wood than woodcrete may account for this difference both because the conductance of water vapour through wooden nestbox walls is greater and also because wooden nestboxes have more open cavities owing to the presence of the frontal slit.

Nestbox type effect on the occupation and predation rate

The deeper and darker inner conditions of woodcrete nestboxes may seem safer for tits because of the greater distance between the nest cup and the entrance hole, and are thus preferred (Browne 2006). This last safety perception may to some extent account for the nestbox type preference shown by tits. Since Great tits are almost double the size of Blue tits, the former is probably more aware of this distance than the latter, as they occupy more volume; but also because heavier species need bigger nests to maintain nest comfort during the breeding stage (Lambrechts *et al.* 2014). In fact, the occupancy analyses revealed that Great tits seemed to prefer woodcrete nestboxes for breeding rather than wooden ones, while Blue tits did not (Browne 2006). Great tits probably cover these two necessities (safety and comfort) better in woodcrete boxes owing to their

depth, as is suggested by the fact that in woodcrete nestboxes the nest height of both species did not differ, but in wooden boxes Great tits constructed significantly shallower nests than did Blue tits; probably in order to maintain a sufficient danger distance from the entrance hole. The possibility that Blue tits could successfully resolve the trade-off between distance-safety and comfort in both types of nestboxes could have led to the lack of occupancy pattern in this species. Another possible explanation is, however, that Blue tits could be displaced by Great tits in woodcrete nestboxes because they are smaller (Dhondt and Adriansen 1999; Barrientos *et al.* 2015). Contrary to nest height, nest volume proved to have an opposite trend in the two nestbox types, probably because of the greater floor area in wooden nestboxes.

In our study area, predation by the ladder snake was significantly higher in woodcrete nestboxes. More open conditions in wooden nestboxes may favour the ventilation of these nests and the smell of the nest and chicks therefore dissipates to a higher degree than in woodcrete nestboxes. If this is the case, woodcrete nestboxes could be acting as an ecological trap, because they seem to be actively chosen by birds, but they are also more attractive to the ladder snake.

Nestbox type effect on the breeding performance

Unlike most previous studies, we have compared the breeding performance of tits in two nestbox types in the same locality, signifying that our results are not hampered by certain biases caused by confounding factors such latitude, climatology or food availability (Møller *et al.* 2014b). In this respect, tits started breeding earlier, laid the same number of eggs but with a smaller volume and had worse breeding success in woodcrete nestboxes. There were no differences in either the parental age or their morphology in both nestbox types. The laying date was about a week earlier in woodcrete nestboxes. This could be related to the warmer and hermetic conditions in those nestboxes, in which the energy saved in thermo-regulation could allow females to get into breeding condition more quickly, as temperature is known to influence the onset of laying (van Balen 1973). Ambient temperature similarly influences the investment in eggs, as females can allocate more energy to egg production, which would otherwise

be used for their self-maintenance (Yom-Tov and Wright 1993). It was therefore expected that the tit eggs laid in woodcrete nestboxes would be bigger, but this did not in fact occur. This could be related to the warmer and wet microclimate inside woodcrete nestboxes, conditions that are beneficial for embryo development and may allow tits to invest less in their eggs (Conway and Martin 2000). Indeed, despite their small size, the cooling rate of both tit species' eggs was lower in woodcrete nestboxes. Clutch size was not different for either nestbox type, not even in the case of Great tits, which have been proved to be a nest size sensitive species (Møller *et al.* 2014a). However, the strength of this relationship may depend on the size range of the different nestbox types used (Sorace and Carere 1996). Despite the differences in the incubation pattern in the two nestbox types (see below), the hatching success was not statistically different. However, we show that breeding success was different depending on the nestbox type, and was significantly worse in woodcrete nestboxes. This effect was probably occasioned by the warmer conditions in woodcrete nestboxes, which could have caused hyperthermia in the nestlings (Mertens 1977; Greño, Belda and Barba 2008). Before fledging, nestlings of both tit species had a shorter wing length in woodcrete nestboxes, suggesting detrimental conditions in these nestboxes, probably owing to hyperthermia.

Nestbox type effect on the incubation performance

Nestbox type also affected the incubation pattern in both species, which may have tremendous consequences as incubation influences the hatchability and phenotype of nestlings, and thus the fitness of both parents and nestlings (Chalfoun and Martin 2007; Nord and Nilsson 2011). The onset of the daily activity of both species was the same in woodcrete nestboxes, but differed significantly in wooden ones. Maintaining adequate conditions in terms of temperature and humidity throughout the continuous nightly incubating period is probably more costly in wooden nestboxes than in woodcrete ones, owing to their lesser degree of airtightness. This energy expenditure is probably more challenging for Blue tits, as they are about half the size of Great tits. The relatively high energy demands of the continuous nightly incubation period for

Blue tits could have forced them to leave the nest for the first time earlier than Great tits to forage and replace calories. Nonetheless, the main nestbox type effect was on attentiveness, which was significantly reduced in woodcrete nestboxes. Providing the optimal conditions for the development of the embryo is very costly (Tinbergen and Williams 2002) and females do so behaviourally through attentiveness (DuRant *et al.* 2013). One possible cause of the reduced attentiveness in woodcrete nestboxes is the warmer and wet microclimate in them, because as the ambient temperatures increase, the costs of maintaining the eggs at the optimal temperature decrease and the embryonic growth during recesses from incubation improves (Haftorn and Reinertsen 1990; Ardia *et al.* 2009). Indeed, in both species, attentiveness was negatively affected by ambient temperature. The females in woodcrete nestboxes might therefore have benefitted from lower attentiveness by improving their own self-maintenance without harming their embryos (Chalfoun and Martin 2007).

In conclusion, this study reinforces the fact that the nestbox type used in avian studies or in conservation programmes is not a trivial issue, as other more ample studies have recently highlighted (Lambrechts *et al.* 2010; Møller *et al.* 2014a,b), and could have huge biological effects on the focus species. Nestbox type significantly affected important biological features, such as the occupation and predation rate, the breeding performance, the incubation behaviour and phenotype of both Blue and Great tits nestlings.

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SUPPORTING INFORMATION

Table S1. Models (GLM, GLZ) showing the effects of forest, year, species and nestbox type on the breeding parameters of Blue tits (*Cyanistes caeruleus*) and Great tits (*Parus major*) in the Montes de Toledo, central Spain. Significant results are highlighted in bold.

Parameter	Effect	Estimate ± SE	Test	*P*
Nest height (mm)	Forest		$F_{2, 356} = 1.95$	0.14
	Year		$F_{2, 354} = 1.18$	0.31
	Species		$F_{1, 359} = 25.36$	**< 0.001**
	Nestbox type		$F_{1, 359} = 12.18$	**< 0.001**
	Nestbox type x Species		$F_{1, 358} = 2.59$	0.11
Nest volume (mm^3)	Forest		$F_{2, 356} = 2.60$	0.08
	Year		$F_{2, 354} = 0.79$	0.45
	Species		$F_{1, 358} = 17.26$	**< 0.001**
	Nestbox type		$F_{1, 358} = 40.97$	**< 0.001**
	Nestbox type x Species		$F_{1, 358} = 4.42$	**0.036**
Safe distance (mm)	Forest		$F_{2, 356} = 1.95$	0.14
	Year		$F_{2, 354} = 1.18$	0.31
	Species		$F_{1, 359} = 25.36$	**< 0.001**
	Nestbox type		$F_{1, 359} = 364.52$	**< 0.001**
	Nestbox type x Species		$F_{1, 358} = 2.59$	0.11
Laying date	Forest		$F_{2, 378} = 13.33$	**< 0.001**
	Year		$F_{2, 378} = 36.12$	**< 0.001**
	Species		$F_{1, 377} = 0.56$	0.45
	Nestbox type		$F_{1, 378} = 9.64$	**0.002**
	Nestbox type x Species		$F_{1, 376} = 0.32$	0.57
Clutch size	Forest		$F_{2, 377} = 7.04$	**< 0.001**
	Year		$F_{2, 377} = 10.36$	**< 0.001**
	Species		$F_{1, 377} = 30.49$	**< 0.001**
	Nestbox type		$F_{1, 376} = 1.27$	0.26
	Nestbox type x Species		$F_{1, 375} = 0.01$	0.98
	Laying date	-0.087 ± 0.007	$F_{1, 377} = 191.7$	**< 0.001**

(continuation)	Effect	Estimate ± SE	Test	*P*
Hatching success (%)	Forest		$\chi^2_2 = 1.36$	0.51
	Year		$\chi^2_2 = 25.39$	**< 0.001**
	Species		$\chi^2_1 = 0.24$	0.63
	Nestbox type		$\chi^2_1 = 1.57$	0.21
	Nestbox type x Species		$\chi^2_1 = 3.39$	0.06
	Laying date	0.0005 ± 0.0008	$\chi^2_1 = 0.36$	0.55
Breeding success (%)	Forest		$\chi^2_2 = 10.26$	**0.006**
	Year		$\chi^2_2 = 128.07$	**< 0.001**
	Species		$\chi^2_1 = 1.55$	0.21
	Nestbox type		$\chi^2_1 = 13.08$	**< 0.001**
	Nestbox type x Species		$\chi^2_1 = 0.44$	0.51
	Laying date	-0.001 ± 0.001	$\chi^2_1 = 1.07$	0.30

Table S2. Models (GLM, GLZ) showing the effect of certain independent variables on the incubation performance of Blue tits (*Cyanistes caeruleus*) and Great tits (*Parus major*) in the Montes de Toledo (Toledo, central Spain). Significant results are highlighted in bold

Parameter	Effect	Estimate ± SE	Test	*P*
Onset of activity (min)	Forest		$F_{2, 135} = 0.68$	0.51
	Year		$F_{1, 137} = 4.61$	**0.033**
	Species		$F_{1, 132} = 0.0009$	0.98
	Nestbox type		$F_{1, 134} = 0.11$	0.74
	Nestbox type x Species		$F_{1, 137} = 10.33$	**0.002**
	Incubation day	-0.001 ± 1.207	$F_{1, 131} < 0.001$	1.00
	Mean temperature (night)	-1.014 ± 0.366	$F_{1, 137} = 7.69$	**0.006**
	Daylight	18.85 ± 5.68	$F_{1, 137} = 11.03$	**0.001**
	Clutch size	0.299 ± 0.877	$F_{1, 133} = 0.12$	0.73
End of activity (min)	Forest		$F_{2, 133} = 0.32$	0.72
	Year		$F_{1, 131} = 0.002$	0.96
	Species		$F_{1, 136} = 0.88$	0.35
	Nestbox type		$F_{1, 135} = 0.84$	0.36
	Nestbox type x Species		$F_{1, 137} = 0.55$	0.46
	Incubation day	3.359 ± 1.696	$F_{1, 139} = 3.92$	**0.049**
	Mean temperature (day)	-0.599 ± 0.442	$F_{1, 138} = 1.84$	0.18
	Daylight	-26.92 ± 8.67	$F_{1, 139} = 9.63$	**0.002**
	Clutch size	-0.428 ± 1.379	$F_{1, 132} = 0.10$	0.76
On-bout duration (min)	Forest		$F_{2, 133} = 0.76$	0.47
	Year		$F_{1, 131} = 0.14$	0.71
	Species		$F_{1, 138} = 29.52$	**< 0.001**
	Nestbox type		$F_{1, 136} = 2.37$	0.12
	Nestbox type x Species		$F_{1, 138} = 4.71$	**0.032**
	Incubation day	0.559 ± 0.446	$F_{1, 135} = 1.57$	0.21
	Mean temperature (day)	-0.041 ± 0.129	$F_{1, 132} = 0.10$	0.75
	Daylight	6.686 ± 2.321	$F_{1, 138} = 8.30$	**0.005**
	Clutch size	0.643 ± 0.346	$F_{1, 137} = 3.44$	0.07
Off-bout duration (min)	Forest		$F_{2, 133} = 0.33$	0.72
	Year		$F_{1, 138} = 3.79$	0.054
	Species		$F_{1, 139} = 79.23$	**< 0.001**
	Nestbox type		$F_{1, 136} = 0.64$	0.42
	Nestbox type x Species		$F_{1, 135} = 0.72$	0.40
	Incubation day	0.151 ± 0.149	$F_{1, 137} = 1.03$	0.31
	Mean temperature (day)	0.196 ± 0.037	$F_{1, 139} = 28.38$	**< 0.001**
	Daylight	0.034 ± 0.867	$F_{1, 131} = 0.001$	0.97
	Clutch size	0.032 ± 0.111	$F_{1, 132} = 0.08$	0.78

(continuation)	Effect	Estimate ± SE	Test	*P*
Number of off-bouts	Forest		$F_{2, 133} = 0.59$	0.55
	Year		$F_{1, 135} = 0.63$	0.43
	Species		$F_{1, 138} = 38.18$	< **0.001**
	Nestbox type		$F_{1, 136} = 0.37$	0.55
	Nestbox type x Species		$F_{1, 138} = 3.92$	**0.049**
	Incubation day	0.052 ± 0.364	$F_{1, 131} = 0.02$	0.89
	Mean temperature (day)	-0.017 ± 0.010	$F_{1, 132} = 0.03$	0.86
	Daylight	-3.714 ± 1.685	$F_{1, 138} = 4.86$	**0.029**
	Clutch size	-0.213 ± 0.254	$F_{1, 137} = 0.70$	0.40
Attentiveness (%)	Forest		χ 22 = 6.61	**0.037**
	Year		χ 21 = 5.32	**0.021**
	Species		χ 21 = 8.23	**0.004**
	Nestbox type		χ 21 = 28.88	< **0.001**
	Nestbox type x Species		χ 22 = 16.13	< **0.001**
	Incubation day	0.001 ± 0.002	χ 21 = 0.54	0.46
	Mean temperature (day)	-0.006 ± 0.0004	χ 21 = 247.78	< **0.001**
	Daylight	1.584 ± 0.528	χ 21 = 9.03	**0.003**
	Squared daylight	-0.052 ± 0.018	χ 21 = 8.31	**0.004**
	Clutch size	0.003 ± 0.001	χ 21 = 6.99	**0.008**
Egg Cooling (ºC min-1)	Forest		F2, 134 = 1.12	0.33
	Year		F1, 130 = 0.04	0.84
	Species		F1, 136 = 1.46	0.23
	Nestbox type		F1, 137 = 4.10	**0.045**
	Nestbox type x Species		F1, 132 = 0.51	0.47
	Incubation day	0.061 ± 0.088	F1, 133 = 0.48	0.49
	Mean temperature (day)	0.016 ± 0.024	F1, 131 = 0.45	0.50
	On-bout duration	0.057 ± 0.016	F1, 137 = 12.98	< **0.001**
	Species x On-bout duration		F1, 137 = 23.71	< **0.001**
	Clutch size	0.289 ± 0.062	F1, 137 = 21.35	< **0.001**

Table S3. Models (GLM) with female and male tarsus length and body condition as response variables in relation to several explanatory variables of Blue tits (*Cyanistes caeruleus*) and Great tits (*Parus major*) breeding in Montes de Toledo, central Spain. Significant results are highlighted in bold.

Parameter	Effect	Estimate ± SE	Test	*P*
Female tarsus length (mm)	Forest		$F_{2, 192} = 5.50$	**0.005**
	Year		$F_{2, 187} = 0.14$	0.87
	Species		$F_{1, 192} = 1535.9$	**< 0.001**
	Nestbox type		$F_{1, 189} = 0.03$	0.86
	Nestbox type x Species		$F_{1, 186} = 0.01$	0.94
	Laying date	-0.001 ± 0.003	$F_{1, 190} = 0.13$	0.71
	Brood size	-0.016 ± 0.013	$F_{1, 191} = 1.46$	0.23
Female body condition (g)	Forest		$F_{2, 185} = 1.68$	0.19
	Year		$F_{2, 187} = 17.22$	**< 0.001**
	Species		$F_{1, 187} = 325.17$	**< 0.001**
	Nestbox type		$F_{1, 182} = 0.35$	0.56
	Nestbox type x Species		$F_{1, 183} = 0.34$	0.56
	Laying date	-0.015 ± 0.004	$F_{1, 187} = 14.66$	**< 0.001**
	Brood size	-0.015 ± 0.020	$F_{1, 184} = 0.55$	0.46
	Female tarsus length	0.278 ± 0.096	$F_{1, 187} = 8.34$	**0.004**
Male tarsus length (mm)	Forest		$F_{2, 152} = 6.70$	**0.002**
	Year		$F_{2, 148} = 0.50$	0.60
	Species		$F_{1, 152} = 1352.3$	**< 0.001**
	Nestbox type		$F_{1, 150} = 0.62$	0.43
	Nestbox type x Species		$F_{1, 147} = 0.20$	0.66
	Laying date	-0.004 ± 0.004	$F_{1, 151} = 1.20$	0.28
	Brood size	-0.008 ± 0.020	$F_{1, 146} = 0.19$	0.66
Male body condition (g)	Forest		$F_{2, 145} = 0.72$	0.49
	Year		$F_{2, 149} = 10.33$	**< 0.001**
	Species		$F_{1, 149} = 2839.8$	**< 0.001**
	Nestbox type		$F_{1, 147} = 0.44$	0.51
	Nestbox type x Species		$F_{1, 144} = 0.12$	0.73
	Laying date	-0.011 ± 0.005	$F_{1, 149} = 4.78$	**0.03**
	Brood size	-0.0001 ± 0.026	$F_{1, 143} < 0.0001$	1.00
	Male tarsus length	0.152 ± 0.102	$F_{1, 148} = 2.20$	0.14

Table S4. Models (GLM) containing nestling morphological measurements (tarsus length, body condition and wing length) at day 13 after hatching as response variables in relation to several explanatory variables of Blue tits (*Cyanistes caeruleus*) and Great tits (*Parus major*) breeding in the Montes de Toledo, central Spain. Significant results are highlighted in bold.

Parameter	Effect	Estimate ± SE	Test	*P*
Nestling tarsus length (mm)	Forest		$F_{2,225} = 1.63$	0.20
	Year		$F_{2,223} = 1.21$	0.30
	Species		$F_{1,227} = 3132.0$	**< 0.001**
	Nestbox type		$F_{1,222} = 0.68$	0.41
	Nestbox type x Species		$F_{1,221} = 0.05$	0.83
	Laying date	-0.006 ± 0.002	$F_{1,227} = 5.80$	**0.017**
	Brood size	0.034 ± 0.013	$F_{1,227} = 6.80$	**0.01**
Nestling body condition (g)	Forest		$F_{2,221} = 0.06$	0.94
	Year		$F_{2,225} = 5.35$	**0.005**
	Species		$F_{1,225} = 149.95$	**< 0.001**
	Nestbox type		$F_{1,224} = 1.79$	0.18
	Nestbox type x Species		$F_{1,220} = 0.002$	0.96
	Laying date	-0.011 ± 0.003	$F_{1,225} = 11.90$	**< 0.001**
	Brood size	-0.008 ± 0.018	$F_{1,223} = 0.18$	0.67
	Nestling tarsus length	0.817 ± 0.090	$F_{1,225} = 83.18$	**< 0.001**
Nestling wing length (mm)	Forest		$F_{2,225} = 11.72$	**< 0.001**
	Year		$F_{2,222} = 0.36$	0.70
	Species		$F_{1,225} = 467.01$	**< 0.001**
	Nestbox type		$F_{1,225} = 4.11$	**0.044**
	Nestbox type x Species		$F_{1,224} = 0.40$	0.52
	Laying date	-0.032 ± 0.014	$F_{1,225} = 4.87$	**0.028**
	Brood size	0.184 ± 0.078	$F_{1,225} = 5.46$	**0.02**

CAPÍTULO III

TRANSMISIÓN CULTURAL Y SU POSIBLE EFECTO EN LA ADAPTACIÓN ACÚSTICA URBANA DEL CARBONERO COMÚN (Parus major)

Bueno-Enciso J, Núñez-Escribano D y Sanz JJ. (2015) Cultural transmission and its possible effect on urban acoustic adaptation of the Great tit *Parus major*. *Animal Biodiversity and Conservation* 38: 221–231.

Abstract

Urban Great tits (*Parus major*) sing with a higher minimum frequency than their forest conspecifics. Cultural processes may account at least in part for the song divergence in city birds as Great tits learn their repertoire from conspecifics and switch to high pitch song types in presence of background noise. However, in small cities, this process of cultural divergence could be constrained because it is likely that these birds have a greater exchange of song types with the outside. We tested this prediction by recording Great tit songs in a small city (Toledo, central Spain) and in a nearby forest. We found that background noise and the peak and the maximum frequency of songs were higher in the city but the minimum frequency did not differ. The pause length was also longer in forest birds. Seventy percent of the song types were shared between Toledo and the nearby forest. These results suggest that the small size of Toledo allows a homogenized cultural wealth, preventing the development of a high pitch song as observed in larger cities.

Introduction

Like many other urban bird species, urban Great tits, *Parus major*, sing with a higher minimum frequency than their forest conspecifics (Slabbekoorn and den Von-Bisser 2006) (see Table 1), highlighting the role of environmental conditions on sound production (i.e. Figure 1 in Laiolo 2010). Urbanization produces extreme novel habitat conditions for animals in and also near cities (Warren 2006). Although urban and rural habitats differ in many aspects that could influence animal acoustic communication (Shochat *et al.* 2006), the loud low-frequency background noise in cities (hereafter referred to as 'anthropogenic noise') has shown to be directly related to the elicitation of high minimum frequency city songs (Slabbekoorn 2013).

The mechanism underlying this spectral shift in urban birds remains unclear (Nemeth *et al.* 2012; Slabbekoorn *et al.* 2012). It has been hypothesized that urban birds increase the minimum frequency of their songs to avoid overlap

Table 1. Summary of the studies that assess the effect of the anthropogenic noise in the minimum frequency of songbirds. All shifts are toward high frequencies in urban songs in relation to forest songs. The type of study is O: observational, E: experimental or both: O / E.

Species	Min. Freq. shift	Type of study	References
Parus major			
Great tit	Yes	O / E	1, 3, 6, 7, 13, 16
Carpodacus mexicanus			
House finch	Yes	O / E	2, 4, 14
Colluricincla harmonica			
Grey Shrike-thrush	Yes	O	5
Rhipidura fuliginosa			
Grey Fantail	No	O	5
Turdus merula			
Common blackbird	Yes	O / E	8, 9, 11, 12, 20
Emberiza schoeniclus			
Reed bunting	Yes	E	10
Trichoglossus haematodus			
Rainbow lorikeet	Yes	O	11
Platycercus eximius			
Eastern rosella	Yes	O	11
Anthochaera carunculata			
Red wattlebird	Yes	O	11
Manorina melanocephala			
Noisy miner	No	O	11
Manorina melanophrys			
Bell miner	Yes	O	11
Strepera graculina			
Pied currawong	No	O	11
Gymnorhina tibicen			
Australian magpie	No	O	11
Cracticus torquatus			
Grey butcherbird	No	O	11
Grallina cyanoleuca			
Magpie-lark	No	O	11
Rhipidura leucophrys			
Willie wagtail	No	O	11
Acridotheres tristis			
Common myna	No	O	11
Agelaius phoeniceus			
Red-winged blackbirds	Yes	O / E	15
Zosterops lateralis			
Silvereyes	Yes	O/E	17, 19, 21
Erithacus rubecula			
European robin	Yes	O / E	18

References: (1) Slabbekoorn y Peet 2003; (2) Fernández-Juricic *et al.* 2005; (3) Slabbekoorn y der Boer-Visser 2006; (4) Bermúdez-Cuamatzin *et al.* 2009; (5) Parris y Schneider 2009; (6) Halfwerk y Slabbekoorn 2009; (7) Mockford y Marshall 2009; (8) Nemeth y Brumm 2009; (9) Ripmeester *et al.* 2010; (10) Gross *et al.* 2010; (11) Hu y Cardoso 2010; (12) Mendes *et al.* 2010; (13) Salaberria y Gil 2010; (14) Bermúdez-Cuamatzin *et al.* 2011; (15) Hanna *et al.* 2011; (16) Hamao *et al.* 2011; (17) Potvin *et al.* 2011; (18) Montague *et al.* 2012; (19) Potvin y Parrish 2012; (20) Nemeth *et al.* 2013; (21) Potvin y Mulder 2013.

with anthropogenic noise. This acoustic shift could be a microevolutionary response or a short-term response (Brumm 2006; Patricelly and Blickley 2006; Slabbekoorn and Ripmeester 2008). This is an important topic, because if the acoustic shift is a microevolutionary response, and because frequency features of the song may be under sexual selection (Halfwerk *et al.* 2011; Halfwerk *et al.* 2012; Huet des Aunay *et al.* 2013; but see Eens *et al.* 2012), the spectral shift would entail genetic inheritance and could promote speciation through reproduction isolation (Slabbekoorn and Smith 2002; Slabbekoorn and Reepmester 2008). However, to be selected, the urban acoustic shift should imply fitness benefits to the singer, but empirical data is lacking (Nemeth *et al.* 2012; Halfwerk and Slabbekoorn 2013; but see Slabbekoorn 2013). Experimental procedures have shown that birds can adjust their acoustic signals as a short-term response to increasing noise levels due to vocal plasticity. These plastic adjustments could be mediated by an active frequency shift (Gross *et al.* 2010; Bermúdez-Cuamatzin *et al.* 2011; Hanna *et al.* 2011; Potvin and Mulder 2013), by switching song types (Cardoso and Atwell 2011a; Halfwerk and Slabekoorn 2009), by increasing the duration of the vocalizations (Montague *et al.* 2012; Potvin and Mulder 2013), or by increasing the amplitude of the songs (Brumm 2004; Shuster *et al.* 2012). These plastic adjustments are not mutually exclusive and two or more may operate concurrently or vary among species (Slabbekoorn *et al.* 2012). Such phenotypic plasticity would impede habitat-dependent selection and therefore speciation (Baker 2006)

Great tits are a good model to study this topic because they are a successful urban songbird and one of the best-studied species in terms of acoustics (Slabbekoorn 2013). It is a closed-ended learning species (Rivera-Gutierrez *et al.* 2011) that achieves a discrete, crystalized repertoire of songs learned from parents and neighbours (McGregor and Krebs 1982; Franco and Slabbekoorn 2009). Thus, the different song types sung by each great tit can be considered cultural traits, referred to hereafter as ‘memes’ (Baker *et al.* 2001; Baker and Gammon 2008; Cardoso and Atwell 2011a) and the learning process in this species is the basis of their ‘cultural transmission’ (Slabbekoorn 2013). Among the plastic

adjustments cited earlier, Great tits have been seen to actively switch to a different meme that transmits better in presence of anthropogenic noise (Halfwerk and Slabekoorn 2009; Slabbekoorn 2013). Thus, cultural evolution (i.e. changes in the expression frequencies of the meme pool over time) may play an important role in city-forest song divergence in this species (Cardoso and Atwell 2011a; Slabbekoorn 2013). Indeed, the process of cultural transmission has led to the establishment of population-specific repertories or ‘dialects’, separated by either geographical distance or barriers that prevent dispersal (O´Loghlen *et al.* 2011; Potvin and Parris 2012). The mechanisms behind this cultural diversity can be classified into two broad categories: (i) stochastic factors such as meme mutation, drift or immigration, and (ii) selective pressures that favour directional changes in the meme pool frequencies such as choice of female, morphological adaptations (e.g., bill/body size), and environmental conditions that affect sound production (Lynch, 1996; Cardoso and Atwell 2011a; O´Loghlen *et al.* 2011; Xing *et al.* 2013). In relation to the latter, and because the anthropogenic noise in cities mask birdsongs (Slabbekoorn and Peet 2003), acoustic signals can reach the receivers degraded in cities and consequently do not fulfil its biological function of communication (Brumm and Naguib 2009). The risk of losing the acoustic message may lead birds to try to match their song with the acoustic properties of the environment to enhance transmission as proposed by the Acoustic Adaptation Hypothesis (AAH) (Morton 1975). The way Great tits seem to do this is by switching memes to a higher minimum frequency or singing them for longer periods (Halfwerk and Slabekoorn 2009). This acoustic shaping to the environment influences cultural transmission and therefore cultural evolution as birds copy undegraded songs differentially during their sensitive period of learning (Peters *et al.* 2012). Thus, under anthropogenic noise, only those memes from the whole repertoire that escape from masking or transmit and reach the receiver more clearly will be correctly and frequently copied (Slabbekoorn 2013). Over time, this may lead to changes in the expression frequencies of the memes sung in a city, increasing those that transmit better under anthropogenic noise, i.e. those with a high minimum frequency (Luther and Baptista

2010) because of their better acquisition by young birds during their sensitive period. This cultural evolution may be responsible, at least in part, for the city-forest song divergence found in Great tits. Over time, it could be culturally and genetically fixed (Price *et al.* 2003; Slabbekoorn 2013). Furthermore, such phenotypic plasticity allows acoustic shifts in frequencies and may account, to some degree, in urban song divergence (Nemeth *et al.* 2012).

The aim of the present study was to compare the typological and spectral song characteristics (Baker 2006) in two Great tit populations, one in a small city, Toledo (central Spain), surrounded by a large rural and forest area, and the other in a nearby forest. The small size of Toledo distinguishes this study from the former studies performed in large cities (i.e. Slabbekoorn and den Von-Bisser 2006; Hamao *et al.* 2010 or Salaberria and Gil 2010). If cultural evolution is responsible, at least in part, for the city song divergence found in this species, the fact that Toledo Great tits may still be learning ‘forest memes’ (i.e. with relative low minimum frequency) from surrounding rural and forest areas may constrain the plastic cultural response of choosing ‘urban memes’ (i.e. with high minimum frequency) from their repertories; as many crystalized memes in their repertories will be those learned from outside of the city. This possible high exchange of memes between Toledo and forest may allow a cultural convergence. We predicted that the differences in the minimum frequency and the typological song characteristics (i.e. percentage of meme type used) between Toledo and the forest would be less marked than those described in previous studies in city-forest pairs in large cities. This comparison of the song features between a relatively small city and the nearby forest could help us to understand the mechanisms involving urban song divergence in Great tits.

Methods

Study areas

Great tit songs were recorded in two, well-differentiated habitats: city (Toledo) and forest (Montes de Toledo). Toledo is a relatively small city (17 Km^2) located 71 km south of Madrid (central Spain, 529 m above sea level), with a population of 80,000 inhabitants. Forest songs were recorded in two nearby areas

of the Toledo mountains (Montes de Toledo, Toledo province): Quintos de Mora (39º 25’N, 4º 04’W) and San Pablo de los Montes (39º 31’N, 4º 21’W), located at 80 and 60 km south of Toledo, respectively (average elevation of both forest areas is 908 m a.s.l.; see Ferrer *et al.* 2012). Both forest areas comprise deciduous forests dominated by Pyrenean Oak *Quercus pyrenaica*, usually relegated to the shady area of the mountains and ravine funds, accompanied by Mediterranean scrublands. Both study areas have a continental Mediterranean climate, with mean values of annual rainfall of 350-450 mm for Toledo and 700-800 mm for the forests, concentrated in the months of autumn and spring. Summer drought and daily thermal oscillation are marked in the whole area.

Song recording

We recorded Great tit songs between March 5 and April 27 in 2011. Day 1 = 1 March. All song recordings were made between 0900 and 1500 hours. To ensure the independence of our samples, we only recorded lone individuals located at least 100 m apart, a distance greater than that considered to be the territory size in this species (Naef-Daenzer and Keller 1999). Urban Great tits began to sing 22 days earlier than their forest conspecifics, possibly due to the “heat island” effect of cities (Shochat *et al.* 2006) and food availability (Isaksson and Andersson 2007). To correct the differences in the breeding cycle advancement between forest and urban Great tits, we considered the day when the first male was heard singing in each study area as the first day. We recorded 56 songs from 37 different Great tit males in Toledo. Four males were recorded three times, eleven males were recorded two times, and the remaining males were recorded once. We recorded 106 songs from 63 males in the forest area; three males were recorded four times, nine males were recorded three times, and sixteen males were recorded two times.

All songs were recorded using an EDIROL R-09HR digital recorder equipped with a Sennheiser unidirectional microphone and headphones, pointing directly toward the singing individual. Individuals were recorded at a distance of between 10 and 15 m and at least 10 strophes per song were recorded. After each recording, background noise data (dB) were measured every second for 5

minutes with a multidirectional sound level meter (SLM; A-weighted, reference level 20 µPa) positioned in a fixed location (1.5 m above the ground) with the aid of a tripod. As a measure of background noise, we used the mean value over this period.

Song analysis

We randomly selected one song recording for each male to analyse the same number of songs for each male. Audio tracks were first converted from stereo to mono with the Audacity 1.3.14-beta program. Five strophes of the song were then selected and exported to the program RavenPro 1.4 (Charif *et al.* 2010), where, before analysis, a band filter was used to remove the typical low frequency background noise after visual inspection of the spectrogram to prevent the unintentional removal of any song element. For spectral description (Baker 2006), we measured: average minimum, maximum and peak frequency (Hz), frequency bandwidth (difference between the maximum and the minimum frequency of the song, Hz), mean strophe length, mean pause length and average number of notes per phrase (no. of notes). Peak frequency was measured automatically; the other variables were measured manually using a Hann window and a fast Fourier transformation (FFT), length of 1024, resulting in a spectral resolution of 43.1 Hz. Minimum and maximum frequencies were measured by precisely placing a selection box in the spectrogram view, similar to Francis *et al.* (2011). Because this manual methodology can entail a bias in the frequency measure of birdsongs (Zollinger *et al.* 2012), we tested the reliability of our measures by reanalysing a subset of song recordings (15 belonging to forest sites and 15 from Toledo) with a power spectra using Avisoft SAS Lab Pro 4.15 (Avisoft Bioacoustics, Schönfließer Str. 83, 16548 Glienicke, Germany). Concretely, we recalculated the minimum and maximum frequency of this subset of song recordings by subtracting 20 dB from the peak amplitude value in the power spectrum. Neither minimum nor maximum frequency differed significantly between the two methodologies used (Student t-test: t= 0.01; d.f.= 58; p= 0.99; manually= 3397.67 ± 66.26 and automatically= 3398.34 ± 71.54; Student t-test: t= -0.51; d.f.= 58; p= 0.61; manually= 5571.48 ± 136.95 and automatically= 5459.90 ±

172.25, for the minimum and the maximum frequency respectively). We also calculated the song-rate (relation between the total number of phrases recorded and the total length of the strophe). For the typological level of description (Baker 2006), we noted the note type following the criteria of McGregor and Krebs (1982) by visual inspection of the sound spectrograms, and we classified each meme-type depending on the number of notes, type, and order in the phrase. All song analyses were performed by DNE and JBE. Meme-type classifications of both observers were compared to obtain a single classification.

Statistical analyses

We analysed the acoustic habitat-dependent divergences between study areas using General Linear Models (GLMs), with the spectral song characteristics as dependent variables and study area as a categorical predictor with two levels (Toledo and forest). Standardized date was incorporated in all models as a covariate. The interaction between study area and date was also included in the full models. Number of notes was analysed using a Generalized Linear Model (GLZ) with a poisson distribution. For post hoc analyses, the Tukey HSD test was used. Differential note type used between Toledo and Forest was analysed using other GLZs with a binomial distribution. In this case, the response variable was the ratio between the 'number of one note type used' (numerator) divided by the 'total number of note types used' (denominator) (Zuur *et al.* 2009), with the study area as categorical predictor and the standardized date as the covariate. The interaction between study area and date was also included in the full models. In the present study, when the interaction between study area and date was not significant, it was eliminated from the final presented models. All these analyses were performed with R (R Core Team, 2014) and the function "glm" of the package lme4 (Bates *et al.*, 2004). Assumptions of the homoscedasticity, proper distribution used and independence were verified graphically with the residuals of the model, following the recommendations of Zuur *et al.* 2010.

Results

Table 2 shows mean ± SE values of background noise and spectral song

characteristics of Great tits recorded in the study areas. Some of the spectral song variables showed significant differences between areas, with the exception of song-rate, strophe length and minimum frequency (Table 3). Neither spectral song variable varied significantly with the date (Table 3).

In relation to the typological level of description, a total of 16 memes were recorded in the two study areas: 13 in Toledo, 2 of which (15%) were exclusive, and 14 in the forest, 3 of which (21%) were exclusive. Eleven of the 13 memes were common to both areas (70% of the memes recorded), but only three o were sufficiently sampled to make comparisons. The remaining 8 memes were recorded for only one or two males in each study area and therefore not considered suitable for comparison. These shared memes were “two-note type” memes and they were named arbitrarily as meme “A” (composed of the notes ‘a’ and ‘b’, see below), shared by 7 males in Toledo and by 11 in the forest, meme “B” (composed of the notes ‘a’ and ‘c’) shared by 3 males in Toledo and 31 in the forest, and meme “C” (composed of the notes ‘b’ and ‘c’) shared by 7 males in both areas (see Figure 1). Apart from presenting a shorter pause length and a quicker song-rate in Toledo than in the forest (GLM: $F_{1,\,64} = 5.89$; p = 0.02 and $F_{1,\,64} = 5.49$; p = 0.02, respectively), these

Table 2. Mean ± SE (n) and range of the spectral song characteristics of great tit (Parus major) male songs in Toledo and in a nearby Forest.

	Toledo		Forest	
	Mean ± SE (37)	Range	Mean ± SE (63)	Range
Background noise (dB)	54.13 ± 0.84	45.10 - 63.66	38.97 ± 0.55	30.89 - 48.90
Nº Notes	2.27 ± 0.10	0.20 - 0.40	2.19 ± 0.07	0.20 - 0.40
Song-rate (St/s)	0.19 ± 0.03	0.03 - 0.37	0.15 ± 0.02	0.01 - 0.35
Pause length (s)	3.14 ± 0.38	1.30 - 6.80	4.98 ± 0.35	1.02 - 12.51
Strophe length (s)	2.83 ± 0.26	1.61 - 8.03	2.67 ± 0.14	1.26 - 5.64
Min. Freq. (Hz)	3389.27 ± 82.41	2495.1 - 4179.6	3256.19 ± 45.29	2288.1 - 3978.1
Peak Freq. (Hz)	4413.24 ± 109.13	3375.0 - 5564.6	4198.82 ± 62.17	3140.6 - 4952.4
Max. Freq. (Hz)	5813.36 ± 146.16	4570.3 - 7944.9	5215 ± 89.78	4318.4 - 7244.3
Bandwidth (Hz)	2483.35 ± 124.99	1275.6 - 4878.5	1925.81 ± 78.77	1039.1 - 3732.8

shared memes did not differ in minimum frequency (GLM: $F_{1,\ 64} = 2.96$; $p = 0.09$) or in peak frequency (GLM: $F_{1,\ 64} = 2.56$; $p = 0.11$); only the maximum frequency differed, being higher in Toledo than in the forest (GLM: $F_{1,\ 64} = 19.82$; $p < 0.001$; Toledo= 5483 ± 117 Hz and Forest= 4956 ± 57 Hz). The difference in maximum frequency of these three shared memes between study areas (527 Hz) was slightly smaller than the difference in maximum frequency when all memes were taken into account (598 Hz, see Table 2). Thus, these three shared memes account for 88% of the magnitude of the overall maximum frequency divergence between the Toledo-Forest pair.

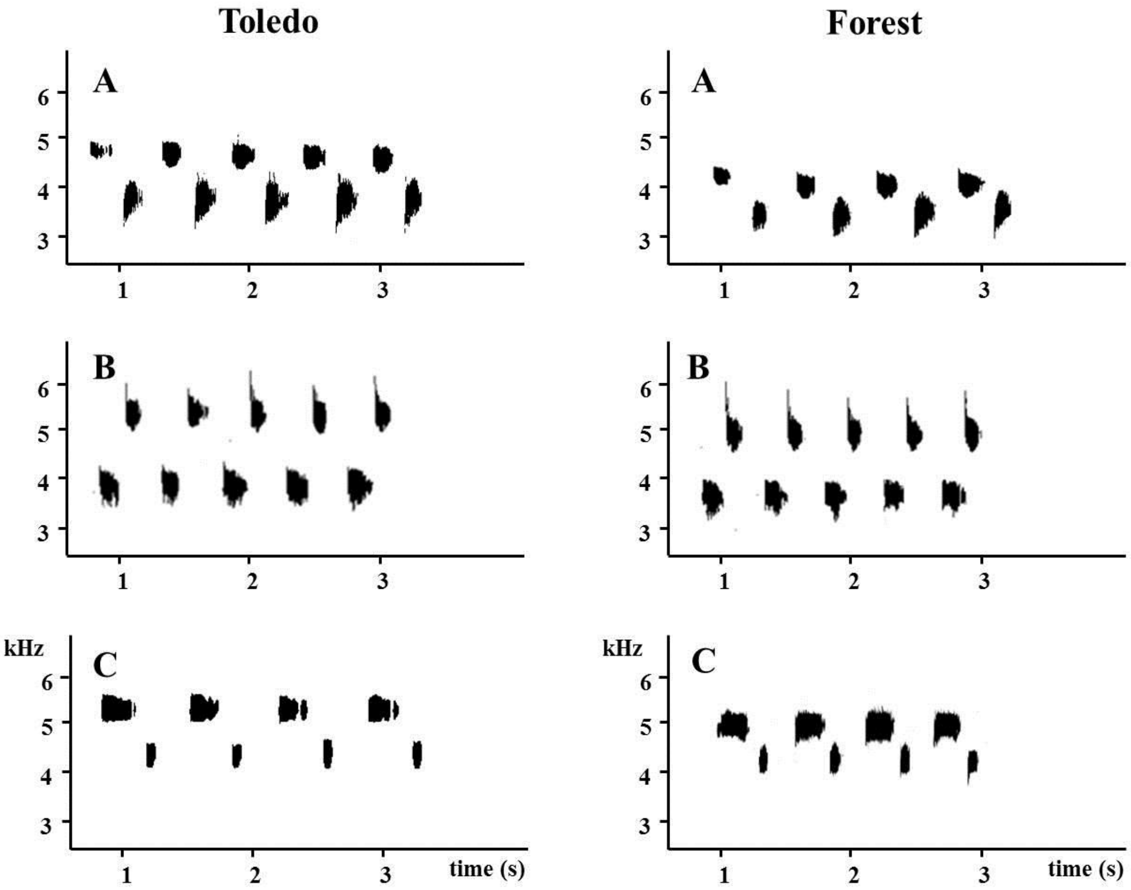

Figure 1. Sonograms of the shared memes most frequently used Toledo and Forest. Each level in the figure represents the same meme elicited in Toledo and Forest (A, B or C).

Table 2. Mean ± SE (n) and range of the spectral song characteristics of great tit (Parus major) male songs in Toledo and in a nearby Forest.

	Test Factor	*P*	Estimate	Test Covariate	*P*
Background noise (dB)	$F_{1, 98}$= 283.7	**<0.001**	0.02	$F_{1, 97}$= 0.16	0.69
Nº Notes	$Z_{1, 98}$= 0.48	0.57	0.001	$Z_{1, 97}$= 5.88	0.89
Song-rate (St/s)	$F_{1, 98}$= 3.16	0.08	<0.001	$F_{1, 97}$= 0.38	0.54
Pause length (s)	$F_{1, 98}$= 9.23	**0.003**	-0.003	$F_{1, 97}$= 0.89	0.35
Strophe length (s)	$F_{1, 98}$= 0.78	0.40	0.014	$F_{1, 97}$= 0.73	0.40
Min. Freq. (Hz)	$F_{1, 98}$= 1.13	0.29	2.86	$F_{1, 97}$= 0.29	0.59
Peak Freq. (Hz)	$F_{1, 98}$= 7.81	**0.006**	-8.42	$F_{1, 97}$= 1.36	0.25
Max. Freq. (Hz)	$F_{1, 96}$= 17.32	**<0.001**	35.3	$F_{1, 96}$= 1.67	0.20
Bandwidth (Hz)	$F_{1, 96}$= 15.39	**<0.001**	30.84	$F_{1, 96}$= 1.30	0.26

The 16 memes observed in Great tit songs were made up of a combination of 11 different note types (Table 4). To test city-forest differences, we used note types that represent at least 10% of the total (Table 3). Three note types were therefore considered and they were named arbitrarily as: ‘a’ (Peak frequency = 3500 Hz), ‘b’ (Peak frequency = 4500 Hz) and ‘c’ (Peak frequency = 5500 Hz).

Table 4. Percentage of occurrence (%) of the 11 different note types used by Great tits in the two study areas.

Note type	Toledo (%)	Forest (%)
a	18.44	38.66
b	36.18	35.53
c	29.4	17.75
d	3.11	3.62
e	1.31	0.82
f	2.68	0.79
g	0	0.81
h	1.8	0
i	3.97	0
j	0	2.01
k	3.11	0

The use of note type ‘a’ and ‘c’ differed significantly between Toledo and forest (GLZ. Note type ‘a’: $Z_{1, 98} = -13.77$; $p < 0.001$ and Note type ‘c’: $Z_{1, 98} = 12.91$; $p < 0.001$), while the use of note type ‘b’ did not differ between study areas (GLZ. Note type ‘b’: $Z_{1, 98} = 0.65$; $p = 0.52$). Note type ‘a’ was less frequently used in Toledo than in forest (Tukey test, $p < 0.001$; Figure 2a), contrary to note type ‘c’ used, which was significantly more frequently used in Toledo (Tukey test, $p < 0.01$; Figure 2c).

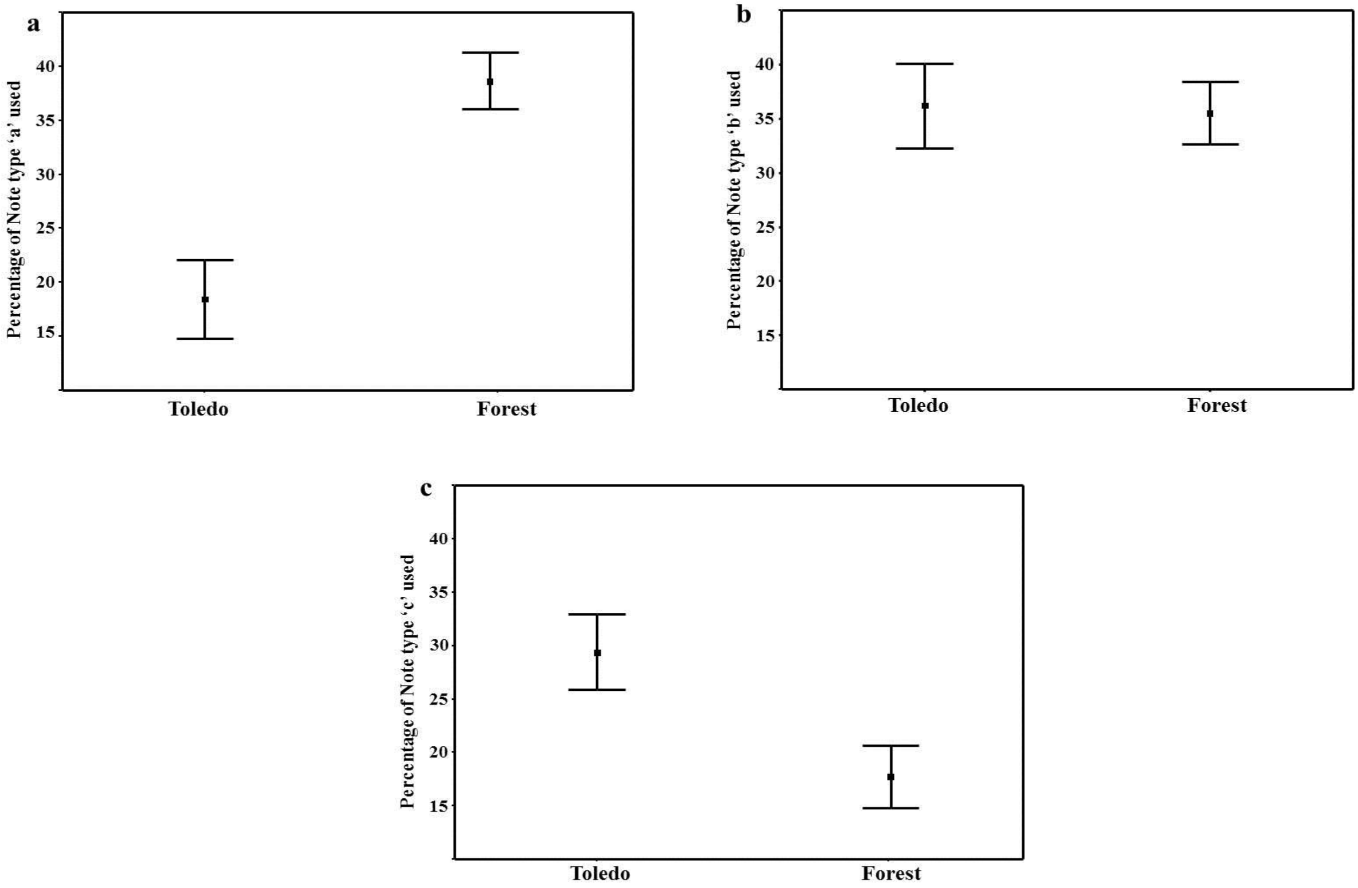

Figure 2. Percentage of note type used by Great tits in Toledo and Forest. Vertical bars indicate SE.

Discussion

Great tits started singing before in Toledo than in Forest, this advancement in the phenology of urban Great tits has also been reported in other city-forest pair comparisons (Partecke and Gwinner 2007; Chamberlain *et al.* 2009). Analysis of song recordings revealed differences in the spectral song characteristics between city and forest birds, probably due to the constraints of background noise in the city, as observed in many other studies (reviewed in Laiolo 2010). The differences observed include the temporal features of the song, such as shorter pauses in Toledo, which help to create a contrast with the background noise and enhance the detectability of the signal (Warren *et al.* 2006; Hanna *et al.* 2011).

Beyond this temporal feature of the song, the main difference between city and forest songs in other city-forest pair studies, is the shift of the minimum frequency toward high frequencies (Table 1). Surprisingly, the minimum frequency of Great tit songs did not differ between Toledo and forest, but the maximum frequency did so significantly, making bandwidth in Toledo wider. The peak frequency was also significantly higher in Toledo. This is in contrast with the strategy of urban Great tits reported previously, which were shown to increase the minimum frequency of their songs (Slabbekoorn and den Boer-Visser 2006; Mockford and Marshall 2009; Montague *et al.* 2012) even in a very close urban Great tit population (Salaberria and Gil 2010), a mechanism that has also been reported in other urban bird species (reviewed in Brumm and Zollinger 2011; Table 1). In our study, the same memes sung in Toledo and forest showed the same frequency shift in the peak and maximum frequency mentioned earlier (Figure 1), suggesting that the singing strategy in Toledo males is a short-term phenotypic response to increase the SNR in presence of anthropogenic noise (Halfwerk *et al.* 2011). This is also seen in other species (Gross *et al.* 2010; Verzijden *et al.* 2010; Potvin and Mulder 2013). This variation in Toledo songs could be a consequence of an active frequency shift (McMullen *et al.* 2014), possibly because these frequency features of the Great tit songs (peak and maximum frequencies) can be more plastically modulated; or it could be a side effect of louder singing, i.e. the Lombard effect (Zollinger and Brumm

2011). It has been shown that the Lombard effect increases the bandwidth of the signal and, to a lesser extent, peak frequency. Changes in minimum frequency, in contrast, are highly independent of the Lombard effect (Cardoso and Atwell 2011b). However, we could not confirm this mechanism as we did not accurately measure song amplitude (Brumm 2004). Both active frequency shift and increased amplitude are short-term plastic adjustments that improve detection and increase the SNR in presence of anthropogenic noise (Nemeth and Brumm 2010; Halfwerk *et al.* 2011).

The low minimum frequency found in Toledo Great tit songs, however, is a surprising result and the main ‘urban song difference’ reported in this study (Table 1). This lack of significant difference in the minimum frequency between Toledo and forest could be a side effect of the small metropolitan area of Toledo and the process of cultural transmission (O´Loghlen *et al.* 2011; Potvin and Parris 2012). Anthropogenic noise might influence cultural transmission by favouring songs that propagate better in the acoustic environment and reach the receiver more clearly (Luther and Baptista 2010), particularly when birds differentially copy undegraded songs in their learning process (Peters *et al.* 2012). Thus, a phenotypic plastic response like sing with a higher minimum frequency in cities (Slabbekoorn and Peet 2003), can be culturally fixed in a scenario where most of the population is under the selection pressure (Price *et al.* 2003). This is also propitiated because high minimum frequency songs transmit better and reach the receiver neatly (Huet de Anuay *et al.* 2013; Potvin and Mulder 2013) and Great tits choose these high memes from their repertoire and sung them for longer duration (Halfwerk and Slabbekoorn 2009). In big cities, with a surface large enough to harbour an almost isolated resident population during all its life span, this scenario can be given. It can therefore be expected that within a few generations, isolated populations present culturally different sets of memes as the result of differences in background noise. This has been observed in San Francisco (California) with the White-crowned sparrow *Zonotrichia leucophrys* (Luther and Baptista 2010) and seems to be occurring in Europe with the Great tit, where a particular set of ‘urban memes’

are sung in cities (Slabbekoorn and Den Boer-Visser 2006). However, the small metropolitan area of Toledo may allow a higher meme exchange between Great tits in Toledo and those in rural and forest areas outside the city, as the high ratio of shared memes suggests; 70% in contraposition with the low ratio of shared memes in other city-forest pair studies (17% in Slabbekoorn and den Boer-Visser 2006 or 8% in Cardoso and Atwell 2011a). In this scenario, Toledo chicks would learn both types of memes: the low minimum frequency memes from outside the city and the high minimum frequency memes developed in the city. This may lead to a homogenized cultural wealth between Toledo and forest, constraining the development of a particular cultural song tradition in Toledo. However, a long-term study marking individuals in Toledo and the surrounding rural areas would be necessary to assess dispersal between these populations.

Although Toledo and forest share 70% of their cultural wealth, a differential note type was used in both study areas (Figure 2). The proportional use of 'high-frequency notes' was higher in Toledo while the proportional use of 'low-frequency notes' was lower (Slabbekoorn and Den Bon-Visser, 2006, see Figure 2). This suggests that memes that were less masked by noise were sung more frequently, as proposed by Halfwerk and Slabbekoorn 2009), and could be a cultural plastic response of Toledo males. The effect of this potential cultural plastic response, however, was not large enough to differentiate the minimum frequency between study areas.

This study suggests that the degree of isolation of a population could influence the city-forest song divergence in Great tits. The size of a city may be an important feature in song divergence, as birds in smaller cities likely have a higher exchange of memes with those in areas outside of the city. In these reduced urban areas, even though Great tits are under high anthropogenic acoustic pressure, the minimum frequency shift could be partially constrained due to cultural wealth with a high proportion of low frequency memes from outside the city. It could be that in this situation, Great tits sing the same memes louder or shift other frequency features of their songs to enhance sound transmission. Further studies comparing city and forest pairs in different sized cities with different city-

forest distances may help to clarify these findings.

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CAPÍTULO IV

LA ESTRUCTURA DEL HÁBITAT INFLUYE EN LAS CARACTERÍSTICAS DEL CANTO DENTRO DE UNA POBLACIÓN DE CARBONEROS COMUNES

Bueno-Enciso J, Ferrer ES, Barrientos R y Sanz JJ. Habitat structure influences the song characteristics within a population of Great tits.

Under review

Abstract

To fulfil its function in sexual selection, birdsongs have to reach the receivers neatly, which is not a simple issue because of the impediments that the acoustic habitat properties impose. This necessity for a suitable transmission may have shaped the evolution of birdsongs, as the Acoustic Adaptation Hypothesis (AAH) proposes. If so, one could expect habitat related variation in song characteristics within populations. The aim of this study was to test if the structure of the habitat influences the song characteristics in a population of Great tits *Parus major*. To do this, during the breeding season of 2012, we recorded Great tit songs from 42 territories in two different days and measured the habitat structure in each territory. We also trapped the males and estimated breeding density around each territory, so we were able to correct the analysis by date, breeding density and male characteristics. The strophe length was positively affected by the ground cover and the song rate; whereas peak and minimum frequency were negatively affected by the shrub cover. Furthermore, these song characteristics showed a relatively higher degree of repeatability within individuals. Peak frequency was also negatively affected by the date, although this was probably an indirect effect of differences in male size along the breeding season. This study shows for first time habitat-related variation in songs within a population due to habitat structure, and suggests that Great tits are capable to adjust their vocalization in each territory presumably to enhance transmission due to vocal plasticity.

Introduction

For many animal species belonging to different taxa, acoustic communication plays an essential role in their lives, as it is involved in the regulation of social relations (reviewed in Bradbury and Vehrencamp 1998). This is particularly true for songbirds, because they intensively rely in acoustic long-range signals to gather information about each other and resolve conflicts over resources such as food, territory or mates, without the need to engage in costly interactions (McGregor and Dabelsteen 1996, Peake *et al.* 2001, Searcy and Beecher 2009, Maynard *et al.* 2012). As a consequence, acoustic communication directly influences their survival and reproductive success (Andersson 1994, Slabbekoorn 2013). Therefore, it is crucial for songbirds getting their message across to potential receivers neatly, in order to fulfil its biological function, i.e. communication (Brumm and Naguib 2009). In view of this, male birdsong constitutes a secondary sexual trait composed for multiples characters, some of which should be subject to some physical constraint to encode stable and honest messages of individual quality and thus, serve the function of sexual selection. Such characters are called performance-related traits (Gil and Gahr 2002). However, most of these performance-related traits are constrained within limits, which allow signallers to modulate its performance adaptively (Searcy and Beecher 2009).

Among these performance-related traits are the spectral features of the birdsong (Baker 2006), being the minimum frequency an important one (Morton 1977). Minimum frequency in songbirds is negatively related to their body size (Ryan and Brenowitz 1985, Wiley 1991). However, this feature could also be a cue of body size within species and consequently, serve as a cue in sexual selection (Gil and Gahr 2002). Indeed, Montezuma oropendolas *Psarocolius montezuma* males significantly diminish the minimum frequency of their songs in vocal contests (Price *et al.* 2006). The minimum frequency of the birdsongs is also related to female fertility and its sexual fidelity (Halfwerk *et al.* 2011, des Aunay *et al.* 2013). Other important feature in terms of sexual selection is the song output, i.e. the amount of song a male produces; because singing may be a costly activity, both in terms of energy expended and in terms of the risk of exposure to predators (Otter *et al.* 1997, Gil and Gahr 2002). Song output can be measured in several ways (Poesel *et al.* 2001), such as the total time spend singing (Welling *et al.* 1995) or strophe length or percentage performance time (Lambrechts and Dhont 1986, 1987). Although the most common measure of song output is the song rate, i.e. number of song units per time unit (Welling *et al.* 1997, Catchpole and Slater 2008). Song rate is positively selected by females (Alatalo *et al.* 1990, Wasserman and Cigliano 1991, Houtman 1992) and it is intensified in male-male competition (reviewed in Baker *et al.* 2012).

However, the impediments that each habitat imposes to sound transmission may force songbirds to modify these spectral song features in order to improve its transmission (Brumm and Naguib 2009); as the capacity of transmission of each spectral song feature is not the same in all of its range of variation. These impediments are delimited by the acoustic properties of each habitat, i.e. the structure of the habitat itself, the background noise and the climate conditions; and can set a trade-off between the message and the transmission of the sound (Patricelli and Bickley 2006, Slabbekoorn and Ripmeester 2008, Halfwerk *et al.* 2011, Luther and Magnotti 2014, McMullen *et al.* 2014). A recent example that has attracted attention of biologists in last years is the frequency shift that some songbird species do in the presence of anthropogenic background noise, presumably to avoid masking (Slabbekoorn and Peet 2003, Slabbekoorn and den Boer-Visser 2006, Redondo *et al.* 2013). Regarding sound transmission, one habitat especially challenging for acoustic communication are the forests, because of the impediments that the vegetation imposes in terms of frequency-dependent attenuation and reverberation, which diminish the active space of the signal (Wiley and Richards 1982). Concretely, forest birdsongs are characterized to possess a slower song rate, a lower minimum frequency and more tonal sounds with slow modulations in amplitude and frequency than in more open habitats (Hunter and Krebs 1979, Wiley 1991, Nemeth *et al.* 2013). This is because dense vegetation scatters and absorbs high frequencies of vocalizations (Aylor 1972, Marten and Marler 1977) and reverberate fast amplitude/frequency-modulated signals, such as trills or sounds

that sweep up and down over a wide frequency range (Slabbekoorn *et al.* 2002).

If effectively, adaptation to acoustic habitat properties is a strong selective force acting upon birdsongs, as the Acoustic Adaptation Hypothesis (AAH) proposes (Morton 1975); then one could expect habitat related variation in song structure also within populations (Hunter and Krebs 1979). Whether most studies have looked for relationships between habitat structure and birdsong structure among populations (reviewed in Boncoraglio and Saino 2007); very few studies have investigated this topic at the population level. Thus, the aim of this study was to test if the habitat structure within a territory affects the song characteristics of the owner in a population of Great tits *Parus major*. Great tits constitute an ideal species to study this topic, because is one of the best-studied species in terms of acoustics (Slabbekoorn 2013), and they predominantly use their song as a long-range signal in territory contexts (Krebs *et al.* 1981). This species cues on song characteristics such as frequency, song rate or strophe length to assess conspecifics by eavesdropping (Peake *et al.* 2001) and distinguish individuals (Weary and Krebs 1992). They also estimate the distance to the sender by song degradation cues (McGregor and Krebs 1984). We hypothesize that the habitat structure within a territory will affect the songs of Great tits, and predict that those Great tits breeding in territories with denser vegetation will perform more ‘forests’ songs, i.e. with a relative lower minimum frequency and slower song rate than conspecifics breeding in more open territories.

Methods

Study area

The present study was conducted in the locality of San Pablo de los Montes (39°32′44″N, 4°19′41″W. Toledo, central Spain). The whole study area presents continental Mediterranean climate, with mean values of annual rainfall of 700-800 mm concentrated in autumn and spring, and pronounced summer droughts with a wide daily thermal oscillation. The study area is constituted by deciduous oak *Quercus pyrenaica* forests accompanied by its typical shrub courtship, mainly formed by strawberry tree *Arbutus unedo*, common hawthorn *Crataegus monogyna*, elmleaf blackberry *Rubus ulmifolius*, terebinth *Pistacia terebinthus*, flax leaved-daphne *Daphne gnidium* and common broom *Cytisus scoparius*. These forests are considered the most suitable breeding habitats for Great tits in the area (Atiénzar *et al.* 2012). However, this landscape has been fragmented due to human activities, as in other regions of the Mediterranean Basin (Blondel and Aronson 1999); and the remaining forest patches can be considered 'habitat islands' dispersed in a matrix of less suitable pasturelands (García-Navas *et al.* 2014). For this study, we selected ten oak patches separated each other by a mean distance of 4.2 km, and provided with a number of nestboxes accordingly to its size, separated each other for at least 30 m. All nestboxes were placed spanning a distance to the forest edge of at least 50 m. Across the study area, background noise is homogeneous because of the reduced urbanisation of the area, with a mean value of 37.41 ± 0.38 dB measured in a previous study (Bueno-Enciso *et al.* 2015).

Field procedure

During the breeding season, from 13-March-2012 to 23-May-2012 (day 1= 1 March) and between 0800 and 1200 hours, we recorded Great tit songs using an EDIROL R-09HR digital recorder equipped with a Sennheiser unidirectional microphone and headphones, pointed directly toward the singing individual. All song recordings were made at a distance of 10 to 25 m.

We visited each forest patch at least three times, and noted on a map with the aid of a GPS, the exact position of the singing male. We identified the individual birds by their territorial behaviour associated with a particular nest box (Doutrelant *et al.* 2000). We only took into account those males recorded on at least two different days, within a radius of 15 m from a nest box occupied by a Great tit pair (Rivera-Gutierrez *et al.* 2010). Due to the regular inspection of the nest boxes, we were able to determine the stage of the reproductive cycle of the Great tit pairs when the recordings were made. Only those songs recorded after the nest was built until the last egg was laid were used in our analyses, coinciding with the fertile period of the female in this species (Mace 1987); because male phenology influences Great tit songs

(Halfwerk *et al.* 2011). It is in this period when males increase their song activity and mate-guarding behaviour to minimize the risk of cuckoldry (Møller 1991), at a slight distance from the nest area and using only a small portion of their territory, as they establish a trade-off between territory defence and mate-guarding (Slagvold *et al.* 1994, Ritschard *et al.* 2011). We captured the males while they were feeding their chicks (8-9 days old), weighed them with a portable digital balance (to the nearest 0.1 g), measured their tarsus length with a digital caliper (0.01 mm) and aged them (yearling or older) according to plumage characteristics. Body condition index of males was obtained from the standardized residuals of a linear regression in which the body weight was the dependent variable and the tarsus length was the predictor. We calculated the density of Great tits in each breeding territory as the number of Great tit breeding pairs in a radius of 50 m around each nest box with the aid of Quantum GIS 2.0.1. We used this number of breeding pairs in the neighbourhood of a territory as an index of the intensity of competition among males, because this is an important factor affecting song performance (Martin-Vivaldi *et al.* 2004, Hamao *et al.* 2011).

All applicable institutional and/or national guidelines for the care and use of animals were followed.

Habitat structure

At the end of the breeding season, we estimated by eye the vegetation structure of the breeding territories in a sampling plot centred on the nest box with a radius of 25 m. This area is hereafter referred to as the territory surrounding the nest box, based on the assumption that it is representative of the habitat structure of the whole territory of breeding pairs (Svensson and Nilsson 1995). The structural variables of the breeding territories included the diameter at breast height (DBH) of oak trees (measured in three random oaks for each territory), and the percentages of tree, shrub and ground cover. Ground cover include the bare soil and the grass cover to simplify analyses, because these two components only affect the birdsongs sung near the ground, which is not the case of the Great tits (Marten and Marler 1977).

Song analyses

As we stated before, two song recordings from different days were analysed for each male, as it was the maximum sample size available for all males recorded. Maximum time gap between two recording days for a male was four days. Following this procedure, 42 different first brood males that held their own territory were selected, which supposes a total of 84 Great tit song recordings employed in the analyses. Great tits have a small discrete repertoire (Lambrechts and Dhont 1986), where each song type is sung in bouts of the same strophe type, the separation between adjacent strophes is called a pause. Each strophe is composed of a repetition of the same phrases, which are in turn composed of notes. For song analyses, we selected 10 different strophes of each song recording, and exported them to the program RavenPro 1.4 (Charif *et al.* 2010). After visual inspection of each audio track, we used a band filter to remove low frequency background noise from the recording without the removing any song components (Mockford and Marshall 2009). We focused on the spectral level of the description of the songs (Baker 2006), and measured each strophe separately. At the end, we averaged the 10 strophes selected of the same song type. The song characteristics measured are the following: Number of notes that compose each phrase, strophe length (s), pause length (s), song rate (ratio between the number of phrases and the strophe length), minimum, maximum and peak frequency (Hz) and the bandwidth (difference between the maximum and the minimum frequency, Hz). The average number of phrases was not used in the analyses, as it was highly correlated with the strophe length (($r = 0.74$; $n = 84$; $P < 0.001$). Peak frequency was automatically measured; the rest of the variables were manually measured using a Hann window and a fast Fourier transformation (FFT), length of 1024, resulting in a spectral resolution of 43.1 Hz. Minimum and maximum frequencies were manually measured by precisely placing a selection box in the spectrogram view, similar to Francis *et al.* (2011).

Statistical analyses

To test whether the structure of the habitat in each territory affects the song characteristics of Great tits, we used each habitat variable as an explanatory variable. Through this way, we included all the variation of the habitat structure in each territory, which allowed us to concretely see which specific habitat component influences each song characteristics. We also included the date of each song recording as an explanatory variable, as the date has been seen to influence the song performance of birds (Lambrechts and Dhont 1986). This could be due to changes in the motivation and singing practice of the males (Vehrencamp *et al.* 2013), but also due to phenotypic differences of singing males along the breeding season (Perrins 1970). Furthermore, in temperate deciduous forests as in this study, the drastic changes that the habitat structure suffers along the breeding season, mainly because of the emergence of leaves, change the acoustic properties of the habitat (Blumenrath and Dabelsteen 2004). Consequently, individuals could change their song performance to match these new environmental acoustic properties (Brumm and Naguib 2009). Other factor that can influence the song characteristics of birds is the breeding density, as it is an index of the intensity of competition among males (Hamao *et al.* 2011). Male characteristics can also affect the song characteristics (Slabbekoorn 2013, Read *et al.* 2014), so we included as explanatory variables the age, body size and body condition of males; because of the singing experience that males acquired with age (Vehrencamp *et al.* 2013, Ota and Soma 2014); the body size constraints (Wiley 1991, Price *et al.* 2006) and the body condition constraints (Gil and Gahr 2002, Juola and Searcy 2011). Therefore, we tried to explain the variance of each song characteristics with these nine explanatory variables: mean recording date, breeding density, mean DBH, tree, shrub and ground cover, male age, tarsus length (as a surrogate of body size) and male condition. To avoid collinearity we designed a two-step statistical analysis procedure, in a similar way as Barrientos (2010).

In a first step, we used a Principal Component analysis (PCA) with the varimax normalized factor rotation, to build a correlation matrix to explore the degree of association among the nine explanatory variables (table 1). For the subsequent model development, and in order to minimize collinearity among independent variables, we selected the most highly correlated variable within each of the PC factors, discarding the

remaining variables with correlations >0.6. This technique allows the direct use of original variables rather than PCA factors, which are sometimes difficult to interpret from an ecological point of view. In addition, variables that are likely correlated can initially be included as the less influential will finally be discarded. Due to the strong associations of the variables selected according to PCA factor loadings, three variables were removed before the modelling process: DBH, tarsus length and male body condition; as they were highly correlated with male age, mean recording date and shrub cover respectively (table 1). For these analyses, we used R (R Core Team 2014) and the function “prcomp” of the package stats (R Core Team 2014). In a second step, we performed Linear Mixed-effect Models (LMMs) with each spectral song feature as dependent variable and the independent variables selected in step 1 as explanatory variables. Number of notes and pause length were analysed performing a Generalized Linear Mixed-effect Models (GLMMs) fitted with a poisson distribution. In all models, the identity of the Great tit male (Male ID) nested in the identity of the forest patch (Patch ID) was included as a random term. Nonetheless, to test whether the variables discarded in step 1 significantly explained some of the variance of the spectral song features of Great tits; we performed a second set of Mixed-effect Models constructed in the same way; but including the discarded variables in step 1. To do this, we removed those variables that were highly correlated with these new variables included to avoid collinearity. We performed all Mixed-effect Models using the function “lme” of the package lme4 (Bates *et al.* 2004).

Table 1. The results of the principal component analysis run prior to the development of the Mixed-effect Models in order to investigate the collinearity among independent variables

	PC1	PC2	PC3	PC4
Recording date	-0.07	0.80	-0.20	0.09
Density	0.03	0.48	0.49	-0.11
DBH (cm)	-0.75	-0.26	0.04	0.04
Tree cover (%)	0.39	0.11	0.56	0.13
Shrub cover (%)	0.18	0.20	-0.23	0.79
Ground cover (%)	-0.10	-0.07	0.83	0.01
Male age	0.89	-0.33	0.09	0.11
Tarsus length (mm)	0.07	0.74	0.21	-0.01
Male condition	-0.08	-0.16	0.31	0.77
Eigenvalue	1.84	1.58	1.39	1.20
% Total variance	20.40	17.52	15.42	13.30

Assumptions of homoscedasticity and normality were verified graphically

with the residuals in each model (Zuur *et al.* 2009). Initial LMMs and GLMMs were constructed with all independent variables fitted and final models were selected following a backward procedure, by progressively eliminating non-significant variables.

For each spectral song feature measured, we also calculated the Intraclass Correlation Coefficient (ICC) (Sokal and Rohlf 1995); as an index of repeatability within individuals. Repeatability is the fraction of total variation in a set of measurements because of the variance among individuals; which is often used to measure the degree of reliability of a behaviour trait (Wolak *et al.* 2012). The ICC is defined by the equation: ICC= $S^2_A / S^2_A + S^2_W$; where S^2_A is the variance-among individuals and S^2_W is the variance-within individuals. If the variance-within individuals of a trait is greater than the variance-among individuals, the ICC takes low values; indicating low repeatability of that trait. Therefore, a spectral song feature affected by the habitat with a high ICC, may suggest that enhancing the transmission of such song feature is biologically important for Great tits. ICCs were obtained with the function "ICCest" of the package ICC (Wolak *et al.* 2012).

Results

The PCA yielded four factors that collectively accounted for 66.64% of the total variance (table 1). Accordingly to the selection criterion in step 1, six independent variables were used to explain the variance of each song characteristic in the first set of Mixed-effect Models (table 2). These analyses revealed that the peak frequency was negatively affected by the recording date, while no other song characteristic was significantly affected by the date. The density of breeding pairs in the surroundings of each territory did not significantly affect any song characteristic, but the structure of the habitat in each territory did. Concretely, the strophe length was positively affected by the ground cover (figure 1), whereas both the song rate and the minimum frequency were negatively affected by the shrub cover (figures 2 and 3 respectively). Finally, the bandwidth of the songs was positively affected by the age of the males, with older males singing with a broader frequency range (table 2).

Table 2. Results of the Mixed-effect Models analyzing the effects of the mean recording date, density, habitat structure and male characteristics on the spectral song features of Great tits *Parus major* in San Pablo de los Montes (Toledo), central Spain. The ID male nested in ID patch is included in all models as the random term. Z value is given with non-parametric distributions. Significant results are highlighted in bold.

	No notes			Song rate			Strophe length (s)			Pause length (s)		
	Estimate ± SE	z value	*P*	Estimate ± SE	t value	*P*	Estimate ± SE	t value	*P*	Estimate ± SE	z value	*P*
Intercept			<0.001			<0.001			<0.001			<0.001
Recording date	0.002 ± 0.004	0.49	0.62	-0.001 ± 0.006	-0.03	0.98	0.003 ± 0.006	0.41	0.69	0.003 ± 0.005	0.66	0.51
Density	-0.001 ± 0.07	-0.01	0.99	-0.02 ± 0.10	-0.22	0.82	0.002 ± 0.10	0.02	0.99	0.12 ± 0.08	1.56	0.12
Tree cover (%)	0.001 ± 0.004	0.32	0.75	-0.002 ± 0.005	-0.42	0.68	-0.001 ± 0.005	-0.11	0.91	-0.005 ± 0.004	-1.24	0.22
Shrub cover (%)	0.002 ± 0.003	0.78	0.44	-0.01 ± 0.004	-2.24	**0.03**	-0.001 ± 0.004	-0.18	0.86	0.001 ± 0.004	0.29	0.77
Ground cover (%)	-0.001 ± 0.002	-0.51	0.61	-0.005 ± 0.003	-1.64	0.10	0.01 ± 0.003	2.87	**0.01**	-0.001 ± 0.003	-0.48	0.63
Male age	-0.01 ± 0.07	-0.20	0.85	0.11 ± 0.10	1.07	0.29	-0.07 ± 0.097	-0.76	0.45	0.15 ± 0.08	1.80	0.07
	Maximum frequency (Hz)			Peak frequency (Hz)			Minimum frequency (Hz)			Bandwidth (Hz)		
	Estimate ± SE	t value	*P*	Estimate ± SE	t value	*P*	Estimate ± SE	t value	*P*	Estimate ± SE	t value	*P*
Intercept			<0.001			<0.001			<0.001			0.01
Recording date	-8.04 ± 5.62	-1.43	0.16	-6.37 ± 3.02	-2.21	**0.03**	-3.55 ± 3.01	-1.18	0.25	1.93 ± 5.25	0.37	0.72
Density	32.93 ± 108.20	0.30	0.76	-17.71 ± 48.10	-0.37	0.72	9.62 ± 59.93	0.16	0.87	52.02 ± 83.73	0.62	0.54
Tree cover (%)	0.38 ± 5.81	0.07	0.95	-1.72 ± 2.53	-0.68	0.50	-2.233 ± 3.04	-0.74	0.47	-2.32 ± 4.63	-0.50	0.62
Shrub cover (%)	-3.53 ± 4.95	-0.71	0.48	-3.63 ± 2.12	-1.71	0.10	-6.71 ± 2.56	-2.63	**0.01**	-3.18 ± 4.04	-0.79	0.43
Ground cover (%)	-6.67 ± 3.57	-1.87	0.07	-1.47 ± 1.68	-0.88	0.39	1.12 ± 1.88	0.59	0.56	-5.28 ± 2.91	-1.82	0.07
Male age	126.28 ± 110.36	1.14	0.26	53.38 ± 49.49	1.08	0.29	-21.26 ± 60.25	-0.35	0.73	251.93 ± 91.30	2.76	**0.01**

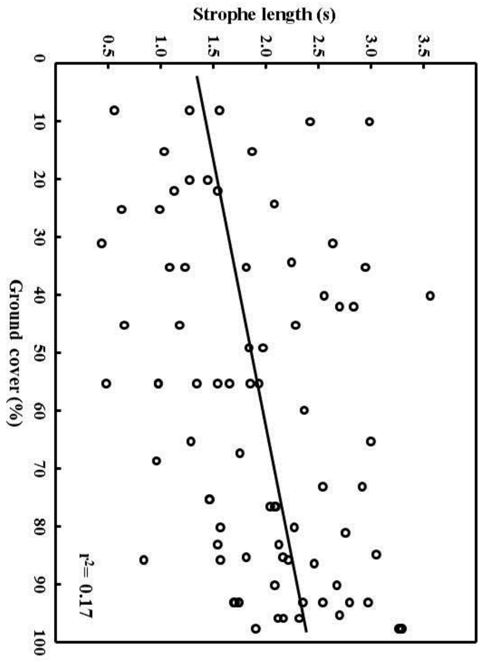

Figure 1. Scatterplot representing the relationship between the strophe length and the percentage of ground cover within each territory in a population of Great tits *Parus major* breeding in San Pablo de los Montes (Toledo), central Spain. Two measures from each individual are represented.

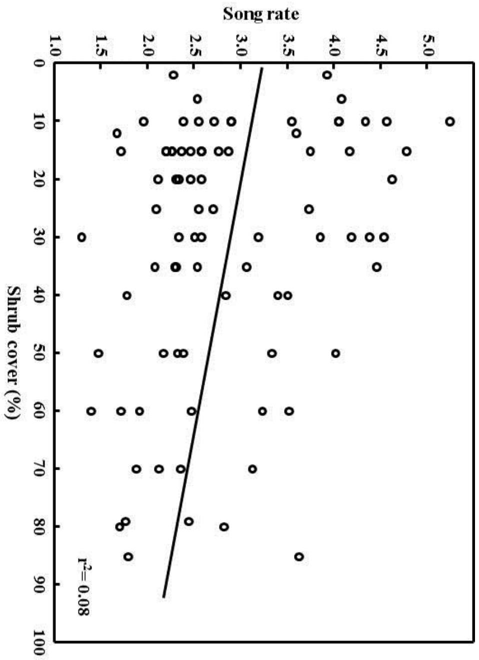

Figure 2. Scatterplot representing the relationship between the song rate and the percentage of shrub cover within each territory in a population of Great tits *Parus major* breeding in San Pablo de los Montes (Toledo), central Spain. Two measures from each individual are represented

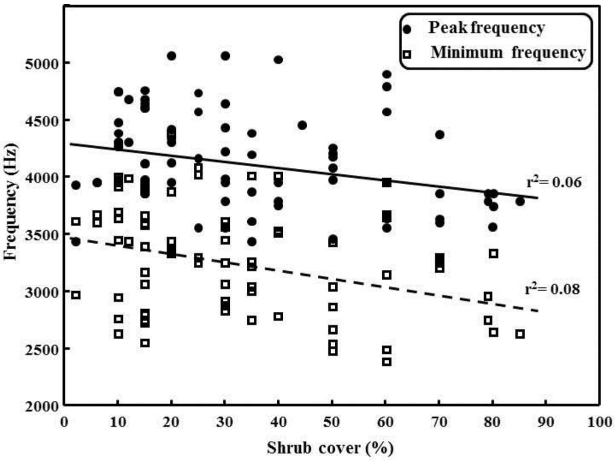

Figure 3. Scatterplot representing the relationships of the peak and the minimum frequency with the percentage of shrub cover within each territory in a population of Great tits *Parus major* breeding in San Pablo de los Montes (Toledo), central Spain. Two measures from each individual are represented

Attending the second set of Mixed-effect Models, the incorporation of the explanatory variables discarded in step 1 practically did not change the results; since most of them did not significantly affect any song characteristic (data not shown for brevity). Concretely, mean DBH did not significantly affect the bandwidth of the songs (LMM: Estimate ± SE= -6.26 ± 9.67; *t* value= -0.65; *P*= 0.52), and the male condition did not significantly affect the song rate or the minimum frequency (LMM: Estimate ± SE= -0.003 ± 0.11; *t* value= -0.25; *P*= 0.80 and LMM: Estimate ± SE= 53.41 ± 62.40; *t* value= 0.86; *P*= 0.40 for the song rate and the minimum frequency respectively). The only exception was the peak frequency, which was negatively affected by the tarsus length (LMM: Estimate ± SE= -186.35 ± 70.11; *t* value= -2.66; *P*= 0.01; figure 4). It is worth noting to remark that in this model, the shrub cover also negatively affected the peak frequency of Great tit songs (LMM: Estimate ± SE= -4.54 ± 2.08; *t* value= -2.18; *P*= 0.03; figure 3). However, in the same way as with the song rate and the minimum frequency, the male body condition did not significantly affect the peak frequency (LMM: Estimate ± SE= -25.19 ± 49.65; *t* value= -0.51; *P*= 0.62).

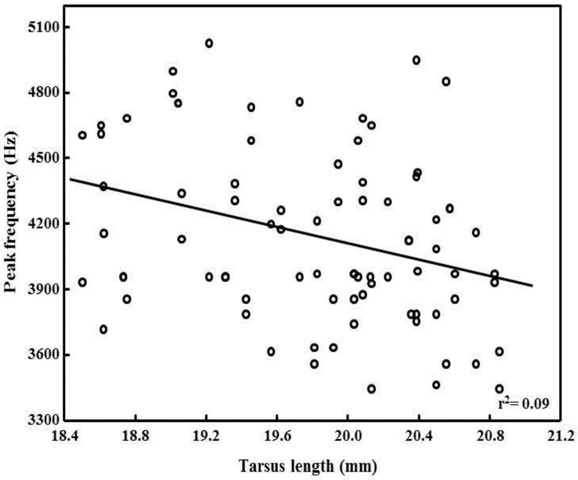

Figure 4. Scatterplot representing the relationship between the peak frequency and the male tarsus length in a population of Great tits *Parus major* breeding in San Pablo de los Montes (Toledo), central Spain. Two measures from each individual are represented.

Most of the spectral song characteristics showed a low ICC, with higher variance-within individuals than variance-among individuals (table 3). The only exception was the minimum frequency. The mean ICC for all spectral song features was 0.34 ± 0.05 (mean ± SE). Nonetheless, those spectral characteristics that were significantly affected by some explanatory variable, present on average a significantly higher ICC than those song characteristic whose variance were not significantly explained by neither of the explanatory variables (Mann-Whitney U Test: Z= -2.09; *P*= 0.04; table 3).

Table 3. Intraclass Correlation Coefficients (ICC) calculated for the spectral song features measured in a population of Great tits *Parus major* breeding in San Pablo de los Montes (Toledo), central Spain. n is the number of individuals, k is the number of measures for each individual, S^2_W is the variance-within individuals and S^2_A is the variance among individuals.

	ICC	n	k	Range	S^2_W	S^2_A
No notes	0.17	42	2	-0.14 - 0.44	0.38	0.08
Phrase rate	0.43	42	2	0.15 - 0.65	0.49	0.37
Strophe length (s)	0.49	42	2	0.21 - 0.69	0.34	0.32
Pause length (s)	0.21	42	2	-0.13 - 0.49	3.33	0.90
Maximum frequency (Hz)	0.25	42	2	-0.05 - 0.51	485390.5	165047.2
Peak frequency (Hz)	0.42	42	2	0.14 - 0.64	107358.8	78652.87
Minimum frequency (Hz)	0.55	42	2	0.30 - 0.73	98733.57	120646.5
Bandwidth (Hz)	0.38	42	2	0.09 - 0.61	386374.9	236807.5

Discussion

The peak frequency of Great tit songs resulted negatively affected by the recording date. One way by which the date could affect the song structure in deciduous forests that particularly concerns this study, is indirectly because of the emergence of leaves; which change the acoustic properties of the habitat (Blumenrath and Dabelsteen 2004). In their acoustic transmission experiment, the former authors showed that the active space of an acoustic signal is half reduced with the budding due to the absorption and attenuation from the broadleaved foliage. In our study area, the phenology of oaks is almost synchronized among forest patches, because of the small variation in altitude among them and the reduced geographical extension that they occupy, which imposes homogeneous climate conditions. Consequently, Great tits could be behaviourally adjusting their vocalizations to shape the changing acoustic properties of deciduous forests (Brumm and Naguib 2009), as lower frequencies are less prone to scattering and attenuate from broadleaved foliage (Aylor 1972, Marten and Marler 1977, Wiley and Richards 1982). However, the fact that none song characteristic was significantly affected by the tree cover challenges this explanation, as the environmental change predominantly occurs at high altitudes in the canopy of deciduous forests (Blumenrath and Dabelsteen 2004). A possible explanation for the temporal pattern in the peak frequency of Great tit songs could be due to changes in male characteristics along the breeding season (Perrins 1970). Indeed, the size of males significantly varied along the breeding season, with bigger males breeding later; as it can be appreciated in the PC1 (table 1). Interestingly, the peak frequency was also negatively affected by the tarsus length (figure 4), as the second set of Mixed-effect Models revealed. Hence, the temporal pattern of the peak frequency could be indeed, due to differences in the size of Great tit males along the breeding season and no because of temporal changes in the acoustic properties of the habitat. As it happens with the minimum frequency of birdsongs (Wiley 1991, Price *et al.* 2006), low-peak frequency songs could be body-sized constrained if they are performed near the physiological limit (Gil and Gahr 2002, Searcy and Beecher 2009). Peak frequency contains the maximum power of the song (Charif *et al.* 2010), so it is expected that reaches the greater distances without attenuation. Thus, encoding an honest message of body size on it would be advantageous (Gil and Gahr 2002). For example, in Great frigatebirds *Fregata minor* the peak frequency is negatively related to the size of their gular pouch (Joula and Searcy 2011).

As we stated before, neither spectral song feature was significantly affected by the tree cover. Blumenrath and Dabelsteen (2004) estimated that a bird can counteract the effects of the broadleaved foliage on sound transmission by diminishing the height of their singing perch and sing beneath the canopy, because of a sound 'channel' between the canopy and the undergrowth. This change in the sender position would prevent changes in the song structure due to the inevitable emergence of leaves, which would be adaptive if changes in the song characteristics would entail changes in the message (Patricelli and Bickley 2006, Slabbekoorn and Peet 2008, Halfwerk *et al.* 2011, Luther and Magnotti 2014, McMullen *et al.* 2014). This behavioural response could be happening in our population, although we could not confirm it as we did not measure the singing height of the individuals. Supporting this, the only habitat variables that significantly affected Great tit songs were the shrub and ground cover. These variables are almost constant along the breeding season as most of the species compounding the undergrowth are evergreen and its growth rate is not rapid enough to vary within a season. Thus, Great tits face the impediments that the habitat structure impose to sound transmission beneath the tree canopy from the beginning of the breeding season, i.e. DBH, shrub and ground cover; and could therefore, adjust their vocalizations to match these invariable acoustic habitat properties shortly after settling in their territories. Concretely, Great tits sung with a slower song rate and a lower peak and minimum frequency in territories with high shrub cover (figures 2 and 3). As can be appreciated in PC4 (table 1), male body condition was highly correlated with the shrub cover in each territory. However, the body condition of males did not significantly affect any spectral song feature, which leads the shrub cover as the most feasible factor shaping the former spectral features of Great tit songs. In such territories singing fast may be disadvantageous, because rapid and consecutive sound waves can easily intermingle among them due to the echoes and reflections off the tree trunks and shrub vegetation, causing a rapid degradation and a drastic decrease of the active space of the signal (Wiley and Richards 1982, Slabbekoorn *et al.* 2002). In the same way, singing high-frequency songs in such territories may entail transmission problems in terms of frequency-dependent attenuation (Wiley and Richards 1982), because higher frequencies suffer a higher degree of attenuation than lower ones from vegetation (Aylor 1972, Marten and Marler 1977). Thus, in denser habitats is advantageous to diminish the song frequency to avoid attenuation, above all in the peak and minimum frequency because these are the frequencies that carry more energy (Boncoraglio and Saino 2007). Additionally, Great tits sung with longer strophes in territories with higher ground cover; probably because in such territories the risk of degradation due to echoes and reflections is lower, so Great tits could be able to include more phrases in their songs.

The density of breeding Great tits in the surroundings of each territory did not significantly affect any song characteristic, besides that the intensity of competition among males in this species has been seen to affect some aspects of their songs (Hamao *et al.* 2011). Nonetheless, the former study was conducted in a city, where typically the density of breeding birds is higher than in forests (Marzluff 2001); so it could be that the breeding density in our study area remains low enough to do not significantly affect the song characteristics in our population of Great tits. Finally, the bandwidth of the song was also affected by the age of Great tits, with older males singing with a broader bandwidth than younger ones. These age-related changes in song characteristics have been seen in other closed-learned species and are related to an enhancement of the singing skills with age (Vehrencamp *et al.* 2013). Concretely, Ota and soma (2014) also observed an increase of the bandwidth with age in the Java sparrows (*Lonchura oryzivora*), only achieved by an increase of the coordination between the syringe muscles and the air flow (Podos 1977). Nonetheless, as Ota and Soma (2014) pointed out, this increase in the bandwidth with age could be a side effect

of singing louder, as the bandwidth is dependent on sound pressure (Zollinger *et al.* 2012).

The repeatability of the song characteristics within individuals were low, with ICC values for almost all spectral song features below the average ICC for behavioural traits (mean= 0.48, range= -0.20 – 1; reviewed in Wolak *et al.* 2012). Except the minimum frequency, the variance within individuals was greater than the variance among individuals for the rest of song characteristics, a result anticipated in the literature (i.e. Salaberria and Gil 2010, Slabbekoorn and den Boer-Visser 2006). However, it is noteworthy that those song characteristic that were significantly affected by the habitat structure or male characteristics, i.e. song rate, strophe length, peak and minimum frequency and the bandwidth, had an ICC significantly greater than the rest of spectral features whose variance were not significantly explained by any independent variable. This result suggests that Great tits are more conservative performing those song characteristics that transmit better or signal their quality.

This study suggests that habitat structure can influence the song of Great tits even within a population, despite that the habitat structure has been seen not to be a shaping force as strong as the background noise modelling birdsongs (Brumm and Naguib 2009, Proppe *et al.* 2012). Indeed, variation in song characteristics within a population because of the acoustic properties of the habitat has only been assessed with different levels of background noise (i.e. Slabbekoorn and Peet 2003, Salaberria and Gil 2010). This study shows that changes in the habitat due to human activities, such as habitat fragmentation or degradation, can affect wildlife in several ways, even the performance of birdsongs. These vocal adjustments, although seem to improve transmission, could entail fitness costs in the sender and receiver if the message is compromised (Patricelli and Blickley 2006, Warren *et al.* 2006, Halfwerk *et al.* 2011, Read *et al.* 2014).

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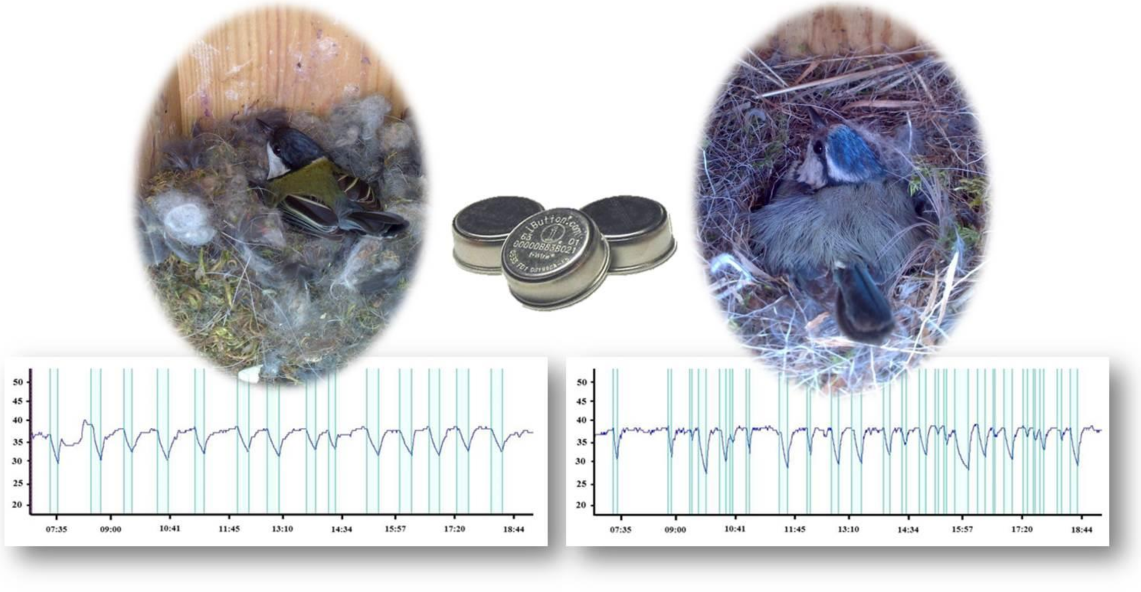
CAPÍTULO V

DIFERENCIAS EN EL COMPORTAMIENTO DE INCUBACIÓN ENTRE DOS POBLACIONES SIMPÁTRICAS DE AVES QUE ANIDAN EN OQUEDADES



Bueno-Enciso J, Barrientos R y Sanz JJ. Differences in incubation behaviour as regards two sympatric populations of cavity nester bird species.

Under review

Abstract

The incubation stage in avian reproduction could be as costly as the nestling rearing stage. This is particularly true in the case of uniparental incubation, during which both current and future breeding attempts may be compromised. Discovering the proximate effects that condition the incubation costs in free-living bird populations is therefore, of great importance as regards understanding the evolution of birds' life history and the variation of incubating behaviours within and among species. In this study, we have spent two years assessing the incubation rhythms of Blue (*Cyanistes caeruleus*) and Great tits (*Parus major*) that inhabit the same Mediterranean area in central Spain through the usage of i-button data-loggers. This i-button methodology has proved to be reliable, and has shown that the incubating behaviour of Great tits resembles that reported for other southern populations of Great tits, i.e. with a relatively low attentiveness and a short active day. Furthermore, we describe the incubation behaviour of Blue tits for first time. The fact that Blue tits' eggs are smaller probably explains why the nest i-button cools at a higher rate than in Great tits' clutches, which strongly influences the incubation behaviour in this species. However, the fact that Great tits' eggs are larger allows the species to be more flexible in its incubation behaviour and presumably utilise a less costly incubation strategy than that of Blue tits, i.e. longer but fewer bouts. Ecological factors affect both species in a similar manner, although the advantage of the relatively lower cooling rate of Great tits' eggs allows them to obtain more benefit from favourable conditions such as warmer ambient temperatures than Blue tits.

Introduction

The reproduction of birds has classically been divided into three well differentiated stages: egg-production, incubation and care of the nestlings (Nilsson *et al.* 2008); although it has subsequently been proposed that the stage of nest construction should also be included (Mainwaring and Hartley 2013). Until recently, researchers' attention has been focused almost exclusively on the last stage (care of nestlings), because it was considered to be the most expensive in terms of energy expenditure (Williams 1996). However, egg-production could be as expensive as both nestling rearing (Ward 1995) and the incubation period, during which the metabolic rates of parents incubating eggs can exceed the metabolic rate of birds thermoregulating at cool temperatures by 40-50% (reviewed in Tinbergen and Williams 2002). This is particularly true in the case of those species in which only one parent incubates, usually the female (Conway and Martin 2000a, Matysioková and Remeš 2010, Cooper and Voss 2013). The highly costs of uniparental incubation compromise not only current breeding attempts but also those of the future (Williams 1996, Reid *et al.* 2000a, Tinbergen and Williams 2002); and because optimal embryo developing conditions are provided by the female through incubation, this stage is also crucial for the hatchability of eggs and the phenotype of nestlings (Cooper *et al.* 2005, Chalfoun and Martin 2007, Nord and Nilsson 2011).

The optimal incubation conditions are provided by the females in a behavioural manner; this behaviour is reflected in the incubation rhythm, which is the result of the balance between the needs of the female and the needs of the embryos (Cooper and Voss 2013). Females need to leave the nest (mainly to forage) during daylight, and the temperature of their eggs starts to decrease according to the cooling rate of the eggs and ambient temperature; these periods away from the nest during which the eggs are left unattended are called off-bouts. When the females return, they rewarm the eggs and maintain the optimal incubation temperature until they need to leave again; the periods during which the eggs are actively warmed are called on-bouts. The duration and number of these bouts define each species' incubation behaviour and also determine attentiveness during daylight, which is the

percentage of time during which females provide their clutches with optimal conditions (Chalfoun and Martin 2007). However, many factors (abiotic and biotic) affect the costs of incubation and thus influence the incubation behaviour itself. Knowing these factors is essential as regards not only understanding the evolution of certain life history traits in birds, such as clutch size (Monaghan and Nager 1997, Reid *et al.* 2002a) or nest construction (Reid *et al.* 2002b) because of the costs that incubation entails and its effects on parental and nestling fitness, but also understanding the proximate effects underlying the high variation within and among species' incubation behaviours (Conway and Martin 2000b).

The aim of this study has been to describe the incubation behaviour of Blue (*Cyanistes caeruleus*) and Great tits (*Parus major*) in a Mediterranean area in central Spain. The climatic conditions in this region differ greatly from those in the majority of studies carried out with these species, usually at higher latitudes, at which the temperatures are colder and there is less daylight, features that are likely to influence species' incubation behaviour (Ricklefs and Brawn 2013). Furthermore, this is the first time that a comprehensive view and a description of the incubation behaviour of the Blue tit has been provided because, despite being one of the most frequently studied species and the fact that various studies tackle certain aspects of its incubating rhythm (Haftorn and Reinertsen 1985, Haftorn 1988, Tatner and Bryant 1993, Nilsson *et al.* 2008, Deeming and Du Feu 2011, Vedder *et al.* 2012), no study has as yet described its incubation behaviour. Both species inhabit the same area and share a similar ecology, i.e. both species breed in the same wooden nest boxes provided by us, construct similar nests made mostly of moss, and feed predominantly on lepidopteran caterpillars (Perrins 1991). This allowed us to compare their incubation behaviours which were controlled by means of confounding factors such as latitude, temperature, food supply, predation risk or nest type (Conway and Martin 2000b). This is, to the best of our knowledge, the first and most complete comparison of the incubation behaviour of two free-living species in the same area, which is necessary to clarify the proximate effects underlying the variation in incubation behaviour among species (Chalfoun and Martin 2007).

Methods

Study area

The present study was conducted in the locality of San Pablo de los Montes (39°32′44″N, 4°19′41″W. Toledo, central Spain). This region is situated on a mountain ridge called the Toledo Mountains which has a mean altitude of 1400 masl. The area has a continental Mediterranean climate, with a mean annual temperature of 12.9 ºC and a mean annual precipitation of 841.5 mm. However, the mean temperature during spring is 15.6 ºC. Its landscape comprises ten patches of oak (*Quercuspyrenaica*) forest in addition to Mediterranean scrubland consisting principally of butcher´s broom (*Ruscusaculeatus*), flax-leaved daphne (*Daphne gnidium*), dog-rose (*Rosa* sp.), common broom (*Cytisusscoparius*) and Heather (*Erica* sp.). In autumn 2010 we hung 330 wooden nest boxes on the branches of oak trees at a height of 2.5-3 meters, and at a distance of 30 m from each other.

Field work

The field work was carried out in the springs of 2012 and 2013. From 1st April (day = 1) onwards we visited each nest box on a daily basis in order to obtain the basic reproductive parameters of our population: laying date, clutch size, hatching date and brood size. Once we had found the first eggs laid, we measured the height of the nest with a rule, and then multiplied this height by the area of the nest box as a surrogate of the nest volume (nest box area: 11.5 x 13 cm). After the nestlings had fled, we removed the old nests from the nest boxes to ensure that every nest measured was of recent construction. When the nestlings were 8 days old we used spring traps to catch the females while feeding their nestlings measured their tarsus length with an electronic calibre (0.01 mm) and weighed them (0.01 g) with a portable electronic balance.

The tits' incubation behaviour was measured by placing two thermometers inside each nest box. One was located among the eggs (nest data-logger) while the other was stuck to an inner side of the nest box with the aid of brown medical tape (control data-logger), as reported in the work of Ardia *et al.* (2009). In total, we measured the incubation behaviour of 33 Blue tits and 30 Great tits in 2012 and 53 Blue tits and 22 Great tits in 2013 (see Table 1). The thermometers were

DS1922L Thermochron Data Logger i-buttons (size= 17.35 x 5.9 mm and weight= 3.3 g), which are able to record temperatures ranging between -40 ºC and +85 ºC with an accuracy ±0.5 ºC in the range -10 ºC to +65 ºC. All data-loggers were placed the day before the measurements were carried out and retrieved the day after. The nest data-loggers were also covered with brown medical tape to prevent the female from recognising them, as i this makes them less showy, and a transparent line was also tied to each one. We then introduced the nest data-logger through the bottom of the nest up to the lower side of the nest cup, just below the eggs, and tied the free end of the line to the front lid of the nest box to prevent the birds from throwing the i-button out of the nest box if they detected it. We validated the ability of the nest data-loggers to perceive sudden changes in temperature with a subset of 47 nests (25 Blue tit nests and 22 Great tit nests). A handy-cam (Sony DCR-SR290E) was placed in these nests and fixed with the aid of a tripod 5 m away from the nest box. The entrance of the nest box was then filmed for 2 hours at midday, when ambient temperatures are highest, on the same day that we measured the temperature with the data-loggers. Once we had obtained the video recordings and the temperature recordings, we compared the departures and entrances of the females (assessed with the handy-cams) with the changes in eggs-temperature (assessed using the nest data-loggers), to discover whether both methodologies coincided. Both nest and control data-loggers were programmed to start recording temperatures at 00:01 a.m. and finish two hours after sunset on the same day, obtaining one piece of temperature data every 11 sec. All i-buttons were located between days 6 and 11 of incubation; but 85 % of the incubation rhythms of Blue tits and 76% of those of Great tits were measured on days 7, 8 and 9 of incubation. Owing to the skew that different incubation days can set in the analyses (Cooper and Voss 2013), we included the incubation day as a co-variable in the analyses (see statistical analyses). We downloaded the temperature recordings using the Eclo Express-Thermo software 2007 (www.eclo.pt/expressthermo). The programme rhythm 1.0 (Cooper and Mills 2005) was used to convert the text files of the temperature into audio tracks, and were imported to the RavenPro 1.5

programme (Charif *et al.* 2010). We adjusted the programme rhythm 1.0 with a minimum off-bout duration of 2 min and a minimum off-bout change in temperature of 2 ºC, after a visual rechecking of some trial outputs in the RavenPro 1.5 programme. This programme was also used to measure the incubation rhythm variables, which were: 1. Onset - minutes after sunrise that the nest was first left; 2. Offset - minutes before sunset that the nest was last entered; 3. Active day - difference in minutes between the offset and the onset; 4. On-bout - average duration in minutes of the periods during which the females actively warmed the eggs; 5. Off-bout - average duration in minutes of the periods during which the females were off the nest; 6. No off-bout - number of times that the females left the nest per day and 7. Attentiveness - percentage of time in the active day during which the females were actively incubating. As a surrogate of the cooling rate of the clutches, we averaged the ratio between the decreases in temperature during each off-bout and the length of each off-bout. For the analyses we took into account only those incubation rhythms with a nest attentiveness of above 60%, which is far below the lowest nest attentiveness registered for Great tits (Álvarez and Barba 2014a). We decided to remove these atypical low attentiveness rhythms as we considered that they did not respond to the general incubation behaviour of the species, but responded rather to the energy constraints of the incubating females and were not thus representative of the general incubation rhythms in our population (Haftorn 1988, MacDonald *et al.* 2013).

Statistical analyses

We compared each variable of the incubation rhythm between species using a t-Student test. In order to discover the daily variation of the incubation rhythm in each species, we divided the daily hours into three blocks: morning: from 6 am to 11 am; afternoon: from 12 pm to 16 pm and evening: from 17 pm to 21 pm. We produced General Linear Models (GLM) in which the variables of the incubation rhythm were used as response variables, the year and day block was used as a categorical predictor and the day of measurement was used as a co-variable. The interaction between the two categorical factors was first included in all the models.

The effects of the ecological factors on the incubation behaviour of our population of tits were examined by creating General Linear Mixed Models (GLMM) in which the variables of the incubation rhythms were the response variables, the ID of the forest patch was the random term, and the year and species were categorical predictors. The factor ‘species’ was included in order to confirm that the differences between the species assessed in the first analysis with the t-Student test (see above) were maintained once they had been corrected using the various ecological factors described below. These ecological factors were included as co-variables and were: 1) ambient temperature, which is the most important abiotic factor to affect the incubation behaviour of bird species (Conway and Martin 2000a, Ardia *et al.* 2009), as it is negatively related to the cooling rate of eggs (Webb 1987, Turner 2002) and hence allows longer off-bouts (Conway and Martin 2000a). The temperature also negatively affected the costs of rewarming and maintaining the temperature of the egg (Haftorn and Reinersten 1985, Heij *et al.* 2008, Nord *et al.* 2010); 2) Daylight, which is also an important abiotic factor because it marks the time available to forage, which would otherwise be used to care for the eggs. Daylight, for example, limits attentiveness at lower latitudes because there are fewer hours (Ricklefs and Brawn 2013, Álvarez and Barba 2014a); 3) Incubation day (i.e. age of the embryo), because the cooling rate of the eggs increases as the embryos develop. The females are aware of these changes and consequently respond to them (Cooper and Voss 2013); 4) Clutch size, because the costs of warming larger clutches are relatively higher: Biebatch (1984) estimated that an increase of one egg in the clutch size of starlings (*Sturnus vulgaris*) increases energy expenditure by 3-5%, while Haftorn and Reinertsen (1985) estimated that the increase of three eggs in the clutch size of Blue tits increases energy expenditure by 18%. The clutch size also affects the cooling rate of eggs by reducing it (Reid *et al.* 2000b, Boulton and Cassey 2012); 5) Nest volume, because of its capacity to maintain an optimal microclimate around the eggs, in terms of temperature and water vapour (Reid *et al.* 2002b, Deeming 2011, Deeming *et al.* 2012) and 6) The females’ body condition, as the energy reserves of the females have been correlated with the length of the on-bouts (Weathers and Sullivan 1989, Chaurand and Weimerskirch 1994, Ardia *et al.* 2009).

The females' body condition was obtained from the standardised residuals of a linear regression in which the body weight was the dependent variable and the tarsus length was the predictor. All models were fitted with normal distribution after the visual inspection of the residuals (Zuur *et al.* 2009); and were initially constructed with all the explanatory terms fitted, including interactions. The final models were then selected by means of a backward procedure during which non-significant terms were progressively eliminated starting with the interactions. The final models were therefore those that explained the most variance with the fewest number of explanatory variables. All analyses were performed in R (R Core Team 2014) with the lme4 package (Bates *et al.* 2014). The mean ± SE (n) is shown in the results.

Results

Validation of the i-button data-loggers

The reliability of the i-buttons as regards assessing the incubation behaviour in our population of tits was validated by placing video-cams in front of the entrance hole to the nest boxes. In the case of both species, the total amount of time that the females spent outside the nest as assessed by the video recordings was highly correlated with the total amount of time that occurred between a sudden descent and the consecutive abrupt increase in temperature assessed by the i-buttons (Figure 1).

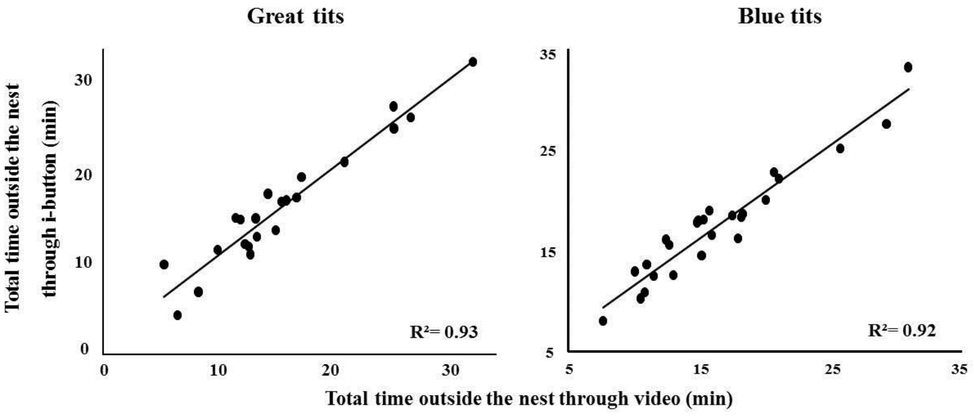


Figure 1. Lineal correlation between the total time outside the nest assessed by means of visual departures of the females using video recordings and sudden decreases in the clutch temperature assessed using i-buttons data-loggers placed in the nest cup among the eggs for two species of tits: Blue (*Cyanistes caeruleus*) and Great tits (*Parus major*).

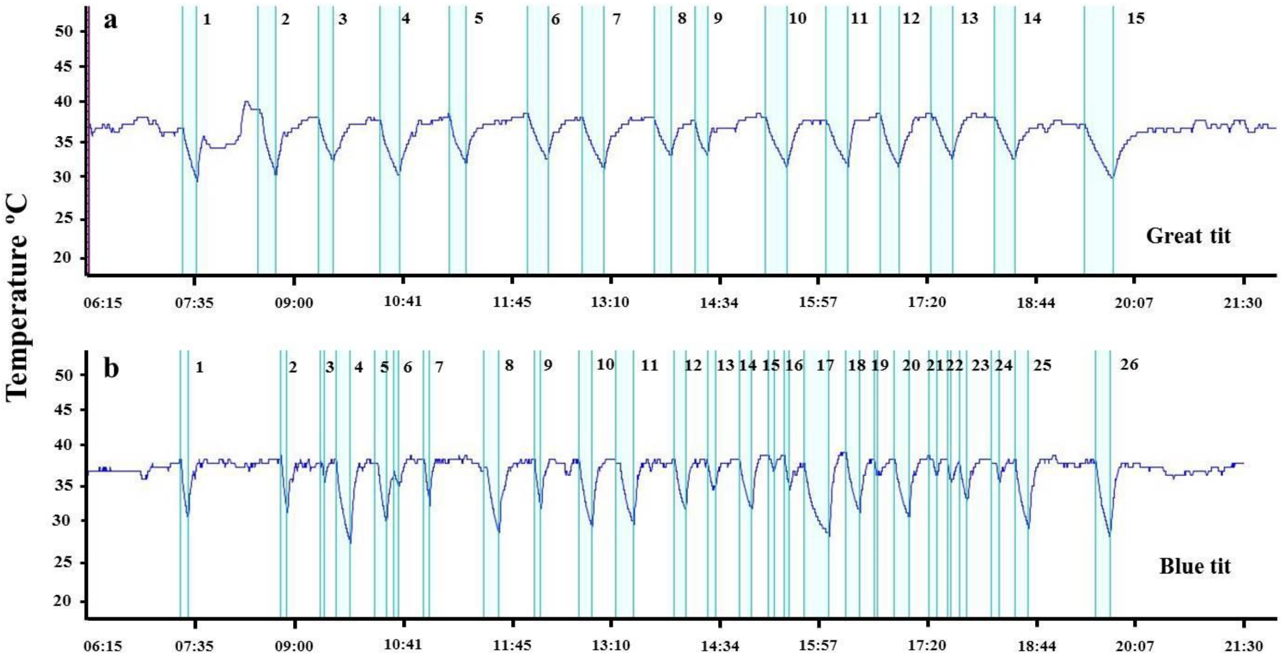


Figure 2. Diagrams of the incubation rhythm during daylight of a) Great tits (*Parus major*) and b) Blue tits (*Cyanistes caeruleus*) in a population in the Toledo Mountains (Toledo, central Spain). Incubation rhythms were obtained by placing i-button data-loggers among the eggs. Off-bouts are highlighted in blue, and their order number is shown in the upper part of the diagrams.

Incubation rhythm

The date on which the incubation rhythms were measured was the same for both species (GLMM: Estimate ± SE= -1.61 ± 1.83, $F_{1,\ 133}$= 0.78, *P*= 0.38) and in both years (GLMM: Estimate ± SE= -1.08 ± 1.81, $F_{1,\ 133}$= 0.36, *P*= 0.55). The incubation behaviour of the Blue and Great tits in the Montes de Toledo is shown in both Figure 2 and Table 1, itemised in the two years of study. In general, both species started the active day after sunrise, with the exception of 5% of the Blue tits (n= 86) and 10% of the Great tits (n= 53). All the females finished their active day before sunset (Table 1). There was no significant correlation between the onset and the offset for either of the two species (Blue tits: r= -0.08, n= 86, *P*= 0.45 and Great tits: r= 0.16, n= 53, *P*= 0.25). In both species attentiveness was negatively correlated with the mean duration of the off-bouts (Blue tits: r= -0.51, n= 86, *P*< 0.001 and Great tits: r= -0.56, n= 53, *P*<

0.001) and positively correlated with the duration of the on-bouts (Blue tits: r= 0.52, n= 86, *P*< 0.001 and Great tits: r= 0.50, n= 53, *P*< 0.001). However, the number of off-bouts negatively affected the attentiveness of Blue tits (r= -0.24, n= 86, *P*= 0.02) but not that of Great tits (r= -0.25, n= 53, *P*= 0.07). The temperature loss of nest i-buttons positively affected both species' attentiveness, that is, the higher the temperature loss the higher the attentiveness (Blue tits: r= 0.26, n= 86, *P*= 0.01 and Great tits: r= 0.30, n= 53, *P*= 0.03). The duration of both species' on-bouts was positively correlated with the duration of their previous off-bouts (Blue tits: r= 0.24, n= 1087, *P*< 0.001 and Great tits: r= 0.21, n= 594, *P*< 0.001).

Comparison between species

Both species proved to have very different incubation behaviours (Table 2). Blue tits had a significantly more active day than Great tits - around 16 min longer - although the onset and offset of each species did not differ significantly (Table 2). Besides the duration of the active day, the Blue tits' on- and off-bout periods proved to be significantly shorter than those of the Great tits, but the relationship between the duration of the on- and off-

Table 1. Description of the incubation behaviour of Blue tits (*Cyanistes caeruleus*) and Great tits (*Parus mayor*) in the Toledo Mountains (Toledo, Central Spain) during the two years of study. Year 2012: Blue tits, n= 33 and Great tits, n= 30; year 2013: Blue tits, n= 53 and Great tits, n= 22. Date is the calendar day on which the i-button data-loggers were placed (day 1= 1April).

	Blue tit				**Great tit**			
	2012		**2013**		**2012**		**2013**	
	Mean ± SE	**Range**	**Mean ± SE**	**Range**	**Mean ± SE**	**Range**	**Mean ± SE**	**Range**
Date	49.76 ± 1.60	28 - 67	51.28 ± 1.52	35 - 81	50.57 ± 2.24	28 - 69	47.82 ± 2.17	34 - 72
Time of first leaving	07:22 ± 0.04	06:51 – 08:00	07:22 ± 0.04	06:58 – 08:30	07:32 ± 0.06	06:55 – 08:29	07:21 ± 0.05	06:54 – 07:57
Time of last entering	20:41 ± 0.07	19:10 – 21:35	20:31 ± 0.06	19:28 – 21:24	20:27 ± 0.09	19:16 – 21:19	20:32 ± 0.11	19:25 – 21:33
Active day (min)	797.79 ± 5.52	697.02 - 844.78	792.57 ± 4.69	691.92 - 872.32	770.15 ± 5.59	711.18 - 822.79	789.33 ± 7.32	714.87 - 853.45
Onset (min)	24.49 ± 2.49	-5.58 - 52.7	21.79 ± 2.77	-9.00 - 83.10	35.09 ± 3.69	-7.73 - 75.52	19.01 ± 3.25	-4.30 - 55.07
Offset (min)	-45.29 ± 5.75	-133.03 - 17.52	-54.49 ± 3.60	-122.68 - (-0.73)	-62.09 ± 5.90	-124.58 - (-6.23)	-55.21 ± 6.06	-117.62 - (-9.4)
On-bout (min)	22.09 ± 0.84	12.38 - 31.42	22.97 ± 0.82	12.73 - 38.18	39.09 ± 1.64	23.97 - 55.95	33.82 ± 2.20	18.68 - 57.15
Off-bout (min)	9.04 ± 0.41	5.53 - 15.75	8.58 ± 0.25	4.03 - 13.20	15.97 ± 0.74	8.30 - 29.92	11.41 ± 0.43	8.02 - 14.65
No Off-bouts	27.45 ± 1.03	19 - 43	26.94 ± 0.81	16 - 41	15.27 ± 0.56	11 - 21	19.23 ± 0.96	11 - 29
Attentiveness (%)	69.89 ± 0.85	60.50 - 79.25	71.47 ± 0.76	60.27 - 81.43	69.22 ± 1.09	62.39 - 80.62	72.83 ± 1.17	63.13 - 80.69

bouts was the same for both species: the duration of the on-bout was about 2.5 times the duration of the off-bout (Table 2). Blue tits compensate for the shorter duration of their bouts by having a significantly higher number of them - about 10 off-bouts per day more than Great tits, which eventually leads to the same attentiveness in both species (Figure 2). Another important trait that significantly differed between species was the cooling rate of their eggs, which was greater in Blue tits (Table 2; Figure 3). With regard to daily variation in the incubation rhythm, the interaction between categorical predictors (year and day block) did not prove significant in any of the models, and neither did the co-variable (day of measurement). Only Great tits were affected by the year as regards the duration of their bouts and the number of them, which were shorter and more numerous in 2013 ($GLM_{On\text{-}bout}$: $F_{1, 157}$= 6.05, P= 0.01; $GLM_{Off\text{-}bout}$: $F_{1, 155}$= 32.03, P< 0.001 and $GLM_{No\ Off\text{-}bouts}$= $F_{1, 155}$= 55.40, P< 0.001; see following section and Table 4)

Table 2. Results of the t-Student tests comparing the incubation rhythm of Blue tits (*Cyanistes caeruleus*) n= 86, and Great tits (*Parus major*) n= 52, in the Toledo Mountains (Toledo, Central Spain). Significant results are highlighted in bold type.

Response variable	Blue tit mean ± SE	Great tit mean ± SE	t-value	d.f.	*P*
Onset (min)	22.84 ± 1.95	28.29 ± 2.75	-1.66	136	0.10
Offset (min)	-50.96 ± 3.15	-59.18 ± 4.25	1.57	136	0.12
Active day (min)	794.60 ± 3.57	778.27 ± 4.62	2.81	136	**<0.01**
On-bout (min)	22.63 ± 0.60	36.86 ± 1.36	-10.89	136	**<0.001**
Off-bout (min)	8.76 ± 0.22	14.04 ± 0.56	-10.23	136	**<0.001**
No. Off-bout	27.14 ± 0.63	16.95 ± 0.58	10.93	136	**<0.001**
Temperature loss (ºC)	0.39 ± 0.03	0.25 ± 0.02	3.10	136	**<0.01**
Attentiveness %	70.87 ± 0.57	70.75 ± 0.83	0.09	136	0.93

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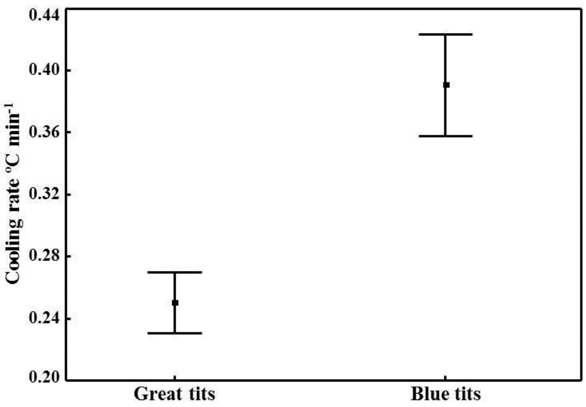

Figure 3. Difference between the cooling rate of clutches of Great tits (*Parus major*) and Blue tits (*Cyanistes caeruleus*). Vertical bars indicate SE.

Apart from the yearly variation in Great tits, there were some differences between species as regards their daily incubation behaviours (Table 3). Blue tits performed significantly shorter on-bouts in the morning than during the rest of the day, while the duration of the Great tits' on-bouts did not vary throughout the day (Table 3). With regard to the off-bouts, both species showed a similar pattern. The duration of the off-bouts continued to lengthen as the day progressed; and the frequency with which these off-bouts took place was the opposite, with the highest number of off-bouts occurring in the morning, and the lowest number occurring in the evening (Table 3). However, there were greater size differences as regards the duration of the Great tits' off-bouts in the evening as opposed to the rest of the day in comparison to those of the Blue tits (Table 3). This may have led to the significant decrease in the Great tits' nest attentiveness in the evening, while the Blue tits did not modify their attentiveness throughout the day (Table 3).

Table 3. Results of the GLMs analysing the daily variation in the incubation behaviour of Blue tits (*Cyanistes caeruleus*) and Great tits (*Parus major*). Results are Mean ± SE. Average ranks of levels with different superscripts are significantly different (Tukey HSD test < 0.05); factors: Mo= Morning, Af= Afternoon and Ev= Evening. Significant results are highlighted in bold type

	Morning	**Afternoon**	**Evening**	**F**	**d.f.**	***P***	**Tukey HSD test**
			Blue tits				
On-bout (min)	23.87 ± 0.87	27.42 ± 1.32	28.36 ± 1.69	3.08	243	**0.04**	Mo^1 < Ev^2 , Af$^{1, 2}$
Off-bout (min)	8.23 ± 0.26	9.47 ± 0.33	10.15 ± 0.36	9.16	243	**<0.001**	Mo^1 < Af^2 =Ev^2
No Off-bouts	9.42 ± 0.29	8.98 ± 0.30	7.05 ± 0.29	18.93	243	**<0.001**	Mo^1 = Af^1 >Ev^2
Attentiveness (%)	71.41 ± 0.70	72.95 ± 0.83	71.70 ± 0.84	3.29	243	0.32	Mo^1 = Af^1 =Ev^2
			Great tits				
On-bout (min)	39.82 ± 2.35	39.61 ± 2.14	37.82 ± 2.30	0.25	147	0.78	Mo^1 = Af^1 =Ev^2
Off-bout (min)	12.51 ± 0.76	14.54 ± 0.65	16.24 ± 0.85	7.99	147	**<0.001**	Mo^1 < Ev^2 , Af$^{1, 2}$
No Off-bouts	6.32 ± 0.30	6.08 ± 0.25	4.55 ± 0.24	14.83	147	**<0.001**	Mo^1 = Af^1 >Ev^2
Attentiveness (%)	71.51 ± 1.15	72.14 ± 0.99	68.32 ± 1.39	3.16	147	**0.04**	Mo^1 = Af^1 >Ev^2

Incubation behaviour in an ecological frame

The Blue tits' behaviour did not in either year, but the Great tits' did. The Great tits' onset occurred later in 2012 than it had in 2013 (GLMM: Estimate ± SE= -18.34 ± 5.19, t= -3.53, *P*< 0.001; Table 1), and it was also later than that of the Blue tits in 2012 (GLMM: Estimate ± SE= 14.60 ± 4.53, t= 3.23, *P*= 0.002; Table 1). The onset of both species was, however, the same in 2013. The temperature negatively affected the onset of both species (Estimate ± SE= -0.95 ± 0.25), that is, on colder days the tits left the nest for first time significantly later (Table 4) while daylight positively affected the onset (Estimate ± SE= 0.27 ± 0.09). With regard to the offset, there was a significant interaction between the incubation day and the species (Table 4). The offset of Blue tits was positively affected by the incubation day, signifying that as they got closer to the hatching day, they entered the nest for the last time significantly later and consequently also significantly lengthened their active day (Blue tits: GLMM: Estimate ± SE= 9.06 ± 2.27, t= 3.98, *P*< 0.001 and GLMM: Estimate ± SE= 10.38 ± 3.02, t= 3.44, *P*< 0.001 for the offset and the active day respectively), while Great tits did not (Great tits: GLMM: Estimate ± SE= -0.97 ± 2.10, t= -0.46, *P*= 0.64 and GLMM: Estimate ± SE= -3.13 ± 2.95, t= -1.06, *P*= 0.29 for the offset and the active day respectively). Daylight negatively affected both species' offset (Estimate ± SE= -0.63 ± 0.17), as did nest volume (Estimate ± SE= -0.04 ± 0.01) (Table 4). With regard to the active day, there was also the same interaction between species and incubation day as in the offset model (Table 4), and its length was also negatively affected by nest volume in the case of both species (Estimate ± SE= -0.05 ± 0.01) (Table 4).

The duration of the Great tits' on-bouts was significantly different in the two years studied since it was shorter in 2013. However, the duration of the Blue tits' on-bouts was the same in both years (significant interaction between species and year in Table 4; GLMM: Estimate ± SE= -6.34 ± 2.20, t= -2.88, *P*= 0.005 and GLMM: Estimate ± SE= 0.33 ± 1.65, t= 0.20, *P*= 0.84 for Great and Blue tits, respectively). The temperature negatively affected the duration of the on-bouts (Estimate ± SE= -0.22 ± 0.11; Table 4). As with the on-bouts, the Great tits' off-bouts were significantly shorter in 2013,

while the Blue tits' were not (significant interaction between species and year in Table 4; GLMM: Estimate ± SE= -4.80 ± 0.73, t= -6.58, *P*< 0.001 and GLMM: Estimate ± SE= -0.44 ± 0.55, t= -0.81, *P*= 0.42 for Great and Blue tits, respectively). But in this case, the temperature positively affected both the duration of both species' off-bouts (Estimate ± SE= $9.82e^{-2}$ ± $3.40e^{-2}$) and the female's body condition (Estimate ± SE= $3.99e^{-1}$ ± $1.91e^{-1}$). With regard to the number of off-bouts, those of the Great tits were again significantly different in the two years studied, with a great number of off-bouts in 2013, while the Blue tits did not differ (significant interaction between species and year in Table 4; GLMM: Estimate ± SE= 4.95 ± 1.61, t= 3.07, *P*= 0.003 and GLMM: Estimate ± SE= -0.12 ± 1.21, t= -0.10, *P*= 0.92 for Great and Blue tits, respectively).

The cooling rate of the clutches was significantly different for the two species (Table 2 and 4) and was negatively affected by the temperature and the clutch size (Estimate ± SE= -$8.81e^{-3}$ ± $3.56e^{-3}$and -$2.69e^{-2}$ ± $1.10e^{-2}$ respectively). Finally, attentiveness was negatively affected by the temperature (Estimate ± SE= -$4.91e^{-3}$ ± $7.37e^{-4}$) and

Table 4. Results of the GLMMs analysing the effects of the year, species [Blue (*Cyanistes caeruleus*) and Great tits (*Parus major*)] and the ecological factors on the incubation behaviour of tits in the Toledo Mountains (Toledo, central Spain). All the interactions between species and each explanatory variable were initially included in the models, but only significant interactions are shown. Mean effects of the factors or co-variables included in the significant interactions were not considered (even when *P*< 0.05). The ID of the forest patch was included in all models as the random term. The degrees of freedom in all models were 138. Significant results are highlighted in bold type.

	Onset		**Offset**		**Active day**		**On-bout**		**Off-bout**		**No. Off-bout**		**Temperature loss**		**Attentiveness**	
	Test	*P*	Test	*P*	Test	*P*	Test	*P*	Test	*P*	Test	*P*	Test	*P*	Test	*P*
Intercept		0.02		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		n.s.
Year	F= 14.57	<0.001	F= 0.59	0.45	F= 0.49	0.48	F= 4.71	0.03	F= 24.59	<0.001	F= 3.43	0.07	F=0.28	0.60	F=1.62	0.21
Species	F= 2.14	0.15	F= 5.77	0.02	F= 6.21	0.01	F= 127.21	<0.001	F= 126.49	<0.001	F= 114.26	<0.001	F=7.23	**0.008**	F=0.24	0.63
Incubation day	F= 0.07	0.79	F= 4.73	0.03	F= 3.27	0.07	F= 0.51	0.48	F= 0.35	0.54	F= 0.65	0.42	F=1.47	0.23	F=2.24	0.14
Temperature	F= 13.65	**<0.001**	F= 2.67	0.11	F= 0.06	0.81	F= 4.11	**0.04**	F= 19.68	**<0.001**	F= 1.22	0.27	F=6.09	**0.01**	F=44.43	**<0.001**
Day-light	F= 8.76	**0.004**	F= 15.24	**<0.001**	F= 0.81	0.37	F= 1.19	0.28	F= 2.20	0.14	F= 0.45	0.46	F=0.62	0.43	F=10.68	**0.001**
Clutch size	F= 0.44	0.51	F= 0.86	0.35	F= 0.99	0.32	F= 0.82	0.37	F= 1.62	0.21	F= 0.77	0.38	F=5.95	**0.02**	F=6.37	**0.01**
Nest volume	F= 1.38	0.24	F= 7.62	**0.003**	F= 13.17	**<0.001**	F= 2.32	0.13	F= 3.67	0.07	F= 0.22	0.64	F=0.72	0.79	F=0.62	0.43
Body condition	F= 1.81	0.18	F= 0.18	0.67	F= 1.96	0.16	F= 0.73	0.39	F= 9.12	**0.003**	F= 0.61	0.44	F=0.75	0.39	F=1.66	0.20
Sp*Year	F= 5.24	**0.02**					F= 4.21	**0.02**	F= 70.59	**<0.001**	F= 4.75	**0.01**				
Sp*Inc. Day			F= 6.35	**0.002**	F= 6.48	**0.002**										

positively affected by daylight and clutch size ((Estimate ± SE= $9.81e^{-4}$ ± $3.00e^{-4}$ and $6.48e^{-3}$ ± $2.57e^{-3}$, respectively).

Discussion

Upon viewing the videos we confirmed that the sudden decreases in the temperature of the eggs were indeed due to the departures of the incubating female (Figure 1). The use of the i-button methodology to assess the incubation behaviour of these tit species therefore proved to be reliable, as has been shown with other species (Ardia *et al.* 2009, Hanna and Emmery 2009, Zangmeister *et al.* 2009, O´Connor and Ritchison 2013, Meugeot *et al.* 2014).

The incubation behaviour of our population of Great tits resembles that described for other southern populations in Europe at a similar latitude (Álvarez and Barba 2014a), characterised by the fact that they have a relatively low attentiveness and shorter active day in relation to the more northern populations. This is mainly because there are fewer hours of daylight at lower latitudes and less time is consequently available to care for the eggs, although the time needed to forage presumably remains constant, which results in a lower attentiveness during the day at lower latitudes (Álvarez and Barba 2014a, Shaw and Cresswell 2014). However, the birds compensate for the relative lower dedication during the day with a longer attendance overnight, signifying that the time spent heating the eggs after the day is similar throughout the latitudinal range of species (Ricklefs and Brawn 2013). The mean duration of the on- and off-bouts in our population of Great tits was the longest registered for this species; more specifically, the on-bouts were on average two minutes longer than the duration of those described for the population of Wytham Woods (Cresswell and Mcleery 2003), while the off-bouts were on average two minutes longer than those described for the population of Sagunto (Álvarez and Barba 2014a). The relatively long off-bouts in southern populations when compared to northern ones (present study and Álvarez and Barba (2014a) could reflect a general pattern, because the warmer temperatures at lower latitudes may allow females to have longer off-bouts (Conway and Martin 2000b), as the main factor to influence incubation is temperature (Haftorn 1984; Weathers and

Sullivan 1989; Conway and Martin 2000a). In our population the duration of a particular on-bout was positively correlated with the duration of the previous off-bout, as occurred with the Norwegian population studied by Haftorn (1981), which may explain the relatively long on-bouts periods registered in this study. This is probably owing to the fact that the female is able to intake a higher quantity of food during long off-bouts and then use this energy to spend more time caring for the eggs (Conway and Martin 2000b, Kovarík *et al.* 2009). The relatively higher temperatures at lower latitudes may also account for this variation, as the costs of maintaining the eggs at the optimal temperature are negatively correlated with ambient temperature (Haftorn and Reinertsen 1985, 1990, Nord *et al.* 2010). Nonetheless, although Álvarez and Barba (2014a) did not provide the correlation between the duration of the off-bouts and the subsequent on-bouts in their study, the huge difference between the duration of our population's on-bouts and that of Sagunto is striking (Álvarez and Barba 2014a). The duration of the on-bouts is set by the females cuing on their own energy reserves, rather than on egg temperature (Chaurand and Weimerskirch 1994, Reid *et al.* 1999, Ardia *et al.* 2009). This variation may therefore depend on differences in habitat quality and consequently on the availability of energy in terms of food supply (Bryan and Bryant 1999), because the quantity of food ingested over a given time depends on foraging success (Londoño *et al.* 2008). In fact, the study area in Sagunto is an extensive orange (*Citrus aurantium*) monoculture, which constitutes a suboptimal habitat for tits and the Great tits breeding there are thus limited as regards the availability of food (Barba *et al.* 2004). This is not the case of our population, which breeds in a deciduous oak forest, considered the preferred Mediterranean habitat for tits (Atiénzar *et al.* 2012). It is therefore feasible that the duration of the on-bouts in Sagunto may be constrained by energy budgets (Weathers and Sullivan 1989, Eikenaar *et al.* 2003, Londoño *et al.* 2008). What did vary in our population with regard to the others was the number of off-bouts, which were rather fewer. However, the longer duration of the off-bouts may compensate for this low number, and the attentiveness was even higher than that of Sagunto, although it is still lower than of

more northern populations (Álvarez and Barba 2014a). Other traits that were different in our population and that of Sagunto was the time of the first leaving and the time of last entering with regard to sunrise and sunset, respectively. In our population, the onset was 21 minutes later and the offset was about 15 minutes earlier than those of the population of Sagunto; nonetheless the active day was about 28 minutes longer. This variation is probably owing to differences in the phenology between these two populations. Sagunto is a much more thermic area situated at 30 masl and only fifteen nests were used in the study, with laying dates that comprehended between day -5 and 15. Our population, however, is situated at around 1400 masl and we used 53 Great tit nests, comprehending a period between days 5 and 65. This led to an average delay in the phenology of nearly 30 days between our population and that of Sagunto, signifying that there are 72 more minutes of daylight in San Pablo de los Montes. As occurs at higher latitudes (Haftorn 1981, Álvarez and Barba 2014a), the Great tits in our population delay their onset and advance their offset as the days become longer (Table 4). This is why the increase in the active day is not as marked as the differences between the increases in daylight in Sagunto and our study area, as occurs throughout the latitudinal gradient of species (Haftorn 1988).

Unlike the case of Great tits, no study has described the incubation behaviour of Blue tits and we were thus unable to compare our study with that of other populations of Blue tits. Nonetheless, the comparison with the incubation rhythm of Great tits helped to clarify some proximate effects behind the large interspecific variation in the incubation behaviours (Conway and Martin 2000b, Chalfoun and Martin 2007). Both Blue and Great tits had very different incubation strategies (Table 2). However, both species' attentiveness was very similar, which reinforces the idea that the global pattern in nest attentiveness, with a relatively high clutch attendance in northern temperate bird species in comparison with tropical and southern temperate species, is owing to evolutionary processes (Martin *et al.* 2000, Chalfoun and Martin 2007). What did vary between species was the duration of the on- and off-bouts and the number of them. These traits express the adjustments that incubating females make

in order to balance their needs and the requirements of the developing embryos (Cooper and Voss 2013). In this respect, having less but longer off-bouts, rather than many short ones, may be beneficial for females in terms of energy savings, as the number of times that they have to rewarm the eggs decreases and consequently the associated costs (Conway and Martin 2000b). However, Blue tits may have to constrain this strategy because their eggs are smaller (about 40% less volume than those of Great tits; our own unpubl.data), signifying that their cooling rate increases (Turner 2002, Chalfoun and Martin 2007) and they have less resources that could protect them from inclement conditions (Martin 2008). If they do not wish the temperature of their eggs to fall below the Physiological Zero Temperature (PZT) they must therefore return to their nests more quickly to rewarm them (Haftorn 1988, Conway and Martin 2000a). The higher cooling rate of Blue tit clutches can be appreciated in Figure 2, which shows a sharper decline of temperature in the incubation rhythm of Blue tits in comparison with that of Great tits. The shorter off-bouts may also constrain the intake of food and possibly the duration of the following on-bout (Eikenaar *et al.* 2003, Londoño *et al.* 2008). The limitation as regards the duration of the Blue tits' off-bouts may force them to carry out a greater number of them in order to achieve their energy needs. This could lead to the longer active day of Blue tits with regard to Great tits (Table 2). Although both species showed similar daily patterns, there were some differences (Table 3). The Blue tits' shorter off-bouts, together with the fact that their body mass is about half that of Great tits may, be a greater handicap as regards this species' continuous nightly incubating period. This may result in a significantly lower duration of their on-bouts in the morning (Table 3), while the opposite probably occurs during the evening with the Great tits, when the warmer temperatures inside nest boxes at this time, together with the relative lower cooling rate of their clutches, probably allow them to significantly diminish their attentiveness in the evening (Table 3).

With regard to the factors that influenced the incubation rhythm in both species, one notorious aspect was the yearly variation in Great tits when compared to that of Blue tits (Table4). In 2012 the Great tits delayed their onset by

about 15 minutes with regard to 2013 and the onset of Blue tits in both years, and they also significantly lengthened their bouts and significantly reduced their number (Table 1). The reason for this may be the fact that the conditions in 2012 were warmer than in 2013 (mean temperature inside nest boxes provided by control data-loggers were in year 2012= 19.90 ± 0.83 ºC, and in year 2013= 16.17 ± 0.62 ºC; t-value= 2.86, d.f.= 136, *P*= 0.005). This difference of about four degrees more in 2012 may have reduced the costs of incubating (Bryan and Bryant 1999, Nord *et al.* 2010), thus allowing the Great tits to start their activity later and perform longer bouts. However, the Blue tits' response may again have been constrained because of the higher cooling rate of their clutches, which may impede the lengthening in excess of their off-bouts. The relatively lower cooling rate associated with bigger eggs may therefore allow species to be more flexible in their incubating behaviour, while the relative higher resources in bigger eggs may also protect the embryo from inclement conditions to a higher degree (Martin 2008) thus possibly explaining the differential effect that the incubation day had on the incubation rhythm of each species (Table 4). Cooper and Voss (2013) showed that the cooling rate of eggs increases as the embryo ages (Boulton and Casey 2012, Cooper and Voss 2013), but it is possible that Blue tits are more susceptible to this thermal change of eggs than are Great tits owing to their smaller eggs.

The factor that affected most traits in both species' incubation behaviour was, in addition to these interactions, temperature (Table 4). This is mainly because ambient temperature is closely related to the costs of incubation (Haftorn and Reinertsen 1985, Heij *et al.* 2008, Nord *et al.* 2010) and the cooling rate of eggs (Webb 1987, Conway and Martin 2000a, Turner 2002). In general terms, the temperature negatively affected attentiveness. This effect has been seen in both other observational studies (Haftorn 1981, 1988, Matysioková and Remeš 2010) and thermal manipulation experiments (Haftorn and Reinertsen 1990, Londoño *et al.* 2008, Álvarez and Barba 2014b). This reduction in attentiveness was achieved by a significant shortening of on-bouts and a significant lengthening of off-bouts (Table 4). The temperature also negatively affected the onset of tits, so at

warmer dawns the females left the nest for first time earlier. This is probably because the costs of the continuous nightly incubating period are expensive, and even more so for those populations that inhabit southern latitudes at which this period is longer (Ricklefs and Brawn 2013). What is more, females could leave the nest earlier to forage because the cooling rate is lower in these warmer conditions (Conway and Martin 2000a). Daylight positively affected the onset and attentiveness, and negatively affected the offset of tits (Table 4). This temporal pattern in our study area is similar to that found in a latitudinal gradient, at which birds compensate for the fewer nightly hours by spending more time in the nest in the early morning and in the late evening; the energy savings made during the shorter nights are employed during the day, thus increasing attentiveness, but the time spent heating the eggs after 24 h remains constant (Ricklefs and Brawn 2013, Álvarez and Barba 2014a). The clutch size negatively affected the cooling rate of clutches; this result was previously shown by Reid *et al.* (2000b) and Boulton and Cassey (2012), and is explained by the fact that the neighbouring eggs buffer the heat loss caused by convection in large clutches. Nonetheless, the clutch size positively affected attentiveness (Table 4), a result also obtained by Matysioková and Remeš (2010), probably because the increasing energy demands of large clutches (Biebach 1984, Haftorn and Reinertsen 1985, Reid *et al.* 2002a) may force females to invest more time on the eggs. Nonetheless, three other possible causes may be operating: 1. In spite of the beneficial effect of the reduced cooling rate in large clutches, more time is needed to rewarm these clutches (Reid *et al.* 2000b), which could increase the duration of the on-bouts, although not significantly (Table 4), but at the end of the active day accounts for the increased attentiveness in those clutches; 2. It may be that, when stimulated by larger clutches, the males feed the incubating females more frequently (Sanz 1996, Heij *et al.* 2008), which may help to increase attentiveness (Lifjeld *et al.* 1987) and 3. As stated previously, incubating large clutches entails severe increasing costs for females and may constrain the optimal clutch size (Monaghan and Nager 1997). Thus, only the best females may have been able to lay large clutches and provide them with optimal care.

With regard to the nest volume, it negatively affected the active day because those females with bigger nests entered the nest for the last time relatively earlier (Table 4). It could be that the enhanced thermoregulatory properties of big nests allowed females to regulate the nest microclimate more efficiently (Reid *et al.* 2000a) and start the continuous nightly incubation period earlier than in small nests, where females seem to need to capitalise more on daylight in order to forage. Another unexpected result was that female body condition positively affected the duration of the off-bouts rather than the on-bouts, which have been seen to be dependent on the female's hunger levels (Chaurand and Weimerskirch 1994, Reid *et al.* 1999). It could be that females with a better body condition are able to rewarm and provide their clutches with optimal conditions more efficiently, and are thus able to leave the eggs to cool more frequently so as to forage more extensively, as rewarming these cooled clutches is not such a big deal for them.

Conclusions

This study reinforces the latitudinal pattern of nest attentiveness suggested in other studies (Ricklefs and Brawn 2013, Álvarez and Barba 2014a) and for first time describes the incubation behaviour of Blue tits. The most remarkable result is that an endogenous cause i.e. egg size, constrains the incubation behaviour of species. This suggests that bigger eggs, owing to their relatively lower cooling rate, and a higher number of resources allow more flexible incubation rhythms and thus help embryos to endure inclement condition and females to benefit more from favourable conditions.

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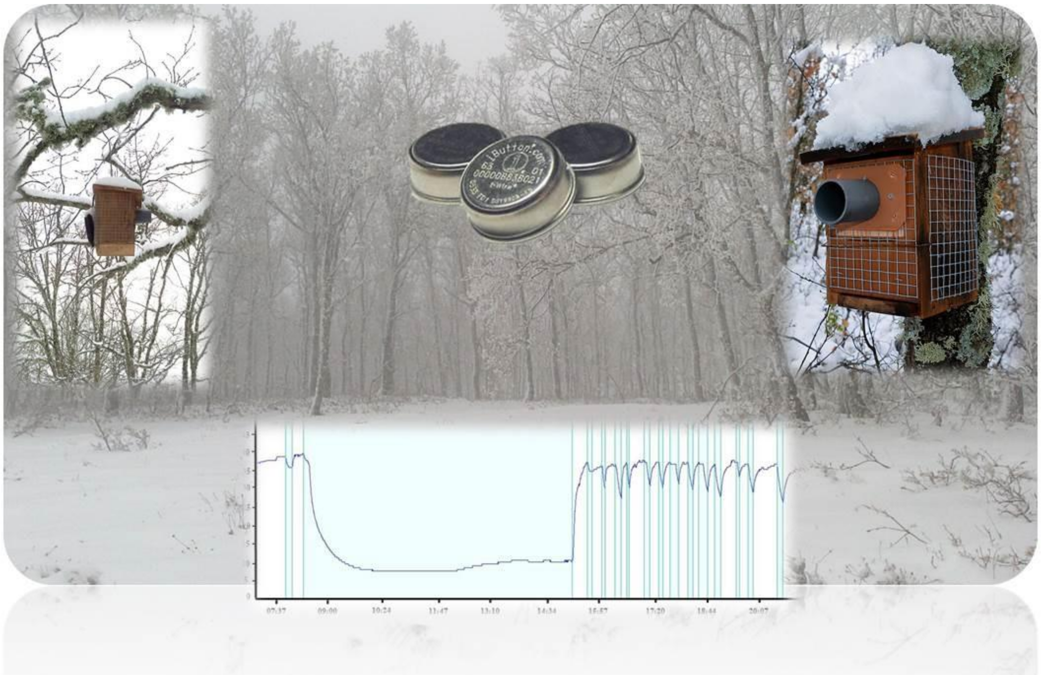
CAPÍTULO VI

¿TIENE EFECTOS ECOLÓGICOS LA REALIZACIÓN DE RECESOS EXTENSOS DURANTE LA INCUBACIÓN EN DOS POBLACIONES DE PÁRIDOS?



Bueno-Enciso J, Barrientos R, Ferrer ES y Sanz JJ. Do extended recesses during incubation in two hole-nesting birds carry fitness costs?

Under review

Abstract

The occurrence of extended recesses, although with a low prevalence, seems to be part of the normal incubation behaviour of passerine species. However, these recesses have been treated as outliers in the majority of incubation studies and thus overlooked. Nonetheless, due to their ubiquity in natural passerine populations, it is important to evaluate them in an ecological context and identify their possible fitness effects. With this aim and over two years, we monitored the incubation behaviour of Blue (Cyanistes caeruleus) and Great tits (Parus major) through the use of i-button data-loggers in central Spain. We classified a recess in the incubation behaviour of tits as an 'extended recess' when recesses lasted more than four times the mean recess duration of each species in each year. Thereafter, we classified the nests based on the type of incubation pattern exhibited, as either a 'neglect incubation pattern' (if they incurred at least one extended recess) or a 'normal incubation pattern' (with no extended recesses). The prevalence of neglect incubation patterns was higher in the year in which females presented lower body condition and in the woodcrete nestboxes. Blue tits showed neglect incubation patterns more frequently than Great tits, and in both species, these patterns usually occurred after a cold and long night. Nest attentiveness over the entire incubation pattern was lower when at least one extended recess was performed as was mean temperature loss of those clutches. The incubation period was about 4 days longer in the nests that underwent at least one extended recess, although no other fitness trait was significantly affected by the type of incubation pattern. Overall, these results suggest that extended recesses are performed due to energy constraints on the incubating females in passerine species and that they could entail fitness costs.

Introduction

Bird embryos are ectothermic, and thus depend entirely on the heat provided by their parents to maintain their optimal developmental temperature (White and Kinney 1974). Parents provide an adequate microclimate for the eggs through nest attentiveness (DuRant et al., 2013). In those species in which the female incubates the eggs alone, they must leave the nest regularly for feeding and self-maintenance (Deeming 2002). Consequently, their daytime incubation pattern is a succession of drops and increases in the temperature of the eggs, relative to the female departures and entrances to the nest. The duration of the recesses can be constrained by the temperature loss of the eggs. In passerine species, females try to maintain the egg temperatures within the assumed physiological requirements for embryonic development (Haftorn 1988). Thus, they should return to reheat the eggs before their temperature falls below the physiological zero temperature (PZT) (Conway and Martin 2000). On the other hand, the duration of the on-bouts is constrained by energy budgets of the females (Ardia et al. 2009). As a consequence, the incubation pattern of species with uniparental incubation reflects a trade-off between the self-maintenance of the females and the care of the eggs (Cooper and Voss 2013).

One scenario that may indicate a complete switch to self-maintenance in the females in the above-mentioned trade-off is when females abandon their clutches for extremely long periods of time (Nord and Williams 2015). During these extended recesses the egg temperature falls far below the PZT (reviewed in MacDonald et al. 2013). For some species this behaviour seems to be part of their normal incubation routine, such as in the case of seabirds (Boersma and Wheelwright 1979; Blight et al. 2010). Nonetheless, for passerine species this behaviour has been related to the energy constraints of parents, usually under prolonged inclement weather conditions (Haftorn 1988; MacDonald et al., 2013). The extreme of this situation is when incubating birds enter the so-called ‘emergency life history stage’, and are forced to abandon the current reproduction attempt when further incubation investment would result in the immediate death of adults (Wingfield 2003).

There have been several studies examining cases of slight but continuous energy costs in incubation, such as during reductions in the ambient temperature (e.g. Hepp et al. 2006; Nord and Nilsson 2011). However, the fitness costs to young due to extended recesses and the associated prolonged periods of hypothermia experienced by the embryos are poorly understood (Nord and Williams 2015). Most studies assessing fitness consequences of egg neglect during incubation have found that extended recesses do not impair the hatchability of eggs. Thus, it has been suggested that passerine embryos are well-adapted to these long periods of cooling (Haftorn 1988; Nord and Williams 2015). However, MacDonald et al. (2013) found that the hatchability of eggs in Horned larks (Eremophila alpestris) was reduced by about 10 % in those clutches that suffered at least one extended recess. Other fitness consequences of egg neglect during incubation include an extension of the period of incubation, because the development of embryos stops below the PZT (Webb 1987). Longer incubation periods increase the risk of predation (Martin 2002; Martin et al., 2007), but also the energy expenditure of the embryos in their development (Eiby and Booth 2009), such that their phenotype can be negatively affected (DuRant et al., 2013). Despite the fact that egg neglect during incubation has been suggested to be a normal behaviour pattern in small passerines (Haftorn 1988), these extended recesses have been considered outliers in the majority of general incubation studies with passerines, and thus overlooked (MacDonald et al., 2013). However, due to their ubiquity in natural passerine populations, it is important not only to evaluate such extended recesses in an ecological context, but to identify their consequences for embryonic development, growth and post-hatching performance (Nord and Williams 2015).

With this purpose, we monitored the incubation behaviour in two populations of Blue tits (Cyanistes caeruleus) and Great tits (Parus major), both female-only incubation species, breeding in a Mediterranean area in central Spain for two years. Our aim was to determine the prevalence of extended recesses in such populations, determine the causes that trigger this behaviour and its fitness consequences. We predict that: 1. The prevalence of extended recesses will be higher in colder years, because of the extra costs that low temperatures impose on incubation (Haftorn and Reinertsen 1985; Nord et al., 2010); 2. The prevalence of extended recesses will be lower in Great tits than in Blue tits, because the greater weight of Great tit clutches reduces the cooling rate of their clutches in comparison with those of Blue tits (Boulton and Cassey 2012). A slower cooling rate reduces the costs of incubation (Webb 1987; Conway and Martin 2000). 3. Those clutches that suffer extended recesses may incur in some fitness cost (Nord and Williams 2015).

Methods

Study area

The present work was conducted in the locality of San Pablo de los Montes (39°32′44″N, 4°19′41″W), which is situated in a mountainous region in southern Toledo province (central Spain). The study area consisted of ten oak (Quercus pyrenaica) forest patches with a mean altitude of 1400 masl. The region has a continental Mediterranean climate, characterized by pronounced summer droughts and a high daily thermal oscillation, with mean values of annual rainfall of 700-800 mm. In all forest patches, we attached wooden nestboxes to oak branches at 2-3 m above the ground, separated from each other by 30 m. In 3 forest patches we also hung 20 woodcrete nestboxes at 30 m intervals next to the wooden nestboxes at the same height and density. The data on the material, shape and size of these nestboxes can be found in García-Navas et al. (2008). All nestboxes were placed in the study area in the winter of 2010.

Field work

During the breeding seasons of 2012 and 2013 (day 1= 1 April), we inspected the nestboxes daily to obtain the basic reproductive parameters of our population of tits, such as laying date, clutch size, hatching date and brood size. We defined the incubation length as the number of days between the onset of full incubation (the day on which the female was found incubating or the eggs were found uncovered and warm) and the first signs of hatching, in the same way as reported in Sanz and García-Navas (2009). On day 22 after the hatching date, we revisited the nestboxes to assess the number of nestlings that had fledged.

Females were captured with spring traps when feeding their nestlings (8-9 days old). We weighed them in a portable balance (0.01 g) and measured their tarsus length with an electronic calliper (0.01 mm). We also weighed the nestlings at the age of 13 days and measured their tarsus length.

The incubation pattern of tits was measured by placing two thermometers inside each nestbox, one among the eggs (nest data-logger) and the other attached with brown medical tape to an inner side of the nestbox (control data-logger), in the same way as reported in Ardia et al. (2009). The thermometers were DS1922L Thermochron Data Logger i-buttons (size= 17.35 x 5.9 mm and weight= 3.3 g), which are able to record temperatures ranging from -40 ºC to +85 ºC, with an accuracy ±0.5 ºC in the range -10 ºC to +65 ºC. 93 % of the pairs of i-buttons were placed between days 6-10 of incubation (range= 4-12). All i-buttons were placed between days 6 and 11 of incubation; but 85 % of the incubation rhythms of Blue tits and 76 % of those of Great tits were measured on days 7, 8 and 9 of incubation. Owing to the skew that different incubation days can create in the analyses (Cooper and Voss 2013), we included the age of the embryo as a co-variable in the analyses (see statistical analyses). Both nest and control data-loggers were placed the day before the measurement of the incubation pattern. I-buttons were programmed to start recording temperature at 00:01 a.m. and finish two hours after sunset on the same day, collecting one temperature data point every 11 sec. We downloaded the temperature recordings using the Eclo Express-Thermo software 2007 (www.eclo.pt/expressthermo) and used the program Rhythm 1.0 (Cooper and Mills 2005) to select the recesses from nest-temperature recordings. We adjusted the program Rhythm 1.0 to select a recess when a change of at least 2 ºC in the temperature among eggs occurred for at least 2 min. We then used RavenPro 1.5. (Charif et al., 2010) to visually recheck the output and confirm the recesses automatically selected by the program 1.0, as this is advised by the developers of the program. This allowed us to determine the duration of each on-bout and recess and their number. With this data, we searched for atypical extended recesses, which result in substantial egg-cooling and therefore are likely to be ecologically significant for embryos

(MacDonald et al. 2013). Consequently, we considered an extended recess to be those that exceeded by at least four times the mean recess duration of each species in each year. In the majority of the cases, tits only performed one extended recess in each incubation pattern, with the only exceptions being two Blue tit incubation patterns–one registered in 2012 with 3 extended recesses and the other in 2013 with 2 extended recesses (see Table 1). Those nests for which we measured the incubation pattern and that successfully hatched (i.e. they were not deserted or predated) were classified for analytical purposes according to the incubation pattern registered. Thus, we classified the nests as having a 'neglect incubation pattern' (those with at least one extended recess) and nests having a 'normal incubation pattern' (those that do not have extended recesses), as described in MacDonald et al. (2013).

Table 1. Number of incubation patterns and recesses recorded in each species in the two years of study in a population in San Pablo de los Montes (Toledo, central Spain). NIP is 'neglect incubation pattern' and ER is extended recess. Mean ± SE (SD) refers to the mean duration (min) of the recesses in each species and year.

	Blue tits	
	2012	2013
No incubation patterns	83	80
No NIP	19	7
NIP Prevalence (%)	22.9	8.75
No recesses	1992	2086
No ER	21	7
ER Prevalence (%)	1.05	0.34
Mean (min) ± SE (SD)	11.38 ± 0.59 (26.18)	8.76 ± 0.17 (7.66)
	Great tits	
	2012	2013
No incubation patterns	74	44
No NIP	6	1
NIP Prevalence (%)	8.11	2.27
No recesses	1276	1000
No ER	7	1
ER Prevalence (%)	0.55	0.1
Mean (min) ± SE (SD)	14.95 ± 0.62 (22.19)	11.64 ± 0.23 (7.20)

Statistical analyses

From all incubation patterns registered, 11 Blue tit females and 6 Great tit females were repeated between years. We repeated the analysis without one of these duplicated females and the results did not change. Therefore, we treated each incubation pattern as independent (MacDonald *et al.* 2013). We compared mean daily ambient temperature between study years using a one-way ANOVA. We then looked for patterns in the prevalence of neglect incubation patterns between years or species. To do this, we performed a Generalized Linear Mixed Model (GLMM) fitted with a binomial distribution, where the response variable

was the type of incubation pattern (0= normal incubation pattern and 1= neglect incubation pattern) and the study year, species and nestbox type were categorical factors. We included nestbox type as a fixed factor (woodcrete vs. wooden) to correct for the possible effects that the different thermal properties of each nestbox type may have on female incubation behaviour. In this analysis, we also included the length of the night (min) before the measurement of the incubation pattern, the mean temperature during the night (measured with the control data-logger for each nest) and the clutch size as covariates. We included the length of the night to correct the model for calendar date (as both variables were highly correlated, R2= 0.96, P< 0.001), but also because in our latitude the continuous nightly incubation period is highly energy demanding (Álvarez and Barba 2014). Mean night temperature and clutch size were included since both variables can influence the costs of incubation (Haftorn and Reinerstend 1985, Nord et al. 2010).

To test if the type of incubation pattern affects either the nest attentiveness provided by the females to their clutches or the mean temperature loss of the clutches that were measured with the nest data-logger, we performed two GLMMs. One was fitted with a binomial distribution, in which the numerator of the response variable was the total amount of time in which the female was actively incubating and the denominator was the duration of daylight (nest attentiveness). The other GLMM was fitted with a Poisson distribution, in which the response variable was the average temperature decreases of each off-bout assessed by the nest data-logger in each incubation pattern. While we did not measure egg temperature loss, it is expected that the thermal conditions experienced by the embryos were related to the temperature in their immediate environment. The measure of mean temperature loss for each incubation pattern by the nest data-loggers is important because it is expected that only if egg temperature loss in neglect incubation patterns is significantly higher than in normal incubation patterns, will there be fitness consequences due to extended recesses (Nord and Williams 2015). In both models, the study year, species, nestbox type and incubation type (neglect or normal) were included as fixed factors. As covariates, the day on which the pattern of incubation was recorded, the age of the embryos on this day, the mean temperature on this day (measured with the control data-logger) and the clutch size were included.

To test whether the incubation type has a fitness effect in the studied hole-nesting birds, we performed several GLMMs with the same fixed factors as the two previous models and the hatch day and clutch size as covariables. The responses variables were the length of the incubation period (days), the hatching and breeding successes (analysed with a binomial distribution in which the numerator was the number of nestlings hatched or fledged, respectively, and the denominator was the clutch size), the female and nestling body condition (by adding the tarsus length as an additional covariate) and the tarsus length of the nestlings.

All models were fitted with a Gaussian distribution unless otherwise stated. In all models we included the identity of the forest patch (ID patch) as the random term. Homoscedasticity, proper distribution employed and dependence were graphically verified with the residuals (Zuur et al., 2009). All models were first constructed with all explanatory terms fitted including interactions. Final models were selected following a backward procedure, by progressively eliminating non-significant terms starting with the interactions. Thus, final models were those that explained the most variance with the least number of explanatory variables. All analyses were performed in R (R Core Team 2014) with the package ‘lme4’ (Bates et al., 2014). For non-parametric distributions, the Wald chi-square statistic ($\chi 2$) from package ‘car’ (Fox and Weisberg 2011) is given. Mean ± SE (n) is given in the results.

Results

During both study years, the incubation activity of 163 Blue tit females and 118 Great tit females were recorded. In all incubation patterns, females spent the prior night on the nest actively incubating the eggs. In total, we measured 4078 and 2276 recesses by Blue and Great tits, respectively. Twenty-eight of the Blue tit recesses were extended, and 8 recesses were extended in Great tits. Thus, the prevalence of extended recesses in Blue tits was 0.68 % and 0.35 % in our population of Great tits (Table 1). Fig. 1

shows an example of two incubation patterns with extended recesses in Blue and Great tits. Mean daily temperatures on the recording date significantly differed between study years (ANOVA: F1, 279= 13.98, P< 0.001; year 2012= 16.30 ± 0.47 ºC and year 2013= 13.92 ± 0.40 ºC), with 2013 the colder study year.

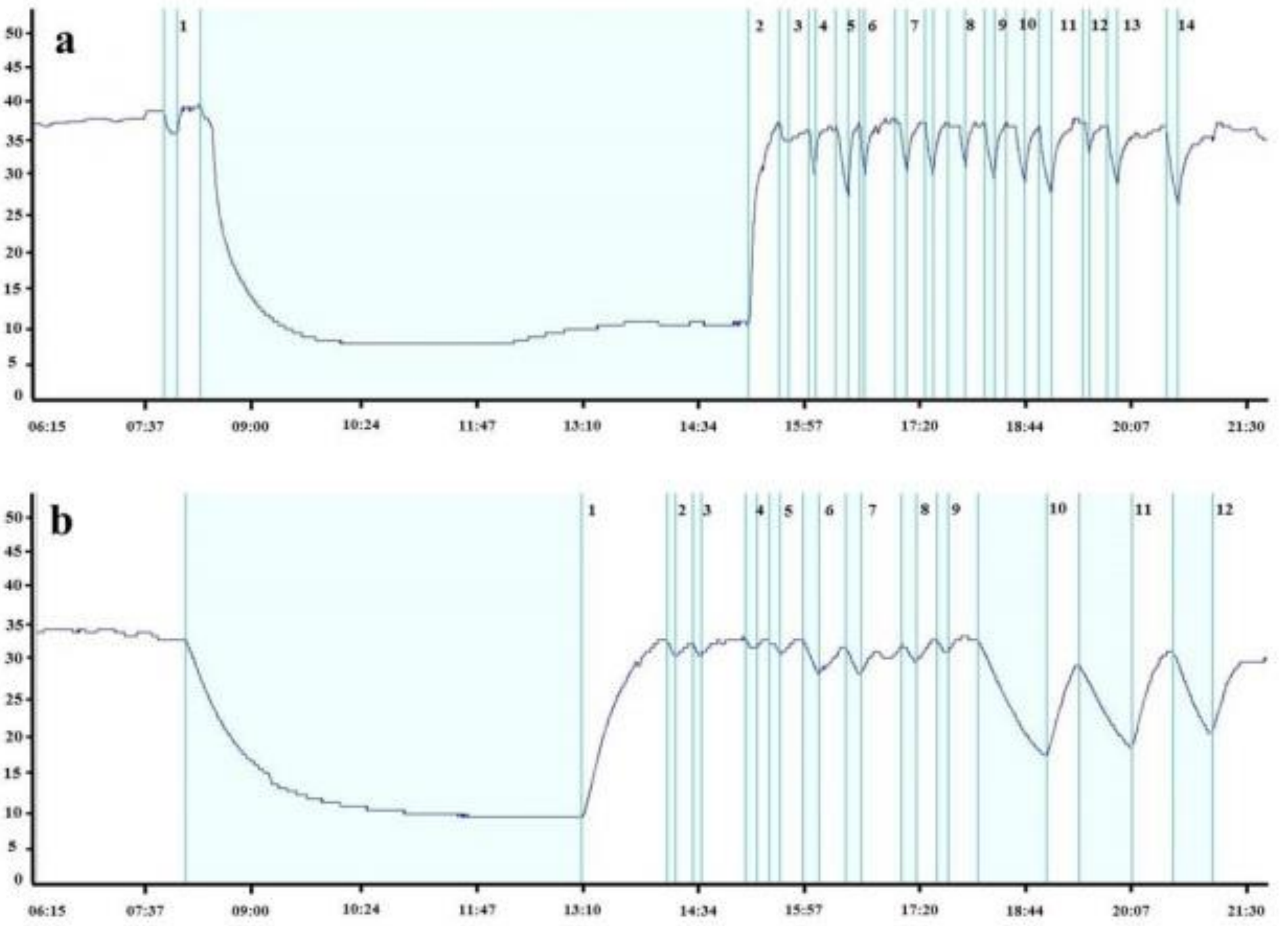


Figure 1. Example of two neglect incubation patterns of (a) Blue tit (*Cyanistes caeruleus*) and (b) Great tit (*Parus major*), where an extended recess can be appreciated in Montes de Toledo. Incubation patterns were obtained through the placement of i-button data-loggers among the eggs. Off-bouts are highlighted in blue, and the order number is shown in the upper part of the diagrams. The Y-axis represents clutch temperature and the X-axis represents daily hours.

Regarding nest categorization, the prevalence of neglect incubation patterns was significantly higher in 2012 than in 2013 (GLMM: χ21, 274= 4.69, P= 0.03; year 2012= 15.92 ± 2.92 % and year 2013= 6.45 ± 2.22 %) and in woodcrete nestboxes than in wooden ones (GLMM: χ21, 274= 7.41, P= 0.01; woodcrete= 19.18 ± 4.64 % and wooden= 9.13 ± 2.00 %) for both species. The prevalence of neglect incubation patterns was significantly higher for Blue tits than for Great tits (GLMM: χ21, 274= 9.17, P= 0.002; Blue tits= 15.95 ± 2.18 % and Great tits= 5.93 ± 2.18 %). The length of the night before the incubation pattern was recorded was positively related to the prevalence of neglect incubation patterns in both species (GLMM: χ21, 274= 13.41, Estimate ± SE= 5.87 ± 1.60, P< 0.001). On the other hand, mean night temperature was negatively related to the prevalence of neglect incubation patterns (GLMM: χ21, 274= 7.98, Estimate ± SE= -0.16 ± 0.06, P= 0.005). Finally, clutch size did not affect the prevalence of neglect incubation patterns in either of these hole-nesting species (GLMM: χ21, 273= 0.34, Estimate ± SE= -0.08 ± 0.14, P= 0.56).

Nest attentiveness provided by the females to their clutches was significantly lower in the neglect incubation patterns than in the normal patterns in both species (GLMM: χ21, 270= 2421.10, P< 0.001; Great tits, neglect= 40.66 ± 8.81 % and normal= 69.55 ± 0.59 %. Blue tits, neglect= 42.89 ± 2.79 % and normal= 69.94 ± 0.51 %. Fig. 2), although this pattern was significantly stronger in Great tits than in Blue tits (significant interaction term between species and type of incubation pattern, GLMM: χ21, 270= 11.00, P< 0.001; Great tits: Estimate ± SE= -0.97 ± 0.03 and Blue tits: Estimate ± SE= -0.86 ± 0.02). In both species, nest attentiveness was significantly lower in 2012 than in 2013 (GLMM: χ21, 270= 282.29, P< 0.001; Great tits, 2012= 66.20 ± 1.41 % and 2013= 70.60 ± 0.96 %. Blue tits, 2012= 61.38 ± 1.65 % and 2013= 70.04 ± 0.82 %). Nest attentiveness also differed between nestbox types, being significantly lower in woodcrete nestboxes in both species (GLMM: χ21, 270= 109.19, P< 0.001; Great tits, Woodcrete= 62.99 ± 2.38 % and Wooden= 70.23 ± 0.73 %. Blue tits, Woodcrete= 61.85 ± 2.55 % and Wooden= 66.23 ± 1.04 %). However,

nest attentiveness did not differ between species (GLMM: χ21, 270= 1.28, P= 0.26; Great tits= 67.84 ± 0.97 % and Blue tits= 65.63 ± 0.99 %). All covariates in this model significantly affected nest attentiveness in the same way in both species: the day of measurement was positively related to nest attentiveness (GLMM: χ21, 270= 37.02, Estimate ± SE= 0.003 ± 0.001, P< 0.001), nest attentiveness was higher as the embryos got older (GLMM: χ21, 270= 99.73, Estimate ± SE= 0.03 ± 0.003, P< 0.001), ambient temperature was negatively related to nest attentiveness (GLMM: χ21, 270= 15.49, Estimate ± SE= -0.003 ± 0.001, P< 0.001), and the clutch size was positively related to nest attentiveness (GLMM: χ21, 270= 8.72, Estimate ± SE= 0.01 ± 0.002, P= 0.003).

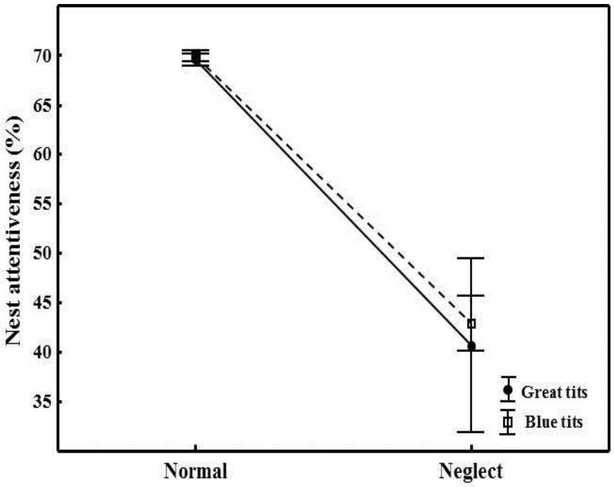


Figure 2. Mean nest attentiveness provided by female Blue tits (*Cyanistes caeruleus*) and Great tits (*Parus major*) in relation to the type of incubation pattern (normal vs. neglect) in Montes de Toledo. Vertical bars indicate ± SE.

The mean temperature loss as recorded by the nest data-loggers in each incubation pattern was significantly affected by the type of the incubation pattern. In neglect incubation patterns, the mean temperature loss was significantly higher than in normal incubation patterns in both species (GLMM: χ21, 274= 35.67, P< 0.001; Great tits, neglect= 7.14 ± 1.61 ºC and normal= 3.36 ± 0.15 ºC. Blue tits, neglect= 6.14 ± 0.53 ºC and normal= 2.87 ± 0.16 ºC. Fig. 3). Mean temperature loss significantly differed between species, being higher in Great tits (GLMM: χ21, 274= 9.17, P= 0.002; Great tits= 3.59 ± 0.18 ºC and Blue tits= 3.39 ± 0.19 ºC). The temperature loss recorded by the nest data-loggers did not significantly differ between study years or nestbox types (GLMM: χ21, 273= 0.38, P= 0.53, GLMM: χ21, 272= 0.14, P= 0.71, respectively). All covariates, with the exception of the day of measurement (GLMM: χ21, 271= 0.25, Estimate ± SE= 0.001 ± 0.003, P= 0.62), significantly affected the temperature loss as recorded by the nest data-loggers. The age of the embryos was negatively related to temperature loss (GLMM: χ21, 274= 4.24, Estimate ± SE= -0.04 ± 0.02, P= 0.04), as were the ambient temperature

and the clutch size (GLMM: χ21, 274= 9.79, Estimate ± SE= -0.02 ± 0.005, P= 0.002 and GLMM: χ21, 274= 18.60, Estimate ± SE= -0.07 ± 0.01, P< 0.001 respectively).

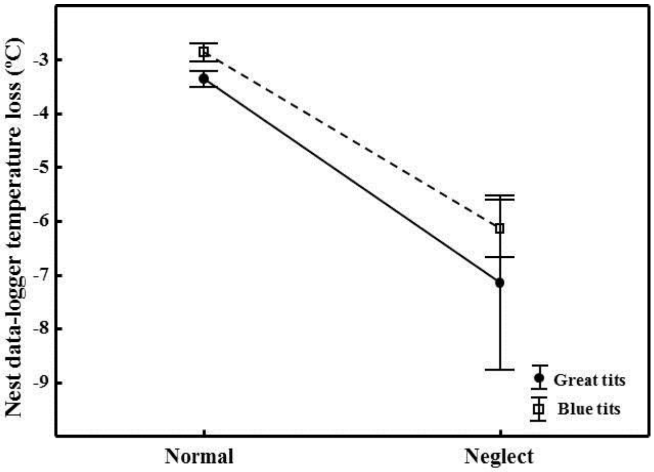


Figure 3. Mean temperature loss recorded by the nest data-loggers placed within the clutches of Blue tits (*Cyanistes caeruleus*) and Great tits (*Parus major*) in relation to the type of incubation pattern (normal vs. neglect) in Montes de Toledo. Vertical bars indicate ± SE.

Regarding the possible fitness effects of the type of incubation pattern in the studied hole-nesting species, the length of the incubation period was significantly longer for those clutches that suffered at least one neglect incubation pattern in both species (Great tits, neglect= 18.14 ± 1.62 days and normal= 13.56 ± 0.28 days; Blue tits, neglect= 17.32 ± 0.47 days and normal= 14.02 ± 0.22 days. Table 2 and Fig. 4). Additionally, the length of the incubation period was negatively related to hatching date and clutch size (Table 2). In relation to hatching success, the two species showed different trends in regards to the type of incubation pattern (significant interaction term between species and incubation type in Table 2. Great tits: Estimate ± SE= -0.74 ± 0.29 and Blue tits: Estimate ± SE= 0.51 ± 0.21). However, the post-hoc test showed that a statistically significant difference was only achieved between species with normal incubation patterns (Unequal N HSD test P< 0.01. Normal incubation pattern, Great tits: 84.45 ± 1.65 % and Blue tits= 76.26 ± 1.82 %). No other independent variable was significantly related to hatching success in either tit species (Table 2). Regarding breeding success, the two species showed different trends with respect to the type of incubation pattern (significant interaction term between species and incubation type in Table 2. Great tits: Estimate ± SE= -0.92 ± 0.34 and Blue tits: Estimate ± SE= 0.76 ± 0.22). Nonetheless, the post-hoc test of this interaction revealed that there were no statistically significant

differences among groups (Unequal N HSD test P> 0.05). Breeding success significantly differed between study years and nestbox types in the same way in both species, being lower in 2012 and in woodcrete nestboxes, respectively (Great tits, 2012= 65.17 ± 4.32 % and 2013= 79.72 ± 3.68 %. Blue tits, 2012= 62.43 ± 3.99 % and 2013= 72.39 ± 3.35 %. Great tits, woodcrete= 59.49 ± 8.34 % and wooden= 73.63 ± 3.21 %. Blue tits, woodcrete= 62.85 ± 5.50 % and wooden= 68.98 ± 2.94 %).

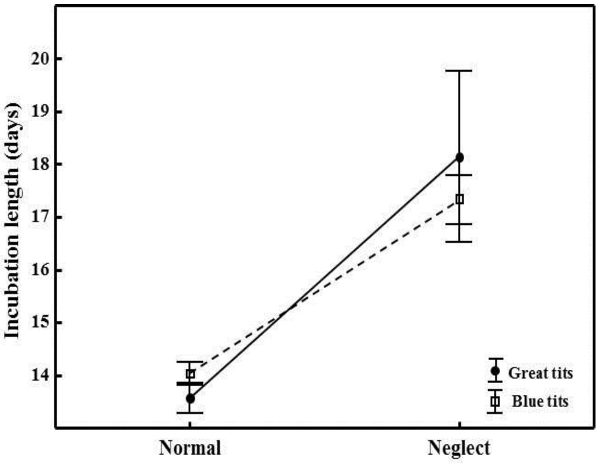


Figure 4. Mean length of the incubation period of Blue tits (*Cyanistes caeruleus*) and Great tits (*Parus major*) in relation to the type of the incubation pattern (normal vs. neglect) in Montes de Toledo. Vertical bars indicate ± SE.

The body condition of females when they were feeding nestlings did not differ between those that performed a neglect incubation pattern and those that performed a normal incubation pattern, in either species (Table 2). However, female body condition differed between study years, being significantly worse in 2012 (Table 2. Great tits: 2012 = 16.21 ± 0.15 g, 2013 = 16.67 ± 0.14 g. Blue tits: 2012 = 9.13 ± 0.12 g, 2013 = 9.54 ± 0.06 g). Female body condition in both species was negatively and positively related to hatching date and clutch size, respectively (Table 2). Regarding nestling phenotype, no nestling characteristic differed between types of incubation patterns in either tit species (Table 2). Nestling body condition and tarsus length were worse in 2012 than in 2013. Nestling phenotype in both species was negatively related to the hatching date (Table 2). Only nestling body condition was negatively related to brood size (Table 2).

Table 2. Results of the GLMMs analyzing the breeding performance in a population of Blue tits (*Cyanistes caeruleus*) and Great tits (*Parus major)* situated in San Pablo de los Montes (Toledo, central Spain). Estimates ± SE are only shown for covariates and when the intercept is statistically significant. Only significant interactions are shown and the main effects of the factors involved in them were not considered (even when P< 0.05). Nestbox type has two levels (Woodcrete vs. wooden) and incubation type has two levels (neglect vs. normal). ID of the forest patch was included in all models as the random term. Significant results are highlighted in bold.

	Incubation length (days)				**Hatching success (%)**				**Breeding success (%)**		
	Estimate ± SE	Test	*P*		Estimate ± SE	Test	*P*		Estimate ± SE	Test	*P*
Intercept	2.39 ± 0.23			Intercept	1.28 ± 0.16			Intercept			
Year		$F_{1,\,256}= 1.63$	0.20	Year		$\chi^2_{1,\,256}= 3.68$	**0.04**	Year		$\chi^2_{1,\,170}= 27.48$	**<0.001**
Nestbox type		$F_{1,\,254}= 0.10$	0.75	Nestbox type		$\chi^2_{1,\,255}= 1.28$	0.26	Nestbox type		$\chi^2_{1,\,170}= 11.91$	**<0.001**
Species		$F_{1,\,255}= 0.98$	0.32	Species		$\chi^2_{1,\,257}= 15.57$	<0.001	Species		$\chi^2_{1,\,170}= 10.91$	<0.001
Incubation type		$F_{1,\,257}= 15.63$	**<0.001**	Incubation type		$\chi^2_{1,\,257}= 2.67$	0.10	Incubation type		$\chi^2_{1,\,170}= 6.93$	0.008
Hatch day	-0.17 ± 0.01	$F_{1,\,257}= 176.08$	**<0.001**	Hatch day	-0.002 ± 0.01	$\chi^2_{1,\,254}= 0.08$	0.78	Hatch day	-0.01 ± 0.006	$\chi^2_{1,\,169}= 3.77$	0.05
Clutch size	-0.15 ± 0.07	$F_{1,\,257}= 4.69$	**0.03**	Clutch size	-0.02 ± 0.03	$\chi^2_{1,\,253}= 0.19$	0.66	Clutch size	0.01 ± 0.03	$\chi^2_{1,\,168}= 0.11$	0.73
Incubation type * Species				Incubation type * Species		$\chi^2_{2,\,257}= 12.90$	**<0.001**	Incubation type * Species		$\chi^2_{2,\,170}= 17.68$	**<0.001**
	Female condition (g)				**Nestling condition (g)**				**Nestling Tarsus length (mm)**		
	Estimate ± SE	Test	*P*		Estimate ± SE	Test	*P*		Estimate ± SE	Test	*P*
Intercept				Intercept	-4.38 ± 2.07			Intercept	16.33 ± 0.22		
Year		$F_{1,\,188}= 24.84$	**<0.001**	Year		$F_{1,\,160}= 34.53$	**<0.001**	Year		$F_{1,\,163}= 4.77$	**0.03**
Nestbox type		$F_{1,\,187}= 2.70$	0.10	Nestbox type		$F_{1,\,159}= 1.98$	0.16	Nestbox type		$F_{1,\,161}= 3.62$	0.06
Species		$F_{1,\,188}= 239.14$	**<0.001**	Species		$F_{1,\,160}= 49.45$	**<0.001**	Species		$F_{1,\,163}= 2046.15$	**<0.001**
Incubation type		$F_{1,\,186}= 0.21$	0.65	Incubation type		$F_{1,\,158}= 0.01$	0.92	Incubation type		$F_{1,\,159}= 1.79$	0.18
Hatch day	-0.02 ± 0.005	$F_{1,\,188}= 11.82$	**<0.001**	Hatch day	-0.03 ± 0.006	$F_{1,\,160}= 19.94$	**<0.001**	Hatch day	-0.01 ± 0.003	$F_{1,\,163}= 12.04$	**<0.001**
Clutch size	2.04 ± 2.04	$F_{1,\,188}= 5.25$	**0.02**	Brood size	-0.07 ± 0.03	$F_{1,\,160}= 5.81$	**0.02**	Brood size	0.03 ± 0.02	$F_{1,\,160}= 3.21$	0.08
Tarsus length	0.57 ± 0.09	$F_{1,\,188}= 35.48$	**<0.001**	Tarsus length	0.99 ± 0.13	$F_{1,\,160}= 61.03$	**<0.001**				

Discussion

The prevalence of extended recesses was less than 1 % of total recesses performed in both tit populations, a value similar to that obtained by MacDonald et al. (2013) in an alpine population of Horned larks. This behaviour is probably a last resort for some females that cannot afford the costs of incubation and are forced to completely switch to self-maintenance in a trade-off with taking care of eggs, as the prevalence pattern of neglect incubation patterns suggests (see below). The fact that this behaviour occurred in both tit species and occurs in many other passerine species (reviewed in MacDonald et al. 2013) reinforces the suggestion of Haftorn (1988) about the habitual nature of this behaviour in free-living passerine populations. However, the low prevalence of this behaviour may explain why, in the majority of general incubation studies, it has been overlooked. Nonetheless, the fact that the performance of one extended recess affects global nest attentiveness and clutch temperature loss for the entire incubation pattern suggests the importance of considering this behaviour in an ecological context. In this regard, the prevalence of neglect incubation patterns in both species was higher in 2012 than in 2013, and Blue tits showed this behaviour more frequently than Great tits. These results support the idea that the performance of extended recesses in passerines is due to energy constraints of the females (Haftorn 1988). While 2012 was significantly warmer than 2013, reducing the costs of incubation (Nord et al. 2010), the body condition of females in both species was significantly worse in 2012. The limitation in energy reserves probably made it more difficult to cope with the costs of incubation, resulting in a higher prevalence of neglect incubation patterns in this year and in the species that presented poorer body condition. Similarly, Wiebe and Martin (1997) found that extended incubation recesses in the White-tailed ptarmigans (Lagopus leucura) were inversely related to the body condition of females. Both tit species also showed more frequent neglect incubation patterns in woodcrete nestboxes than in wooden ones, possibly because of the different thermal properties of each nestbox type. In a previous study, García-Navas et al. (2008) showed that woodcrete nestboxes, despite being on average warmer than

wooden ones, insulate less effectively from ambient temperatures because woodcrete nestboxes conduct heat faster than wooden ones. This feature increases the costs of incubation in woodcrete nestboxes under inclement weather conditions, such as cold nights, because the chamber of the nestbox is less insulated from the exterior. This may account for the higher prevalence of neglect incubation patterns in those nestboxes. As expected, the prevalence of neglect incubation patterns was positively affected by the length of the prior night and negatively affected by its mean temperature. Both longer nights and lower temperatures increase the costs of incubation (Ricklefs and Brown 2013; Nord et al., 2010) and coincide more frequently at the beginning of the breeding season. In this period, the availability of food in the forest has not yet reached its peak, limiting the female's energy budget (Eikenaar et al. 2003, Londoño et al. 2008). Therefore, the availability of food could be another non-exclusive factor that may account to some degree for the prevalence patterns of neglect incubation patterns.

As mentioned above, the performance of one single extended recess in an incubation pattern significantly decreased the nest attentiveness provided to those clutches (Fig. 2). The degree of nest attentiveness is set by females cuing on their own energy reserves, rather than on egg temperature (Chaurand and Weimerskirch 1994, Reid et al. 1999, Ardia et al. 2009), which may explain why nest attentiveness was lower in 2012 than in 2013. Nest attentiveness also differed between nestbox types, being lower in woodcrete nestboxes. The average warmer microclimate in woodcrete nestboxes may allow females to reallocate the incubation energy savings to other functions, such as self-maintenance (Chalfoun and Martin 2007). As the breeding season advances, nest attentiveness increases, probably due to increased availability of food with advancing calendar date. Females also provided more nest attentiveness as the embryos got older. This is likely because the care that embryos need varies over the course of their development (Cooper and Voss 2013) and females respond behaviourally by modulating the degree of nest attentiveness. Clutch size also positively affected the female nest attentiveness. In this respect, the effect of the clutch size on the energy expenditure

during incubation is currently discussed in the literature. Despite the fact that there are several studies that assess increases in the incubation metabolic rate of females with enlarged clutches, it is not clear that these increases translate into a significant increase in the field metabolic rate of incubation (Nord and Williams 2015). This is probably due to the energy savings of females incubating larger clutches due to their lower cooling rate (Conway and Martin 2000; Boulton and Cassey 2012). This was partly supported by the fact that the temperature loss recorded by the nest data-loggers during the recesses in this study was negatively affected by clutch size.

One key fact that could lead to fitness consequences in the young due to extended recesses is whether the overall mean temperature experienced by the embryos over the course of the incubation patterns is significantly lower in neglect than in normal incubation patterns (Nord and Williams 2015). The mean temperature loss recorded by the nest data-loggers placed within the clutches suggests that this may be the case, as the clutches under neglect incubation periods suffered from a higher degree of hypothermia over the course of the entire incubation pattern than the clutches under normal incubation periods (Fig. 3). The fact that the temperature loss recorded by nest data-loggers placed in Great tit clutches was higher than in Blue tit clutches could be due to the different incubation strategies exhibited by each species, with Great tits performing fewer but longer recesses (Bueno-Enciso et al. in prep). Apart from the type of incubation pattern, species and clutch size, mean temperature loss was negatively influenced by the mean ambient temperature, probably reflecting the negative relationship between the cooling rate of eggs and the ambient temperature (Conway and Martin 2000). The age of embryos also negatively affected the temperature loss; this is likely because the heat produced by the embryos increases with their age (Cooper and Voss 2013), buffering the temperature loss recorded by the nest data-loggers.

Regarding the fitness consequences of the type of incubation pattern, the length of the incubation period was about 4 days longer in those clutches where at least one extended recess was performed in both species (Fig. 4). This result was probably due to

the more extreme and prolonged periods of hypothermia experienced by the embryos in the nests that underwent a neglect incubation pattern, which may have slowed their development. This lengthening of the incubation period was also suggested by MacDonald et al. (2013), although not statistically. It may carry fitness costs associated with an increase in the risk of predation (Martin 2002; Martin et al., 2007) and with an increase in the energy expenditure by embryos during development, resulting in a worse phenotype in nestlings (Eiby and Booth 2009). However, no phenotypical characteristics in the nestlings in either population of tits were significantly affected by the type of incubation pattern. This reinforces the idea that passerine embryos are probably well-adapted to prolonged periods of egg neglect (Nord and Williams 2015). The phenotype of nestlings was affected by study year, being worse in 2012 than in 2013, and was also negatively affected by the hatching date. These patterns in the phenotype of nestlings probably respond to constraints in their growing conditions principally due to the availability of food. Similar to nestling phenotypic characteristics, the hatching success was not affected by the type of incubation pattern, although a different trend related to the type of incubation pattern was apparent in both species. The lack of effects of the type of incubation pattern on the hatching success is contrary to the findings of MacDonald et al. (2013), where they found a reduction of 10 % in those clutches that suffered at least one extended recess. Our results are in line with those found by Haftorn (1988) in the Great tit. Breeding success in our study species was not affected by the type of incubation pattern, although it was significantly worse in 2012 and in woodcrete nestboxes in both species. These results indicate that 2012 was a more limiting year for breeding tits, and that breeding in woodcrete nestboxes is more challenging than in wooden ones.

In conclusion, this study reinforces the idea that the performance of extended recesses is a common behaviour in free-living passerine populations, carried out by females as a last resort when they cannot afford the costs of incubation. The fact that the performance of one unique extended recess affects the entire incubation pattern, diminishing total nest attentiveness and increasing mean

temperature loss for the eggs, makes these recesses ecologically significance, despite their low prevalence (less than 1 %).

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Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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DISCUSIÓN GENERAL

Discusión general

Esta tesis pone de manifiesto que, efectivamente, el fenotipo de nuestras poblaciones de Carbonero y Herrerillo común está influenciado por el medio ambiente en el que viven. La plasticidad fenotípica que presentan dichas poblaciones les ayuda a adaptarse a las distintas condiciones ambientales con las que se enfrentan, sin embargo en muchos casos, su eficacia biológica se ve reducida. Esto significa que cambios drásticos producidos en las condiciones medio ambientales pueden tener consecuencias sobre su persistencia a largo plazo.

En esta tesis hemos abordado distintos procesos de transformación del hábitat que llevan asociados cambios en las condiciones medio ambientales y, a posteriori, hemos comparado distintos parámetros de la biología reproductiva entre individuos de las dos especies de estudio criando a lo largo de dichos gradientes medio ambientales (a veces discretos, ej. tamaño de parche forestal o tipo de caja nido) para ver las repercusiones en su eficacia biológica y fenotipo.

Debido a que en cada capítulo de esta tesis hemos discutido ampliamente los resultados obtenidos, en esta sección sólo nos vamos a centrar sobre los efectos medio ambientales en la eficacia biológica y fenotipo de nuestras poblaciones de páridos; una síntesis de este apartado se puede encontrar en la tabla 2.

Tabla 2. Síntesis de los distintos procesos medioambientales estudiados en esta tesis y sus efectos sobre nuestras poblaciones de Herrerillo (*Cyanistes caeruleus*) y Carbonero común (*Parus major*).

Proceso medioambiental	Cambio de las condiciones ambientales	Respuesta fenotípica	Efecto sobre la eficacia biológica	Efecto sobre el Fenotipo
Reducción del tamaño del parche forestal	Efecto borde Disminución de recursos cruciales	Estrategia forrajeadora	Tasa de reclutamiento	Características parentales Características de los pollos
Tipo de caja nido	Microclima del nido Espacio útil para nidificar Percepción de seguridad frente a la depredación	Porcentaje de ocupación Arquitectura del nido Cambios en la fenología Patrón de incubación	Porcentaje de depredación. Éxito reproductivo	Volumen de los huevos Características de los pollos
Urbanización	Condiciones medioambientales Propiedades acústicas por aumento del ruido ambiente	Frecuencias del canto Porcentaje de uso de notas	*	Características del canto
Degradación del habitat	Condiciones medioambientales Propiedades acústicas por cambios en la estructura del hábitat	Frecuencias del canto Longitud de las estrofas del canto	*	Características del canto
Latitud	Variación climática (horas de luz y temperatura)	Patrón de incubación		Atención al nido Actividad diaria

*****Posible coste en la eficacia biológica si el mensaje codificado en el canto queda comprometido.

1

El principal proceso ambiental que se puede encontrar en nuestra zona de estudio y que afecta a las especies forestales, es el ocasionado por la fragmentación de los melojares incrementada por la acción del hombre, debido al cual la superficie de hábitat óptimo se ha reducido enormemente, quedando relegada en muchas ocasiones a parches forestales de no más de 2 ha de superficie rodeados por pastos y cultivos (véase Tabla 1 de la Introducción). Las condiciones ambientales que experimentan las aves durante la reproducción en dichos parches difieren sustancialmente de las condiciones ambientales en parches de bosque más grandes. Estas diferencias no son sólo microclimáticas debidas a una mayor proporción de ecotono en los parches forestales más pequeños y a sus efectos asociados, es decir al efecto borde (Robinson *et al.* 1995, Chalfoun *et al.* 2002, Batáry *et al.* 2014); si no que también son consecuencia de una reducción en la disponibilidad de recursos cruciales causada por la disminución de hábitat *per se*, tales como sitios adecuados para anidar (Barrientos *et al.* 2015) o alimento (Møller 1991, Didham *et al.* 1996, Laurance *et al.* 2002, Zannete *et al.* 2000). Comparando las medidas morfo-métricas de los pollos de ambas especies entre aquellos que habían sido criados en parches forestales pequeños y en parches grandes (**capítulo I**), observamos que los pollos de Carbonero común presentaban una mayor longitud de tarso y los pollos de Herrerillo común una mejor condición corporal en los parches forestales grandes que sus conspecíficos criados en parches forestales pequeños. En las aves altriciales, donde los pollos dependen enteramente del suministro de alimento aportado por sus padres para desarrollarse, su fenotipo está altamente influenciado por la calidad y cantidad de alimento que reciben (Van Noordwijk *et al.* 1988). El fenotipo de los pollos al abandonar el nido es muy importante para ellos, ya que condiciona su supervivencia y reclutamiento posterior (Tinbergen y Boerlijst 1990); lo que tiene consecuencias sobre la eficacia biológica de los pollos y padres. El análisis de reclutamiento de los pollos confirmó esto en cierta medida, ya que su tasa de reclutamiento fue mayor para aquellos pollos que nacieron en parches forestales grandes (**capítulo I**). Al comparar la dieta de los pollos de ambas especies entre

parches forestales grandes y pequeños, algunas pistas relacionadas con las diferencias fenotípicas entre tipos de parches forestales salieron a la luz. A pesar de que ambas especies comparten una ecología trófica similar, depredando mayoritariamente sobre larvas de lepidópteros (Perrins 1991), los Herrerillos comunes presentan un nicho trófico más amplio que los Carboneros comunes (Matthysen *et al.* 2011); incorporando en su dieta una mayor variedad de larvas de lepidópteros y sus imagos, además de otros tipos de invertebrados como arañas, coleópteros u ortópteros (**capítulo I**). Los Carboneros comunes por su lado, están altamente especializados en orugas de lepidóptero de gran tamaño, principalmente noctuidos en nuestra zona de estudio (García-Navas *et al.* 2013). El resultado del análisis de la dieta de los pollos sugiere que los Carboneros comunes no fueron capaces de conseguir la misma dieta en parches forestales grandes y pequeños. Por tanto, se vieron forzados a modificar fenotípicamente su estrategia trófica en los parches pequeños incorporando una mayor proporción de presas secundarias a expensas de su preferida, los noctuidos. Aunque esta plasticidad fenotípica les permitió mantener la misma tasa de cebas en ambos tipos de parches forestales, el tarso más corto de los pollos en los parches pequeños sugiere que no consiguieron proporcionar las mismas condiciones nutricionales en dichos parches. Otra posibilidad es que la diferencia fenotípica de los pollos de Carbonero común entre tipos de parches forestales fuese como consecuencia de la diferencia de edad de los machos adultos criando en unos parches o en otros. La proporción de machos jóvenes fue mayor en los parches forestales pequeños, lo que pudo afectar a su estrategia forrajeadora debido a la inexperiencia; aunque un estudio anterior con esta especie no encontró efectos de las categorías de edad sobre las estrategias de forrajeo (Barluenga *et al.* 2001). El caso de los Herrerillos comunes fue distinto. Esta especie presenta ya de por sí un nicho trófico amplio, lo que les permite no depender excesivamente de ningún tipo de presa en concreto. La dieta de sus pollos por tanto no se vio afectada por el tamaño del parche forestal donde fueron criados, posiblemente porque en ambos tipos de parches dispusieron de la cantidad mínima de cada tipo de presa que necesitaban. No obstante, tal y como

se ha comentado anteriormente, la condición corporal de sus pollos fue peor en los parches forestales pequeños. Esta diferencia fenotípica pudo ser debida a cambios sutiles en la dieta incluso a nivel de especie entre tipos de parches forestales, ya que cada especie de oruga presenta un valor nutricional distinto (Arnold *et al.* 2010, Eeva *et al.* 2010).

Además de por el tamaño del parche forestal, el fenotipo de los pollos también se vio afectado por el tipo de caja nido (cemento o madera) donde fueron criados, así como la biología reproductiva de los padres (**capítulo II**). Esto se debe a que en el interior de cada tipo de caja nido se originaba unas condiciones microclimáticas diferentes por las distintas propiedades aislantes del material de cada tipo de caja nido y por el distinto grado de estanqueidad de cada una de ellas. Las aves que criaron en las cajas nido de cemento experimentaron cambios más bruscos de temperatura y humedad, además de unas condiciones más cálidas y húmedas durante una mayor parte del día. Junto a estas diferencias microclimáticas en el interior de cada tipo de caja nido, el distinto tamaño y forma de cada una de ellas también afectó a la biología reproductiva de las aves que anidaron en ellas, pues condiciona el espacio disponible para hacer el nido (Møller *et al.* 2014) y la percepción de seguridad frente a la depredación (Mazgajski y Rykowska 2008) respectivamente. Estas diferencias medio ambientales entre cada tipo de caja nido (oquedad para nidificar) probablemente no pasaron desapercibidas para las aves, tal y como sugiere el hecho de que el porcentaje de ocupación fue significativamente mayor en las cajas nido de cemento para las dos especies. Sin embargo, cuando tomamos en consideración la disponibilidad de cada tipo de caja nido en el bosque, sólo los Carboneros comunes parecieron preferir anidar en las cajas nido de cemento. Ambas especies construyeron nidos más altos en las cajas nido de cemento, posiblemente porque la mayor profundidad de este tipo de cajas nido les permitió hacerlo aumentando incluso la distancia de peligro; es decir, la distancia que hay entre la superficie del nido y la abertura de la caja nido (Mazgajski y Rykowska 2008, Lambrechts *et al.* 2010). Debido a que los Carboneros comunes son más grandes que los Herrerillos comunes, son probablemente más susceptibles a esta distancia, ya que tanto

ellos como sus pollos ocupan un mayor volumen. Ésta puede ser la razón de que mientras que en las cajas nido de cemento la altura del nido de ambas especies no difirió, en las cajas nido de madera los Carboneros comunes construyeron nidos significativamente más bajos que los Herrerillos comunes, posiblemente en un intento por mantener una distancia de peligro óptima para ellos en este tipo de cajas menos profundas. La distinta distancia de peligro que necesita cada especie podría estar detrás del distinto grado de preferencia mostrado por cada especie para cada tipo de caja nido, como se ha comentado anteriormente. No se puede descartar tampoco, que la ausencia de preferencia por un tipo u otro de caja nido en los Herrerillos comunes no sea como consecuencia de su desplazamiento de las cajas nido de cemento por parte de los Carboneros comunes. Además de la arquitectura del nido, ambas especies ajustaron fenotípicamente algunos aspectos de su biología reproductiva probablemente en función de las condiciones microclimáticas reinantes en cada tipo de caja nido. Así, las condiciones más cálidas en las cajas nido de cemento pudieron posibilitar que las hembras de ambas especies adelantasen su fecha de puesta en dichas cajas nido, ya que la energía ahorrada en termorregulación pudo ser empleada en comenzar antes la reproducción (Lack 1966, van Balen 1973). Otra opción alternativa, es que la fecha de puesta se retrasase en las cajas nido de madera debido a que las primeras aves en reproducirse en dichas cajas nido fuesen aquellas que fracasaron en conseguir una caja nido de cemento. Las condiciones cálidas junto con un mayor grado de humedad en las cajas nido de cemento pudieron permitir que las hembras invirtiesen menos recursos en la formación de sus huevos y que les proporcionasen una menor atención durante la incubación, ya que dichas condiciones protegen y preservan mejor a los embriones frente a la hipotermia y deshidratación (Conway y Martin 2000, Deeming 2011). Sin embargo, estas condiciones microclimáticas *a priori* beneficiosas para las hembras en términos de ahorro energético en las etapas previas a la eclosión, pudieron ser negativas posteriormente debido a un mayor grado de hipertermia sufrido por los pollos debido a que la temperatura ambiente se incrementa durante la primavera con el paso de los días. Esto explicaría el peor

éxito reproductor y la menor longitud de las alas de los pollos en las cajas nido de cemento. Conviene destacar el efecto del tipo de caja nido sobre el éxito reproductor de las aves por cuestiones exógenas a las cajas nido, como el hecho de que las cajas nido de cemento sufrieron una tasa de depredación mayor debido a que fueron más fácilmente detectadas por la culebra de escalera (*Rhinechis scalaris*). Nuestro estudio indica que el tipo de caja nido utilizada en los programas de manejo y gestión de especies nidícolas puede tener consecuencias a largo plazo sobre la dinámica poblacional de estas especies.

El medio ambiente, además de a la biología reproductiva de estas especies y al fenotipo de sus pollos, afectó también a aspectos cruciales de su biología como es el canto (**capitulo III y IV)**. En estas especies el canto tiene una función principal en la selección sexual (Catchpole y Slater 2008, Ferrer *et al.* 2015). Tanto es así que cuando se producen alteraciones en el medio ambiente, concretamente en sus propiedades acústicas, muchas especies de aves ven reducida su abundancia o desaparecen al no poder comunicarse eficazmente (Francis *et al.* 2011, Proppe *et al.* 2013). La urbanización es un proceso cada vez más extendido y de entre todas las alteraciones medio ambientales que provoca, el aumento del ruido ambiental es sin duda la más limitante para el proceso de comunicación de las aves (Brumm y Slabbekoorn 2005). Poseer una plasticidad fenotípica que permita alterar las características del canto y poder comunicarse en presencia de elevado ruido ambiental, es por tanto crucial para el mantenimiento de las especies de aves a corto plazo en las ciudades (Gross *et al.* 2010). El Carbonero común es una especie que ocupa hábitats urbanos satisfactoriamente y los biólogos que han estudiado su canto en las ciudades han observado que su estrategia principal es incrementar la frecuencia mínima del canto en comparación con poblaciones forestales, presumiblemente para evitar solaparse con el ruido ambiente concentrado a bajas frecuencias; aunque los mecanismos de dicho ajuste permanecen actualmente bajo debate (Nemeth y Brumm 2010, Nemeth *et al.* 2012, Slabbekoorn *et al.* 2012). En el **capítulo III**, comparamos el canto de Carboneros comunes macho cantando en Toledo (población urbana) y en San Pablo de los Montes y Quintos de Mora

(poblaciones forestales) y observamos que, pese a que la intensidad de ruido ambiental fue significativamente mayor en Toledo, la frecuencia mínima del canto del Carbonero común no difirió entre dicha población urbana y las forestales. Por lo tanto podría estar ocurriendo algún proceso que estuviese constriñendo la respuesta de los Carboneros comunes de elevar la frecuencia mínima de su canto en Toledo (**capítulo III**). Los Carboneros comunes poseen un repertorio de tipos de canto pequeño, aprendido de sus padres y vecinos cuando están en el nido y durante su primer año de vida (McGregor y Krebs 1982). Consecuentemente la transmisión cultural de los tipos de cantos en esta especie es importante, como prueba la existencia de dialectos cada vez más diferentes conforme aumenta el aislamiento entre distintas poblaciones (Fayet *et al.* 2015). La forma que esta especie tiene de afrontar el aumento de ruido ambiental es cambiando a tipos de canto de su repertorio con una frecuencia mínima elevada y cantarlos durante más tiempo, más que cambiar plásticamente la frecuencia del mismo tipo de canto (Halfwerk y Slabekoorn 2009, Slabbekoorn 2013). La ausencia de un dialecto acústico en Toledo con una frecuencia mínima elevada en comparación con las poblaciones forestales cercanas podría ser una consecuencia indirecta del pequeño tamaño de dicha ciudad, que permitiría un flujo elevado de individuos, y por tanto de tipos de canto, entre el exterior de la ciudad y el interior, y provocaría una homogenización cultural en toda la zona. Pese a que no podemos confirmar este escenario ya que no marcamos los individuos ni estudiamos su genética, el elevado porcentaje de tipos de cantos compartidos entre las poblaciones estudiadas sugiere un intercambio cultural elevado o un aislamiento reciente. No obstante, el porcentaje de uso de notas con una frecuencia pico más elevada fue mayor en Toledo. A diferencia de la frecuencia mínima, la frecuencia pico y máxima sí que se diferenciaron entre los cantos de la población de Toledo y el de las poblaciones forestales, siendo mayores en Toledo (**capítulo III**). Esta estrategia podría ser un cambio activo de dichas frecuencias (McMullen *et al.* 2014), o podría ser un artefacto de cantar más fuerte en Toledo (Cardoso y Atwell 2011); es decir, del efecto Lombard (Zollinger y Brumm 2011).

Estos ajustes vocales según las propiedades acústicas del medio ambiente, se pusieron también de manifiesto cuando estudiamos las características del canto de los Carboneros comunes en relación a la estructura del hábitat del territorio que cada macho ocupaba (**capítulo IV**). La estructura del hábitat de los territorios en la zona de San Pablo de los Montes no es homogénea, pese a que todas las cajas nido las colgamos dentro de robledales. Esto se debe a la heterogeneidad natural de los hábitats, pero también a la degradación de dichos robledales debido a procesos antrópicos asociados con la fragmentación del hábitat, tal y como se explica en el **capítulo I**. Así pues, los machos que defendían territorios con una mayor cobertura de matorral cantaron más despacio y con una frecuencia mínima más baja que los machos defendiendo territorios más ‘abiertos’; y los machos que defendían territorios con una mayor superficie de suelo desnudo aumentaron la longitud de sus estrofas. Estos ajustes del canto posiblemente fueron hechos para mejorar la transmisión del canto en cada territorio (Catchpole y Slater 2008). Conviene destacar que la frecuencia pico del canto de los Carboneros comunes estuvo relacionada negativamente con la longitud de su tarso. Esto puede ser debido a que la producción a ciertos niveles de la frecuencia pico está constreñida por el tamaño del animal, por lo que podría constituir una señal honesta de su tamaño (Gil y Gahr 2002), tal y como sucede en otras especies (Joula y Searcy 2011). En relación a esto, cambios medioambientales que modifiquen el fenotipo de las aves, como hemos visto que sucede con la longitud del tarso de los pollos de Carbonero común criados en parches forestales pequeños (**capítulo I**) podrían disminuir su eficacia biológica si esto les impidiese cantar con una frecuencia pico competitiva a la hora de defender un territorio o de atraer pareja.

La situación latitudinal de nuestras poblaciones de Carboneros y Herrerillos comunes cercana a su límite de distribución meridional (Cramp y Perrins 1993) presenta unas peculiaridades climáticas que las diferencia de poblaciones más norteñas, soportando en general temperaturas más elevadas durante la época de reproducción y un número menor de horas de luz diarias. Estas condiciones climáticas condicionan en gran medida un aspecto crítico de su biología

reproductiva: la incubación (DuRant *et al.* 2013). Esto se debe a que la temperatura es el principal factor que afecta al gasto energético de los padres de mantener calientes los huevos (Haftorn 1978, Haftorn y Reinertsen 1985, Reid *et al.* 2000, Nord *et al.* 2010) y las horas de luz diarias condicionan el tiempo disponible de los padres para conseguir alimento (Ricklefs y Brawn 2013, Shaw y Cresswell 2014). Debido a esto se sabe que las especies modifican fenotípicamente su comportamiento de incubación según las condiciones climáticas en cada latitud (Álvarez y Barba 2014). El análisis del comportamiento de incubación de nuestra población de Carboneros comunes confirmó este patrón latitudinal (**capítulo V**), ya que redujeron la actividad diaria y la atención al nido en comparación con poblaciones más norteñas. La atención al nido es una variable muy importante del periodo de incubación, porque se refiere al porcentaje de tiempo que las hembras están activamente calentando los huevos, y por lo tanto proporcionando las condiciones óptimas de desarrollo de sus embriones (Webb 1987). La reducción de la atención al nido a bajas latitudes podría estar relacionada con el hecho de que en dichas latitudes, el gasto energético de mantener los huevos calientes durante la noche es mayor debido a su mayor duración; por lo que las hembras de Carbonero común se verían forzadas a disminuir la atención al nido debido a su necesidad de alimentarse y recuperarse del gasto energético durante la noche. El hecho de que además la temperatura ambiental aumente conforme disminuye la latitud puede facilitar dicho patrón latitudinal de atención al nido, ya que la pérdida de calor de los huevos es inversamente proporcional a la temperatura ambiente, por lo que se disminuye el riesgo de hipotermia a bajas latitudes (Conway y Martin 2000). Los Herrerillos comunes pese a presentar un patrón de incubación completamente diferente al de los Carboneros comunes ejecutando un número mayor de salidas y entradas pero con una duración menor de las mismas, proporcionaron a sus puestas el mismo porcentaje de atención al nido. Este resultado sugiere que pese a que las necesidades de atención al nido de los embriones en distintas especies sean parecidas, la forma en que dichas especies cubren las necesidades de sus embriones puede variar por causas endógenas a ellas mismas, como diferencias en las propiedades térmicas de sus huevos.

El patrón de incubación de una especie es relativamente constante en un rango latitudinal concreto, respondiendo a las condiciones climáticas de dicha latitud (principalmente temperatura y horas de luz disponibles). Pero esta constancia no es exacta, y las diferencias en los patrones de incubación dentro de una misma especie y localidad, reflejan el conflicto de interés (trade-off) entre las necesidades de la madre y de sus embriones (Cooper y Voss 2013). En este sentido, las madres intentan optimizar las condiciones de desarrollo de sus embriones pero sin poner en peligro su propia supervivencia (Wingfield 2003). Durante la monitorización del comportamiento de incubación de nuestras poblaciones de páridos (**capítulo V**), encontramos diversos patrones anómalos, caracterizados por poseer recesos (ausencias en el nido de la hembra) muy largos. En dichos recesos extensos, los huevos soportaban temperaturas muy por debajo del límite fisiológico cero (límite térmico a partir del cual el desarrollo embrionario se detiene por completo) durante periodos muy prolongados de tiempo. En el **capítulo VI** analizamos la prevalencia de estos recesos extensos y, pese a que fue muy baja en las dos poblaciones de páridos (menos del 1 % del total de recesos), los patrones de incubación que contenían al menos uno, presentaban un nivel de atención al nido menor y una pérdida media de temperatura de los huevos mayor que los patrones en los que no había recesos extensos. Esto nos llevó a categorizar los patrones de incubación en ‘anómalos’ (si presentaban al menos un receso extenso) y en ‘normales’ (si no presentaban recesos extensos). El análisis de prevalencia de dichos patrones ‘anómalos’ sugirió que este comportamiento de abandonar el nido por periodos extensos de tiempo puede ser un último recurso efectuado por las hembras cuando no pueden soportar los costes energéticos impuestos por la incubación (Williams 1996, Nord y Williams 2015). En este sentido, la mayor prevalencia de patrones ‘anómalos’ se dio el año en que las hembras presentaban la peor condición corporal (2012) y también en las cajas nido de cemento, donde debido a su menor grado de aislamiento (**capítulo II**) el coste energético de la incubación durante periodos climáticos adversos es mayor. Los Herrerillos comunes realizaron patrones de incubación ‘anómalos’ en mayor proporción que los Carboneros comunes, posiblemente debido a que las hembras de Herrerillo común presentaron una condición corporal peor que las hembras de Carbonero común; además de que el menor tamaño de sus huevos hace que la estrategia de incubación sea más costosa en los Herrerillos que en los Carboneros comunes (**capítulo V**). El hecho de que el mayor porcentaje de patrones ‘anómalos’ se produjera justo después de las noches más largas y frías refuerza la idea de que este comportamiento es debido a limitaciones energéticas de las hembras. Además estas condiciones (noches más largas y frías) se dan al principio de la temporada de cría, cuando la disponibilidad de alimento en el bosque es menor, por lo que las hembras ven dificultada la posibilidad de recuperar la energía gastada durante la noche. Sin embargo este comportamiento ‘anómalo’ no tuvo consecuencias en la eclosión ni en el fenotipo de los pollos, lo que hace suponer que en ambas especies los embriones están bien adaptados a periodos prolongados de tiempo en hipotermia durante la incubación. Lo que sí se vio afectado por la realización de recesos extensos fue el periodo de incubación, probablemente debido a un desarrollo más lento de los embriones en dichas puestas por soportar temperaturas más frías (Webb 1987). Por lo que las hembras que efectuaron recesos extensos sí que pudieron comprometer en cierta medida su eficacia biológica, debido al aumento en el riesgo de depredación que supone un alargamiento del periodo de incubación (Martin *et al.* 2007).

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CONCLUSIONES

Conclusiones

I. Los cambios producidos en el medio ambiente afectaron a distintos aspectos de la biología reproductiva de nuestras poblaciones de Herrerillo y Carbonero común, tales como su éxito reproductor, el fenotipo de sus huevos, padres y pollos o el canto.

The environmental changes affected different traits of the breeding performance of our populations of Blue and Great tits, such as their breeding success, egg volume, father and nestling phenotype or Great tit song.

II. Ambas especies respondieron plásticamente a los cambios medioambientales y amortiguaron sus efectos, mediante cambios en las estrategias de forrajeo, su fenología, la arquitectura del nido, el comportamiento de incubación o las características del canto.

Both species plastically responded to the environmental changes and managed to buffer their effects by means of changes in their foraging strategies, nest architecture, incubation behaviour or song characteristics.

III. Las respuestas fenotípicas de ambas poblaciones de páridos frente a los cambios medioambientales no siempre neutralizaron sus efectos, produciéndose en algunos casos una disminución de su eficacia biológica.

The phenotypic responses of both populations of tits to environmental changes did not always neutralize its effects, in some cases resulting in a decrease in their fitness.

IV. La fragmentación del paisaje forestal en parches de bosque de distinto tamaño afectó a las poblaciones de Herrerillo y Carbonero común, posiblemente debido a cambios en la disponibilidad de alimento en los parches forestales más pequeños.

The fragmentation of the forest landscape into forest patches of different sizes affected the populations of both Blue and Great tits, possibly owing to changes in the availability of food in the smallest forest patches.

V. El efecto del tamaño del parche fue diferente en cada especie debido a las distintas estrategias tróficas mostradas por cada una de ellas. De este modo, poseer una estrategia trófica generalista ayuda a hacer frente a cambios en la disponibilidad de alimento con mayor eficacia.

The effect of forest patch size effect was different for each species owing to the different trophic strategies employed by each one. In this respect, having a generalist trophic behaviuor would appear to help them cope with changes in the availability of food.

VI. El tipo de caja nido utilizado en estudios de biología es un factor importante a tener en cuenta debido a las diferentes condiciones medioambientales que cada tipo de caja nido origina en su interior, lo que tiene consecuencias sobre la biología reproductiva de las especies de aves criando en ellas.

The type of nestbox used in biological studies is an important factor to consider owing to the different environmental conditions that are created within each nestbox type, which have consequences in the reproductive biology of the species nesting in them.

VII. La transmisión cultural de tipos de cantos en el Carbonero común puede tener un papel importante en la divergencia acústica entre las poblaciones urbanas y forestales en dicha especie.

The cultural transmission of song types could be an important factor leading to the acoustic divergence between urban and forest Great tit populations.

VIII. La necesidad de transmitir nítidamente el canto es importante para los Carboneros comunes, tal y como sugiere el hecho de que dentro de una misma población, las características de su canto varíen en función de la estructura del hábitat en cada territorio.

The need to clearly transmit the message encoded in Great tit songs is important for the species, as is suggested by the fact that the song characteristics change within a population, thus shaping the habitat structure in each territory.

IX. Peculiaridades propias de cada especie como el tamaño de los huevos podría explicar en parte la gran variedad de comportamientos de incubación que hay entre las especies, debido a las diferencias en las propiedades térmicas de los huevos asociadas a su tamaño.

The endogenous peculiarities of each species such as the size of their eggs could, in part, explain the great variation in the incubation behaviour among species, owing to the different thermic properties of the eggs associated with their size.

X. Episodios de negligencia prolongados en el cuidado de los huevos durante la incubación parecen ser comunes en las poblaciones de aves paseriformes, como consecuencia de restricciones energéticas de los padres.

The neglect of eggs during incubation seems to be common in free living passerine populations, as a consequence of the parents' energetic restrictions.

XI. Las poblaciones naturales no son estáticas y el fenotipo de ellas que observamos está altamente influenciado por el medio ambiente donde se desenvuelven. Por tanto, para entender profundamente las características de cada población hay que tener en cuenta las presiones que el medio ambiente ejerce sobre ellas.

Natural populations are not static and their phenotype is highly influenced by the environment in which they live. In order to understand the characteristics of each population, we therefore need to understand the environmental pressure exerted on them.

RESÚMENES DE LOS CAPÍTULOS

Resúmenes de los capítulos

Capítulo I. La fragmentación del hábitat influye en el crecimiento de los pollos de Carbonero y Herrerillo común mediterráneos.

El tamaño del parche forestal donde las aves crían es una variable importante que influye en su éxito reproductivo. Sin embargo, los efectos próximos que empeoran el éxito reproductor en los parches pequeños no se conocen con certeza, y se ha sugerido que la disminución de recursos clave en dichos parches podría ser una causa responsable. Con el objetivo de profundizar en este aspecto, monitoreamos el ciclo reproductivo de dos poblaciones de Herrerillos y Carboneros comunes criando en tres parches forestales 'grandes' (de entre 26,5-29,6 ha de superficie) y doce parches forestales pequeños (de entre 1,1-2,1 ha de superficie) en el centro de España durante tres años (2011-2013). También grabamos la dieta de los pollos dentro de las cajas nido con la ayuda de cámaras de video especialmente dispuestas. Sólo los machos difirieron significativamente entre los dos tipos de parches forestales. Ningún parámetro reproductivo varió entre tipos de parches forestales, pero la condición corporal de los pollos de Herrerillo común y la longitud del tarso de los pollos de Carbonero común sí que difirió, siendo mejor y más larga en los parches forestales grandes respectivamente. La tasa de reclutamiento también fue mayor en los parches forestales grandes, aunque esta tendencia sólo fue significativa en los Herrerillos comunes. Atendiendo a la dieta de los pollos, los Herrerillos comunes no difirieron pero los Carboneros comunes sí, aportando una mayor cantidad de orugas en los parches forestales grandes. La mayor variación en los parámetros reproductivos se dio entre años, posiblemente debido a las diferentes condiciones climáticas en cada uno de ellos. Este estudio sugiere que la limitación en la disponibilidad de alimento en los parches forestales pequeños podría estar empeorando el éxito reproductor de las aves en dichos parches.

Capítulo II. Efectos del tipo de caja sobre la biología reproductiva de dos especies paseriformes que anidan en agujeros.

El uso de cajas nido para estudiar especies de aves que anidan en agujeros naturales ha mejorado enormemente nuestro conocimiento en relación con diversos campos de las

ciencias ambientales. El objetivo de este estudio ha sido comparar la biología reproductiva de Carboneros y Herrerillos comunes en dos tipos de cajas nido que se diferencian en su forma y propiedades térmicas, prestando especial atención a la arquitectura del nido y a la fase de incubación. Ambos tipos de cajas nido fueron colocadas en la misma área y años para evitar posibles sesgos debidos a la geografía, el clima o la disponibilidad de alimento. Hubo diferencias significativas entre los valores extremos de temperatura y humedad relativa obtenidos en ambos tipos de cajas nido, y también en el patrón diario de dichas variables. Ambas especies de aves parecieron preferir anidar en las cajas nido de cemento. La depredación por la culebra de escalera fue, sin embargo, mayor en las cajas nido de cemento. La altura de los nidos fue significativamente mayor en las cajas nido de cemento y también la distancia de peligro entre la superficie del nido y la entrada de la caja nido, lo que pudo contar por el hecho de que los Carboneros comunes prefiriesen criar en dichas cajas nido. La fecha de puesta fue anterior en las cajas nido de cemento, pero el tipo de caja nido no influyó en el tamaño de puesta. Sin embargo, los huevos de ambas especies fueron más pequeños en las cajas nido de cemento. El éxito reproductor fue peor en las cajas nido de cemento. El tipo de caja nido también afectó al patrón de incubación de ambas especies, y la atención al nido disminuyó en las cajas nido de cemento. Este estudio refuerza la idea de que el tipo de caja nido utilizado en los estudios con aves no es trivial y puede tener fuertes efectos biológicos sobre las poblaciones de aves. Es importante tener esto en cuenta cuando las cajas nido se utilizan como medida de conservación de aves.

*Capítulo III. Transmisión cultural y su posible efecto en la adaptación acústica urbana del Carbonero común (*Parus major*).*

Los Carboneros comunes (*Parus major*) urbanos cantan con una frecuencia mínima mayor que sus conspecíficos forestales. Procesos culturales podrían estar detrás de esta divergencia acústica, ya que dichas aves aprenden sus cantos de los vecinos y cambian a tipos de canto con alta frecuencia en presencia de ruido de fondo. Sin embargo, en las ciudades pequeñas este proceso de divergencia cultural podría estar limitado, ya que en dichas ciudades es esperable un alto grado de intercambio de tipos de canto con el exterior. Nosotros testamos esta predicción grabando cantos de Carbonero común en una ciudad

pequeña (Toledo, España) y en un bosque cercano. El ruido de fondo fue más alto en la ciudad, al igual que la frecuencia 'pico' y máxima de los cantos, pero la frecuencia mínima no difirió. La longitud de la pausa fue mayor en el bosque. El setenta por ciento de los tipos de canto se compartieron entre la ciudad y el bosque. Estos resultados sugieren que el pequeño tamaño de Toledo impide el establecimiento de una tradición de cantos particular en Toledo con una frecuencia alta.

Capítulo IV. La estructura del hábitat influye en las características del canto dentro de una población de Carboneros comunes.

Para cumplir su función, los cantos de las aves tienen que llegar a los receptores nítidamente. Esto no es una cuestión sencilla debido a los impedimentos que las propiedades acústicas de cada hábitat imponen. La necesidad de una transmisión adecuada posiblemente a moldeado la evolución de los cantos de las aves, tal y como la Hipótesis de la Adaptación Acústica (HAA) propone. De ser así, uno esperaría variaciones en las características del canto relacionadas con el hábitat dentro de las poblaciones. El objetivo de este estudio fue comprobar si la estructura del hábitat influencia las características del canto en una población de Carboneros comunes (Parus major). Para ello, durante la temporada de reproducción del año 2012, grabamos los cantos de Carbonero común provenientes de 42 territorios en dos días diferentes, y medimos la estructura del hábitat en cada territorio. Dichos machos fueron capturados y estimamos la densidad reproductora alrededor de cada territorio, por lo que fuimos capaces de corregir los análisis por la fecha, densidad reproductora y características de cada macho. La longitud de la estrofa y la velocidad del canto estuvieron positivamente relacionadas por la cobertura de suelo desnudo, mientras que la frecuencia pico y mínima estuvieron negativamente relacionadas por la cobertura de matorral. Dichas variables mostraron un grado mayor de respetabilidad entre individuos. La frecuencia pico se relacionó negativamente con la fecha, aunque posiblemente esto fue debido a consecuencia de la variación del tamaño de los machos a lo largo de la temporada de reproducción. Este estudio muestra por primera vez variaciones en el canto relacionadas con la estructura del hábitat dentro de una misma población de

Carboneros comunes, y sugiere que dicha especie es capaz de ajustar su canto en cada territorio posiblemente debido a su plasticidad fenotípica.

Capítulo V. Diferencias en el comportamiento de incubación entre dos poblaciones simpátricas de aves que anidan en oquedades.

La etapa de incubación puede ser tan costosa como la etapa de cría de los pollos. Esto es particularmente cierto en el caso de las especies con incubación uniparental, debido a que las tentativas de reproducción presente y futuras pueden verse comprometidas. Conocer los efectos próximos que condicionan los costes de incubación es, por tanto, de gran importancia para entender la historia evolutiva de las aves y la variación en el comportamiento de incubación dentro y entre las especies. En este estudio, monitorizamos el comportamiento de incubación durante dos años de dos poblaciones de Herrerillo común (*Cyanistes caeruleus*) y Carbonero común (*Parus major*) criando en la misma zona del centro de España mediante la colocación termómetros i-Button. Esta metodología resultó ser fiable y mostró que el comportamiento de incubación de los Carboneros comunes se asemejaba al comportamiento de otras poblaciones de Carboneros comunes sureñas, es decir con una baja atención al nido y una actividad diaria corta. Más aún, por primera vez se describe el comportamiento de incubación del Herrerillo común. El hecho de que esta especie posea huevos más pequeños que los Carbonero comunes posiblemente explica por qué la tasa de enfriamiento de sus huevos es mayor, lo que probablemente influye en su estrategia de incubación. Sin embargo, los huevos más grandes de los Carboneros comunes permiten a esta especie ser más flexible en su comportamiento de incubación y utilizar una estrategia de incubación menos costosa, caracterizada por realizar menos salidas pero más largas. Las condiciones ambientales afectaron a ambas especies de forma similar, pero la menor tasa de enfriamiento de los huevos de Carbonero común les permitió beneficiarse en mayor medida de las condiciones ambientales favorables.

Capítulo VI. ¿Tiene efectos ecológicos la realización de recesos extensos durante la incubación en dos poblaciones de páridos?

La ocurrencia de recesos extensos, aunque con baja prevalencia, parece ser parte del comportamiento de incubación normal de las especies de paseriformes. Sin embargo, dicho comportamiento ha sido considerado como atípico en la mayoría de estudios de incubación general y por tanto, pasado por alto. No obstante, debido a la generalidad de este comportamiento es importante evaluarlo en un contexto ecológico e identificar sus posibles efectos sobre la eficacia biológica. Con este objetivo y durante dos años, monitorizamos el comportamiento de incubación de dos poblaciones de Herrerillo (*Cyanistes caeruleus*) y Carbonero común (*Parus major*) en el centro de España mediante la colocación de termómetros i-buttons. Clasificamos los recesos durante la incubación como ‘extensos’ cuando duraban más de cuatro veces la duración media de los recesos de cada especie en cada año. A continuación, clasificamos los nidos en función del patrón de incubación que tuvieron en ‘atípicos’ (si apareció al menos un receso extenso) y ‘normales’ (si no apareció ningún receso extenso). La prevalencia de patrones atípicos fue mayor el año que las hembras presentaron una peor condición corporal, y también en las cajas nido de cemento. Los Herrerillos realizaron patrones atípicos con más frecuencia que los Carboneros comunes, y en las dos especies normalmente ocurrieron después de noches largas y frías. La atención al nido disminuyó en los patrones atípicos, así como la pérdida media de temperatura de los termómetros situado entre los huevos. El periodo de incubación fue en promedio 4 días más largo en las puestas con patrones atípicos, pero ningún otro parámetro de eficacia biológica fue afectado por el tipo de patrón de incubación. En general, estos resultados sugieren que los recesos extensos son consecuencia de limitaciones energéticas de las hembras y que pueden acarrear costes en su eficacia biológica.

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