Estrategias antidepredatorias de adultos y pollos en el mirlo común: Implicaciones evolutivas y ecológicas

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TESIS DOCTORAL

2010

Editor: Editorial de la Universidad de Granada
Autor: Juan Diego Ibáñez Álamo
D.L.: GR 2379-2010
ISBN: 978-84-693-1298-8

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Estrategias antidepredatorias de adultos y pollos en el mirlo común: Implicaciones evolutivas y ecológicas

Memoria presentada por el licenciado Juan Diego Ibáñez Álamo para optar al título de Doctor en Biología, dirigida por Manuel Soler Cruz de la Universidad de Granada.

Granada, enero de 2010

El Doctorando

Vº Bº del Director

Juan Diego Ibáñez Álamo

Manuel Soler Cruz

A Olivia, mi otra mitad.

A mis padres, Blas y Manoli, mis modelos a seguir.

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RESUMEN

La depredación es considerada una de las fuerzas selectivas más importantes de la naturaleza. Esta relevancia es incluso mayor en el caso de las aves, ya que es un grupo con un largo periodo de cuidados parentales que, en muchos casos, implica la permanencia de las crías en una localización concreta (el nido) durante todo o gran parte de su desarrollo. De hecho, la depredación es considerada la principal causa de pérdidas de nidos en las aves. La presente tesis explora este tema desde el doble punto de vista de los padres y los pollos usando el mirlo común (*Turdus merula*) como especie modelo. El doble enfoque es importante ya que los depredadores pueden usar no sólo las pistas producidas por la actividad de sus presas (p.e. petición de alimento por parte de los pollos) para localizar y depredar el nido, sino también las producidas por los padres que los cuidan (p.e. visitas al nido).

En este trabajo hemos utilizado tres poblaciones de mirlos no investigadas con anterioridad. Debido a ello, nuestro primer paso fue estudiar los parámetros reproductivos de nuestra especie modelo en cada localización. Encontramos diferencias significativas entre poblaciones tanto en la presión selectiva dominante como en varios rasgos reproductivos. Dado que nuestras tres zonas varían en su influencia humana, la urbanización podría ser la responsable de las diferencias halladas. Nuestros resultados también destacan la idoneidad del uso de síndromes de estrategia vital en lugar de rasgos aislados en los estudios evolutivos.

Los cuidados parentales pueden atraer a los depredadores al nido, por lo que hemos investigado las estrategias usadas por los padres para minimizar este riesgo. Estos cuidados pueden variar en los diferentes periodos de desarrollo de las crías, razón por la cual hemos estudiado estas estrategias tanto en la fase de incubación como en la fase de pollo. De esta manera, hemos confirmado directamente que las hembras de mirlo pueden reducir su actividad durante la incubación en presencia de un depredador para disminuir las posibilidades de revelar la ubicación del nido, lo que apoya la hipótesis de Skutch. Sin embargo, éstas también pueden usar otras estrategias antidepredatorias con objeto de aumentar la ocultación del nido. El uso de una u otra estrategia posiblemente depende de la presión de depredación de nidos a que está sometida cada población. Más aún, ambos sexos responden de manera similar al riesgo de depredación durante el periodo de estancia de los pollos en el nido, aunque no para

todos los rasgos analizados (p.e. duración media de las visitas). Y la existencia o no de estas respuestas antidepredatorias parece depender también del riesgo de depredación ambiental. Además, hemos hallado que los padres pueden responder al riesgo de depredación sin comprometer la alimentación de sus pollos, el principal costo asociado a este comportamiento, modificando otras actividades no relacionadas con la alimentación como el empolle. Estos resultados destacan la complejidad de las respuestas antidepredatorias en aves y sugieren una reconsideración de la teoría establecida en este campo.

Los depredadores también pueden usar directamente la actividad de las crías como pista para localizar y depredar el nido. La petición de alimento es una de las principales actividades realizadas por los pollos de las especies nidícolas. Varios estudios han destacado que la señal acústica asociada a este comportamiento puede atraer la atención de los depredadores de nidos. Sin embargo, estas evidencias han sido criticadas por problemas metodológicos debidos, principalmente, al uso de nidos artificiales. Aquí hemos usado nidos naturales y activos de mirlo para investigar si el comportamiento de petición incrementa la depredación. Nuestros resultados apoyan esta hipótesis indicando que el coste en depredación implica una importante restricción a la evolución del comportamiento de petición de alimento.

En relación a esto, sería adaptativo que los pollos detectaran la proximidad de un depredador cerca del nido y se comportaran de una manera más críptica. De hecho, existen varias evidencias que apoyan esta modificación del comportamiento en los pollos. En esta tesis se ha explorado, por primera vez, este tema desde una perspectiva hormonal. Nuestros resultados muestran un cambio hormonal (corticosterona y testosterona) como respuesta al riesgo de depredación del nido. Aunque, las discrepancias en los resultados a largo y corto plazo del riesgo de depredación parecen sugerir la existencia de diferentes mecanismos para enfrentarse a situaciones de estrés crónico o inmediato. Mientras que los efectos a corto plazo podrían explicarse con objeto de reducir la actividad de petición de alimento, los cambios inducidos a largo plazo podrían producirse bien como una estrategia hormonal de los pollos para favorecer un crecimiento rápido en la población con la mayor presión de depredación, o bien como una respuesta indirecta de las crías al riesgo de depredación mediada por los padres. Estos hallazgos destacan la importancia de estudiar la depredación de nidos desde el inusual punto de vista de los pollos y resalta los beneficios de incluir variables fisiológicas en el estudio de las interacciones depredador-presa.

Finalmente, nos hemos centrado en el estudio del efecto de las actividades de investigación en el éxito reproductivo de las aves desde dos puntos de vista: local y general. Los investigadores han estado preocupados desde hace tiempo por los efectos que tienen sus propias actividades y cómo éstas pueden afectar a sus resultados. La idea tradicional destaca que la actividad científica tiene una influencia negativa en el éxito reproductivo de las especies de aves estudiadas a través de un incremento de la tasa de depredación de los nidos. Sin embargo, los resultados de nuestro estudio muestran que el incremento de la tasa de visitas al nido favorece la reproducción de los mirlos a través de una reducción en la depredación de sus nidos. Más aún, este efecto positivo se mantiene entre poblaciones a pesar de diferencias en su comunidad de depredadores. Desde una perspectiva comparativa, usando un meta-análisis, hemos encontrado que, contrariamente a lo que se pensaba, las actividades investigadoras no afectan a la depredación de nidos en aves. Esta relación es heterogénea entre órdenes de aves, y sorprendentemente, en el caso de los Paseriformes, resulta significativa y positiva. Esta nueva perspectiva de que el efecto de los investigadores no es tan negativo como se pensaba podría tener importantes implicaciones en trabajos sobre Ecología y Evolución, puesto que las especies de Paseriformes son, con frecuencia, utilizadas como modelos de estudio para comprobar hipótesis generales. Además, esta nueva interpretación puede tener también importantes repercusiones en el campo de la Biología de la Conservación.

SUMMARY

Predation is considered one of the most important selective forces in nature. This relevance is even greater in the case of birds because this group has a long period of parental care which, in many cases, involves the offspring permanence in a certain location (the nest) during their development. Indeed, nest predation is considered the main reason of avian nest loses. This thesis explores this topic from the double point of view of the parents and their chicks, using the common blackbird (*Turdus merula*) as the model species. The double focus is important because nest predators can use cues produced by their preys' activities (i.e. chicks' begging) to locate and predate the nest, but also cues provided by parents who care them (mainly nest visits).

We used three blackbird populations that had not been investigated previously. Thus, as a first step, we studied the breeding parameters of our model species in each location. As predicted we found significant differences among populations in their main selective pressures and several reproductive traits. Given that our three studied locations differ in their human influence, urbanization might be behind the differences found. Our results also highlight that it is better (and critical) to use life-history syndromes instead of single traits in evolutionary studies.

Because parental care behavior can attract nest predators to the nest we have explored the strategies used by parents to minimize this risk. Parental care can change during the different developmental stages of the offspring at the nest, thus, we investigated this antipredator strategies for the incubation and chick's period. We have confirmed that female blackbirds can reduce their activities during incubation in the presence of a predator, which decreases the possibilities of revealing the location of their nest according to Skutch's hypothesis. However, females can use other antipredator strategies in order to enhance nest concealment. The use of one or another strategy possibly depends on the population specific nest predation risk. Moreover, both sexes respond similarly to the risk of nest predation during the chick's stage, although not for all traits analysed (i.e. mean visit duration). And the existence or not of antipredator responses seems to depend on the environmental risk of nest predation. In addition, we have found that parents can respond to the risk of nest predation without compromising the food delivered to their nestlings, the main cost associated to this behavior, modificating other non-feeding behaviors like brooding. These results

highlight the complexity of antipredator responses in birds and suggest the re-consideration of established theory in this field.

But, predators can use directly offspring activities to locate and predate the nest. Begging is one of the main activities delivered by nestlings. Several studies indicate that the acoustic signal associated with this behavior could attract the attention of nest predators. However, these evidences have been criticized because of methodological problems, mainly the use of artificial nests. Here, we use natural and active blackbird nests to investigate whether begging behavior increases nest predation. Our findings support this hypothesis indicating that this predation cost imposes an important restriction to the evolution of begging behavior.

In relation to this, it would be adaptive for chicks to detect the presence of the predator near their nest and change to a more cryptic behavior. Indeed, there are several evidences that support this behavioral modification in nestlings. We have explored this topic, for the first time, from a hormonal perspective and our results have showed hormonal changes in response to nest predation risk. However, we have found unmatched results between short and long-term nest predation risk, which seems to point out the existence of different mechanistic pathways to cope with acute and chronic stressful situations. While its short-term effects could be explained by a reduction in begging activity, long-term induced changes suggested whether a hormonal strategy to favor a fast growth in chicks of the population with the higher predation pressure or the offspring indirect response to nest predation risk mediated by parents. These findings highlight the importance of studying nest predation from the unusually considered chick's perspective and underline the benefits from including physiological variables in the study of predator-prey interactions.

Finally, we focused on the study of the effect of investigation activities in avian breeding success from two points of view (local and general). Researchers have long been worried about the effects of their own activities and how they could influence their findings. The traditional idea stresses the negative influence that scientific disturbance has on breeding performance by increasing nest predation rate of species studied. However, the results in our study locations showed that the increase in nest-visiting rates favours blackbird's breeding success through a reduction of nest predation. Moreover, this positive effect is maintained between populations despite the differences in the community of nest predators. From a comparative perspective, using a meta-analysis, we have found that research activities do not affect avian nest predation, contrary to the traditional idea. This relationship is heterogeneous among avian orders, and surprisingly, significantly positive for passerines. This new perspective on the effect of investigators could have important implications in several fields such as Ecology and Evolution because passerines are widely used to test general hypotheses. This conclusion will be also important in other fields like management and conservation.

INTRODUCCIÓN

La Ecología es la ciencia que estudia las interacciones de los seres vivos con su ambiente y, como parte de ellas, aborda el estudio de las relaciones de los organismos entre sí como el parasitismo, el mutualismo o la depredación. La evolución juega un papel vital en el entendimiento de esta ciencia (Moreno 2008), y la Ecología del Comportamiento, o Etología Evolutiva como también se la conoce en la actualidad, ha sido la disciplina que ha contribuido de una forma más decidida a darle ese enfoque evolutivo al estudio de las relaciones entre especies (Carranza 2008). La presente tesis se ha centrado en el estudio evolutivo de una de esas interacciones entre individuos, la depredación, desde el prisma del comportamiento animal.

La depredación es una interacción biológica por la cual un organismo (depredador) se alimenta de otro (presa) con objeto de obtener la energía necesaria para su mantenimiento, crecimiento y reproducción ante la incapacidad de crearla por sí mismo. El hecho de que normalmente esa alimentación implique la muerte de la presa hace que la depredación sea considerada como una de las fuerzas selectivas más importantes en la naturaleza (Lima y Dill 1990; Lima 1998). Por tanto, no es de extrañar que haya mantenido un puesto predominante en la Biología Evolutiva desde sus inicios y siga siendo en la actualidad uno de los temas protagonistas de la investigación en este campo, aunque todavía se desconozca el significado adaptativo de muchos comportamientos mostrados por las presas ante los depredadores (Caro 2005).

La importancia de la depredación es si cabe todavía mayor en el caso de las aves, pues poseen un periodo prolongado de cuidados parentales, que en muchas ocasiones implica la permanencia de los pollos en un lugar concreto (el nido) durante todo o gran parte de su desarrollo, por lo que el riesgo de depredación es elevado. Así, la depredación es considerada como el factor responsable de la mayoría de las pérdidas de nidos en aves (Ricklefs 1969; Martin 1995). Sin embargo, no ha sido hasta los últimos años que la depredación de nidos ha pasado a considerarse como uno de los principales mecanismos en la evolución de las estrategias vitales de las aves. Esto es debido a la aparición de nuevas evidencias que subrayan su especial importancia, tanto desde el punto de vista de los adultos (p.e. Ferretti et al. 2005; Eggers et al. 2005, 2006, 2008; Zanette et al. 2006), como de los pollos (p.e. Platzen y Magrath 2004, 2005; Madden et al. 2005 a, b; Magrath et al. 2007), que han supuesto una revolución en este campo. Hoy día se asume que la limitación de alimento, considerada la principal causa de las modificaciones en las estrategias vitales de las aves (Lack 1947) hasta hace poco, no es la única importante. Los últimos descubrimientos han puesto de manifiesto que la depredación es, al menos, tan influyente como la disponibilidad de alimento.

Estos trabajos que han conseguido que se reconsidere la importancia de la depredación de nidos se basan eminentemente en un enfoque particular: la capacidad de los depredadores de localizar la ubicación del nido y las estrategias utilizadas por las aves para evitarlo. En general, los depredadores pueden localizar a sus presas a través de diversos mecanismos, siendo uno de los principales el uso de la propia actividad de las presas (Maier 2001; Caro 2005). Esto, también es aplicable a los depredadores de nidos, ya que pueden utilizar no sólo las pistas proporcionadas por la actividad de sus presas, los pollos, sino también las facilitadas por los padres que cuidan de ellos. Uno de los principales objetivos de esta tesis ha sido precisamente profundizar en este aspecto: el estudio de las pistas utilizadas por los depredadores para localizar el nido y las estrategias antidepredatorias usadas por los pollos y sus padres como defensas.

Cuidados parentales

Los cuidados parentales son una adaptación evolutiva por la cual un organismo reproductor no se limita a producir copias de su material genético, sino que además emplea parte de su tiempo y energía en aumentar las posibilidades reproductivas de esas copias, incluso a costa de su reproducción futura (Carranza 2003). En sentido amplio consisten en todo aquello que los padres hacen para favorecer el éxito de las crías (Clutton-Brock 1991), teniendo especial relevancia los comportamientos parentales tras la producción de los hijos. Estos comportamientos, en muchos casos necesarios para la supervivencia de las crías (p.e. alimentación de los hijos; Figura 1), crean una serie de pistas que pueden ser usadas por los depredadores para localizar y depredar el nido. El primero en proponer esta relación fue Alexander Skutch (1949), que sugirió que los depredadores podrían usar las visitas de los padres para detectar la ubicación del nido. Recientemente la hipótesis de Skutch ha recibido bastante atención consiguiendo un respaldo unánime entre la comunidad científica (p.e. Martin et al. 2000 a, b; Muchai y Du Plessis 2005; Rastogi et al. 2006). Sin embargo, aún se desconocen los costes asociados a tal comportamiento. Se suele asumir que la reducción en visitas al nido impuesta por la presencia de un depredador conlleva unos costes importantes puesto que los pollos reciben menos alimento durante ese periodo. Y está demostrado que una baja tasa de alimentación reduce la calidad de los hijos, y por tanto las probabilidades de supervivencia y reproducción futura de éstos (Daan et al. 1990; Richner 1989, 1992; Nilsson 1990; Metcalfe y Monaghan 2001). De hecho, se han propuesto varios mecanismos que permitirían compensar esta disminución en la tasa de alimentación de los pollos: (i) reducir el tamaño de puesta (p.e. Eggers et al. 2006; Zanette et al. 2006; Kleindorfer 2007; Olsen et al. 2008); (ii) maximizar el tamaño de las cebas (p.e. Martin 1996; Martin et al. 2000 a); (iii) evitar la colaboración de ayudantes en especies con cría cooperativa para disminuir el número de visitas al nido (Strickland & Waite 2001); y (iv) compensar la reducción en visitas durante situaciones de alto riesgo con un aumento de visitas en situaciones de bajo riesgo de depredación del nido (Eggers et al. 2005). Sin embargo, sólo un estudio ha investigado directamente los costes asociados a esta disminución de las visitas. En él, Martin et al. (2000 a) hallaron que aquellas especies con un elevado riesgo de depredación presentaban un menor número de visitas al nido, y consecuentemente una menor tasa de alimentación a los pollos, algo que no conseguían compensar totalmente con un mayor tamaño de las cebas. Es sorprendente que no se haya realizado ningún estudio experimental que haya analizado estos costos directamente, aun siendo críticos para comprender la evolución de los cuidados parentales.

Otra área de desconocimiento que presenta importantes lagunas en lo que respecta a la hipótesis de Skutch, y en general a la depredación de nidos, es su relación con el conflicto sexual (Ghalambor y Martin 2002). Los cuidados parentales constituyen uno de los elementos más importantes de este conflicto evolutivo existente entre los dos miembros de la pareja por optimizar su inversión parental en el presente evento reproductor. Machos y hembras pueden diferir en sus estrategias vitales y actividades en relación al nido (Clutton-Brock 1991), lo que hace pensar que cada sexo puede producir diferentes pistas utilizables por los depredadores. Esto implicaría también la posibilidad de que cada sexo pueda responder de forma diferente ante el riesgo de depredación del nido. Sin embargo, esta perspectiva sexual ha sido escasamente estudiada, habiendo sido investigada únicamente durante el periodo de la incubación y de una manera incompleta (p.e. Ghalambor & Martin 2000, 2002; Fontaine y Martin 2006; Massaro et al. 2008). Los estudios durante la fase de pollos, particularmente en especies en las que ambos padres alimenten a las crías, permitirían un enfoque más completo para comprobar la existencia de diferencias sexuales en las estrategias empleadas ante el riesgo de depredación, por ejemplo, en la reducción de visitas.

***Figura 1**. Macho de mirlo común llevando alimento al nido.*

Hasta la fecha, únicamente existen evidencias indirectas de que las hembras respondan ante el riesgo de depredación del nido con una reducción de sus visitas para evitar revelar la localización del nido, aunque en los machos está ampliamente demostrado (p.e. Ghalambor y Martin 2002; Fontaine y Martin 2006). Esas evidencias indirectas, en concreto el mayor tiempo de permanencia en el nido, pueden ser explicadas simplemente para aumentar la ocultación del nido (Montgomerie y Weatherhead 1988; Weathers y Sullivan 1989; Martin 1992; Kleindorfer y Hoi 1997). Esta ambigüedad en la interpretación tiene importantes consecuencias, ya que por un lado la hipótesis de Skutch implicaría que tanto machos como hembras utilizan las mismas estrategias antidepredatorias frente al riesgo de depredación del nido, mientras que la hipótesis de la ocultación conllevaría diferentes estrategias para machos (reducción de las visitas) y hembras (aumento de la ocultación del nido). La investigación de especies sin alimentación de la hembra por parte del macho durante la incubación permitiría resolver esta ambigüedad. En estas especies, las respuestas de las hembras al riesgo de depredación, en caso de existir, serán responsabilidad exclusiva de ellas, y por tanto, más exageradas y fáciles de detectar. Además, la comparación en el tiempo que tarda la hembra en volver a su nido en presencia y ausencia de un depredador potencial de nidos permitiría discernir entre la estrategia antidepredatoria utilizada por las madres. Si las hembras vuelven rápidamente se confirmaría la hipótesis de la ocultación, en cambio, si éstas retrasan su vuelta más de lo habitual sería la hipótesis de Skutch la que resultaría apoyada.

Actividad de los pollos

Como se ha comentado anteriormente, los depredadores de nidos también pueden utilizar directamente la actividad de sus presas, los pollos, como una pista para localizar los nidos. Aunque existen otras actividades de los pollos que podrían estar asociadas a la localización y depredación de los nidos de aves (p.e.: emisión de calor, producción de excrementos…), es la petición de alimento la que más facilita la depredación. La petición de alimento está compuesta por una serie de señales diversas dirigidas a los padres, con objeto de conseguir la cantidad de alimento óptima necesaria para el correcto desarrollo del pollo. Esta comunicación incluye señales visuales y auditivas, que se despliegan normalmente cuando los padres están presentes, siendo por tanto bastante limitadas en el tiempo (Kilner 2002; Figura 2). Las primeras suelen estar asociadas a las comisuras y a la cavidad bucal, como la intensidad del color o la reflectancia de ultravioletas (revisado en Kilner 2002). Estas señales son dirigidas muy directamente a los padres y su capacidad de transmisión es escasa. Además el conflicto entre hermanos a este nivel de las señales visuales es muy poco llamativo, ya que los pollos no suelen tener constancia de los valores visuales de sus hermanos, especialmente porque parte de su estancia en el nido la realizan sin poder ver (Gill 1990). Por lo tanto, las probabilidades de que estas señales visuales de la petición puedan ser usadas por los depredadores son muy escasas. Sin embargo, las señales acústicas poseen una capacidad de transmisión mucho mayor y no están tan restringidas en el tiempo (Burford et al. 1998; Price 1998). Además, este componente acústico sí que refleja de manera directa el conflicto entre hermanos. El pollo que más pide es el que recibe el alimento, por lo que la presión selectiva que favorece la evolución de señales exageradas es muy fuerte (Roulin et al. 2000; Roulin 2002). Esto hace que las señales acústicas de la petición de alimento puedan constituir una excelente fuente de información para los depredadores, si estos son capaces de localizar su origen. De hecho, existen varios trabajos que ponen de manifiesto de forma directa el coste en depredación asociado al componente acústico de la petición (p.e. Haskell 1994, 1999; Leech y Leonard 1997; Dearborn 1999; McDonald et al. 2009), mientras que otros, de manera indirecta, permiten inferir la existencia de este coste, como el silenciamiento de los pollos al escuchar a un depredador aproximarse o ante las señales de alarma de los padres frente a situaciones de peligro (p.e. Davies et al. 2004; Platzen y Magrath 2004, 2005; Madden et al. 2005 a, b; Magrath et al. 2007). Sin embargo, las conclusiones de los estudios empíricos directos han sido duramente criticadas por problemas metodológicos como el uso de nidos artificiales o grabaciones sonoras (Haskell 2001). Aun así, se asume que el coste en depredación parece ser uno de los más plausibles como responsable de evitar la escalada contínua de la intensidad de las señales de petición (Wrigth y Leonard 2001; Moreno-Rueda 2007). Sería, pues, muy importante determinar de manera fidedigna y directa si ese coste existe realmente. Una posible manera de determinar su existencia y evitar los problemas metodológicos que se han destacado anteriormente, sería utilizar nidos reales activos en lugar de nidos artificiales y, a la hora de manipular el nivel de petición, emplear pollos reales en lugar de grabaciones emitidas por altavoces.

***Figura 2**. Pollos de mirlo común pidiendo comida.*

Por otro lado, es bien conocido que el sistema endocrino participa muy directamente en el control de la petición (Schwabl y Lipar 2002). El sistema endocrino actúa a través de las hormonas para modificar la fisiología y el comportamiento de los individuos, lo que les permite reaccionar ante cambios en su entorno. Las dos principales hormonas que parecen regular la petición son la corticosterona y la testosterona (Kitaysky et al. 2001, 2003; Goodship y Buchanan 2006, 2007; Quillfeldt et al. 2006; Loiseau et al. 2008). Estas hormonas esteroideas se liberan a través de la activación del eje hipotálamo-pituitario-adrenal (HPA, la corticosterona) o del eje hipotálamo-pituitario-gonadal (HPG, la testosterona) como muestra la figura 3, participando en muchas otras funciones del organismo (p.e. Sapolsky et al. 2000; Landys et al. 2006; Fusani 2008). Este hecho impone importantes restricciones a la elevación de estas hormonas, ya que puede producir alteraciones críticas para la supervivencia de los individuos, especialmente durante el desarrollo, como modificaciones del sistema inmune o movilización de reservas energéticas (Landys et al. 2006). Dada la relación existente entre estas dos hormonas y la petición de los pollos, junto con las evidencias mostradas con anterioridad que destacan el silenciamiento de los pollos ante situaciones de potencial peligro para ellos (ver referencias más arriba), no es descabellado pensar que el riesgo de depredación del nido producirá alteraciones fisiológicas en los pollos. De hecho, un reciente estudio muestra los beneficios de la inclusión de variables fisiológicas en el estudio de las interacciones depredador-presa y recomienda su utilización (Slos et al. 2009). Por lo tanto, el estudio de las modificaciones hormonales inducidas por el riesgo de depredación de nidos permitiría profundizar en el entendimiento de la influencia de la depredación en las respuestas adaptativas de las aves.

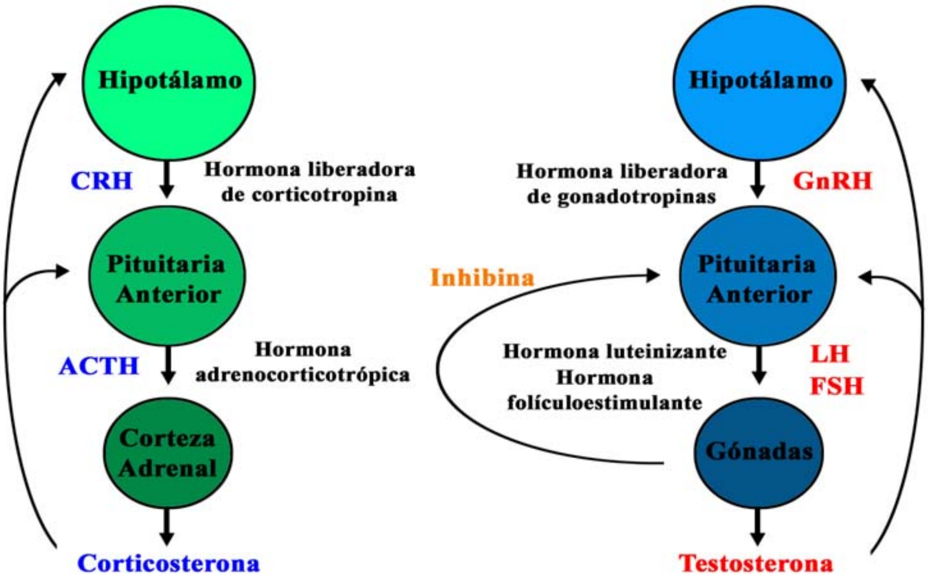


Figura 3. *Diagrama de los ejes hipotálamo-pituitario-adrenal (HPA, verde) e hipotálamo-pituitario-gonadal (HPG, azul) mostrando la cascada de hormonas liberadas tras un estímulo externo.*

El efecto del observador

Los investigadores siempre han estado preocupados por los efectos de sus propias actividades y si éstas podrían influenciar de alguna manera sus hallazgos. Este tema ha recibido especial atención en ciertos campos de la ciencia como la Física, siendo, quizás, el mejor ejemplo de ello el "Principio de Incertidumbre de Heisenberg" (Heisenberg 1927). Sin embargo, es un problema extensible a otras disciplinas como la Biología, particularmente en lo referente a la Ecología del Comportamiento, y en especial al estudio de la depredación. Esto es debido a que los humanos han sido y son importantes depredadores de aves y mamíferos (Robinson y Bennett 2000), y por tanto, la mera presencia de un observador humano puede afectar al comportamiento antidepredatorio de la especie objeto de estudio (Lenington 1979).

Desde hace años los ornitólogos han sido conscientes de este problema metodológico (Johnson 1938), sin embargo, hasta la década de los 60 no se empezaron a diseñar experimentos concretos para evaluar la influencia del observador (p.e. Harris 1964; Choate 1967; Grier 1969). El interés en la comunidad ornitológica ha ido incrementando desde entonces, publicándose numerosos artículos sobre el tema, muchos de ellos mostrando efectos negativos de las actividades de investigación en el éxito reproductivo de las aves (p.e. Anderson y Keith 1980; Cairns 1980; Pierce y Simons 1986; Boellstorff et al. 1988), una idea que se terminó de implantar tras la publicación de la exhaustiva revisión de Götmark (1992). Esto añadió un nuevo problema ético al ya conocido metodológico, especialmente grave en lo referente a estudios de especies amenazadas o vulnerables. En estos últimos veinte años se han publicado nuevos trabajos, la mayoría de ellos sin obtener efectos significativos (p.e. Schaub et al. 1992; Cotter y Gratto 1995; Lloyd et al. 2000; Bêty y Gauthier 2001; Verboven et al. 2001), y los que los obtuvieron, sorprendentemente detectaron efectos positivos de la actividad investigadora sobre el éxito reproductivo de las aves (Mayer-Gross et al. 1997; Ortega et al. 1997; Weidinger 2008). Ante estas evidencias que muestran resultados contradictorios parece clara la necesidad de una nueva revisión y síntesis del tema utilizando las modernas técnicas estadísticas de meta-análisis.

El hecho es que Götmark ya avisaba en su revisión de que el efecto de las actividades de los ornitólogos dependía posiblemente del grupo taxonómico estudiado, existiendo una sobre-representación de ciertos órdenes como Pelecaniformes, Anseriformes o Charadriformes, y una infra-representación de otros como los Falconiformes o los Paseriformes. El caso de los últimos es especialmente relevante, ya que representando casi el 60% de las especies de aves del mundo (Gill 1990), sólo constituían un 19,6% de las especies estudiadas con respecto al efecto de los investigadores (calculado de Götmark 1992). Por regla general, sin encontrar ningún efecto significativo. Esto es si cabe todavía más importante teniendo en cuenta el papel fundamental desempeñado por los Paseriformes como modelos de estudio en trabajos de disciplinas como la Ecología o la Evolución en las últimas décadas. Todo ello hace de vital importancia seguir las recomendaciones previas y centrarnos en el estudio del posible efecto que puedan tener los científicos en los grupos menos estudiados, con especial atención en los Paseriformes.

Lo que sí parece claro es que las alteraciones en el éxito reproductor de las aves debidas a la actividad investigadora se producen a través de cambios en la depredación de nidos, y por lo tanto por modificaciones en la actividad de los depredadores (Götmark 1992). Sin embargo, pocos son los estudios que han considerado directamente el concepto de que la presencia de los científicos afecte a los depredadores, y que éstos puedan tener distinta sensibilidad a la misma (Sandvik y Barret 2001; Sutherland 2007). De hecho, hay estudios que muestran que las aves depredadoras podrían seguir a los investigadores hasta los nidos (Strang 1980; Götmark et al. 1990), mientras otros indican que los mamíferos depredadores evitan los lugares frecuentados por los científicos (MacIvor et al. 1990; Armstrong 1993). Esto hace pensar que la composición de la comunidad de depredadores influirá en el efecto de las actividades de investigación sobre la depredación de nidos, aunque hasta la fecha nadie ha explorado la posibilidad de que pueda ser un factor que explique variaciones en la influencia de los investigadores.

OBJETIVOS

Capítulo 1: Determinar la importancia relativa de las presiones selectivas más importantes (limitación por alimento y depredación de nidos) y analizar los síndromes de estrategias vitales en tres poblaciones de mirlo común con diferente influencia humana.

Capítulo 2: Estudiar los cambios inducidos por el riesgo de depredación en el comportamiento de incubación de las hembras de mirlo común, una especie en la que el macho no alimenta a la hembra durante este periodo, y comprobar si las hembras cumplen la hipótesis de Skutch o la de la ocultación del nido.

Capítulo 3: Determinar si existen diferencias sexuales en los cambios provocados en los cuidados parentales por el riesgo de depredación del nido, y analizar los costes asociados a la modificación de esos comportamientos.

Capítulo 4: Comprobar la hipótesis del coste en depredación de la petición de los pollos con una nueva aproximación al tema, obviando los potenciales problemas metodológicos de la utilización de nidos artificiales y sonidos grabados.

Capítulo 5: Estudiar la existencia de modificaciones fisiológicas en los pollos, a nivel del sistema endocrino (corticosterona y testosterona), inducidas por el riesgo de depredación del nido.

Capítulo 6: Detectar el efecto de la frecuencia de visitas del investigador en el éxito reproductivo del mirlo común, y la posible influencia de la comunidad de depredadores sobre dicho efecto.

Capítulo 7: Comprobar, utilizando la metodología de meta-análisis, la hipótesis de que la influencia del investigador reduce el éxito reproductivo en las aves porque provoca un incremento de la depredación de nidos.

METODOLOGÍA GENERAL

Especie modelo:

El mirlo común (*Turdus merula*) es un ave del orden de los Paseriformes de tamaño medio (85-105 gramos) que presenta un dimorfismo sexual relativamente marcado (Figuras 4 y 5). Se considera una especie politípica de distribución paleártica (hasta 15 subespecies), aunque existe como introducida e invasora en Australia, Nueva Zelanda y otras islas próximas del Pacífico Sur. Se le puede encontrar en una gran cantidad de ambientes, desde bosques densos de árboles caducos o perennes hasta en el interior de las ciudades, pasando por tierras de cultivo, marismas, zonas de riberas, dehesas, brezales o páramos, normalmente asociado a arbustos y árboles de porte medio.

Es una especie sedentaria o migradora, según la latitud. Las poblaciones más septentrionales son consideradas migradoras o migradoras parciales, dejando las zonas de cría en otoño e invierno, sin embargo, en latitudes más meridionales suele ser eminentemente sedentaria (Collar 2005).

***Figura 4**. Macho de mirlo común.*

***Figura* 5**. *Hembra de mirlo común.*

Su alimentación suele incluir principalmente invertebrados (lombrices, caracoles, larvas y adultos de insectos), pero también frutos y semillas, especialmente durante el otoño y el invierno. También puede ingerir diversos alimentos humanos (Collar 2005).

El mirlo común es una especie monógama y territorial. Las parejas suelen formarse al inicio de la temporada de cría y mantenerse como tales hasta que uno de sus miembros muere (Cramp 1988). Su periodo de cría en Europa va desde Febrero a Septiembre, variando según la latitud. Es una especie altricial que construye nidos abiertos. Es la hembra la que se encarga de construir el nido utilizando briznas de broza, ramitas y barro en la base, a una altura que puede oscilar entre 0.5 y 15 m. Su tamaño de puesta es de 2 a 6 huevos, habitualmente 3 a 5, aunque varía con la latitud. La hembra es la única que incuba los huevos durante un periodo de 10-19 días (13 días de media), aunque el macho se ha visto esporádicamente sentado en el nido. Los pollos son alimentados en el nido por ambos padres durante 13-14 días, y tras volar, continúan alimentándolos durante otros 20 días aproximadamente (Collar 2005).

Los nidos de los mirlos sufren elevados niveles de depredación (Snow 1958; Cresswell 1997; Groom 1993; Hatchwell et al. 1996; Tomialojc 1994), siendo la principal causa de fracaso reproductor en esta especie. Buena parte de las depredaciones son atribuidas a depredadores visuales, principalmente córvidos (Groom 1993; Collar 2005), siendo varios los trabajos que relacionan la ocultación de los nidos con el éxito reproductivo (Collar 2005).

Zonas de estudio:

Para la realización de esta tesis se han utilizado tres zonas de estudio ubicadas en la provincia de Granada, separadas entre sí unos 20 km unas de otras (Figura 6). Estas localizaciones presentan poblaciones de mirlo común con densidades de nidificación similares.

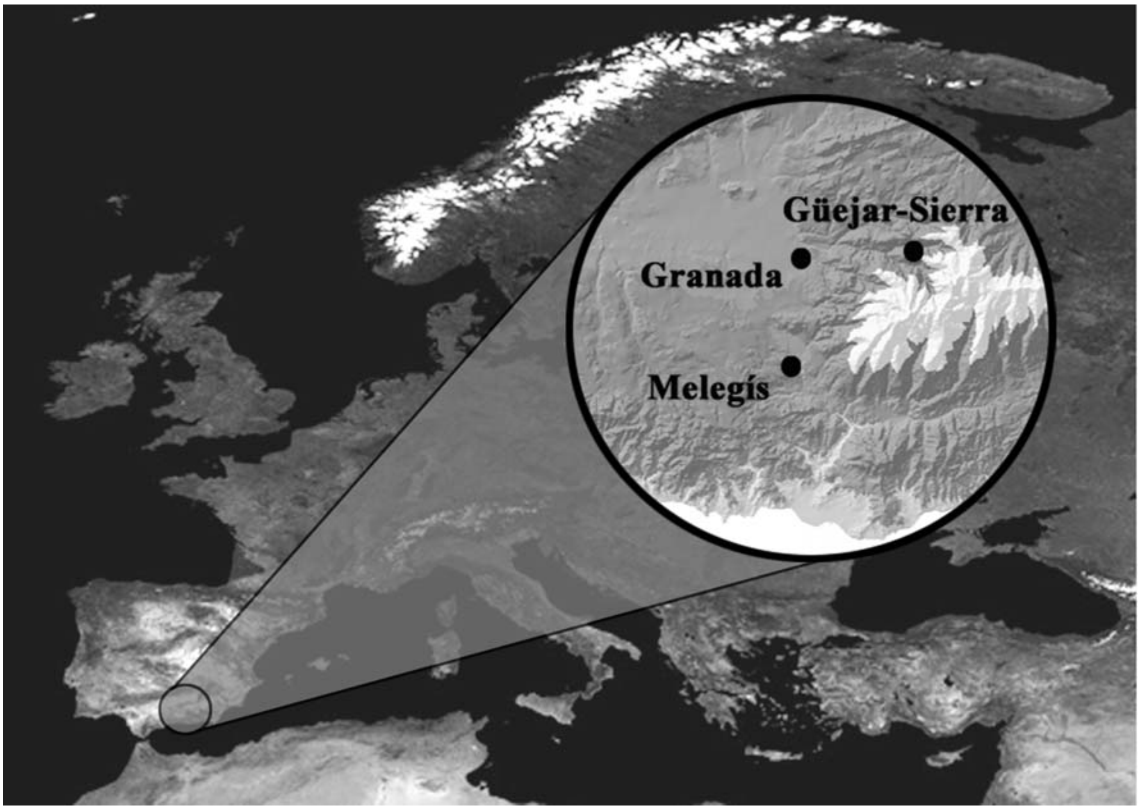


***Figura 6**. Mapa mostrando la localización de las tres poblaciones de mirlo común utilizadas para la realización de esta tesis.*

La primera de ellas se encuentra enclavada en el término municipal de Güejar-Sierra, en el macizo de Sierra Nevada (37º 9’ N, 3º 24’ O; 1050 m). Consiste en un bosque mediterráneo dominado principalmente por la encina (*Quercus rotundifolia*), aunque en las zonas más altas se solapa con individuos de roble melojo (*Quercus pyrenaica*). El bosque está situado en el piso bioclimático mesomediterráneo, con temperaturas medias anuales de 12 a 16 ºC y ombroclima subhúmedo (precipitación anual de 600-1000 mm).

Figura 7. *Fotografía de la zona de estudio de Güejar-Sierra.*

La segunda se localiza en el término municipal de Melegís, en el Valle de Lecrín (37º 17’ N, 3º 59º W; 695 m). Se trata de una zona de cultivo de naranjos (*Citrus sinensis*), con olivos (*Olea europaea*) intercalados en algunas fincas. Estos naranjales están situados en el piso bioclimático mesomediterráneo, presentando temperaturas medias anuales de 12 a 18 ºC y ombroclima seco (precipitación anual de 350-600 mm).

***Figura 8**. Fotografía de la zona de estudio de Melegís.*

La tercera zona de estudio se halla en el término de Granada, concretamente dentro de la matriz urbana de la ciudad del mismo nombre (36º 56’ N, 3º 33’ O; 580 m). Los parques y jardines de la ciudad consisten en una mezcla de especies autóctonas y alóctonas entre las que destacan los álamos (*Populus alba*), naranjos (*Citrus sinensis*), laureles (*Laurus nobilis*) y aligustrones (*Ligustrum vulgare*). La ciudad se encuentra enclavada en el piso bioclimático mesomediterráneo, con una temperatura media anual de 15 ºC y ombroclima seco (precipitación anual de 350-600 mm).

En las tres localizaciones se siguió un patrón de estudio similar, con búsqueda activa de nidos y marcaje con GPS (Garmin Gekko 201). Los nidos eran visitados cada 2-3 días para ver su estado y realizar un seguimiento exhaustivo de los mismos. Para ello se utilizó un espejo montado en un palo y un extensor, que nos permitía ver el interior del nido rápidamente, sin subirse al árbol donde estuviera ubicado el nido. De esta manera se intentó minimizar el tiempo de permanencia cerca del nido y reducir la alteración del ambiente circundante al mismo.

Figura 9. *Fotografía de la zona de estudio de Granada.*

Se realizaron además grabaciones de los nidos con equipos de grabación continua (24 horas), que junto con observaciones directas nos han permitido detectar a los principales depredadores de nidos de las zonas muestreadas:

- **Güejar-Sierra:** Gavilán (*Accipiter nissus*), arrendajo común (*Garrulus glandarius*), garduña (*Martes foina*), culebra de escalera (*Elaphe escalaris*), culebra bastarda (*Malpolon monspessulanus*) y la hormiga *Camponotus cruentatus*. Presentes en la zona y potenciales depredadores también son la urraca común (*Pica pica*), el zorro (*Vulpes vulpes*) y diversas especies de micromamíferos.

- **Melegís:** Ser humano (*Homo sapiens*), gato doméstico (*Felis catus*), Jineta común (*Genetta genetta*). Presentes en la zona y potenciales depredadores también son la comadreja (*Mustela nivalis*), la rata de alcantarilla (*Rattus norvegicus*) y diversas especies de micromamíferos.

- **Granada:** Urraca común (*Pica pica*), gato doméstico (*Felis catus*) y ser humano (*Homo sapiens*). Presentes en la zona y potenciales depredadores también son la rata de alcantarilla (*Rattus norvegicus*) y diversas especies de micromamíferos.

Figura 10. *Secuencia de fotogramas que muestran la depredación de un nido de mirlo por un gavilán común.*

***Figura 11**. Secuencia de fotogramas que muestran la depredación de un nido de mirlo por un arrendajo común. La flecha roja indica la posición de la cabeza del arrendajo, mientras que la flecha azul muestra la localización del huevo.*

CAPÍTULO 1

¿Afecta la urbanización a las presiones selectivas y estrategias vitales de los mirlos comunes?

La depredación de nidos y la limitación por alimento son los dos principales factores propuestos para explicar la evolución de las estrategias vitales. Además, la urbanización, una de las alteraciones humanas del suelo más extremas, está extendiéndose, y el número de especies que se enfrentan a estos cambios aumentando. Sin embargo, resultados contradictorios de estudios previos impiden una interpretación clara de cual de estas dos presiones selectivas es más importante en los hábitats urbanos en comparación con las situaciones naturales, si es que alguna predomina, y si las aves pueden enfrentarse a ellas ajustando sus estrategias vitales. Nosotros investigamos los síndromes de estrategias vitales de tres poblaciones de mirlo común diferenciadas en su influencia humana (urbana, rural y forestal). Analizamos la tasa de depredación de nidos diaria y de inanición de pollos para atribuir la importancia relativa de estas presiones selectivas en cada hábitat. Simultáneamente, varios rasgos de estrategias vitales fueron investigados para determinar si los mirlos están adaptados a su principal fuente de selección. La limitación por alimento fue más importante en la ciudad, mientras que la depredación de nidos fue la fuerza selectiva más importante en el bosque. El hábitat rural estuvo caracterizado por una influencia intermedia de ambos factores. Los síndromes de estrategia vital, entendidos como la covariación de un grupo de rasgos, confirmó estos resultados ya que los mirlos parecían bien adaptados a su principal causa de selección en cada hábitat. Nuestros resultados son consistentes con la idea de que la urbanización impone nuevos retos a las aves, y que éstas pueden responder adaptativamente a los mismos. Adicionalmente, no encontramos diferencias en la tasa de vuelo entre poblaciones. Sin embargo, el hecho de que sólo el 17% de los nidos fueran exitosos en el bosque, pero este valor incrementara hasta el 46% y 53% en las poblaciones rural y urbana respectivamente, evidencian una eficacia biológica significativamente mayor con el incremento de la influencia humana. Esto sugiere que los mirlos se benefician de las actividades humanas, lo que podría ser la principal explicación de por qué esta especie se está expandiendo en las áreas urbanas.

CAPÍTULO 1

Does urbanization affect selective pressures and life-history strategies in common blackbirds?

Juan Diego Ibáñez-Álamo and Manuel Soler

Nest predation and food limitation are the two main factors proposed to explain life-history theory. In addition, urbanization, one of the most extreme land-use alterations, is currently spreading, and the number of species confronting these changes is increasing. However, contradictory results of previous studies impede a clear interpretation of which of these two selective pressures, if any, is more important in urban habitats compared with natural situations, and whether birds can confront them by adjusting their life-history strategies. We investigated life-history syndromes of three common blackbird (*Turdus merula*) populations differing in their human influence (urban, rural and woodland). We analyzed daily nest predation and nestling starvation rates to assess the relative importance of these selection pressures in each habitat. Simultaneously, several life-history traits were investigated to determine if blackbirds seem adapted to their main source of selection. Food limitation was more important in the city, while nest predation was the most important selective force in the forest. The rural habitat was characterized by an intermediate influence of these two factors. Life-history syndromes, as the covariation of a suit of traits, confirmed these results because blackbirds seem well adapted to the main cause of selection in each habitat. Our results are consistent with urbanization imposing new challenges on birds, and that they adaptively respond to them. Additionally, there were no differences in fledging rate among populations. However, the fact that only 17% of nests were successful in woodland, but this increased to 46% and 53% in the rural and urban populations, respectively, showed a significantly higher fitness with increasing human impact. This suggests that blackbirds benefit from human activities, which could be the ultimate explanation why this species has been expanding into urban areas.

Manuscrito

Introduction

Urbanization is currently an area of active investigation as it involves one of the most extreme forms of land-use alteration, generally leading to a complete restructuring of vegetation and species composition (Miller & Hobbs 2002, Sochat et al. 2006). Furthermore, this environmental change will increase as perspectives predict that in less than 20 years 60% of the human population will be concentrated in urban habitats (United Nations 2007). Thus, it is of great interest to study how species cope with such rapid change of environment, especially in terms of life-histories because of their link with fitness (Moreno 2002, Martin 2004). Several factors have been proposed to explain life history variation, including food limitation, nest predation, adult mortality or parasitism (Moreau 1944, Lack 1947, Skutch 1949, Møller 2005). Traditionally, food limitation has been by far the most studied (Martin 2004) and widely considered the major determinant of life-history traits in birds (Martin 1987). However, nest predation has received increasing attention more recently, and both are now considered at least equally important (e.g. Zanette et al. 2003, Ferretti et al. 2005). Urbanization provides an opportunity to investigate life-history theory because environmental change causes modification of selective pressures (Sochat et al. 2006).

Predictions about the relative importance of these pressures (i.e. food limitation versus nest predation) are difficult for urban compared with non-urban landscapes. On one hand, urban habitats have increased food availability compared to natural habitats (e.g. Gaston et al. 2005, 2007), although several studies suggest that direct or indirect human-provided food does not constitute an adequate substitute for natural food (e.g. Shawkey et al. 2004, Chamberlain et al. 2009). Therefore, we could expect urban birds to suffer from food limitation compared with non-urban birds or *viceversa*. On the other hand, studies using artificial nests indicate higher nest predation rates in urban landscapes (Jokimäki and Huhta 2000, Thorington and Bowman 2003), while others have the opposite pattern (Gering and Blair 1999), or even show no differences (Haskell et al. 2001). Thus, it remains unclear whether birds in the cities suffer higher mortality due to higher nest predation than birds in other habitats. Additionally, these findings should be taken with caution as there is a recent trend questioning the validity of nest predation studies using artificial nests because results can be a methodological artifact.

Therefore, only species using natural nests are recommended for this type of studies (e.g. Moore and Robinson 2004).

Food limitation and nest predation have been proposed to explain variation in life-history traits because these two selective pressures greatly influence fitness (Newton 1991). The food limitation hypothesis proposes that the amount of food resources available would be the major constrain to the reproductive effort of the individuals (Lack 1947, Martin 1987). Therefore, greater food limitation has been suggested to give rise to the following predictions: (1) smaller clutch size in order to adjust reproductive effort and maximize survival of chicks (Lack 1947, Martin 1987, Sæther 1994); (2) lower fledging rates as some chicks could die because of starvation (Magrath 1989, Ferretti et al. 2005); (3) lower parental visitation rates to the nest during incubation and (4) nestling periods as parents spend more time looking for food (Martin 1996, Ferretti et al. 2005); (5) longer nestling period, and (6) slower growth of chicks due to reduced energy and nutrients carried to the nest (Martin 1987, McCarty 2001). In contrast, the nest predation hypothesis proposes that this component of extrinsic mortality drives the evolution of reproductive effort mediated by a trade off between present and future reproduction (Skutch 1949, Martin 1995). Thus, greater nest predation causes the following changes in life-history traits: (1) smaller clutch size in order to minimize energy and offspring losses due to predators (Skutch 1949, Slagsvold 1982, Ferretti et al. 2005); (2) no modification of fledging rate as partial clutch or brood predation is very rare (e.g. Pöysä and Pesonen 2007); (3) lower parental visitation rates to incubate and (4) feed young to reduce the number of clues given to predators about the location of the nest (e.g. Martin et al. 2000a, Martin et al. 2000b, Eggers et al. 2008); (5) shorter nestling periods as daily risk of predation increases with increasing time of nestlings in the nest (Bosque and Bosque 1995, Halupka 1998, Remeš and Martin 2002); and (6) higher growth of chicks which would prepare young to leave the nest sooner and/or bigger, thus, increasing the probability of survival under the attack of a predator (Halupka 1998, Remeš and Martin 2002). Therefore, life-history syndromes provide us with contrasting predictions about responses to food limitation versus nest predation (Martin 1996, 2004). In fact, the study of these syndromes has been recommended for a better understanding of the relative importance of these two selective forces (Martin 2004, Ferretti et al. 2005).

However, previous papers that have investigated differences in life-history traits between urban and non-urban habitats have done so comparing only a small suite of traits (e.g.: clutch size and laying date; reviewed in Chamberlain et al. 2009), and it is considered that studies of passerine responses to urbanization often are devoid of important reproductive information (Van Horne 1983). Furthermore, many previous papers analyzing the effects of urbanization have focused on comparisons within the same population (e.g. Kark et al. 2007, Blair and Johnson 2008, reviewed in Fernández-Juricic and Jokimäki 2001), but studies of life-history differences among rather than within populations may provide alternative perspectives to test interactions of the two factors (Ferretti et al. 2005). Finally, the few studies comparing life-history traits of urban and non-urban habitats only used two populations. This could represent a serious problem if different selection pressures act simultaneously in both locations. In this situation it would be impossible to discern whether the differences found were attributed to the effect of nest predation in one or food limitation in the other. Inclusion of a third population would help to surpass this potential problem and to differentiate between their effects more clearly.

In this paper, we simultaneously studied life-history syndromes of three populations that differ in human influence: urban (situation highly altered by humans), rural (semi-natural situation) and woodland (natural situation). We used the common blackbird (*Turdus merula*) as the model species because its urbanization pattern is well known (Evans et al. 2009a, b, c). The main aims of this paper are, first, try to assess the relative importance of each selection pressure (food limitation and nest predation) depending on urbanization; second, test the predictions related to the two main general hypotheses (food limitation and nest predation) proposed to explain life-history evolution; third, contrast the relative information obtained by the most used life-history trait (clutch size) in comparison with life-history syndromes; and, fourth, determine if birds are adapted to the new selection pressures of urbanized areas.

Methods

Data collection

The study was conducted in three breeding populations of common blackbirds in Granada province (South of Spain). One was an urban population settled in gardens and parks of Granada city (37º 17' N, 3º 59º W; 695 m a.s.l., hereafter referred to as Granada), another was a rural population established in orange groves of the Valley of

Lecrín (36º 56' N, 3º 33' W; 580 m a.s.l., hereafter referred to as Lecrín), and the third a population settled in a *Quercus* forest located near Güejar Sierra, in the Natural Park of Sierra Nevada (37º 9' N, 3º 24' W; 1050 m a.s.l., hereafter referred to as Güejar). These populations are separated about 25 km one from each other, almost an order of magnitude greater than the mean adult dispersal distance for this species (3.2 km; Paradis et al. 1998).

We actively searched for nests in each population from the start of the breeding season (early March) until its end (early July) during 2007. Once a nest was located, we monitored it every three days using a pole with a mirror to determine its incubation period, nestling period and reproductive success. Visits allowed us to know the fate of each nest as we registered its contents. Nests were considered as depredated when they were found with biological remains (shells, feathers or blood) or empty before the chicks had the capacity to jump out of the nest (10-12 day-old chicks). Nests were considered successful when at least one chick was 10-11 day-old. Thus, we could assess the relative importance of nest predation pressure for each habitat. Fledging rate was calculated as the mean number of nestlings per nest that managed to successfully leave the nest. Finally, nests were considered as abandoned when eggs remained in the nest 5 days longer than the mean incubation period and were cold. With this procedure we avoided to touch eggs (and the nest) on every visit, thus, minimizing our odor at the nest, a factor that could modify avian behavior (e.g. Amo et al. 2008). Regular visits also allowed us to know if any chick died during the nestling period. Nests in which the smaller chick died before reaching 10 days were assigned to have suffered brood reduction due to starvation. Therefore, we could calculate a nestling starvation rate (number of nests from which at least one chick starved) and assess food limitation for each population as several studies have demonstrated that brood reduction occurs more frequently when food is limited (e.g. Magrath 1989).

Chicks from successful nests were ringed when they were 10-11 day-old with a combination of color and metal rings that would allow us to recognize juveniles in the future. At the same visit we also measured tarsus length with a digital calliper (accuracy 0.01 mm), wing length with a ruler (accuracy 1 mm) and body mass with a digital balance (accuracy 0.1 g) of every chick. We interpreted differences in these variables as evidences of chick growth differences, taking into account that there are no variation for them in blackbird adults between urban and non-urban populations (Evans et al. 2009a). Incubation period was calculated as the period between the laying of the last egg to the day when the first egg hatched as blackbirds usually start to incubate after all eggs are laid for clutch size of 2-3 eggs (Magrath 1992), which are the most common clutch sizes in our populations. Similarly, nestling period was estimated as the period from the hatching of the first egg to the day when the last chick fledged.

Some blackbird nests were filmed during that breeding season in Granada (n = 27) and Güejar (n = 27). We used a Canon MV600 videocamera with a tripod placed near each nest (1.5-2.5 m). We filmed nests for two consecutive hours starting at 8 a.m. (± 30 min), the 11th day (± 1 day) after laying of the first egg for the incubation period and when chicks were 8 days-old (± 1 day) for the nestling period. Parental visits rate for each period and mean food size was extracted from the videos for each nest. Food size was measured for each visit assigning to it one of the following categories: (1) smaller than the bill, (2) similar size as the bill, (3) one time bigger than the bill, and (4) more than twice as big as the bill.

Statistical analyses

To determine differences in breeding parameters among our three populations we carried out general linear models using Statistica 6.0 (Statsoft). Clutch size or brood size was included in the analyses as nominal independent variables when considered relevant (i.e. incubation and nestling period). The assumptions underlying the use of the linear model were systematically checked and the $\log_{10}$-transformation was applied when necessary. Daily predation rates were calculated with the constant model implemented in the program MARK (Rotella 2009) and compared among populations using CONTRAST software (Hines and Sauer 1989). We analysed differences in nestling starvation rates with a generalized linear model. We utilized data from previous years for one variable, incubation period, as sample size for this variable was very small using data from 2007 alone and we have also collected information on this variable in a preliminar study. We previously tested that there were no significant differences among years for each population.

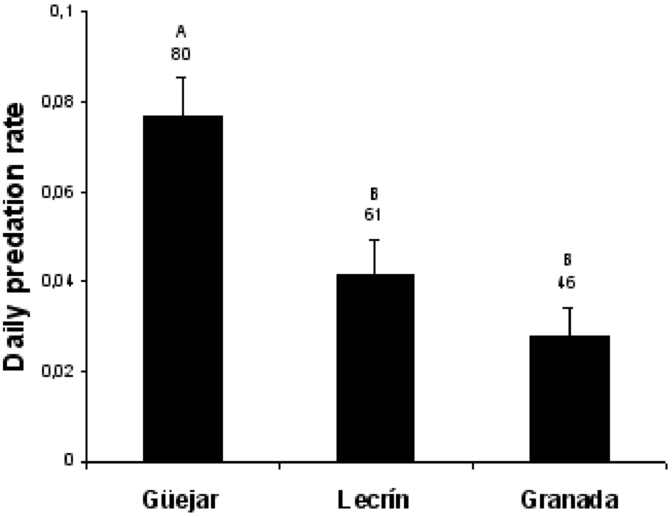
We used mixed linear models with fixed and random effects to analyze data from chicks (nestling weight and tarsus length). As nestlings share genes and the environment (the nest), nest identity was always included in the models as a random factor. The fixed effect considered was population.

Results

We found a total of 209 nests (87 nests in Güejar, 64 in Lecrín and 58 in Granada). Overall nest predation was significantly higher in Güejar than in Granada or Lecrín ($X^2_2 = 19.21$, $p < 0.0001$; Fig. 12a). Conversely, there were no overall significant differences in nestling starvation rate (GLZ binomial, $W_2 = 4.02$, $p = 0.13$). However, when the manually post-hoc comparison was made between locations, we detected a clear difference between Granada and Güejar (LSD test, $p = 0.04$; Fig. 12b). Therefore, it can be concluded that selection pressures differed among populations, especially between the urban and woodland habitats.

With respect to the predictions related to life-history theory, we found significant differences in clutch size among populations (GLM, $N_{\text{Güejar}} = 57$, $N_{\text{Lecrín}} = 32$, $N_{\text{Granada}} = 43$, $F_{2,\ 129} = 6.25$, $p = 0.003$), between Güejar (3.37 ± 0.09 eggs) and Granada (3.09 ± 0.10 eggs; LSD test, $p = 0.037$) or Lecrín (2.88 ± 0.12 eggs; LSD test, $p < 0.001$), but not between Granada and Lecrín (LSD test, $p = 0.15$). These results fit the first prediction suggested by the hypothesis of the food limitation, as the lowest clutch size was that of the population with the highest nestling starvation rate, and, thus, with the highest food limitation. In contrast, the first prediction made by the nest predation hypothesis was not fulfilled, as the largest clutch size was that of the population with the highest daily predation rate. We found no significant differences in hatching rate (GLM, $N_{\text{Güejar}} = 35$, $N_{\text{Lecrín}} = 22$, $N_{\text{Granada}} = 33$, $F_{2,\ 84} = 0.69$, $p = 0.51$) or fledging rate (GLM, $N_{\text{Güejar}} = 10$, $N_{\text{Lecrín}} = 19$, $N_{\text{Granada}} = 24$, $F_{2,\ 45} = 0.36$, $p = 0.70$; Table 1). Thus, our results do not fit with the second prediction made by the food limitation hypothesis, but they are consistent with the nest predation hypothesis. With respect to parental activity at the nest, there were significant differences during incubation between Granada (GLM, $N_{\text{Güejar}} = 13$, $N_{\text{Granada}} = 12$, $F_{1,\ 19} = 6.62$, $p = 0.02$; 2.82 ± 0.47 visits/hour) and Güejar (1.15 ± 0.45 visits/hour). Similarly, parental visitation rate to feed young was significantly higher in Granada (GLM, $N_{\text{Güejar}} = 14$, $N_{\text{Granada}} = 15$, $F_{1,\ 23} = 25.18$, $p < 0.001$; 7.38 ± 0.84 visits/hour) than in Güejar (2.75 ± 0.68 visits/hour), even when controlling for the number of nestlings. Thus, the third and fourth predictions in relation to the nest predation hypothesis are fulfilled as parental activities were the smallest in the population with the highest daily predation rate. Nevertheless, this is not the case for the predictions of the hypothesis of food limitation. Although parental visitation rate in the nestling stage was different, mean food size did not vary significantly between Granada (GLM, $N_{Güejar} = 13$, $N_{Granada} = 15$, $F_{1,21} = 2.37$, $p = 0.14$; 2.96 ± 0.22) and Güejar (3.46 ± 0.22).

(a)



(b)

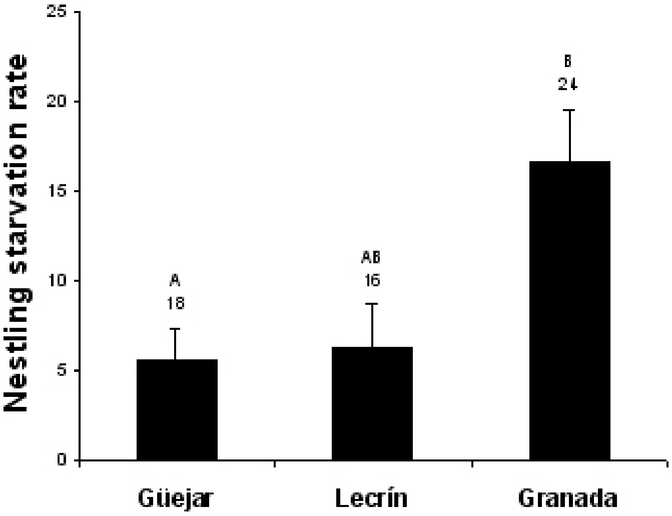


***Figure 12**. Different importance of selective pressures in each blackbird population in 2007: (a) daily predation rate and (b) nestling starvation rate (number of nest from which at least one chick starved). Locations are ordered with increasing human influence. Columns denoted by different letters are significantly different at the 0.05 level according to a post hoc test. Numbers at the top of the bars indicate sample sizes.*

***Table 1**. Hatching rate, fledging rate and wing length obtained in each population. See definitions in methods for further details. Data are means ± S.E.*

	Güejar	**Lecrín**	**Granada**
Hatching rate	2.75 ± 0.25	2.87 ± 0.33	2.52 ± 0.31
Fledging rate	2.23 ± 0.17	2.40 ± 0.15	2.34 ± 0.14
Wing length (mm)	64.16 ± 2.03	62.65 ± 1.74	61.51 ± 1.93

There were no significant differences in incubation period (GLM, $N_{Güejar} = 9$, $N_{Lecrín} = 3$, $N_{Granada} = 13$, $F_{2, 21} = 0.77$, $p = 0.47$) among the populations. If we consider also data from previous years the results are similar to that of 2007 alone, with no significant differences among populations for this variable (GLM, $N_{Güejar} = 41$, $N_{Lecrín} = 58$, $N_{Granada} = 23$, $F_{2, 118} = 1.02$, $p = 0.36$; Fig. 13). However, there were significant differences for the nestling period (GLM, $N_{Güejar} = 10$, $N_{Lecrín} = 18$, $N_{Granada} = 24$, $F_{2, 47} = 4.26$, $p = 0.02$) among populations (Fig. 13). Therefore, our results are consistent with the fifth prediction of both hypotheses, because the nestling period is longer in the population with the highest food limitation and shorter in that with the highest nest predation pressure.

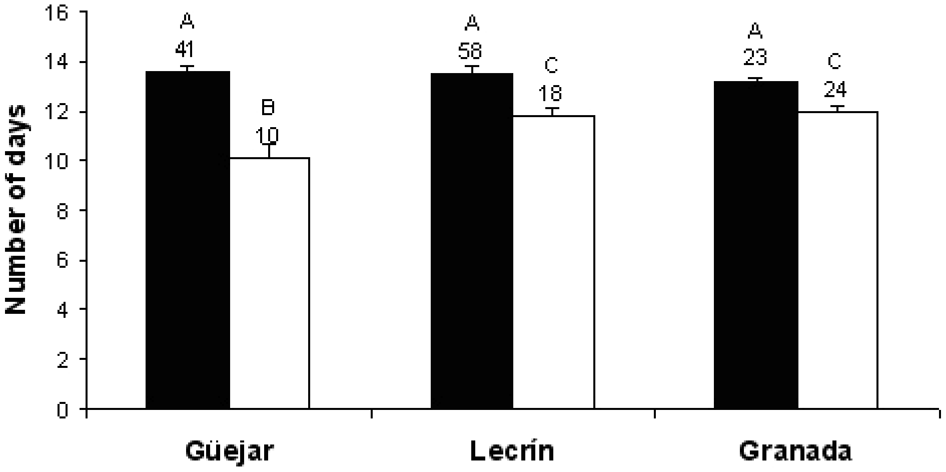


***Figure 13**. Mean (SE) duration of incubation (black bars) and nestling periods (white bars) of blackbirds in different populations. Columns denoted by different letters are significantly different at the 0.05 level according to a post hoc test. Numbers at the top of the bars indicate sample sizes.*

Finally, chicks when 10-11 days old showed significant differences among populations for body mass (GLMM, $N_{Güejar} = 49$, $N_{Lecrín} = 27$, $N_{Granada} = 37$, $F_{2, 23.69} = 5.98$, $p = 0.008$) and tarsus length (GLMM, $N_{Güejar} = 49$, $N_{Lecrín} = 27$, $N_{Granada} = 37$, $F_{2,}$

$_{22.27} = 6.85$, $p = 0.005$; Fig. 14), but not for wing length (GLMM, $N_{\text{Güejar}} = 49$, $N_{\text{Lecrín}} = 27$, $N_{\text{Granada}} = 37$, $F_{2,\ 22.57} = 0.44$, $p = 0.65$; Table 1). These results, in addition to those concerning the nestling period, indicate that nestling growth rate differed among populations, being the lowest in Granada. Thus, the sixth prediction made by the two hypotheses was also fulfilled.

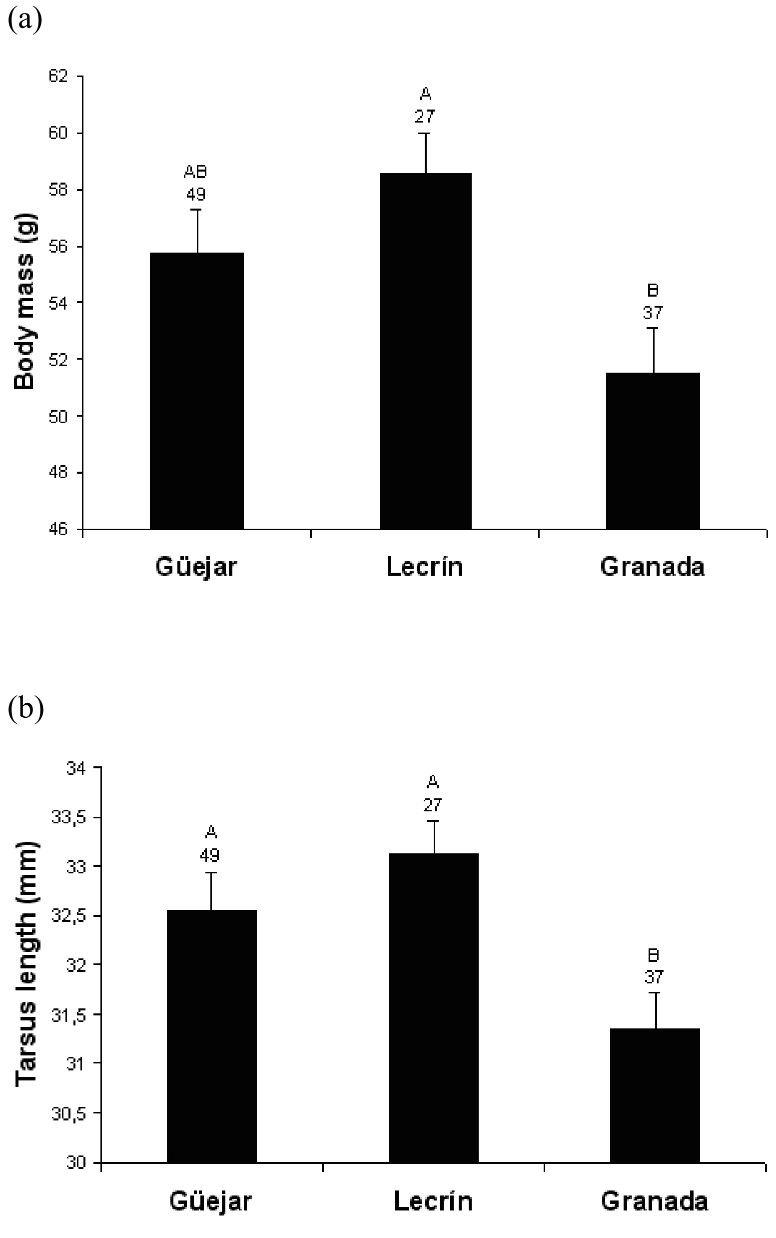


***Figure 14**. Body mass (g) (a) and tarsus length (mm) (b) of 10-11 day-old blackbird nestlings in different populations. Columns denoted by different letters are significantly different at the 0.05 level according to a post hoc test. Numbers at the top of the bars indicate sample size.*

Discussion

Our results showed that the main selection pressure for blackbirds differed among populations (Fig.12), suggesting that urbanization implies lower nest predation, but higher food limitation, than in more natural situations for this species. Gering and Blair (1999) in a study in which predation pressures along an urban gradient was estimated using artificial nests also reported a lower nest predation in the city (but see Jokimäki and Huhta 2000, Thorington and Bowman 2003). Results which are in agreement with those found in the present study, although they were criticized because of the methodology utilized (Haskell et al. 2001). Our results also support the idea that there is a greater food limitation in urban habitats as suggested previously (e.g. Shawkey et al. 2004, Chamberlain et al. 2009). Therefore, supporting that urbanization sets new challenges that must be faced by those species affected by human activities. But, are blackbirds able to cope with them?

To answer this question we are going to focus on the life-history syndromes studied. First, we have to confirm that blackbirds seem adapted to their natural and original habitat. Evans et al. (2009c) proposed the urbanization of this species has occurred independently in each city with an input of individuals from nearby wild populations. It is widely accepted that the original habitat of this passerine is the forest. Therefore, are woodland blackbirds well adapted to cope with their major selective force (nest predation)? According to the nest predation hypothesis, forest blackbirds have no modification in fledging rate (prediction 2; Table 1), a more cryptic parental behavior (lower parental visitation rates to incubate and feed young; prediction 3 and 4), a shorter nestling period (prediction 5; Fig. 13), and a higher growth of chicks compared to urban blackbirds (prediction 6; Fig. 14). The only prediction that was not fulfilled was that about clutch size (prediction 1), but this trait could also be influenced by adult mortality (Moreau 1944, Whittaker 1998). The higher survival rates for adult blackbirds in cities have been suggested in a recent broad scale study (Evans et al. 2009b). Indeed, adult survival in Güejar seemed lower as we observed a mean (± SD) of 4.7 ± 2.5 predation events of blackbird adults per year in Güejar, but only 1.0 ± 1.4 in Lecrín and 0.7 ± 1.2 in Granada, and the forest habitat is the only counting with sparrowhawks (*Accipiter nisus*; personal observation), which is known to be an important predator of adult blackbirds (Millon et al. 2009). Therefore, it seems that this species is very well adapted to its natural, original habitat, and the major selective force acting in it.

In contrast, blackbirds from the urban population, which involved another major selection pressure (food limitation), seem only partially adapted to their new environment. According to the hypothesis of food limitation, urban blackbirds have smaller clutch size (prediction 1), longer nestling periods (prediction 5; Fig. 13) and slower growth of chicks than woodland blackbirds (prediction 6; Fig. 14). But they do not show lower fledging rate (prediction 2; Table 1) or a lower activity at the nest during incubation or nestling period (predictions 3 and 4). The latter two can be understood if we consider not only the quantity of food provided, but also the quality (Shawkey et al. 2004). Some studies stated that available food in cities was not an adequate substitute of that from natural habitats (see references above). Thereby, blackbirds could locate food equally easily in cities than in other more natural habitats, but that from urban locations may have lower energetic value. More detailed studies are necessary to test this hypothesis.

In relation to the utility of single life-history traits (i.e.: clutch size) versus life-history syndromes, our results confirm that clutch size alone could be misleading as suggested previously (e.g. Martin 2004). If we had focused only on clutch size, we would have concluded that blackbirds from Güejar are not well adapted to their major selection pressure (nest predation). However, the broader view given by the syndrome points to the opposite conclusion: they seem well adapted. Thus, we recommend the use of life-history syndromes for the study of life-history evolution.

When testing the two major hypotheses of life-history evolution among three types of habitats we found that there were important differences in several life-history traits between urban and forest populations (i.e.: clutch size, activity at the nest, nestling period and growth rate; Figs. 13 and 14), as well as in selective pressures (nest predation and nestling starvation rate; Fig. 12). In contrast, differences between urban and rural populations concern only one life-history trait (growth rate; Fig. 14). Previous papers have already compared urban and non-urban habitats, but their contrasting results avoided a clear interpretation when summarized (reviewed in Chamberlain et al. 2009). We think that these difficulties to find a clear general pattern, for example in nest predation, are because sometimes these non-urban habitats were rural while other times were woodlands.

Our findings indicate important differences in reproductive success among populations, especially between the forest and the other two habitats. On the one hand, woodland blackbird females seemed to invest much more in a single reproductive event than females of the other two localities (see results for clutch size). However, we did not detect this pattern in fledging rate (Table 1), probably due to variation in the number of eggs hatched compared to eggs laid. On the other hand, breeding success was much lower in Güejar with only 17% of the nests in which fledged at least one young, although this percentage changed to 46% and 53% in the rural and urban population, respectively (Fig. 12a). Thus, blackbirds seem to benefit from human activities, above all by an important reduction in daily predation rates (Fig. 12a), probably due to nest predator avoidance of human altered locations (Ibañez-Álamo and Soler in press). In fact, this could be the ultimate explanation why this species has been constantly and independently expanding through rural and urban habitats (Evans et al. 2009c).

In conclusion, we have found that urbanization implies changes in selection pressures on blackbirds. Food limitation is the main selective pressure in cities while it is nest predation in woodlands, with rural populations experiencing an intermediate situation. Blackbirds change their life-history strategies in accordance with these different pressures, indicating that birds can respond adaptively to this human alteration of the environment. We confirmed that utilization of clutch size alone could be misleading when studying life-history theory and recommend the use of life-history syndromes. Finally, we conclude that the higher reproductive success of blackbirds in urban habitats is the main reason behind the increased abundance of this species.

Acknowledgments

We would like to thank D. Villanueva for their help in the field. A. P. Møller and O. Sanllorente made useful comments that considerably improved this manuscript. This study was partially supported by the Spanish Ministry of Environment (Project 87/2003) to M. Soler and by a FPU predoctoral grant to J. D. Ibáñez-Álamo.

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CAPÍTULO 2

Comportamiento de la hembra inducido por depredadores en ausencia de la alimentación de incubación del macho: Un estudio experimental.

La plasticidad de los cuidados parentales es crítica para comprender la influencia ecológica y evolutiva de la depredación de nidos en las estrategias vitales. En aves, la incubación impone un compromiso entre los requerimientos de la hembra (p.e. alimento) y del huevo (p.e. calor, protección de depredadores). Sin embargo, los estudios sobre este tema son escasos y restringidos a especies en las que el macho alimenta a la hembra mientras incuba, disminuyendo sus costes de incubación. Mientras los machos pueden reducir su actividad en el nido para evitar la detección por parte de los depredadores, no se sabe aún si las hembras responden de forma similar. En este estudio, nosotros manipulamos experimentalmente el riesgo de depredación del nido percibido por mirlos comunes, una especie sin alimentación durante la incubación, mientras incubaban, para estudiar los cambios comportamentales inducidos por el riesgo de depredación de nidos en la hembra. Adicionalmente, llevamos a cabo nuestro experimento en dos poblaciones de mirlos con presiones de depredación de nidos extremadamente diferentes para explorar el efecto del riesgo ambiental de depredación de nidos sobre los cuidados parentales de la hembra inducidos por los depredadores. Nuestros resultados mostraron que las hembras pueden reducir sus visitas al nido en la situación con mayor riesgo de depredación del mismo, minimizando las pistas dadas a los depredadores. Además, las respuestas antipredatorias eran más intensas en la población con mayor presión de depredación de nidos, subrayando la importancia del riesgo específico del hábitat en la modulación del comportamiento plástico inducido por los depredadores de nidos.

CAPÍTULO 2

Predator-induced female behaviour in the absence of male incubation feeding: An experimental study.

Juan Diego Ibáñez-Álamo and Manuel Soler

Parental care plasticity is critical to understand the ecological and evolutionary influence of nest predation on life-history strategies. In birds, incubation imposes a trade-off between the requirements of females (i.e. food) and egg requirements (i.e. heat, protection from predators). However, studies on this topic are rare and restricted to species where the male feeds the incubating female, relaxing her incubation costs. While males can reduce their activity at the nest to avoid detection by predators, it remains untested if females show similar responses. In this study, we experimentally manipulated the perceived risk of nest predation of incubating common blackbirds (*Turdus merula*), a species without incubation feeding, to study female behavioral changes induced by nest predation risk. Additionally, we carried out our experiment in two blackbird populations with extremelly different nest predation pressures to explore the effect of the environmental nest predation risk on predator-induced female parental care. Our results showed that females can reduce their nest visits in the situation with the higher nest predation risk, minimizing clues given to predators. Moreover, antipredator responses were more intense in the population with the higher nest predation pressure, stressing the importance of habitat specific risk in modulating the plastic behaviour induced by nest predators.

Enviado, *Behavioral Ecology*

Introduction

Parental care strategies are thought to evolve due to the existence of a fitness trade-off between the costs and benefits imposed by these behaviours (Clutton-Brock 1991; Stearns 1992; Roff 2002). Given the variable character of most environments, these trade-offs in parental care are constantly changing, and thus, parents must continually adjust their behaviours to maximize their fitness (Roff 2002). Nonetheless, only a few investigations are centered on the phenotypic plasticity perspective in relation to parental care (Ghalambor and Martin 2002; Lima 2009; Martin and Briskie 2009). Incubation, a key form of parental care in bird species, is an appropriate field to study this topic because parents incubating face a dilemma between the needs of their eggs and their own energetic demands. Eggs require a constant temperature for a proper embryonic development and continuous protection from predators (e.g. White and Kinney 1974; Haftorn 1988; Moreno 1989; Weathers and Sullivan 1989; Williams 1996; Conway and Martin 2000). Since environmental temperature and nest predation risk are highly variable, it is expected that parental care plasticity evolved in response to these factors.

Most of the previously published papers on incubation behaviour have focused on the trade-off between ambient temperature and parental energetic demands (White and Kinney 1974; Haftorn 1988; Weathers and Sullivan 1989; Williams 1996), while little attention has been paid to the influence of nest predation (Ghalambor and Martin 2002). However, nest predation is considered a major selection pressure in birds (Martin 1995). Only recently some papers have explored this topic in a few species (e.g. Fontaine and Martin 2006; Massaro et al. 2008), but clearly more studies are needed to understand the evolution of parental care strategies (Martin and Briskie 2009). In all species hitherto investigated the male fed the incubating female (e.g. Ghalambor and Martin 2000, 2002). This behaviour reduces the energetic demands of the female imposed by incubation, which as a result, increases her nest attentiveness and consequently her reproductive success (von Haartman 1958; Lyon and Montgomerie 1985; Smith et al. 1989; Halupka 1994; but see Fontaine and Martin 2006).

Simultaneously, incubation feeding gives more relevance to male compared to female antipredator responses because the majority of the parental activity at the nest during incubation (i.e. nest visits) is due to the male and it is well established that parental activity increases nest predation (e.g. Skutch 1949; Martin et al. 2000; Eggers et al. 2005). In fact, none of the previous studies have detected a reduction in female visits to the nest due to an increase in perceived risk of nest predation, although they have shown a reduction in clutch size (Eggers et al. 2006) or longer duration of on-bouts (e.g. Ghalambor and Martin 2002; Fontaine and Martin 2006). These responses have been interpreted as indirect evidence that females can also reduce their nest visit rate and, therefore, their activity in order to avoid attracting predators' attention to the nest. However, because it has not been demonstrated that females could reduce their nest visits, an alternative explanation is possible for these longer bouts in the presence of predators: females could be simply enhacing nest concealment (Montgomerie and Weatherhead 1988; Weathers and Sullivan 1989; Martin 1992; Kleindorfer and Hoi 1997). Although both interpretations denote phenotypic plasticity, their implications are sustancially different as the first suggests the same antipredator behaviour acting in both sexes while the second indicates different antipredator behaviours in males (reduction in nest visits) and females (nest concealment enhacement).

The study of species without incubation feeding would help to solve this debate because the trade off between female and egg requirements is stressed in these species. Thus, if the response in nest visits exists under a high risk of nest predation, it would presumably be more exagerated and easier to detect in these species. Moreover, time of latency (the time females spend to return to their nests) would be a good variable to differenciate which of the two antipredator strategies is preferentially used by females. If they priorize nest concealment, females should return to their nests sooner under a high risk of nest predation. Alternativelly, if they priorize to avoid attracting attention, females should delay their return as much as possible in the presence of a nest predator near their nests. In addition, given that two recent studies showed strong plasticity of other predator-induced behaviours depending on the environmental nest predation risk (Eggers et al. 2008; Massaro et al. 2008) we expect also female's behaviour to be plastic with respect to the habitat specific risk of predation.

In this study, we increased the perceived risk of nest predation of female blackbirds (*Turdus merula*), a species without male incubation feeding (Cramp 1988). We simulated the presence of a key nest predator, the mapgie (*Pica pica*), nearby active blackbird nests during incubation in two populations with differing nest predation risk to test the following predictions: (i) Blackbird females should reduce their visits to the nest under a high risk of nest predation as an adaptation to avoid attracting attention to the nest by predators. (ii) This reduction is accomplished by females increasing their bouts on the nest to minimize the negative impact on their eggs. (iii) Females should return sooner to their nests in the presence of the higher nest predation risk situation if they priorize nest concealment, or the contrary if the reduction in nest activity is more important to them. Finally, (iv) these antipredator responses should be more intense in the population with the higher nest predation risk.

Material and methods

Study area and experimental design

We studied two breeding populations of common blackbirds in Granada province (South of Spain) from early March to early July 2007. One was an urban population, located in parks of the city of Granada (37º 17' N, 3º 59º W; 695 m a.s.l.) with a low nest predation pressure (30% of the nests predated). The other was a forest population, settled in a *Quercus* forest near Güejar-Sierra, in the Natural Park of Sierra Nevada (37º 9' N, 3º 24' W; 1050 m m a.s.l.) with a high nest predation pressure (80% of the nests predated). See Ibáñez-Álamo and Soler (submitted) for detailed description on these populations.

We manipulated the perceived risk of nest predation by simulating the presence of a magpie in the surroundings of the nest in the 11th day (± 1 day) after the starting of incubation by blackbird females. We chose this day, in the second half of the egg stage, because the reproductive value of the eggs has increased considerably favouring the detection of antipredator responses and lowering the probabilities of abandonment of the nest. Magpies are nest predators present in both study locations (pers. obs.) and known to deeply affect blackbirds' breeding success (Groom 1993; Collar 2005). Nests were exposed for 3 hours to a playback using a recorder (one 15 seconds magpie call per 3 minutes, TDK endless casette, EC-6M) starting at dawn. We alternativelly utilized 7 different magpie call recordings (one different for each nest) to avoid blackbirds get used to them and the potential problem of pseudoreplication. The recorder was placed 20 meters away from the nests and moved every hour to simulate changes in position by predators. This distance allowed us to change the location of the recorder without affecting female incubation behaviour as we could confirm later analyzing the filmed material and observing no apparent behavioral modifications at the time we moved the recorder. After the first hour of playback, when the parents were well aware of the presence of the magpie, a video camera was placed near the nest (1.5-2.5 m) to film nest activity for the following two hours. Females were always frightened away the nests during the placing of the video camera. Two control groups were created: (1) following exactly the same manipulation protocol as for the experimental treatment, but playing no sound (control); and (2), also with the same protocol but presenting woodpigeon (*Columba palumbus*) playbacks (6 different calls) (manipulation control). Nests were exposed to each treatment in consecutive days, with a third of the nests starting with the experimental treatment, another third with the control, and another third with the manipulation control. From the video tapes we extracted the following variables: parental nest visit rate (number of parental visits per hour), time of latency (time since starting filming to the female first visit), mean on-bouts (mean time that a female sits on the nest) and nest attentiveness (measured as percentage of time per hour at the nest).

Statistical analysis

To determine the effect of nest predation risk on parental behaviour we utilized repeated measure ANOVAs using Statistica 6.0 (Statsoft) as the same nest was under different risk of nest predation in consecutive situations (days). Treatment was always included as within-group factors, as well as sex for the analysis of parental visit rate because we measured this variable for males and females in each nest. Population and treatment order were included as between-group factors. The assumptions underlying the use of these analyses were systematically checked and the $\log_{10}$-transformation was applied when necessary, except for percentage of nest attentiveness that was arcsin transformed.

Results

We carried out the experiments at 22 different blackbird nests, of which 11 were located in the low predation risk population while the other 11 were in the high predation risk population. We detected a significant effect in parental nest visits for the interaction sex x treatment x population (Fig. 15; $F_{2, 20} = 8.48$, $p = 0.002$). As expected, males only rarely visited the nests (mean ± SE: 0.25 ± 0.16 visits/hour), and did not respond to the treatment in any population. In contrast, the response of the females to magpie calls depended on the population specific risk and only females in the high risk population showed differences in nest visits among treatments (Fig. 15). Females in this high risk habitat significantly reduced their visits to the nest when exposed to magpie calls in comparison with the control (LSD test, p = 0.0001) or manipulation control treatments (LSD test, p = 0.002).

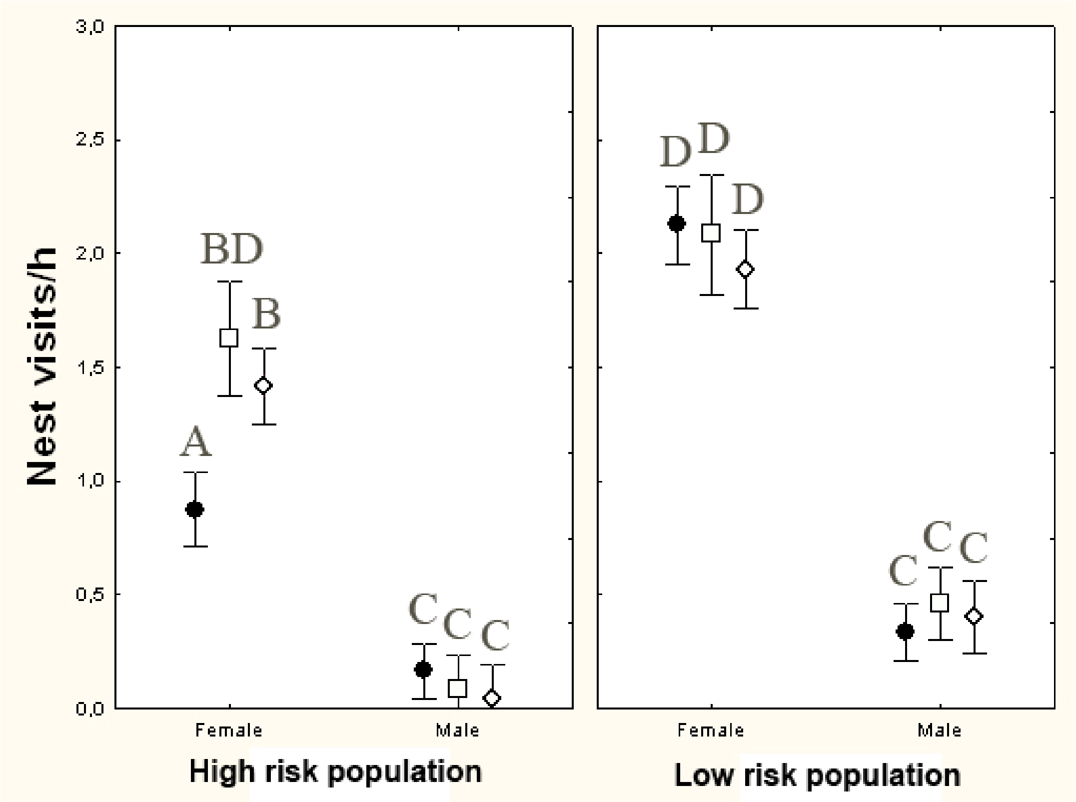

Figure 15. *Female and male nest visit rate (number of visits per hour) in the two studied populations. Simbols correspond to the mean value of one of the following treatments: High nest predation risk (magpie playbacks; circles), low nest predation risk (no sound; squares) and low nest predation risk (woodpigeon playbacks; diamonds). Bars associated to each simbol denote the corresponding standard error. Treatments that share the same letter indicate no significant differences for the LSD post hoc tests at the p<0.05 level.*

In relation to the time of latency, which shows the willingness of females to return to their nests after the placement of the video camera, we found a significant effect between treatment and population (Fig. 16; $F_{2, 14}$ = 11.42, p = 0.001). Females in the high risk population delayed their return to the nest significantly longer when exposed to magpie calls, than females exposed to the control (LSD test, p = 0.03) or manipulation control treatments (LSD test, p = 0.02). In contrast, females in the low risk population showed the opposite pattern returning significantly sooner to the nest in the presence of the magpie calls than in the manipulation control (LSD test, p = 0.005). The control treatment was in an intermediate position and no differences with the other two treatments were observed (Fig. 16; LSD tests, p = 0.13 and p = 0.12 respectively).

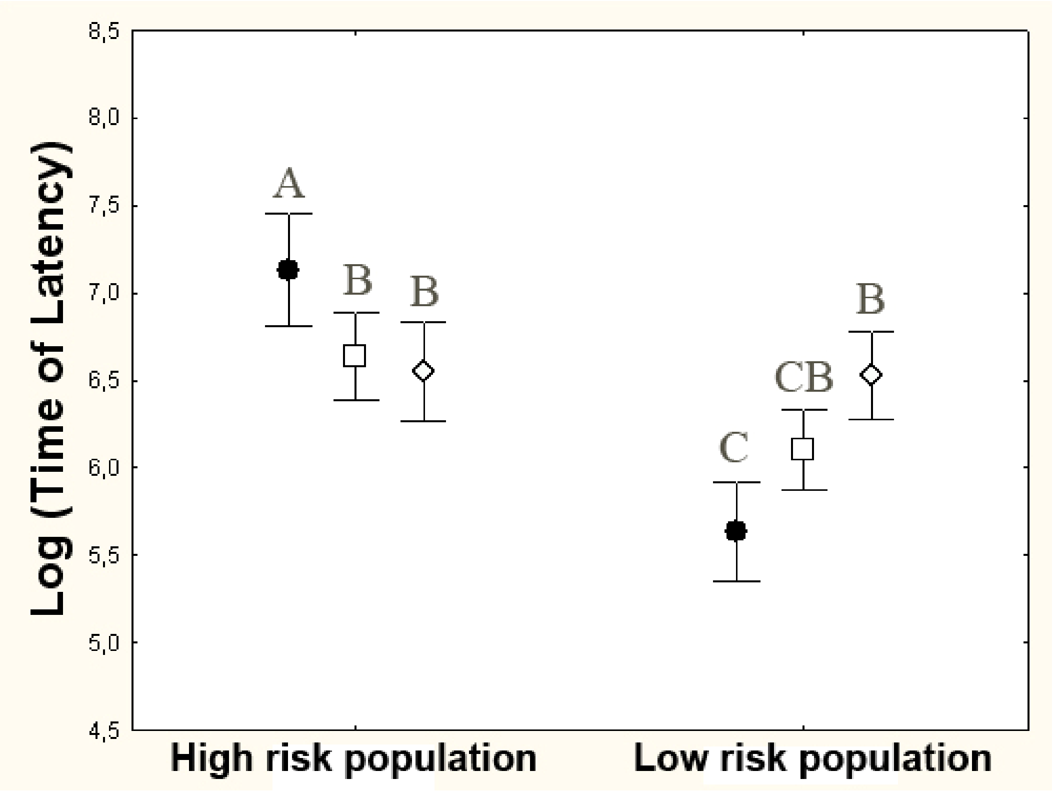

Figure 16. *Time of latency for each treatment and population considered. Simbols correspond to the mean value of one of the following treatments: High nest predation risk (magpie playbacks; circles), low nest predation risk (no sound; squares) and low nest predation risk (woodpigeon playbacks; diamonds). Bars associated to each simbol denote the corresponding standard error. Treatments that share the same letter indicate no significant differences for the LSD post hoc tests at the p<0.05 level.*

With respect to the mean time spent on the nest per incubating bout, females differed depending on the interaction treatment x population (Fig. 17; $F_{2,\ 14}$ = 6.83, p = 0.009). Blackbird females in the high risk population significantly reduced their bouts on the nest under a higher perceived risk of nest predation in comparison with the control (LSD test, p = 0.0009) or manipulation control situation (LSD test, p = 0.003). Similarly to the nest visit rate, these responses did not exist in females inhabiting the low risk habitat (Fig. 17).

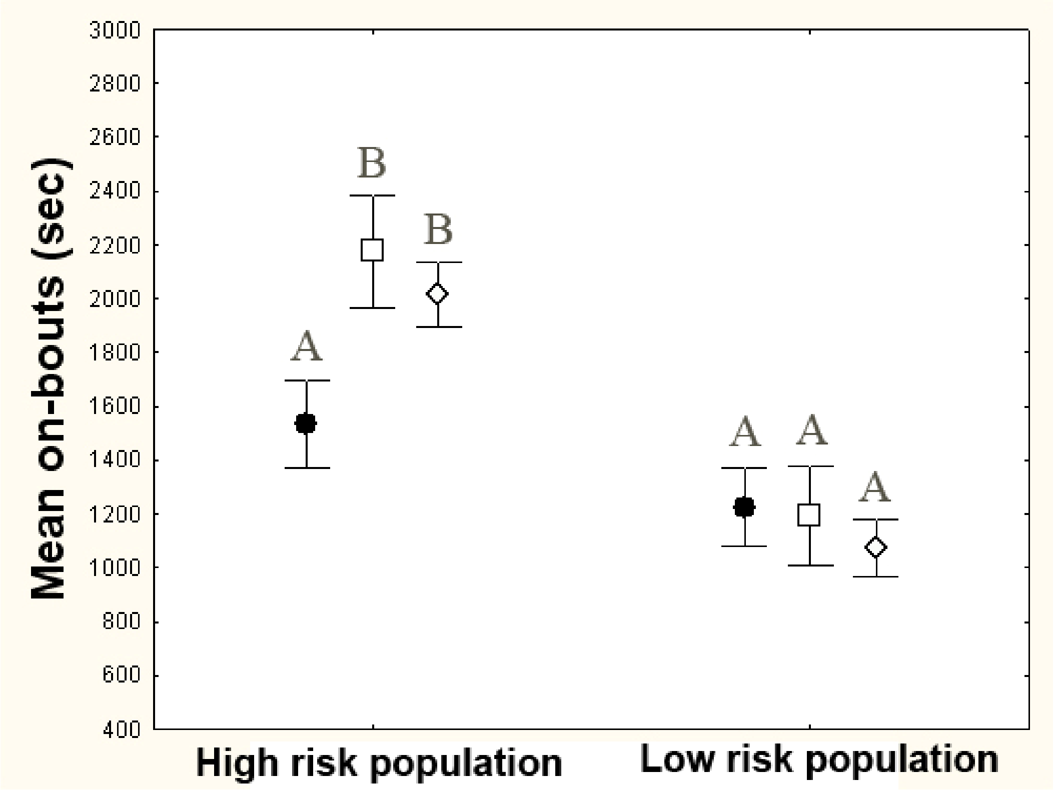

Figure 17. *Mean duration of incubation on-bouts (i.e. length of time, in seconds, that a female sits on the nest in a given bout) for each treatment and population considered. Simbols correspond to the mean value of one of the following treatments: High nest predation risk (magpie playbacks; circles), low nest predation risk (no sound; squares) and low nest predation risk (woodpigeon playbacks; diamonds). Bars associated to each simbol denote the corresponding standard error. Treatments that share the same letter indicate no significant differences for the LSD post hoc tests at the p<0.05 level.*

The percentage of nest attentiveness also showed significant differences in the interaction treatment x population (Fig. 18; $F_{2, 14}$ = 19.32, p = 0.00009). However, for this variable we detected the opposite pattern between both habitats. Females in the high risk population significantly reduced their nest attentiveness as a response to the presence of the magpie (Fig. 18) in comparison with the control (LSD test, p = 0.0008) and manipulation control treatments (LSD test, p = 0.00006). On the contrary, in the low risk population, females increased their nest attentiveness under the same situation (Fig. 18) with respect to the other two treatments (LSD tests, p = 0.02 for both interactions).

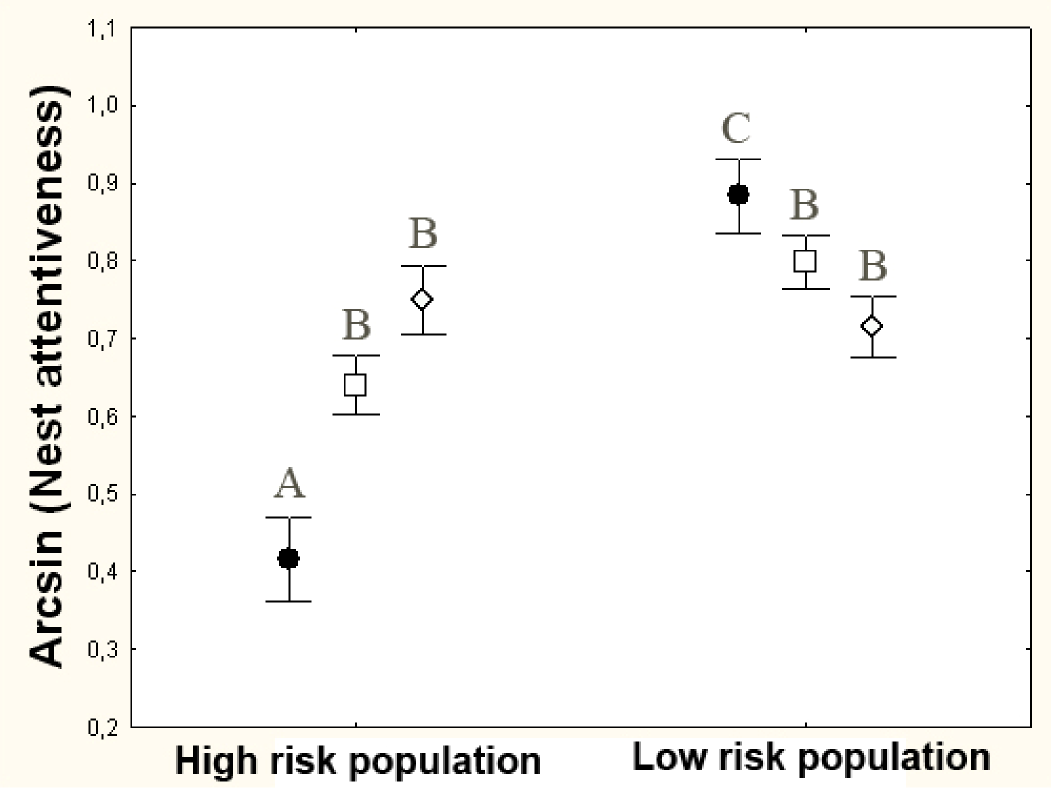

Figure 18. *Nest attentiveness (percentage of time per hour at the nest) for each treatment and population considered. Simbols correspond to the mean value of one of the following treatments: High nest predation risk (magpie playbacks; circles), low nest predation risk (no sound; squares) and low nest predation risk (woodpigeon playbacks; diamonds). Bars associated to each simbol denote the corresponding standard error. Treatments that share the same letter indicate no significant differences for the LSD post hoc tests at the p<0.05 level.*

Discussion

Our results show that females, in a species which lacks incubation feeding by the male, adjust their behaviours depending on the current risk of nest predation in a way that appears adaptive. Females continuously assess the variation in predation risk depending on both the current level of risk and the population specific difference in nest predation risk. Thus, these results are the first experimental demonstration that females can reduce their nest visits as an antipredator response (Fig. 15). Skutch (1949) predicted this behaviour in order to reduce activity at the nest and consequently reduce potential cues used by predators. Earlier studies only provided indirect evidences for the ability of females to adjust their nest visitation pattern depending on nest predation risk (e.g. Ghalambor and Martin 2002; Fontaine and Martin 2006), which did not allow to separate the nest concealment hypothesis from Skutch's hypothesis.

The ability of females to respond to the current nest predation risk is also supported by the results regarding time of latency. Blackbird females in the high risk population delayed their return to the nest in the presence of a nest predator near their nests (Fig. 16), suggesting that they avoided revealing their nest location. However, we can not discard the alternative explanation of enhanced nest concealment because females in the low risk population returned sooner to their nests (Fig. 16). Therefore, our results suggest that the selective pressure related to the cues given by nest visits and that imposed by nest concealment could depend on the population specific and current nest predation risk. This behaviour on two levels could be common in birds as Eggers et al. (2005) found that nest visitation by Siberian jays (*Perisoneus infaustus*) during the nestling stage were different depending on the habitat specific risk and could be altered with playbacks.

In contrast to previous studies (Ghalambor and Martin 2002; Massaro et al. 2008), we found that females in the high risk population reduced their mean on-bouts instead of increasing them (Fig. 17). Although surprising at a first look, there could be two possible explanations for that. On one hand, it has been suggested that a high risk of nest predation could favour to reduce investment in current reproduction in order to accumulate more energy for future reproductive events (Slagsvold 1984; Stearns 1992; Martin 1995; Roff 2002). However, this explanation is unlikely in our study since the high adult blackbird predation in the high risk population lowers the probability of future reproduction, as well as involves an important energy investment in each clutch (Ibáñez-Álamo and Soler submitted). On the other hand, female blackbirds in the high risk population could be incubating more than the minimum necessary for the correct development of embryos. In fact, several papers have demonstrated that an increase in the time females spent incubating shortened their incubation periods (von Haartman 1958; Lyon and Montgomerie 1985; Lifjeld and Slagsvold 1986; Smith et al. 1989; Halupka 1994; Martin 2002, Martin et al. 2007), which means that female blackbirds breeding in the high risk habitat could reduce the time they spent incubating their eggs without compromising the viability of their offspring. Indeed, mean on-bouts were longer (10 more minutes aproximatelly) in the high risk than in the low risk population indicating that a reduced incubation time is possible (Fig. 17). Moreover, females in the high risk habitat reduced their time at the nest to levels similar to that used by urban blackbirds under a high current risk of nest predation. In addition, no significant differences in hatching success were found between experimental and natural nests in the high risk population during that year ($F_{1, 30} = 0.17$, $p = 0.68$), suggesting that even with the reduction in mean on-bouts due to the experimental treatment the same number of eggs managed to hatch.

Percentage of nest attentiveness, consistently with our other results, was reduced in the high risk population in the presence of a nest predator, although the opposite pattern was obtained in the low risk population (Fig. 18). Previous investigations also showed contrasting results in relation to nest attentiveness and risk of nest predation. Some of them indicated increased attentiveness in response to increased predation risk (e.g. Thompson and Raveling 1987; Beissinger et al. 1998; Ghalambor and Martin 2002; Fontaine and Martin 2006), while others presented no such response (e.g. Conway and Martin 2000; Martin 2002; Martin et al. 2007; Massaro et al. 2008). The reason behind our surprising results for the high risk population could be the absence of magpie models accompaning the playbacks. Birds can detect the presence of nest predators only by acoustic cues (reviewed in Lima 2009) and all evidences indicate that female blackbirds became aware of the presence of a potential nest predator in the proximity of their nests. Consecuently, they could invest more time off the nest searching for the predator trying to expel it, as blackbirds can activelly defend their nests (Møller 1989; Cresswell 1997). Interestingly, results on nest attentiveness were different in the low risk population as females increased attentiveness in the presence of the magpie (Fig. 18), stressing the plasticity of this response and indicating that different strategies could be used depending on the habitat specific risk.

Our results showed that blackbirds can use pasive responses to avoid revealing the location of their nests. However, it is also known that blackbirds can defend their nests very efficiently (see references above). Thus, it seems adaptive that parents would use pasive responses as a first option and shift to active defense once the nest is detected by the predator. Indeed this is also the case of other species with cryptic nests such as the Siberian jay (M. Griesser pers. comm.) suggesting that the use of these two strategies could be frequent in this group of birds.

Differences in the costs associated to this active defense between habitats could explain the higher cryptic behaviour observed in the high risk, woodland population. Krams et al. (2007) demonstrated that antipredator mobbing behaviour by parents near the nest increased mustelid nest predation in birds. Thus, considering that mustelids are only present in the woodland (high risk) population (pers. obs.), active responses (i.e.

mobbing) would involved greater costs in the forest than in the city, strongly selecting against the use of active defense and favouring pasive responses in that population.

In conclusion, we have found that females, similarly to males, can respond reducing their activity at the nest, thus supporting Skutch's hypothesis. However, our results do not discard that females can use another antipredator response developed to increase nest concealment. Furthermore, predator-induced plasticity seems to be affected by environmental risk of nest predation, as blackbirds in the high risk population modificated their behaviour more intensively than those in the low risk habitat. Future work should test this on males to know if female and male plasticity are modificated similarly.

Funding

This work was supported by the Spanish Ministry of Environment (87/2003 to MS) and the Spanish Ministry of Science and Education (grant number 2004-5269 to JDI).

Acknowledgments

We would like to thank J. Iglesias, J. Rodríguez and specially D. Villanueva for their help in the field. M. Griesser, J. Martín, J. G. Martínez and O. Sanllorente made useful comments to previous drafts. The Junta de Andalucía for their economic support to the research group Comportamiento y Ecología Animal (RNM-339).

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CAPÍTULO 3

Diferencias sexuales y costes del comportamiento de cuidados parentales inducido por los depredadores en el mirlo común.

Varios artículos recientes apoyan la hipótesis de Skutch, que establece que las aves altriciales constantemente ajustan su comportamiento de visitas al nido dependiendo del riesgo de depredación del mismo. Sin embargo, ningún estudio hasta el momento ha investigado si existen o no diferencias sexuales en esas respuestas antipredatorias pasivas durante el periodo de pollos. Machos y hembras pueden diferir en sus estrategias vitales y, por tanto, la influencia del riesgo de depredación del nido podría también ser diferente para cada sexo. Nosotros manipulamos experimentalmente el riesgo de depredación percibido por mirlos comunes adultos, una especie sexualmente dimórfica, con objeto de comprobar esta hipótesis. Nuestros resultados muestran que ambos sexos responden de manera similar a la presencia de un depredador cerca de su nido, pero no para todos los rasgos considerados (p.e. duración media de las visitas). Esta plasticidad inducida por depredadores era dependiente del hábitat y posiblemente fuera afectada por diferencias a pequeña escala en depredación de nidos. Finalmente, los padres pueden responder al riesgo de depredación sin comprometer el alimento dado a sus pollos, el principal coste asociado a este comportamiento, modificando otros comportamientos no alimentarios como el empolle. Estos resultados subrayan la complejidad de las respuestas antipredatorias en aves y sugieren la reconsideración de la teoría establecida en este campo.

CAPÍTULO 3

Sexual differences and costs of predator-induced parental care behavior in the common blackbird

Juan Diego Ibáñez-Álamo and Manuel Soler

Several recent papers give support to Skutch's hypothesis, which states that altricial birds constantly adjust their nest visitation behavior depending on the current risk of nest predation. However, no study has so far investigated whether there are sexual differences in these antipredator passive responses or not during the nestling period. Males and females could differ in their life-history strategies and, therefore, the influence of the risk of nest predation could also be different for each sex. We experimentally manipulated the perceived risk of nest predation of adult common blackbirds (*Turdus merula*), a sexually dimorphic species, in order to test this hypothesis. Our results showed that both sexes respond similarly to the presence of a nest predator near the nest, but not for all traits considered (i.e. mean visit duration). This predator-induced plasticity was habitat-dependent and possibly affected by small scale differences in nest predation. Finally, parents can respond to the risk of nest predation without compromising the food delivered to their nestlings, the main cost associated to this behavior, modificating other non-feeding behaviors like brooding. These results highlight the complexity of antipredator responses in birds and suggest the re-consideration of established theory in this field.

Enviado, *Proceedings of the Royal Society B: Biological Sciences*

Introduction

Nest predation represents an important source of natural selection (Ricklefs 1969; Martin 1995) and recent studies focusing on life-history strategies stress this relevance in comparison with other selective forces like food limitation (e.g. Ferretti et al. 2005). These studies however centred their investigations mainly around the effect of nest predation in mean expression of traits, paying little attention to its effect on phenotypic plasticity (e.g. Martin 1995). Nevertheless, phenotypic plasticity plays a major role in the evolution of life history traits, especially in relation to highly variable selection pressures such as nest predation (Schlichting & Pigliucci 1998; Martin & Briskie 2009). Skutch's hypothesis about the influence of parental nest visits on nest predation is a good example of this. He proposed that visits by parents could be used by predators to locate and predate the nest, something well demonstrated nowadays (Skutch 1949; Martin et al. 2000a, 2000b; Muchai & DuPlessis 2005; Rastogi et al. 2006). However, parents can ameliorate this pressure reducing their visits under situations of high risk of nest predation (Ghalambor & Martin 2000, 2002; Eggers et al. 2005, 2008; Fontaine & Martin 2006). Therefore, increased plasticity is selected with respect of parental nest visits instead of mean expression of this trait.

In the past few years, some papers have demonstrated the influence of nest predation on phenotypic plasticity for several traits (reviewed in Martin & Briskie 2009), but evidence remains limited to a few papers and species, indicating that further studies on this topic are needed. Even less is known when both sexes are considered separately (Ghalambor & Martin 2002). This is surprising because males and females could differ in their life-history strategies (Clutton-Brock 1991) and, therefore, the influence of nest predation could also be different. This sexual perspective has been partially investigated during incubation (Ghalambor & Martin 2000, 2002; Fontaine & Martin 2006; Massaro et al. 2008; Ibáñez-Álamo & Soler submitted a), but those studies usually focused on different approximations for each sex due to different sexual roles. For example, it is common to measure incubation nest visit rate for males and incubation bout length for females since males feed the incubating female and females are the only sex that incubates (e.g. Ghalambor and Martin 2000; Fontaine and Martin 2006). This makes difficult to compare between sexes in order to look for differences in predator-induced plasticity. While several studies demonstrated a reduction in nest visits when environmental risk of nest predation is higher (e.g. Eggers et al. 2005, 2008; Massaro et al. 2008; Peluc et al. 2008), it remains unknown if the reduction in nest visitation rates differs between sexes. Studies during the nestling period would be the best option to analyse these sexual differences, in particular in species where both male and female feed their chicks, since this allows comparing the response of both sexes (i.e. change in nest visit rate).

A related and important issue is that of costs imposed by this antipredator behavior. Food provisioning has been considered as the major constraint in antipredator behavior during the nestling period (Skutch 1949; Slagsvold 1982; Lima 1987; Martin 1992), because a low provisioning rate could reduce offspring quality, and thus the opportunities of the nestlings to survive and reproduce in the future (Daan et al. 1990; Richner 1989, 1992; Nilsson 1990; Metcalfe & Monaghan 2001). In fact, four mechanisms have been proposed to minimize the food limitation associated to parental antipredator behavior based on the assumption of this trade off: (i) lowering clutch size (e.g. Eggers et al. 2006; Zanette et al. 2006; Kleindorfer 2007; Olsen et al. 2008); (ii) maximizing food load sizes (e.g. Martin 1996; Martin et al. 2000a); (iii) eliminating allofeeding (Strickland & Waite 2001); and (iv) compensating the reduction in feeding during high risk situations with an increase under low risk situations (Eggers et al. 2005). However, there is only one study examining directly the costs associated to changes in nest visitation. Martin et al. (2000a) found that those species with lower visit rate account for lower provisioning rate to nestlings because parents cannot compensate the reduction in nest visits through increased food loads. No experimental demonstration on this exists, nevertheless, this is critical to understand the evolution of these responses, and how species can cope with the costs associated to them.

Here, we manipulated the perceived risk of nest predation of common blackbirds (*Turdus merula*) in order to test if there are sexual differences in predator-induced plasticity. We studied several variables, including nest visit rate and food delivery rate, to try to assess whether these antipredator responses lead to a reduction in nestling food provisioning. Additionally, we investigated the effect of ambient nest predation risk in phenotypic plasticity comparing blackbird responses from two populations with important differences at this level.

Material and methods

Study area and experimental design

The common blackbird is a medium-size passeriform widely spread around Eurasia. Both parents feed their nestlings and are easily distinguishable due to sexual dimorphism in colouration (Cramp 1988). Blackbirds were studied during the spring (March-July) of 2007 in two locations in Andalusia (South of Spain): in the city of Granada (37º 17’ N, 3º 59º W; 695 m a.s.l., hereafter referred to as Granada), a population with a low nest predation pressure (daily nest predation rate of 0.03) and in the Natural Park of Sierra Nevada near Güejar-Sierra (37º 9’ N, 3º 24’ W; 1050 m a.s.l., hereafter referred to as Güejar), a forested area with a high nest predation pressure (daily nest predation rate of 0.08). See Ibáñez-Álamo and Soler (submitted b) for a more detailed information on these populations.

We manipulated the perceived risk of nest predation during the nestling period by simulating the presence of a magpie (*Pica pica*) in the surroundings of the nest when chicks were 8 days-old (± 1 day) to control for nestling development. This corvid is a nest predator present in both study locations (pers. obs.) and it is known to deeply affect blackbird populations (Groom 1993, Collar 2005). Nests were exposed for 3 hours to a playback (one 15 seconds magpie call per 3 minutes) starting at daybreak. The recorder was placed 20 meters away of the nests and changed every hour to simulate changes in position by predators. We used 7 different magpie call recordings to avoid blackbirds get used to them and the potential problem of pseudoreplication. After the first hour of playback a video camera was placed near the nest (1.5-2.5 m) to film nest activity for the following two hours. The control treatment consists in the same manipulation protocol as for the experimental treatment but playing no sound. We did not use a manipulation control treatment as no significant differences were observed between control (no sound) and the manipulation control (woodpigeon playbacks) treatments in a previous study (Ibáñez-Álamo and Soler submitted a). Nests were exposed to each treatment in consecutive days, with half of the nests starting with the experimental treatment and the other half with the control treatment. From the video tapes we extracted the following variables for each sex: nest visit rate (number of parental visits per hour), food delivery rate (number of parental visits with food per hour), time of latency (time since starting filming to the adult first visit), mean visit duration and mean feeding duration (time from the start of feeding the first chick fed to the end of the last nestling fed per each visit).

Procedures used to explore the effect of predation risk usually involve taxidermic mounts of predators, but this can be problematic for several reasons (i.e. group mobbing effects) and the use of playbacks alone is recommended to solve them (Ghalambor & Martin 2001). Indeed, Eggers et al. (2005) demonstrated that the sound alone could be enough to modify parental antipredator behavior in the Siberian jay (*Perisoneus infaustus*). We try to verify if the same was true for the blackbird with a preliminary experiment carried out during 2006 in the Güejar population using the same methodology described above. As expected, our manipulation involved a reduction in parental nest visits under the higher risk of nest predation situation ($F_{1, 15} = 7.80$, $p = 0.01$), which validates our methodology.

Statistical analysis

To determine the effect of nest predation risk on parental behavior we utilized repeated measure ANOVAs using Statistica 6.0 (Statsoft) as the same nest was under different risk of nest predation in consecutive situations (days). Treatment and sex were always included as within-group factors. Population and treatment order were included as between-group factors. The assumptions underlying the use of these analyses were systematically checked and the $\log_{10}$-transformation applied when necessary.

As a general procedure for the variables, in order to increase sample sizes and take advantage of the preliminary data, we first checked if there were significant differences in Güejar between years. Once we confirmed that there were no differences for that variable we conducted an analysis including data from both years. We pooled data across years because we did not find significant differences in Güejar between 2006 and 2007 in parental nest visit rate ($F_{1, 22} = 0.83$, $p = 0.37$), food delivery rate ($F_{1, 22} = 0.90$, $p = 0.35$), time of latency ($F_{1, 11} = 1.39$, $p = 0.26$) and mean feeding duration ($F_{1, 11} = 0.03$, $p = 0.88$). The only variable for which year was significant being mean visit duration ($F_{1, 11} = 5.21$, $p = 0.04$), thus we analyze it taking into account 2007 data alone.

We carried out the experiments in 40 different blackbird nests, of which 15 corresponded to that located in the urban population and 25 in the woodland (including those from the preliminary study). Parental nest visitation rate differed between treatment in interaction with population ($F_{1, 37} = 5.28$, $p = 0.03$), although no sexual differences were observed for the risk of nest predation (Fig. 19; $F_{1, 37} = 0.31$, $p = 0.58$). Both parents in the forest habitat reduced their visits to the nest for the magpie treatment in comparison with the control (female: LSD test, $p = 0.05$; male: LSD test, $p = 0.03$). However, urban blackbirds did not show any differences in nest visits between treatments (Fig. 19). Interestingly, results denoted no significant effect between treatment and population with respect of food delivery rate (Fig. 20; $F_{1, 37} = 3.37$, $p = 0.08$).

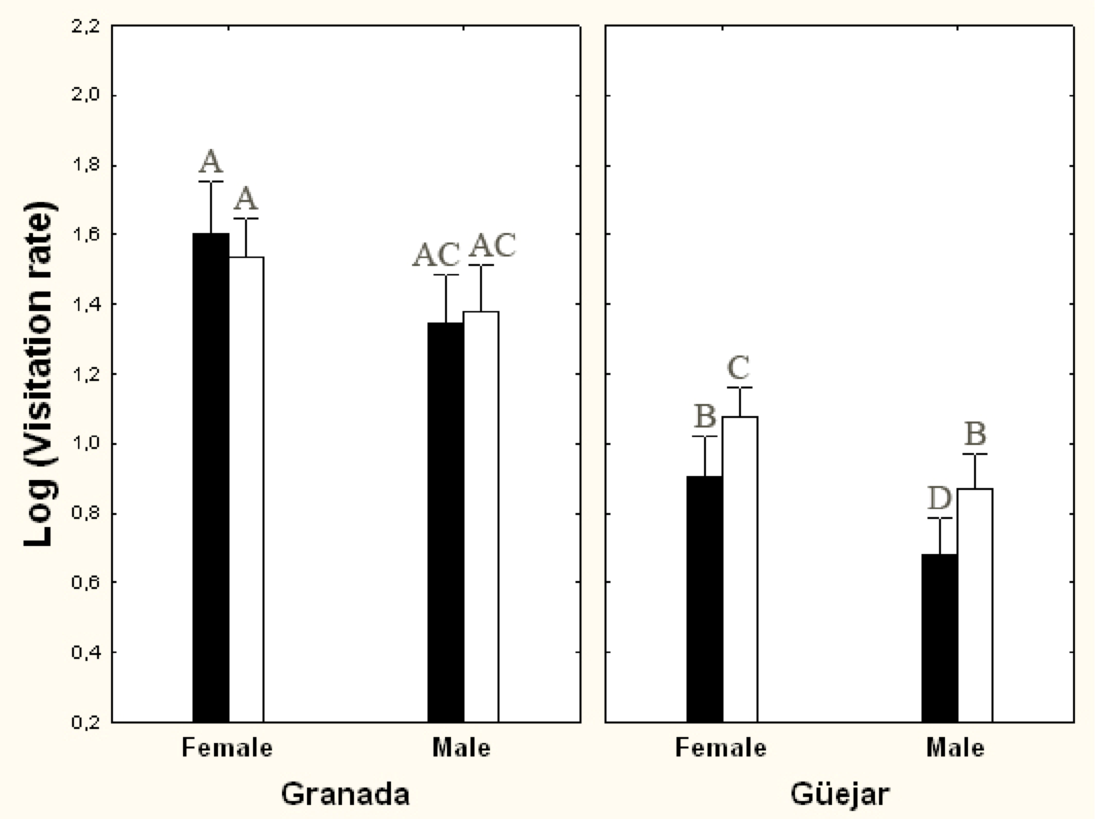

Figure 19. *Female and male nest visit rate (trips per hour) in the studied populations. Black columns correspond to the high nest predation risk treatment (magpie playbacks) and white columns to the low nest predation risk treatment (no sound). Bars associated to each column denote the corresponding standard error. Columns that share the same letter indicate no significant differences for the LSD post hoc tests at the* $p \leq 0.05$ *level.*

In relation to time of latency, which would involve the willingness of parents to return to their nest after the placement of the video camera, we found no significant effect for treatment ($F_{1, 25} = 1.87$, $p = 0.18$), population ($F_{1, 25} = 2.90$, $p = 0.10$), or for the interaction between treatment and population ($F_{1, 25} = 0.62$, $p = 0.44$), although females in general returned on average 13 minutes sooner to their nests than males ($F_{1, 25} = 9.31$, $p = 0.005$), independently of the risk of nest predation ($F_{1, 25} = 2.28$, $p = 0.14$).

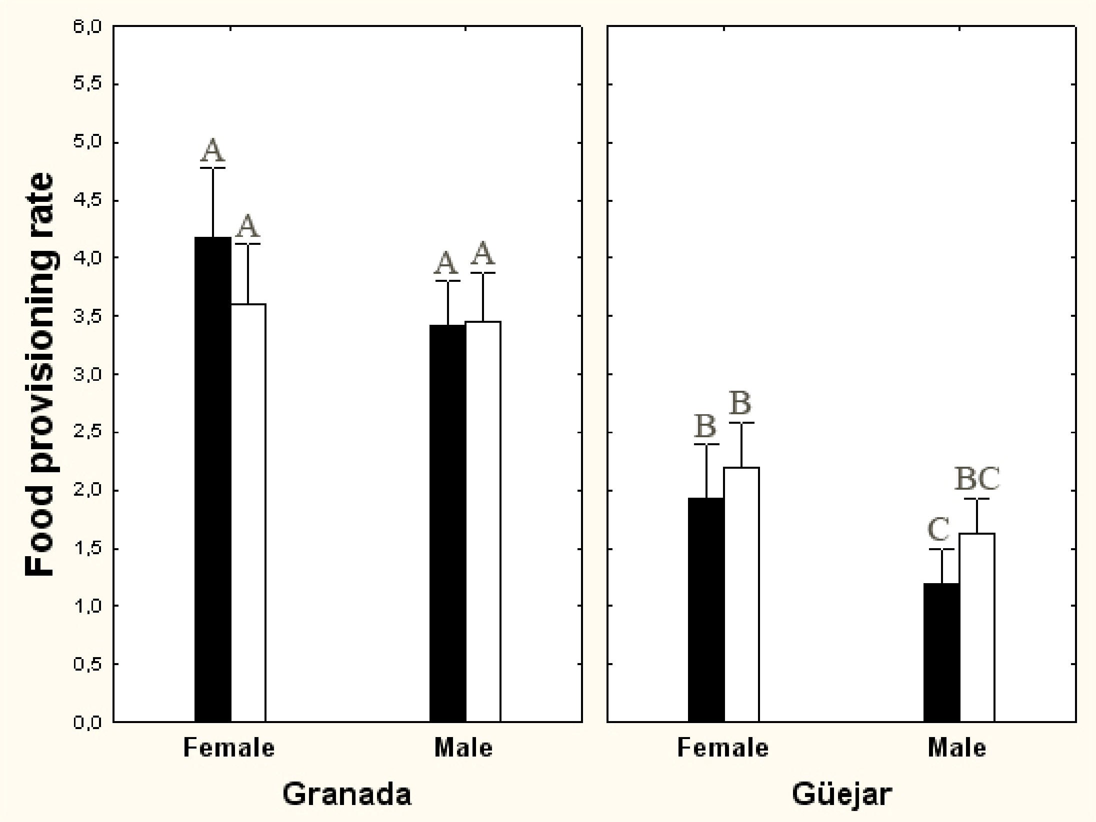

Figure 20. *Female and male food delivery rate (trips in which they fed nestlings per hour) in the studied populations. Black columns correspond to the high nest predation risk treatment (magpie playbacks) and white columns to the low nest predation risk treatment (no sound). Bars associated to each column denote the corresponding standard error. Columns that share the same letter indicate no significant differences for the LSD post hoc tests at the p≤0.05 level.*

With respect of the mean time spent on the nest per visit, we did not observe significant differences between treatment and population ($F_{2, 15} = 0.14$, $p = 0.71$), but we did for the interaction sex x treatment ($F_{1, 15} = 4.48$, $p = 0.05$). Only females reduced the duration of their visits in the presence of a magpie close to their nests (Fig. 21). In accordance with these results, we did not find any significant effect between treatment and population (Fig. 22; $F_{1, 24} = 0.17$, $p = 0.69$) for the mean time spent by parents to feed nestlings. However, neither males nor females changed their mean feeding duration regardless of the risk of nest predation (Fig. 22; $F_{1, 24} = 1.09$, p = 0.31).

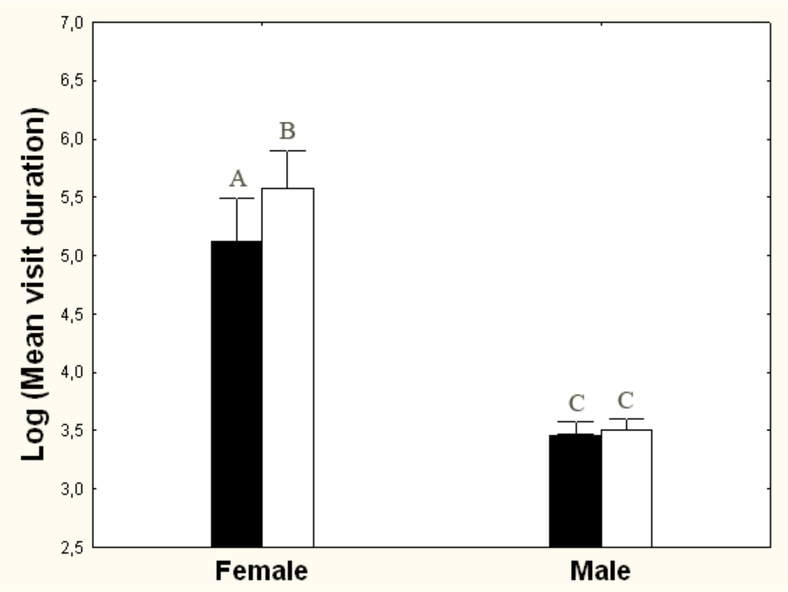

Figure 21. *Mean time spent at the nest per visit for females and males. Black columns correspond to the high nest predation risk treatment (magpie playbacks) and white columns to the low nest predation risk treatment (no sound). Bars associated to each column denote the corresponding standard error. Columns that share the same letter indicate no significant differences for the LSD post hoc tests at the p≤0.05 level.*

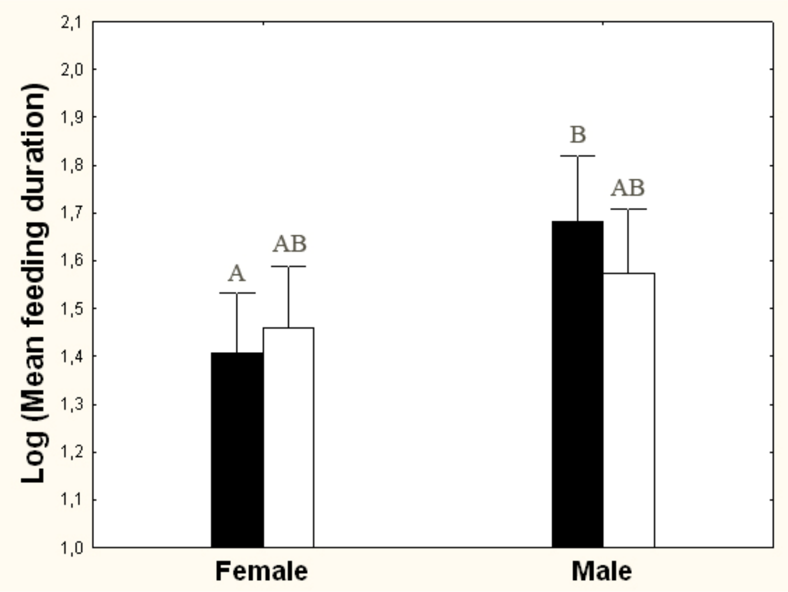

Figure 22. *Mean time spent by females and males feeding their nestlings per visit (from the moment the adult introduced their beak into the mouth of the first chick feeded until the adult finally took it off from the last feeding nestling). Black columns correspond to the high nest predation risk treatment (magpie playbacks) and white columns to the low nest predation risk treatment (no sound). Bars associated to each column denote the corresponding standard error. Columns that share the same letter indicate no significant differences for the LSD post hoc tests at the p≤0.05 level.*

Discussion

Our results show contrasting differences in blackbird male and female responses to changes in risk of nest predation. Moreover, these responses differs between populations: both sexes in Güejar, the population with the highest nest predation pressure, modify their nest visits accordingly with Skutch's hypothesis (Fig. 19). This outcome stresses the importance of clues given to predators by parents' activity. Interestingly, this behavior was not observed in Granada neither for males nor females, suggesting an important habitat-related component influencing similarly these antipredator responses in both sexes. These results indicate that, whether they reduce their nest visits or not, males and females respond in an analogous way (Fig. 19). Although it has been traditionally assumed that both sexes of the same species can respond similarly under the same nest predator stimulus (e.g. Eggers et al. 2005, 2008; Massaro et al. 2008; Peluc et al. 2008), to our knowledge, this is the first experimental study showing it.

We found sexual differences in relation to mean visit duration as blackbird females, but not males, reduced the time spent at the nest when the perceived risk of nest predation increased (Fig. 21). In contrast to visitation rate, we detected no significant variation between populations. These results suggest that this antipredator behavior involves more complexity than considered before, surely, as proposed by Eggers et al. (2008), at least some bird species would be able to employ multiple nest-defense strategies to reduce predator-attracting nest visits. Until now, the usual variable analysed in previous studies about predator-induced plasticity for the nestling period was frequency of nest visits (e.g. Fontaine & Martin 2006; Peluc et al. 2008), however, we have found that adults not only can respond to the risk of nest predation in other ways (i.e. adjusting visit duration) but also that they respond differentially depending on the sex. These sexual differences could be explained by different life-history constraints. Blackbird females are the responsible of performing other activities rather than feeding the chicks (i.e. brooding; Cramp 1988; pers. obs.), thus, they are the only capable of reducing their time at the nest without compromising the food delivered to their nestlings.

Changes in nest visit rate due to risk of nest predation are thought to involve a reduced provisioning rate (e.g. Eggers et al. 2005). However, our results indicate that antipredator responses do not involve necessarily a reduction in food provisioning as adult blackbirds do not reduce the number of feeding visits (Fig. 20) nor the time they dedicate to feed their chicks during each visit (Fig. 22), although the number of visits in general are reduced (Fig. 19) as well as the duration of female nest visits (Fig. 21). This is important because suggests that antipredator responses could be accomplished at the expense of other not so relevant behaviors, such as brooding (when chicks are 8 days-old), and denote that, at least for this species, the trade off between food delivery and antipredator behavior apparently does not exist, or not with the same intensity as for other species.

Our results confirmed that the effect of the risk of nest predation varies between populations. Nest visits are significantly more frequent in Granada, the population with the lower risk of nest predation, than in the forest, replicating the results of the experiments within Güejar in which nest visits were also more abundant in the situation of lower nest predation (Fig. 19). Interestingly, we found that not all antipredator responses were habitat-dependent (i.e. mean visit duration). This could have three possible interpretations: (i) different relation to nest predation for each trait; (ii) different phenotypic plasticity for each trait; and (iii) urbanization influence. With respect of the first option, the cost of some antipredator responses could be different depending on the nest predator community. For example, the existence of passive mechanisms to avoid nest predation (i.e. reduction in nest visits) would exist in Güejar as other possibilities like active defense could involve greater costs (Krams et al. 2007). However, other traits like duration of nest visits could be independent of the type of predator as activities at the nest usually does not increase nest predation (Montgomerie & Weatherhead 1988; Martin 1992). In relation with the second explanation, it has been proposed that not all traits have the same capacity to be plastic (e.g. Fontaine & Martin 2006; Kleindorfer 2007). Many variable forces modulate visitation rate (i.e. food, predators, temperature...), thus, it could be adaptive to maintain this trait highly plastic to adjust it depending on the situation. In contrast, duration of visits are assumed to be mainly restricted by feeding events (e.g. Powell 1984), then it would be necessary a lesser plasticity for this trait. Thirdly, it has been proposed that urban habitats impose a greater food limitation but a smaller nest predation pressure (Chamberlain et al. 2009; Ibáñez-Álamo and Soler submitted b), then, the absence of modification in the visit pattern in Granada could indicate that the cost of reducing the rate of food delivery may be higher than the costs imposed by the risk of nest predation. Anyway, our results highlighted and confirmed that the risk of nest predation imposes changes in bird strategies.

To sum up, both sexes responded to the risk of nest predation in a similar way (Fig. 19), although not for all traits analysed (i.e. mean visit duration; Fig. 21), indicating that in general males and females adjust their behaviors depending on the risk of nest predation. Moreover, this predator-induced plasticity seems influenciated by the level of environmental nest predation risk of each population. All of this suggests that antipredator bird responses are more complex than they were previously considered. Furthermore, our results indicate that adult birds can respond to the presence of predators near their nests without compromising the food delivery to their nestlings (Fig. 20 and 22), thus without important costs for the offspring.

Acknowledgments

We would like to thank J. Iglesias, J. Rodríguez and especially D. Villanueva for their help in the field. M. Griesser, J. Martín and O. Sanllorente made useful comments to previous drafts. This study was partially supported by the Spanish Ministerio de Educación y Ciencia (research project CGL2007-61940/BOS) and by a FPU predoctoral grant to J. D. Ibáñez-Álamo.

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CAPÍTULO 4

Evidencia experimental del coste en depredación de la petición usando nidos activos.

La existencia de los costes de la petición de alimento por parte de los pollos durante su periodo de estancia en el nido, actualmente es uno de los temas que centra la atención de los estudios evolutivos del conflicto entre padres e hijos. Evidencias indirectas parecen apoyar la existencia de un coste en depredación del nido para la petición. Sin embargo, las evidencias empíricas directas de que la petición de los pollos podría atraer la atención de los depredadores de nidos han sido criticadas debido a problemas metodológicos, principalmente por el uso de nidos artificiales. Con objeto de evitar ese problema, en este estudio hemos usado nidos naturales y activos de mirlo común para crear experimentalmente nidos de alto y bajo nivel de petición introduciendo un pollo de críalo europeo (*Clamator glandarius*) o de mirlo respectivamente. Los nidos de alto nivel de petición, los que incluían al pollo de críalo, una especie parasita de cría cuyos pollos presentan un comportamiento petitorio exagerado, fueron significativamente más depredados que los de bajo nivel de petición en los que se había introducido el pollo de mirlo. Encontramos que los nidos de alta petición fueron depredados dos veces más que los de baja petición. Por tanto, nuestro hallazgo apoya la hipótesis de que este coste impone una importante restricción a la evolución del comportamiento de petición.

CAPÍTULO 4

Experimental evidence for a predation cost of begging using active nests.

Juan Diego Ibáñez-Álamo, Laura Arco and Manuel Soler

The existence of begging costs currently focuses the attention of evolutionary studies on the parent-offspring conflict. Indirect evidence seems to support the existence of a nest predation cost of begging. However, empirical direct evidence that begging by nestlings could attract the attention of nest predators has been criticized because of methodological problems, mainly the use of artificial nests. In order to avoid this problem, we used natural and active common blackbird (*Turdus merula*) nests to experimentally create high and low begging nests by introducing a great spotted cuckoo (*Clamator glandarius*) or blackbird chick respectively. Nests showing the higher level of begging were significantly more predated than those presenting lower level of begging. We found that high begging nests were predated twice more than low begging nests. Therefore, our findings support the hypothesis that this cost imposes an important restriction to the evolution of begging behavior.

Manuscrito

Introducción

Begging by nestlings has focused the study of evolutionary conflicts of interest within families in the last two decades, especially begging in altricial birds (Wright & Leonard 2002). The initial aproximation to these conflicts was theoretical (Stamps et al. 1978; Macnair and Parker 1979; Harper 1986; Eshel and Feldman 1991; Godfray 1991, 1995a, b), with the majority of models proposed assuming begging behavior to be costly (review in Johnstone & Godfray 2002). Predation is one of the costs suggested capable of maintaining a honest begging signal (reviewed in Wright & Leonard 2002, Moreno-Rueda 2007), assuming that begging calls may attract predators to the nest (Haskell 1994, 1999, Leech and Leonard 1997, Dearborn 1999).

Indirect evidences of the relevance of this cost are numerous. On one hand, to fall silent is a common response of chicks to parental alarm calls in birds (Davies et al. 2004, Madden et al. 2005 a, b) suggesting that otherwise nests could be detected and predated. For example, Platzen & Magrath (2004, 2005) found that nestlings of the white-browed scrubwren (*Sericornis frontalis*) fell silent in response to alarm calls from their parents indicating especific danger to them. Interestingly, this behavior disappeared when chicks got out of the nest (Magrath et al. 2006). These responses from nestlings seemed not only restricted to the intermediation of parents, but also could be established directly between predator-chicks interactions as Magrath et al. (2007) demonstrated that nestlings of the white-browed scrubwren fell silent when they listened the sound of a nest predator approaching. On the other hand, comparative analyses indicate that species subject to greater nest predation have begging calls with higher pitch and lower volume, thus more difficult to locate by predators (Briskie et al. 1999). Additionally, several studies have found increased nest predation due to brood parasitism, usually explaining their results because of the begging behavior of the parasitic chick (Dearborn 1999, Ortega and Ortega 2003, Kosciuch and Sandercock 2008, Hannon et al. 2009).

Additionally, all previous empirical studies seemed to support the existence of predation costs (Haskell 1994, 1999, Leech & Leonard 1997, Dearborn 1999, McDonald et al. 2009), but their conclusions should be treated carefully because important and, up to date, unsolved methodological problems. All experimental studies focusing on predation costs utilized playback procedures on artificial nests (see references above). These methods have several restrictions: (1) do not take into account parental activities (i.e. active defense of the nest; but see McDonald et al. 2009), (2) pseudoreplication as the sound used for the playbacks corresponds to one or few individuals or nests (i.e.: Haskell 1994, Leech and Leonard 1997), (3) unnaturally duration of begging calls played also during night (i.e.: Dearborn 1999), and (4) differences between nest predation in natural and artificial nests. The last is especially important to the light of recent studies that clearly demonstrated misleading findings in nest predation due to the use of artificial nests (Weidinger 2001, Zanette 2002, Moore and Robinson 2004).

Therefore, nest predation costs of begging seem to exist although adequate experimental evidence is lacking. The best way to test directly whether these costs really exist would be performing experiments with natural nests taking into account not only parental behavior but also real begging from chicks.

In this article, we examined the existence of predation costs of begging with the manipulation of European blackbird (*Turdus merula*) natural nests. We created experimentally high begging activity nests with the introduction of a great spotted cuckoo (*Clamator glandarius*) chick and low begging activity nests introducing a blackbird chick as control.

Methods

Study sites

The experiments were conducted in the Valley of Lecrín in southern Spain (36º 56’ N, 3º 33’ W; 580 m a.s.l.), from late April to late May 2008 and 2009. The location is dominated mainly by orange groves in which blackbirds usually nest..Great spotted cuckoo chicks used in the study were collected from magpie (*Pica pica*) nests found in Hoya de Guadix (37º 18’ N, 3º 11’ W; 1000 m a.s.l.), an area located 55 kms far away from Lecrín. Great spotted cuckoo are very common in this population involving a high incidence of parasitism on magpie hosts, reaching 100% in some areas, and high number of multiparasitized nests (Soler 1990; Soler et al. 1998).

Experimental design

Both blackbird and magpie populations were studied actively searching for nests. Once a magpie nest with a great spotted cuckoo egg was located it was regularly visited in order to know the exact date of hatching. When the cuckoo chick was one day-old we transported it to a blackbird nest with all its chicks of the same age (± a day) than the cuckoo. As soon as two other blackbird nests hatched at the same time, we took a one day-old blackbird chick from one and introduced it in the other following the same procedure.

The methodological procedure to handle chicks has long been utilized by our group to carry out many other cross-fostering experiments (e.g. Soler et al. 1999). It basically consists on taking a cuckoo chick from a magpie nest, placing it in an artificial cotton nest maintaining the temperature between 25-30 ºC, and transporting it to the blackbird nest. The same procedure was followed to do the blackbird chicks manipulation. No chick died during the process and no adult deserted nests from which chicks were taken or introduced.

This way we created experimental nests with high begging activity (cuckoo introduced) and control nests with low begging activity (blackbird introduced). Previous studies have shown that parasite chicks have a more conspicious begging than that of their host chicks (Gochfeld 1979, Davies et al. 1998, Dearborn 1999). This is also true for the great spotted cuckoo, whose chicks emit more begging calls and begged for much longer than its host (magpie) chicks (Soler et al. 1999). This higher conspicuosness of cuckoo chicks acoustic signals is maintained when comparing with blackbird chicks (pers. obs.). Just after the introduction of the aloctonous chick, we filmed the nest using a Canon MV600 videocamera with a tripod placed near each nest (1.5-2.5 m). We filmed experimental nests for two consecutive hours. Parental visit rate was extracted from the videos for each nest.

Experimental nests were visited every two days to check for nest predation and take biometrical data from every chick (cuckoo and blackbird). Nests from which a chick was taken were similarly visited to check for predation and possible desertion from parents. Nests were considered as predated when they were found with biological remains (feathers or blood) or empty before the chicks had the capacity to jump out of the nest (11 day-old chicks for blackbird nestlings). Nests were assigned as successful when chicks were still alive into the nest when 11 day-old.

Ethical note

According to the predicted predation cost of begging, the effect of nest predation on experimental nests was dramatic (see results). For this reason, we took some measures in order to avoid unnecessary death of chicks and get the maximum scientific profitability following ASAB guidelines for behavioural research (ASAB 2006). For instance, the experimental manipulation has been used not only to study, for the first time, the predation cost of begging in natural nests, but also to test other hypotheses related to chick recognition and food delivery by parents (Soler et al. in prep a, b). We conducted the minimum number of experiments involving cuckoo manipulation (11) that allowed us to test our hypothesis with certain statistical power. Additionally, in order to reduce our impact in the cuckoo and blackbird populations due to our manipulations we distributed the experiments in two years. Only 5.5% of the 200 cuckoo chicks controled in the magpie population were used for the experiments assuring that the impact on great spotted cuckoo population was minimal. The methods used in this study complied with Spanish Animal Care guidelines.

Statistical analyses

Previous studies have found that parents can respond to the increase of begging at their nest by increasing nest visits, and therefore increasing their activity at the nest (reviewed in Kilner and Johnstone 1997). The relation between the latter and nest predation is well established (e.g. Martin et al. 2000, Ghalambor and Martin 2002, Eggers et al. 2008), thus, it is important to know if our treatment has an effect on parental activity that could be partially responsible of an increase in nest predation in the cuckoo nests. To determine this effect we used mixed linear models with fixed and random effects using parental visit rate as the dependent variable. As nests share the same environmental conditions during each year, this variable was included in the model as a random factor. Begging treatment and brood size were included as fixed factors.

Generalized linear models (GLZ) were used to test the effect of our treatment (high or low begging) on nest predation. Nest predation rate was fitted to a binomial distribution with logit as link function (JMP, Version 7. SAS Institute Inc., Cary, NC, 1989-2007). Treatment, year and their interaction (treatment x year) were included as nominal independent variables.

Results

We monitored a total of 234 magpie and 331 blackbird nests. As predicted, experimental manipulation provoked a higher predation rate in blackbird nests in which one great spotted cuckoo chick was included. Only 27% (n = 11) of these nests survived. In contrast, 75% (n = 12) of the control nests, those including an extra blackbird chick, were succesful (Fig. 23). Thereby, nest predation was significantly higher for experimental than for control nests (Table 2). We assumed our results reflect natural nest predation properly given that nest predation rate of control nests (25.0%) was very similar to that of the entire blackbird population in the years of study (30.6%).

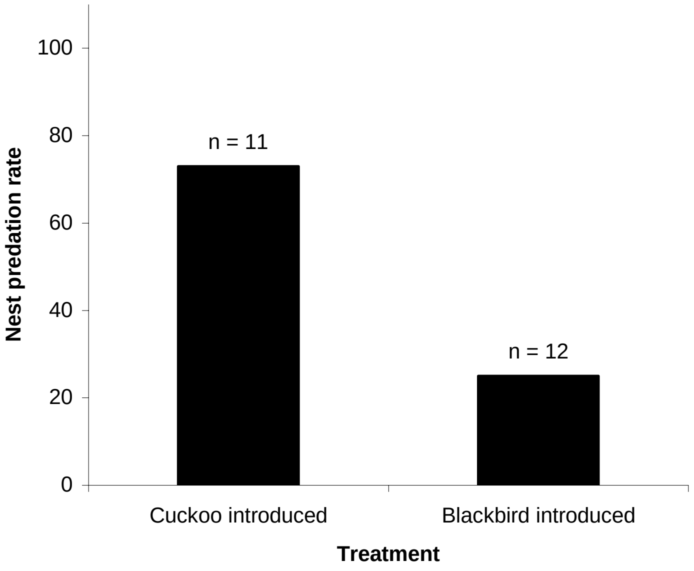

Figure 23*. Percentage of nest predation in relation to treatment. Cuckoo introduced refers to those blackbird nests including a great spotted cuckoo chick, while blackbird introduced corresponds to nests containing only blackbird chicks.*

Table 2*. Results of the generalized lineal model for the probability of nest predation.* $p<0.05$, ** $p<0.01$.*

Variable	**df**	χ^2
Year	1	5.25*
Treatment	1	7.07**
Year x Treatment	1	1.23

We found no significant differences for parental activity at the nest depending on the treatment ($F_{1,\,1.02} = 0.78$, p = 0.54). Therefore, we can be sure that all differences detected in nest predation should be due to differences in begging activity between treatments, not to an increase in parental activity.

Discussion

Our manipulation involved a drastic increase in nest predation for the cuckoo introduced nests, but not for the controls (Fig. 23). Accordingly, Dearborn (1999) found that begging calls from indigo bunting (*Passerina cyanea*) nests parasitized by brown-headed cowbird (*Molothrus ater*) increased nest predation rate, but not begging calls from unparasitized nests. However, this study was made using artificial nests and playbacks, with the consequent problems derived from this methodology (see introduction). To our knowledge, this is the first experimental study demonstrating the existence of a nest predation cost of begging using natural nests and chicks.

These results also reinforce the findings of other series of studies that: (1) state the importance of alarm calls and their effect on chicks silence (Davies et al. 2004, Madden et al. 2005 a, b , Platzen and Magrath 2004, 2005, Magrath et al. 2006); (2) demonstrate direct chick silence to predator sounds as an antipredatory response (Magrath et al. 2007); (3) indicate acoustic structure modification of begging calls in response to predator pressure (Briskie et al. 1999); all of them assuming that this begging cost exists.

However, although we were able to detect the increase of nest predation due to begging activity, we do not know which variable of begging was responsible of this increase. Soler et al. (1999) demonstrated that great spotted cuckoo chicks delivered more and longer begging calls than their magpie host chicks, which is also true when compared to blackbird chicks (unpubl. data). Therefore, begging of cuckoo chicks is more conspicuous and could attract nest predators more easily than begging of blackbird chicks. Differences in the acoustic structure of cuckoo and blackbird begging also could be important. It has been demonstrated that those species with higher nest predation rates have evolved adaptations that minimize detection due to begging (Briskie et al. 1999, but see McDonald et al. 2009). The blackbird is a species with a very high nest predation rate (>60%; e.g. Cresswell 1997; Groom 1993; Hatchwell et al. 1996; Tomialojc 1994), much higher than the magpie's (25%; extracted from Soler et al. 1998), the usual host of the great spotted cuckoo. Furthermore, brood parasite chicks present begging displays more exaggerated than those of host chicks. Thus, great spotted cuckoo chicks surely have more locatable begging calls than those of blackbird chicks.

Possible confounding factors such as nest detectability are probably of small relevance in this study as previous papers demonstrated that blackbird nest characteristics do not influence nest predation when parents are present (Moller 1990, Cresswell 1997), surely due to the defensive behavior of adults. The absence in the study area of visually oriented nest predators like corvids (pers. obs.) also suggests that nest detectability would not influence nest predation.

The level of cost needed to maintain honesty in begging has been recently the center of intense discussion (Johnstone & Godfray 2002) and theoretical analyses indicated that a substantial cost of begging is required for the maintenance of honesty. This assumption was not supported because previous studies suggested variability in the relevance of this cost, as the increase in predation due to begging activity was relatively small in some studies (Dearborn 1999) but large in others (Haskell 1994, 1999). Our experimental results obtained in natural conditions, avoiding methodological problems that have affected previous studies, reflect a dramatic increase in nest predation due to begging activity (Fig. 23). Therefore, the importance of this pressure in the evolution of begging behavior could be very high, imposing great constrains to the increase of the sound begging component. Even more, these costs could be shared by parents if the predator attracted can predate adults as well. We detected an adult predation event along with all the chicks of the nest in 1 of the 8 nests which were predated (12.5%), but none in the control nests.

In conclusion, our results, obtained using a methodology that avoids the limitations associated with artificial nests and the use of playbacks, clearly show that nest predation cost of begging is important and could be large enough to impose significant restriction to the evolution of begging.

Acknowledgments

O. Sanllorente made useful suggestions to previous drafts. L. de Neve helped with the field work. This study was partially supported by the Spanish Ministerio de Educación y Ciencia (research project CGL2007-61940/BOS) to M. Soler and by a FPU predoctoral grant to J.D. Ibáñez-Álamo.

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CAPÍTULO 5

¿Presentan los pollos respuestas adaptativas según el riesgo de depredación? Una perspectiva hormonal.

Los pollos de las aves altriciales pueden percibir el riesgo de depredación por medio de pistas sonoras producidas por los depredadores. Esta capacidad conlleva cambios hacia un comportamiento menos conspicuo con objeto de evitar ser detectado y depredado. Sin embargo, no se sabe nada sobre los mecanismos fisiológicos subyacentes a esta capacidad. Los mecanismos potenciales pueden implicar a la corticosterona y la testosterona ya que ambas hormonas están relacionadas con la petición, cuyas señales acústicas podrían ser usadas por los depredadores para localizar los nidos. Aunque dada la especificidad de especie de esta relación es difícil hacer predicciones particulares. En este estudio manipulamos el riesgo de depredación percibido por el mirlo común y analizamos los niveles de esas hormonas en el plasma de los pollos para ver cómo les afecta el riesgo de depredación a corto plazo. Este experimento se ha llevado a cabo en dos poblaciones de mirlo que presentan diferencias extremas en sus presiones de depredación de nidos, lo que nos ha permitido explorar los efectos del riesgo ambiental (a largo plazo) de depredación de nidos en las respuestas hormonales de los pollos. Nuestra manipulación mostró cambios hormonales en respuesta al riesgo de depredación del nido. Sin embargo, encontramos resultados dispares para el riesgo de depredación a corto y largo plazo, lo que parece indicar la existencia de diferentes mecanismos para enfrentarse a situaciones estresantes puntuales y crónicas. Mientras los efectos a corto plazo pueden ser explicados con objeto de reducir la actividad de petición, los cambios inducidos a largo plazo sugieren bien una estrategia hormonal para favorecer un rápido crecimiento en la población con la mayor presión por depredación, bien la respuesta indirecta de los pollos al riesgo de depredación del nido mediada por los padres. Estos hallazgos resaltan la importancia de estudiar la depredación de nidos desde el inusual punto de vista de los pollos y subraya los beneficios de incluir variables fisiológicas en el estudio de las interacciones depredador-presa.

CAPÍTULO 5

Do nestlings present adaptive responses according to predation risk? A hormonal perspective.

Juan Diego Ibáñez-Álamo, Olivier Chastel and Manuel Soler

Chicks of altricial birds may perceive predation risk by acoustic cues produced by predators. This capacity involves changes to a less conspicuous behavior to avoid being detected and predated. However, nothing is known about the physiological mechanisms underlying this capacity. Potential mechanisms may involve corticosterone and testosterone, two hormones related to begging, an acoustic signal used by predators. However, given the species-specificity of this relationship it is difficult to make particular predictions. We manipulated perceived risk of nest predation in common blackbird (*Turdus merula*) and analyzed nestlings' plasma levels of these hormones to look for short-term nest predation risk effects. Additionally, we carry out our experiment in two blackbird populations with extreme differences in their nest predation pressure to explore the effects of environmental (long-term) nest predation risk on chicks' hormonal responses. Our manipulation showed hormonal changes in response to nest predation risk. However, we have found unmatched results between short and long-term nest predation risk, which seems to point out the existence of different mechanistic pathways to cope with acute and chronic stressful situations. While their short-term effects could be explained to reduce begging activity, long-term induced changes suggested whether a hormonal strategy to favor a fast growth in chicks of the population with the higher predation pressure or the offspring indirect response to nest predation risk mediated by parents. These findings highlighted the importance of studying nest predation from the unusually considered chick perspective and underlined the benefits from including physiological variables in the study of predator-prey interactions.

Enviado, *Hormones and Behavior*

Introduction

Nest predation is one of the main selective forces modeling avian life-histories (Martin, 1995). Several studies have demonstrated that adult birds adaptivelly respond to the risk of nest predation by modifying their behavioral patterns (reviewed in Lima, 2009; Martin and Briskie, 2009). For example, currently it is well established in birds that parents turn into a less conspicious behavior (i.e. reducing their visits to the nest) in the presence of a potential nest predator (e.g. Ghalambor and Martin, 2002; Eggers et al., 2005, 2008; Massaro et al., 2008; Peluc et al., 2008). Additionally, other papers highlight parental physiological changes like modifications in corticosterone (the main stress hormone in birds) levels to acute or chronic threats to the nests (e.g. Silverin, 1998; Fowler, 1999; Scheuerlein et al., 2001; Walker et al., 2006; Cyr and Romero, 2007; but see Butler et al., 2009).

However, much less is known about the changes induced in nestlings (Rubolini et al., 2005) despite the fact that nest predation has greater fitness consequences on chicks (being predated) than on parents (losing a single reproductive event). Recent studies showed that nestlings also respond to nest predation risk by changing their behavior helped by their parents, which can provoke their silence in the presence of a threat (a "switch off" strategy) or their begging calls under all-clear situations (a "switch on" strategy; e.g., Davies et al., 2004; Madden et al., 2005a, 2005b; Platzen and Magrath, 2004, 2005). Even more, at least the chicks of one species, the white-browed scrubwren (*Sericornis frontalis*), seem to assess the risk of nest predation directly from its source and independently of their parents as nestlings of this species fell silent when they listened to the sound of a nest predator approaching (Magrath et al., 2007). This framework of behavioral changes in chicks induced by nest predation risk suggests that physiological alterations in offspring as well as in adults could exist. In fact, recent evidences suggest the benefits of including physiological variables in the study on predator-prey interactions (Slos et al., 2009). Nevertheless, to our knowledge no one has explored this possibility so far.

Hormones are good candidates to study these physiological changes as they are mediators of many of these modifications and are linked with several behaviors (e.g., Landys et al., 2006; McEwen and Wingfield, 2003). Nestlings of many species modificate their corticosterone (Cort) levels in response to stressful situations simulating a nest predation event (i.e. handling by researchers; e.g., Kitaysky et al., 1999, 2001; Love et al., 2003; Saino et al., 2003; Gil et al., 2008). This steroid hormone promotes survival *via* mobilization of endogenous energy and other physiological changes (reviewed in Landys et al., 2006; Sapolsky et al., 2000). Then, if chicks identify a potential nest predator as a threat to their survival, their cort levels should increase accordingly (prediction 1a). Alternatively, high levels of this hormone are also related to begging behavior (Kitaysky et al., 2001, 2003; Loiseau et al., 2008, but see Vallarino et al., 2006). Thus, considering that part of this behavior implies an acoustic signal used by predators to locate the nest (Haskell, 1994, 1999; Leech and Leonard, 1997; Dearborn, 1999; McDonald et al., 2009), a reduction in nestlings' cort levels could be predicted too (prediction 1b). In addition, testosterone (T) is considered to control also part of the mechanism underlying begging activity, although the proposed relationship between T levels and begging is quite variable. Some studies detected a positive relationship showing that high levels of T increased begging (e.g., Goodship and Buchanan, 2006, 2007; Quillfeldt et al., 2006) while others obtained the opposite pattern (e.g., Groothuis and Meeuwissen, 1992; Groothuis and Ros, 2005). Then, we could expect an elevation (prediction 2a) as well as a reduction (prediction 2b) in T levels simultaneously to the changes in cort in order to reduce the acoustic cues given to predators through begging.

Most of the common blackbird (*Turdus merula*) populations suffer from a very high nest predation pressure (e.g., Tomialojc, 1994; Hatchwell et al., 1996; Cresswell, 1997; Ibáñez-Álamo and Soler, submitted a), making this species an excelent model to study the effect of this selective force on chick's behavior and their associated physiological changes. To test the effect of short-term nest predation risk on nestling's hormonal responses we simulated the presence of a magpie (*Pica pica*) in the vicinity of the nests using playbacks. In addition, we carried out the experiments in two populations with extreme differences in nest predation pressure in order to assess the influence of environmental (long-term) risk of nest predation on these responses.

Material and methods

Study area and species

The study was conducted on two common blackbird breeding populations in Granada province (South of Spain), from early March to early July 2007. One was a urban population, settled in parks of the city of Granada (37º 10’ N, 3º 36’ O; 738 asl) with a low nest predation pressure (30% of nest predated), and the other was a woodland population, settled in a *Quercus* forest in the Natural Park of Sierra Nevada (37º 9’ N, 3º 24’ O; 1050 m asl), with a high nest predation pressure (80% of nest predated). We actively searched for nests in both locations (n = 150). Once a nest was located we visited it regularly (every 3 days) until fledge or predation to assess laying date, haching date and number of fledging. See Ibáñez-Álamo and Soler (submitted a) for a more detailed information on these populations.

Manipulation of the nest predation risk and chicks sampling

When chicks were 8 days-old (± 1 day) the perceived risk of nest predation was manipulated by simulating the presence of a magpie (*Pica pica*) in the surroundings of the nest. This corvid is a nest predator present in both study locations (pers. obs.). For this purpose, nests were exposed for 3 hours to a playback (one 15 seconds magpie call per 3 minutes, TDK endless casette, EC-6M) starting at daybreak. The recorder was moved every hour to simulate change in position by the predator. We used 7 different magpie call recordings to avoid blackbirds get used to them and the potential problem of pseudoreplication. The control treatment consisted in the same manipulation protocol as for the experimental treatment but playing no sound as we had previously tested that there are no significant differences for blackbird’s reaction to woodpigeon (*Columba palumbus*) or no sound playbacks (Ibáñez-Álamo and Soler, submitted b). Nests were exposed to control or experimental treatments in consecutive days, with aproximatelly half of the nests starting with the control (n = 20) and the other half with the experimental treatment (n = 15).

After the three hours, half of the brood was sampled the day the nest was exposed to the control treatment and the other half the day with the experimental treatment. Thus, all chicks were sampled at the same time of the day controling for this potential cofounding factor. Chicks sampled for each treatment were chosen randomly, usually sampling 1-2 chicks at a time depending on the brood size of a particular nest (mean brood size ± SE = 2.68 ± 0.18 chicks). Two blood samples (100-180 µl each) were collected from the brachial vein using sterile needles and heparinized microcapillary tubes. The first sample was taken immediately after the end of the playback period, and the second after 30 minutes. We chose 30 min as the time for the second sample because previous studies on this species have shown that it is a good proxy for the maximum corticosterone levels (Partecke et al., 2006). Between sampling, chicks were kept unmolested in a cloth bag. All first blood samples were taken within 3 min following chicks' removal from the nest. Each nestling was weighted using an electronic balance (± 0,1 g) after blood sampling for each treatment (day).

Blood samples were kept in a portable fridge at 4 ºC for as long as 3 hours. Then, blood samples were centrifuged at 13000 r.p.m. for 1 min. Plasma samples were stored at -40 ºC until radioimmunoassay analyses.

Hormone assays

Cort and T levels in plasma were determined by radio immunoassay as detailed in Lormée et al. (2003) and Chastel et al. (2005) at the Centre d'Etudes Biologiques de Chizé (CEBC). Intra-assay coefficients of variation were 1,42% for cort and 3,08% for T (n = 3 duplicates). The lowest concentration detectable was 195 pg mL^{-1} and 68 pg mL^{-1} for cort and T, respectively.

Statistical analyses

We used mixed linear models with fixed and random effects (Statistica 6.0). As nestlings share genes and the environment (the nest), the nest identity was always included in the models as a random factor. The predation risk treatment and the location were included as fixed factors and covariates (body mass, brood size and hatching date) were also added. Baseline cort level was also added as a covariate for the analysis of one variable (maximum cort level). The assumptions underlying the use of the linear model were systematically checked and the $\log_{10}$-transformation was applied when necessary.

Results

We analyzed hormonal levels in 90 chicks from 35 different blackbird nests, of which 16 corresponded to the low risk population and 19 to the high risk one.

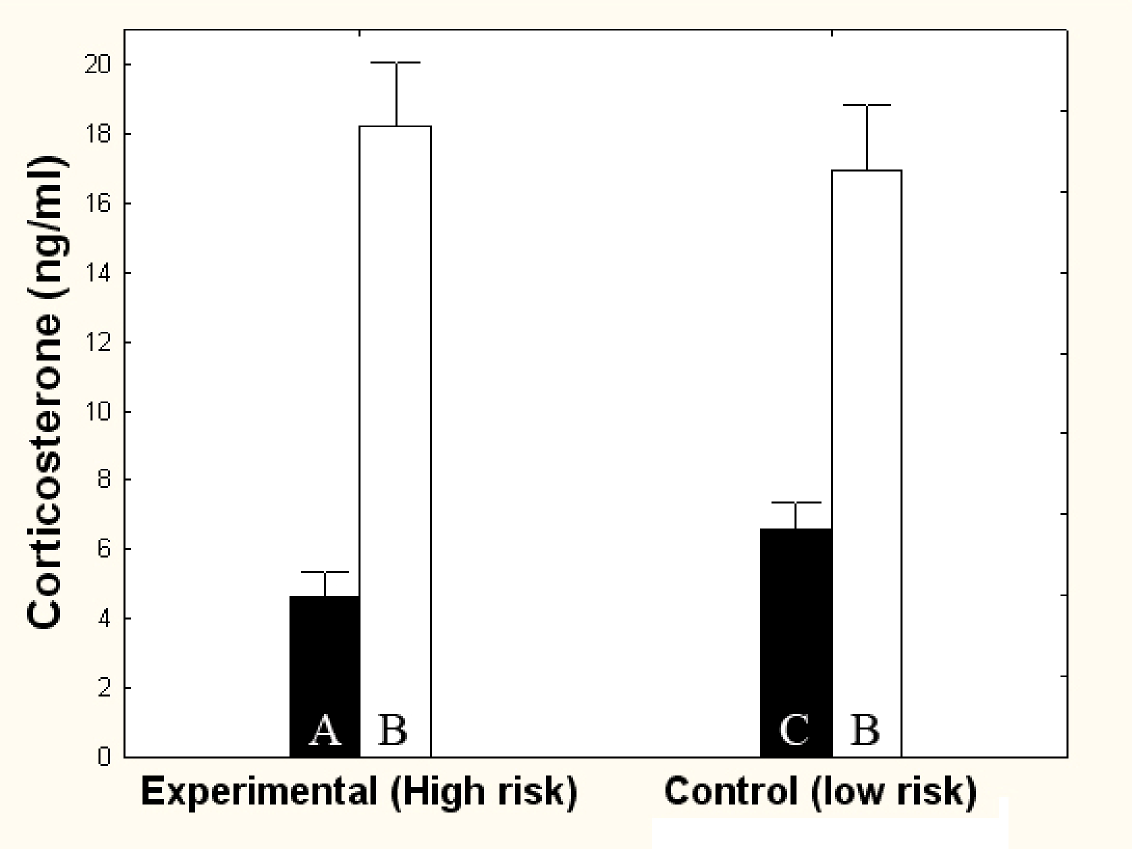

Figure 24. *Nestling baseline (black columns) and maximum (open columns) corticosterone levels for each treatment. Bars associated to each column denote the corresponding standard error. Columns with different letters indicate significant differences at the p<0.05 level.*

Baseline cort levels were significantly influenced by the treatment ($F_{1,\ 29.5}$ = 4.64, p = 0.04) being lower in the experimental treatment (high risk situation) than in the control (low risk situation) fitting to prediction 1b (Fig. 24). In contrast, we observed no significant differences for the maximum cort levels ($F_{1,\ 24.1}$ = 0.44, p = 0.52; Fig. 24), which on the contrary seemed to be positively explained by the baseline cort level ($F_{1,\ 45.4}$ = 7.41, p = 0.009) and the nestling weight ($F_{1,\ 31.2}$ = 5.14, p = 0.03). Brood size did not affect neither baseline cort ($F_{1,\ 28.5}$ = 0.72, p = 0.41) nor maximum cort ($F_{1,\ 25.9}$ = 0.001, p = 0.98). With respect of the T levels, again we detected a significant effect of the treatment on plasma levels of this steroid ($F_{1,\ 21.9}$ = 4.55, p = 0.04) showing the opposite pattern found for baseline cort levels: higher T levels in the experimental treatment (high risk situation) than in the control (low risk situation), thus, fitting to prediction 2a (Fig. 25). Additionally, we did not detect a significant brood size effect on T levels ($F_{1, 20.8} = 0.02$, $p = 0.88$).

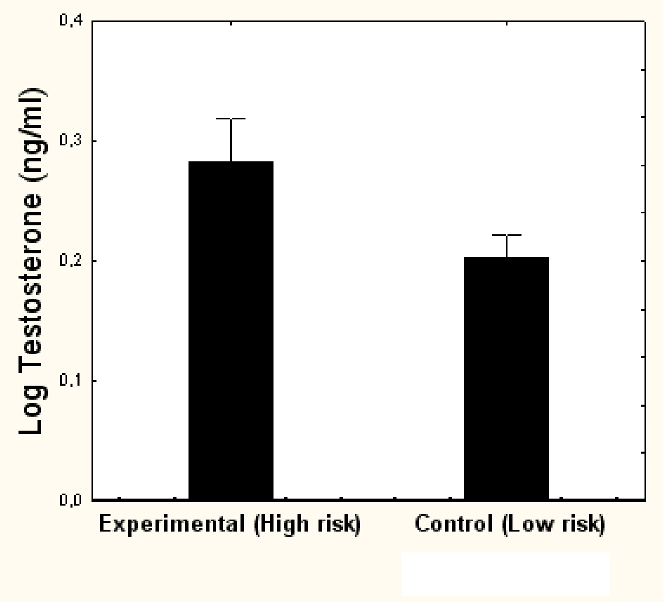

Figure 25. *Nestling testosterone levels for each treatment. Bars associated to each column denote the corresponding standard error.*

In relation to variance in hormonal levels between populations, which would reflect the effect of long-term nest predation risk, we observed significant differences for the baseline cort levels ($F_{1, 29.1} = 4.60$, $p = 0.04$). However, the results showed the opposite trend of that found for the experiment: baseline cort was higher in the high risk location (Güejar) than in the low risk one (Granada; Fig. 26). No differences were detected for the maximum cort ($F_{1, 29.9} = 0.16$, $p = 0.69$; Fig. 26) or the T levels ($F_{1, 21.1} = 0.002$, $p = 0.96$; Fig. 27).

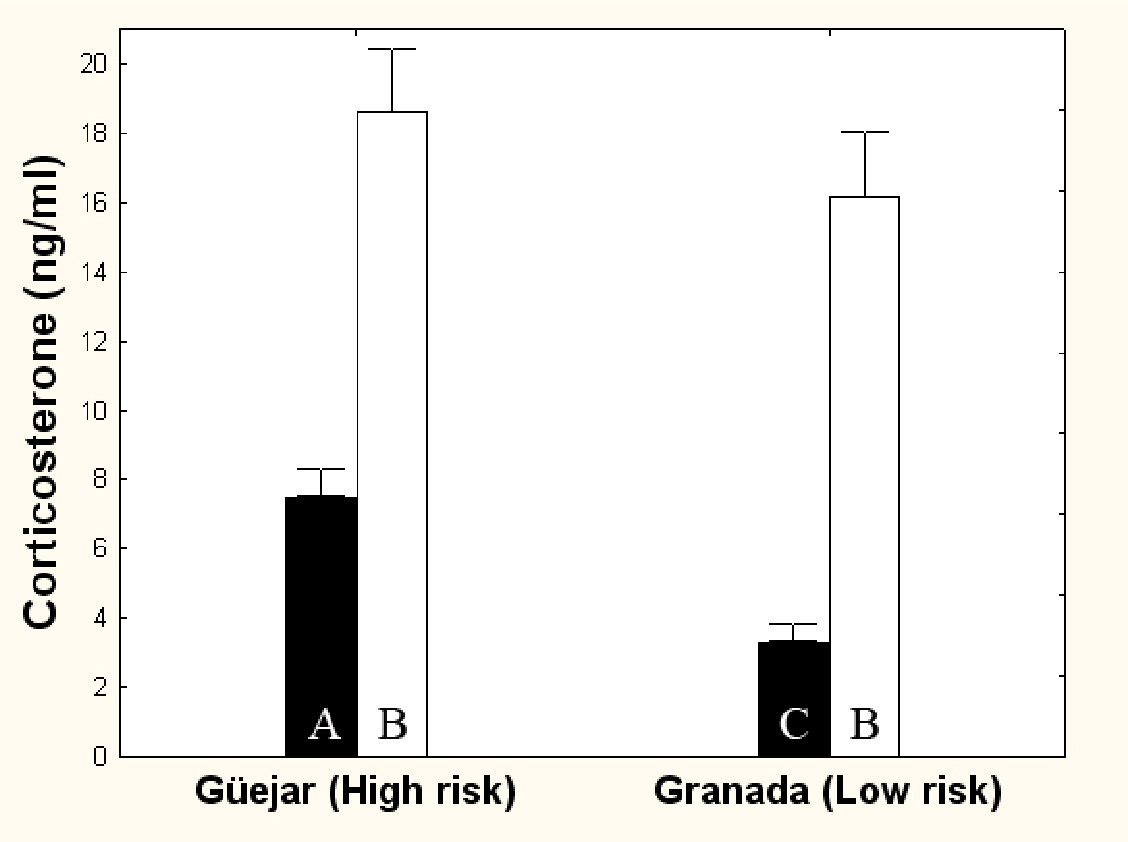

Figure 26. *Nestling baseline (black columns) and maximum (open columns) corticosterone levels for each population. Bars associated to each column denote the corresponding standard error. Columns with different letters indicate significant differences at the $p<0.05$ level.*

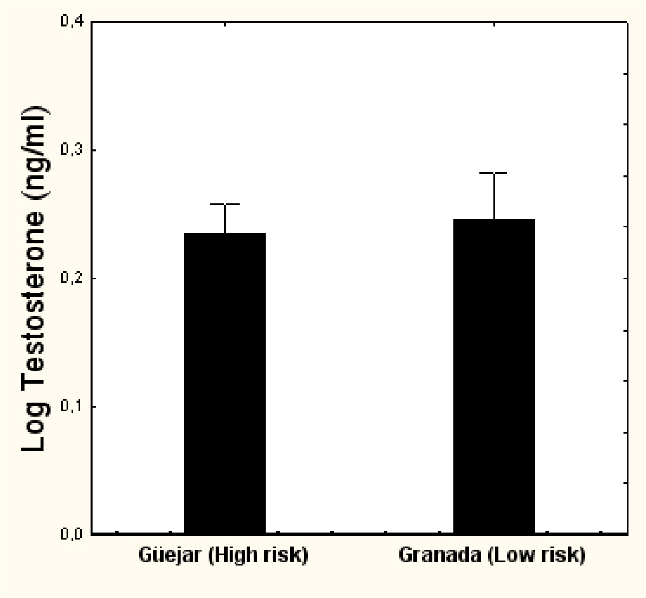

Figure 27. *Nestling testosterone levels for each population. Bars associated to each column denote the corresponding standard error.*

Discussion

The results of this study show that 8 days-old chicks modify their hormonal levels (cort and T) depending on the short and long-term nest predation risk. This suggests that not only adults but also nestlings can adaptively alter their physiology in response to the threat of a potential predator.

Short-term effect of nest predation risk on cort and T levels

Contrary to prediction 1a, the increased nest predation risk situation simulated with the magpie sounds induced a reduction in nestlings' baseline cort level and not a rise (Fig. 24). This is not surprising given the important costs in chicks' growth, immune response and cognition associated with the cort increase that would compromise future survival (e.g. Klasing et al., 1987; Saino et al., 2003; Kitaysky et al., 2003, 2006), specially if the frequency of the events that triggered this increase (i.e. listen to a potential predator) is high. Thus, it seems likely that chicks will rise their cort levels only in situations involving direct and imminent threats to their survival (i.e. a nest attack) when the benefits surpass the costs. This is supported by the nestling large increase in cort in response to handling stress (Fig. 24), which would simulate a predation event.

In contrast, the reduction in baseline cort level under the high risk treatment matches perfectly with prediction 1b, that explained this modification to occur in order to silence the chicks. Begging is known to attract predators to the nests due to the acoustic cues given by nestlings when performing this behavior (Haskell, 1994, 1999; Leech and Leonard, 1997; Dearborn, 1999; McDonald et al., 2009). Moreover, high levels of cort have been related to increased begging (Kitaysky et al., 2001, 2003; Loiseau et al., 2008; but see Vallarino et al., 2006). Therefore, the down-regulation in cort levels associated with the presence of a potential predator (i.e. magpie) near the nest could reduce the number of acoustic cues produced, thus reducing nest conspicuousness and enhancing chicks' survival.

The observed increase in T levels for the experimental treatment fit to prediction 2a indicating that T modification could also be attributed in order to reduce begging activity (Fig. 25).

This is supported by the findings of some studies that showed a substantial decrease in begging due to experimental increases in T (e.g., Groothuis and

Meeuwissen, 1992; Groothuis and Ros, 2005). This is a likely possibility despite other papers have shown the opposite pattern: increased T levels increase begging intensity (Goodship and Buchanan, 2006, 2007; Quillfeldt et al., 2006) because the action of T seems species-specific (Gil et al., 2008). The rise in T levels could partially explained the reduction in cort levels observed in blackbird nestlings too, as both steroid hormones bind with the same binding globulin (Deviche et al., 2001). This indicates the possibility that the down-regulation observed in cort is a consequence of the increase in T rather than a response to the modification of nest predation risk itself. Indeed, no negative effects of T on growth or survival have been reported (e.g., Henry and Burke, 1999; Sockman and Schwabl, 2000; Andersson et al., 2004; Tschirren et al., 2005; Rubolini et al., 2006) and its effects on immunity are variable, with some studies reporting no effect while others finding some, and the possible influence of subtile effects (e.g. Duffy et al., 2000; Roberts & Peters, 2009). This suggests that the costs for the same response would be much lower for the T than for the cort, considering the pleiotropic effects associated to the latter (see above). This cost perspective is especially important in developing organisms, like chicks, as even short-term changes in hormonal levels may alter the proper development and have important fitness consequences (Lindström, 1999).

These hormonal responses apparently support previous findings that suggest the capability of nestlings to detect the presence of a nest predator and alter their behavior accordingly. Nevertheless, the modification of the nest predation risk also induces changes in parental behavior (i.e. giving alarm calls) to minimize the possibilities of nest detection by predators (e.g., Davies et al., 2004; Madden et al., 2005a, 2005b; Platzen and Magrath, 2004, 2005). Because parents can also influence chicks' behavior in this way, it is difficult to discern whether nestlings respond directly to the predator presence or through mediation of their parents. Unfortunately we did not measure parental alarm calls to test this alternative hypothesis. However, independently of the acoustic signal used to trigger nestlings' responses, the relevant fact is that chicks adaptively modify their physiology in response to the current risk of nest predation.

Long-term effect of nest predation risk on cort and T levels

We also studied the effect of chronic (long-term) nest predation risk comparing two populations with extreme differences in nest predation pressure. We detected significant differences in baseline cort levels between nestlings from the high risk (Güejar) and low risk (Granada) location (Fig. 26) although these differences did not exist for the maximum cort or T levels (Fig. 27). These results contrast markedly with that found in our experiment in which we obtained the opposite pattern for cort and significant differences for T (Figs. 24 and 25). These unmatched results might be explained by the existence of different mechanisms controlling the response to an impending unpredictable threat and the hormonal modifications against environmental predictable changes during the developing stage as suggested by the allostatic hypothesis (Landys et al., 2006; McEwen and Wingfield, 2003). Anyway, there are two possible explanations for the populational differences in baseline cort levels observed. First, because elevated cort levels are known to stimulate locomotor activity (Angelier et al., 2007), the increased baseline cort levels in the high risk population (Güejar) may show a strategy of woodland chicks to leave the nest as soon as possible given the extreme nest predation pressure in this location. This is supported by the shorter nestling periods and faster growth of blackbird nestlings from Güejar in comparison with those from Granada (Ibáñez-Álamo and Soler, submitted a).

Second, the mediation of the parents in chicks' responses. Parental visitation rate in the lower risk population (Granada) is twice higher than in the high risk population (Güejar), which minimize clues given to predators of the nest location (Ibáñez-Álamo and Soler, submitted a). Thus, it is likely that the period between visits and hence food intake will be longer in Güejar than in Granada and taking into account several studies showing that a lower frequency of visits increase cort levels (e.g., Kitaysky et al., 1999, 2001; Nuñez de la Mora et al., 1996), this could also explain the higher baseline cort levels in the high risk population (Güejar). Alternatively, Partecke et al. (2006) proposed genetic differences to underlie hormonal differences between urban and woodland populations, however, the absence of variation in nestlings' maximum cort levels argue against this possibility and suggest the action of environmental factors instead.

To sum up, our results show that blackbird chicks adaptively adjust their hormonal patterns of cort and T depending on the short-term nest predation risk, probably in order to reduce their begging activity and to avoid being detected by predators. However, it is not clear yet whether these hormonal changes are triggered directly by predators or their parents (i.e. giving alarm calls). In addition, unmatched results between short (within-population) and long-term (between population) nest predation risk seem to point out the existence of different mechanistic pathways to cope with chronic and acute stressful situations and could have important consequences in previous assumptions about the effect of urbanization in adult physiology. These findings highlighted the importance of studying nest predation from the usually disdain chick point of view and underlined the benefits from including physiological variables in the study of predator-prey interactions.

Aknowledgements

We would like to thank D. Villanueva and J. Rodríguez for their help in the field. We are also grateful to S. Dano, C. Trouvé, A. Lacroix and specially R. Dhennin for their excellent technical assistance in hormones assays. O. Sanllorente made useful comments to previous drafts. This study was partially supported by the Spanish Ministerio de Educación y Ciencia (research project CGL2007-61940/BOS) and by a FPU predoctoral grant to J. D. Ibáñez-Álamo.

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CAPÍTULO 6

Las actividades del investigador reducen la depredación de nidos en el mirlo.

El efecto de la perturbación del investigador ha sido considerado tradicionalmente perjudicial para el éxito reproductivo de las aves por aumentar la depredación de nidos. Sin embargo, esta conclusión está basada en un grupo de especies sesgado taxonómicamente y, por tanto, el estudio de este efecto en más especies es esencial para adquirir una conclusión más firme. Además, aunque se ha sugerido que el efecto de las visitas al nido podría depender también de la comunidad de depredadores, ningún estudio ha comprobado esa hipótesis hasta el momento. Intentando detectar la posible influencia de la tasa de visitas al nido y la comunidad de depredadores en el éxito reproductivo visitamos nidos de mirlo común con dos tasas experimentalmente diferentes en dos poblaciones que diferían considerablemente en su comunidad de depredadores y tasas de depredación de nidos naturales. Contrariamente a la idea tradicional, nuestros resultados no sólo mostraron que la perturbación de los investigadores reduce significativamente la depredación de nidos, sino que esa reducción se mantiene en ambas poblaciones a pesar de las diferencias en la comunidad de depredadores de nidos. Debatimos nuestros hallazgos y sugerimos que los depredadores, especialmente mamíferos, podrían evitar los lugares altamente frecuentados por los investigadores.

CAPÍTULO 6

Investigator activities reduce nest predation in blackbirds.

Juan Diego Ibáñez-Álamo and Manuel Soler

The effect of investigator perturbance has been traditionally considered detrimental for avian nesting success in terms of enhanced nest predation. This conclusion was however based on a taxonomically biased group of species and, thus, the study of that effect in additional species was essential for reaching a more firm conclusion. Furthermore, although it has been suggested that the effect of nest visiting could also depend on nest predator community, no study has so far tested this hypothesis yet. Trying to detect possible influence of nest-visiting rates and predator community on nesting success we visited European blackbird (*Turdus merula*) nests at two different experimental rates in two populations that considerably differ in the composition of their nest predator communities and natural nest predation rates. Contrary to the traditional ideas, our results not only show that investigator disturbance significantly reduces nest predation, but also that this reduction is maintained in both populations despite the difference in the community of nest predators. We discuss these findings and suggest that predators, especially mammals, might avoid those places highly disturbed by investigators.

En prensa, *Journal of Avian Biology*

Introduction

Human perturbation is one of the most striking processes that influence avian breeding performance (Beale and Monaghan 2004). Not only because its direct effect on reproductive success by increasing nest predation (e.g. Giese 1996, Rodway et al. 1996), but also because humans' presence might indirectly influence behavioural responses (Frid and Dill 2002) or modify habitat characteristics (e.g. Marzluff et al. 2001, Sochat et al. 2006). Among different types of human direct perturbation, investigator activities seem to be the most intense (Carney and Sydeman 1999). Since many years, ornithologists has pay attention to the possible effects of research activities (Johnson 1938), but after some pioneering scientists started to design experiments to evaluate it directly in the 1960s (e.g. Harris 1964, Choate 1967, Grier 1969) the interest for this subject had even increase. Numerous papers appeared in these decades claiming mainly negative effects of research activities on avian nesting success (e.g. Anderson and Keith 1980, Cairns 1980, Pierce and Simons 1986, Boellstorff et al. 1988), and Götmark (1992) with his extensive review on the subject concluded that investigator disturbance has an overall negative effect. Nisbet (2000), however, suggested that the hypothetical negative effects of research activities on breeding success of birds are far from clear, and, we have pointed out in a detailed revision, that it could be even positive in a considerable number of cases, especially in certain avian taxa like passeriforms (Ibáñez-Álamo and Soler submitted).

Although it is difficult to establish an overall response of bird populations to research activities, it is likely that this effect depends on the taxonomic group studied (Götmark 1992, Ibáñez-Álamo and Soler submitted). Some groups have concentrated the majority of papers on the subject (e.g. pelecaniforms, anseriforms and charadriforms) whereas others have been poorly studied (e.g. falconiforms and passeriforms). The case of passerines is especially relevant as it represents almost 60% of bird species (Gill 1990) and is the focus of a huge amount of studies published in the last decades on many different subjects. However, passerine species in which the effect of investigator disturbance has been examined represent only 19.6% of the species studied (calculated from Götmark 1992) usually finding no effect. To the light of this data it seemed necessary to change the focus towards those groups less studied such as passeriforms, as recommended previously (Götmark 1992).

Another important issue that might mediated the effect of research activity on nest predation risk is the predator community that may follow or avoid researcher clues directing to nest location. Nest predators could be also disturbed by researcher (Sandvik and Barret 2001, Sutherland 2007), but different nest predators might have different sensitivity to research activities. Some papers claimed that avian predators can follow field worker clues preying those nests visited by investigators (Strang 1980, Götmark et al. 1990, but see O'Grady et al. 1996), while others stated that mammalian nest predators avoided places that are frequently visited by investigators (MacIvor et al. 1990, Armstrong 1993). However, although it is clear that the potential effects of researchers visiting nests are mediated mainly by nest predator (Götmark 1992), variation in nest predator communities between different studied populations of birds has never been explored as a factor explaining possible influence of research activity on nest predation risk.

In this paper, we present results of an investigator effect experiment using the European blackbird (*Turdus merula*) as the model species. The goals of the study were two-fold. First, we explored the relationship between nest visiting frequencies and the probability of nest predation. Secondly, we checked whether this relationship changes between habitats with different nest predator communities. To do this, we modificated our frequency of visits in two different populations that differ in their nest predator community and nest predation rate.

Material and methods

Study sites

The study was conducted in two breeding populations of European blackbirds in Granada (South of Spain). One was a rural population, settled in orange groves in the Valley of Lecrín (36º 56' N, 3º 33' O; 580 m a.s.l., hereafter referred to as Lecrín), and the other was a population settled in a *Quercus* forest close to Güejar Sierra in the Natural Park of Sierra Nevada (37º 9' N, 3º 24' O; 1050 m a.s.l., hereafter referred to as Güejar). We carried out this study from early March to early July 2007 in both populations, but only in Lecrín during 2008. Nest predator community for each population was identified previously as part of another long-term study using time-lapse infrared videocameras (unpubl. data). Güejar had a higher diversity of nest predators

(Sparrowhawk *Accipiter nissus*, European Magpie *Pica pica*, Eurasian Jay *Garrulus glandarius*, Stone Marten *Martes foina*, Common Genet *Genetta genetta*, Ladder Snake *Elaphe escalaris*, and the ant *Camponotus cruentatus*) than Lecrín (Stone Marten, Common Genet, weasel *Mustela nivalis*, and domestic cat *Felis catus*).

Experimental design

Four areas with similar size were selected for each population. The size was chosen as the appropriate to find 10-15 nests with the same search effort. Each area was randomly assigned to one of the two treatments: High or low investigator activity. Nests were visited every 3 days in high activity areas and every 14 days in low activity areas. Visits allowed us to know the fate of each nest as we registered their contents. Nests were considered as predated when they were found with biological remains (shells, feathers or blood) or empty before the chicks had the capacity to jump out of the nest (11 day-old chicks). Nests were assigned as successful when at least one chick was 11 day-old. Nests were considered as abandoned when eggs remained in the nest 5 days longer than the incubation period. Laying date was calculated when not known counting 15 days back from hatching date (based on our data on blackbird's breeding biology collected during the previous two years). For nests found after hatching, chicks were weighted and hatching date was estimated according to data on growth rate of chicks of the same population in previous years. These nests were visited when chicks were estimated to be 11 day-old to assess the fate of the nest although it meant not to follow strictly the general visit procedure described above. As we found nests in different developmental stages we tested statistically if there were differences for these stages (day found in relation to day of first egg laying) between areas and treatments to be sure that our results are not an artifact of our search method. We found no significant effect neither for areas (GLM X^2=11.30; df= 7; p=0.13) or treatments (GLM X^2=0.42; df=1; p=0.52).

In order to discern between our disturbance effect and that due to the presence of different territorial predators in the experimental areas, we gave the same treatment (high activity one) to all areas in Lecrín during 2008. If there is an effect of our visits this would disappear when all areas are visited equally. We decided not to do it in Güejar because of the greater diversity of nest predators, including many non territorial predators like corvids or serpents, that dilute this effect.

Statistical analyses

To determine the differences in nest predation between our treatments (high or low investigator activity) and between both populations, we used generalized linear models fitting nest fate as a binary dependent variable (JMP, Version 7. SAS Institute Inc., Cary, NC, 1989-2007). Treatment, population, area, and year were included as nominal independent variables. Laying date was included as a continuous independent variable. We nested the area within population and treatment for the analysis of 2007 as each area is included only in one population and contains only nests for one treatment.

To discern between our disturbance effect and that of the presence of different territorial predators in the experimental areas, we used generalized linear models fitting nest fate as a binary dependent variable. Area and year were included as nominal independent variables. Laying date was included as a continuous independent variable.

Results

We monitored 156 blackbird nests during our study in both years. Out of them only 6 (4%) nests were abandoned, 7 (4%) were destroyed by humans and 1 (0.6%) was found with chicks dead at the nest by unknown causes.

Overall nest predation in 2007 was significantly higher at Güejar (80.5%, n = 41) than at Lecrín (48.9%, n = 47; Table 3). The proportion of predated nests in both populations was comparable to that found in other years (unpubl. data). Regarding the effect of the treatment, those nests in high activity areas, which were visited on average 6.2 ± 0.3 times, were significantly less predated in both populations than those in low activity areas, visited on average 1.3 ± 0.1 times (Fig. 28, Table 3).

***Table 3**. Results of the generalized lineal model for the probability of nest predation in the year 2007.* $p<0.05$, ** $p<0.01$, *** $p<0.001$*

Variable	**df**	χ^2
Population	1	3.96*
Treatment	1	18.40***
Population x Treatment	1	4.754^{e-12}
Laying date	1	0.49
Area (Population, Treatment)	4	5.80

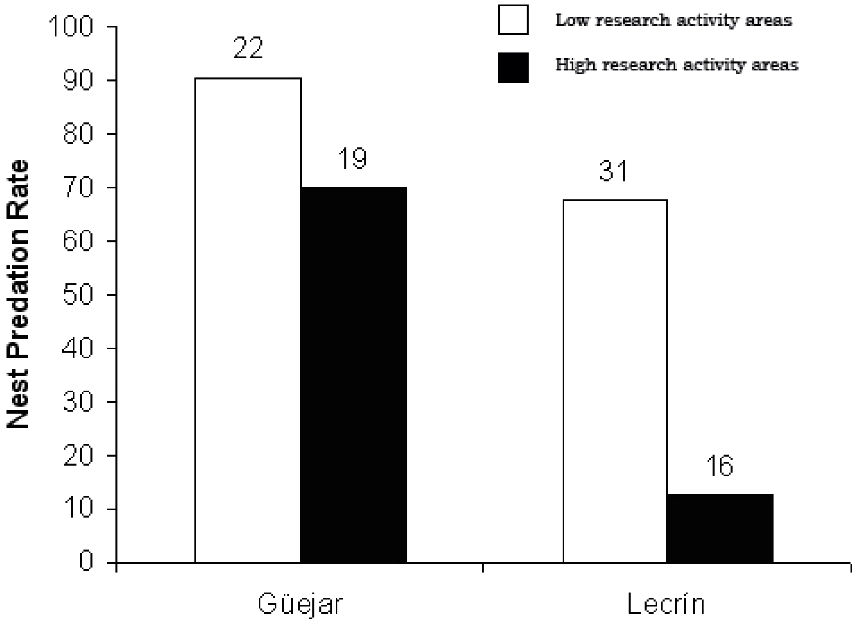

***Figure 28**. Percentage of nest predation in relation to population and treatment. Numbers at the top of the bars indicate sample size.*

In the comparison between years for the population of Lecrín, there is a significant effect in the interaction of year with area (Table 4). Figure 29 shows that those areas that were given a low research activity treatment in 2007 but a high research activity one in 2008 reduce their nest predation.

***Table 4**. Results of the generalized lineal model for the probability of nest predation in the population of Lecrín for 2007 and 2008. **p<0.01.*

Variable	**df**	χ^2
Year	1	0.44
Area	3	5.48
Year x Area	3	12.38**
Laying date	1	2.72

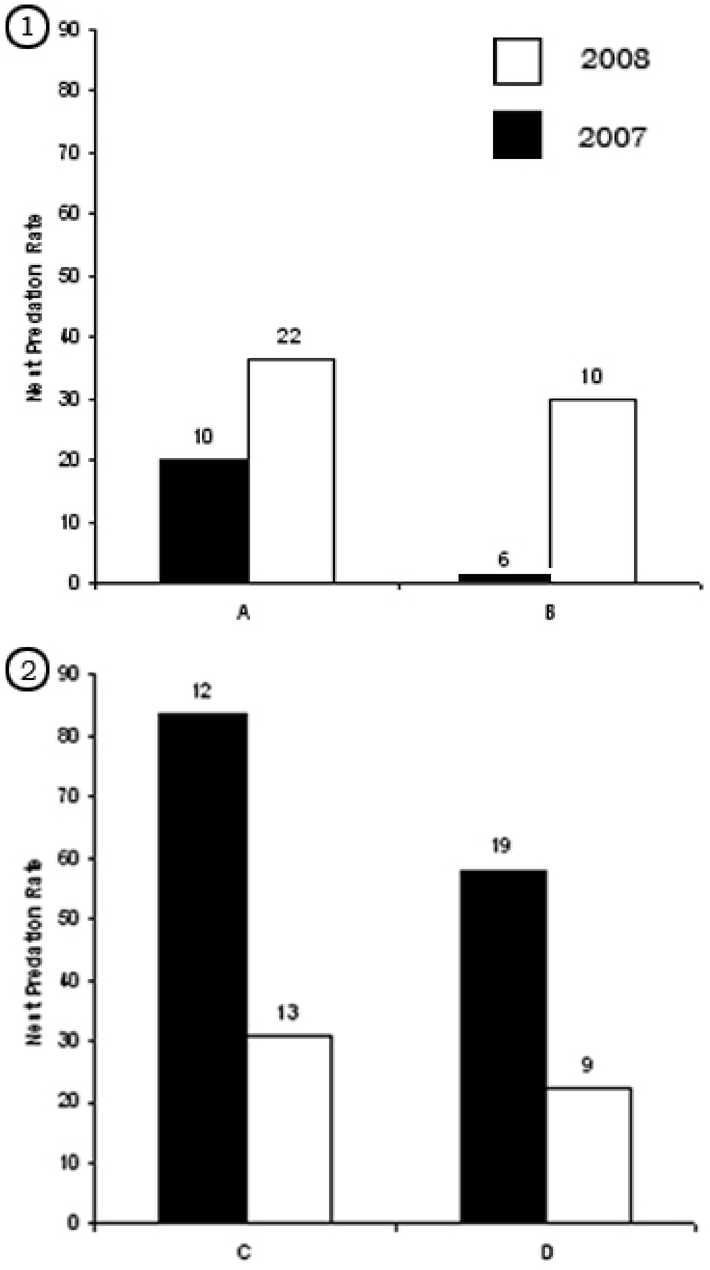

Figure 29. *Percentage of nest predation in relation to area and year at the population of Lecrín. (1) represent those areas (A and B) which were given a high research activity treatment both years. (2) represent those areas (C and D) which were given a low research activity treatment in 2007 but a high one in 2008. Numbers at the top of the bars indicate sample size.*

Discussion

Nest predation rates of nests that were visited more frequently was significantly lower than those of less frequently visited nests during 2007 (Fig. 28, Table 3). Two descriptive studies found similar results with respect to human effect (Osborne and

Osborne 1980, Tarvin and Smith 1995), one of them for the same species. When Osborne and Osborne compared the success of blackbird nests depending on the proximity to human frequented places (paths or buildings), they found that those nests closer to human paths or buildings had higher breeding success than those located further away. These results suggest that blackbirds are beneficiated by human activity which provokes a significant decrease of predation rate. This beneficial effect of human activity on blackbird reproductive performance could explain the successful colonization of urban habitats by this species (Luniak et al. 1990) and possibly is an important factor involved in the process of urbanization itself.

The fact that the beneficial effect of our visits in 2007 disappeared for the population of Lecrín in 2008, when all nests were visited frequently (Fig. 29), suggests that our visit rate could be responsible of the effect found and it is not due to the presence of territorial predators in the experimental areas.

In our two populations, mammalian nest predators (Stone marten and Common genet) are responsible of a great part of nest losses, especially in Lecrín where there are no corvids and almost all nest predations were due to mammals (unpubl. data). In both populations, mammals could avoid those places more frequently visited by scientists (MacIvor et al. 1990, Armstrong 1993) as an attempt to minimize contact with humans. Indeed, humans sistematically hunt mammals both in Güejar and Lecrín (pers. obs.). This avoidance could enhance breeding success of the blackbirds (and possibly other passerine species) given that their nest predation rate is reduced. However, the mechanism that underlies this avoidance is not clear. Possibly mammals avoid preferently those territories with fresh human odours or simply direct contact with humans. Further experiments should be necessary to discriminate between both possibilities.

Our results support the recently emphasized trend claiming that investigator disturbance is not as negative as it has been considered traditionally (Nisbet 2000, Ibáñez-Álamo and Soler submitted). Even more, we clearly show that the effect of research activities could be beneficial for this species. This assumption has important implications, especially for conservation, as many threatened species could present the same pattern. However, more passerines species should be studied (Götmark 1992) to test if this is the generalized trend for this group as suggested by Ibáñez-Álamo and Soler (submitted) or it is a single result for this species due to their particular characteristics such as extremely high nest predation rate or being prey of a wide variety of nest predators.

To our knowledge this is the first time that the effect of investigator activities has been simultaneously studied in two populations with different predation rate. Up to date, papers on the subject usually investigated only the species of one location (e.g. Armstrong 1993, Giese 1996, O'Grady et al. 1996, Sandvik and Barret 2001). Mayer-Gross et al. (1997) also tried to compare the effect of research disturbance on nest predation between populations finding contrasted results. However, some species and treatments were restricted only to some locations and differences between species were not tested. This probably explains the discrepancy in their results and avoids a clear interpretation of them.

The variation in nest predator community between Güejar and Lecrín could explain the significant differences found in nest predation rate for each population (Table 3). Usually corvids such as the European magpie or Eurasian Jay are the main responsible of the high nest predation rates recorded in blackbird populations (Groom 1993, Del Hoyo et al. 2003). Then, the absence of corvids in Lecrín may explain the lower nest predation rate found in this population. These differences in nest predators could also explain why the reduction of nest predation rate is greater in Lecrín than in Güejar (Fig. 28), as the "avoidance effect" is restricted to mammalian nest predators (Miller and Hobbs 2000).

Some papers (e.g. Westmoreland and Best 1985) stated that different nest predator communities could modify the effect of investigator disturbance. However, in this study we have found that the investigator effect is very similar in two European blackbird populations presenting different nest predator communities. This can be explained if we take into account the relative abundance of mammalian versus avian nest predators in predator communities. Those habitats in which mammals are predominant will be more prone to have positive effects than those with more abundance of birds as nest predators. This could have important implications for conservation when we consider the impact of human activities. For example in the tropics, where mammals are the dominant predators, an increase in nesting success would be more easily expected than in temperate regions, where corvids are the dominant nest predators (Skutch 1949, Ricklefs 1969).

In conclusion, the results of this study suggest that a moderately high frequency of investigator visits to European blackbird nests favour their breeding success by reducing nest predation rates. This effect could be explained by mammalian avoidance of the areas more frequented by researchers as a way to avoid human contact. This positive effect could be maintained between populations with moderately different nest predator communities.

Aknowledgements

L. de Neve, A. González-Megías, J.G. Martínez, O. Sanllorente and J.J. Soler made useful suggestions to previous drafts. L. Arco and D. Villanueva helped to collected field data. This study was partially supported by the Goverment of Spain (Project 87/2003) to MS and by a FPU predoctoral grant to JDIA.

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CAPÍTULO 7

¿Realmente los científicos incrementan la depredación de nidos en aves? Un meta-análisis.

El efecto de las actividades de los investigadores en el éxito reproductivo de las aves ha recibido importante atención puesto que los científicos llevan tiempo preocupados por los posibles efectos negativos de sus propias actividades (p.e. favoreciendo la depredación de nidos). Tradicionalmente los estudios empíricos parecen apoyar la hipótesis de que la perturbación del investigador tiene un efecto negativo en el éxito reproductivo en general, incrementando la tasa de depredación de las especies estudiadas. Sin embargo, hasta la fecha nadie ha investigado las evidencias generales de tal relación. En este estudio hemos realizado un meta-análisis de los resultados publicados para investigar si las actividades de los investigadores incrementan o no la depredación de nidos de las aves estudiadas. El análisis se ha basado en 20 estudios experimentales incluyendo 26 especies diferentes. Nuestros resultados muestran la ausencia de un efecto conjunto significativo, reflejando que las actividades investigadoras no afectan a la depredación de nidos a nivel general, lo que contradice la idea aceptada tradicionalmente. Más aun, esta relación es heterogénea entre órdenes de aves, y sorprendentemente, es positiva y significativa para los Paseriformes. La posible explicación para esas diferencias entre órdenes podría ser debida a restricciones filogenéticas que implicarían diferentes comunidades de depredadores de nidos. Esta nueva perspectiva sobre el efecto de los investigadores podría tener importantes implicaciones en varios campos como la Ecología y la Evolución, donde los Paseriformes son ampliamente usados para comprobar hipótesis generales, así como en otros campos como la conservación y el manejo de espacios naturales. También detectamos una tendencia significativa en el tiempo mostrando que los artículos recientes sobre el tema encuentran más fácilmente que las actividades investigadoras incrementan el éxito reproductor de las aves. Esta tendencia podría ser explicada principalmente por el cambio en metodologías a través de los años y la falta de un marco teórico para apoyar los efectos humanos positivos.

CAPÍTULO 7

Do scientists really increase avian nest predation? A meta-analysis.

Juan Diego Ibáñez-Álamo and Manuel Soler

The effect of researcher activities on avian nesting success has received substantial attention, as researchers have long been worried about the possible negative effects of their own activities (i.e. favouring nest predation). Traditionally empirical studies seem to support the hypothesis that investigator disturbance has an overall negative effect on breeding success by increasing predation rate of the species studied. However, up to date no one has investigated the overall empirical evidence for such a relationship. We performed a meta-analysis of published results to investigate whether researcher activities increase or not nest predation of birds studied. The analysis was based on 20 experimental studies involving 26 different species. Our results show the lack of an overall significant effect, reflecting that researcher activities in general did not affect nest predation, contrary to the traditional idea. Even more, this relationship is heterogeneous among avian orders, and surprisingly, significantly positive for passerines. The possible explanation for these differences among orders could be intrinsically due to phylogenetical restrictions that would involve different nest predator communities. This new perspective on the effect of investigators could have important implications in several fields such as Behaviour, Ecology and Evolution, where passerines are widely used to test general hypothesis, as well as in other fields like management and conservation. We also detected a significant trend in time showing that recent papers on this subject found more easily that research activities increase avian breeding success. This trend could be explained mainly by the change in methodologies through the years and the lack of a theoretical background to support positive human effects.

Manuscrito

Introduction

Researchers have always been worried about the effects of their own activities and how they could influence their findings. This subject has received especial attention in some fields of science like Physics. The best example of its relevance is probably Heisenberg's uncertainty principle (Heisenberg 1927). However, this problem can be also applied to Biology. Lenington (1979) summarized its implications with her "uncertainty principle" in ornithology, which states that the behavior of birds under study could not reflect their natural behavior as we can alter it simply by our presence in the field. This is a really important issue as birds in general, and especially passerines, are the model group most used to test general hypothesis involving extrinsic mortality (i.e. nest predation) in several fields such as Ecology and Evolution. If we, as investigators, modify nest predation in a way or another, our findings in these fields should be taken with caution, thus imposing great restrictions to its interpretations. This is a methodological problem long known by ornithologists (Johnson 1938). However, it was not until 40 years ago that scientists started to design experiments to evaluate this problem directly (e.g. Harris 1964; Choate 1967; Grier 1969). Interest in the subject has increased since then and many papers subsequently have reported a negative effect of research activities on avian nesting success (e.g. Anderson and Keith 1980; Cairns 1980; Pierce and Simons 1986; Boellstorff et al. 1988). This adds a clear ethical problem to the methodological one, especially relevant in studies of endangered or vulnerable species.

It was Götmark (1992) with his extensive review on the subject who finally established that investigator disturbance had a general negative effect on avian breeding success by increasing nest predation rate of the investigated species. Many studies on the subject have been published since then, most of them reporting no effect (e.g. Schaub et al. 1992; Cotter and Gratto 1995; Lloyd et al. 2000; Bêty and Gauthier 2001; Verboven et al. 2001) or, surprisingly, even a positive effect in some cases (e.g. Mayer-Gross et al. 1997; Ortega et al. 1997; Weidinger 2008; Ibáñez-Alamo and Soler in press). However, their results seemed to go unnoticed as the scientific collective still promotes the traditional idea that research perturbation has a negative effect on the reproductive success in birds. Moreover, recent articles show a clear reluctance to consider positive effects of investigator disturbance despite their results (e.g.

Domínguez and Vidal 2003) or simply mentioning positive effects papers in the same way as no effect ones (e.g. Schiegg et al. 2007).

Although previous narrative reviews highlight the negative impact that research perturbance has on breeding success (Götmark 1992; Carney and Sydeman 1999), up to date no one has investigated the overall empirical evidence for such a relationship. Therefore, to the light of recent works that stress positive effects of research perturbance and the considerable number of articles that found no effects, a quantitative research synthesis is needed.

In this article, we first update the findings about effects of research activities based on meta-analytic procedures to evaluate the overall evidence in favour of a negative association between investigator perturbance and nest predation. We also analyse if there is variability among avian orders for this research impact. Then we test the effect of the different methodologies used to study this subject as well as whether there is a time-trend for the influence of researchers on avian breeding success. After that, we discuss the implications of our findings, and finally we give some conclusions and recommendations for future research on the subject.

Methods

Literature search methods

We first checked all articles included in Götmark's review (1992) assuming that this review had included all the usable studies published prior to 1992. We then search the Web of Science database for all articles related to the subject using "research*", "investigat*", "perturbance", "disturbance" and "nest predation" as key words. Literature cited in relevant papers was also examined. In this review we have considered only experimental studies and not those referring to anecdotal data or reporting the effect of research activities without a well-designed experiment (e.g., not asigning experimental and control nests randomly). We have based this review on papers focused on nest predation, which has been determined as the main cause of reduction of nesting success due to investigator disturbance (Götmark 1992). Only those studies involving natural nests were included in the review, as it has been demonstrated that artificial nests can result in misleading findings in respect of nest predation (Weidinger 2001; Zanette 2002; Moore and Robinson 2004).

Statistical analyses

In order to test for the effect of investigator perturbance in nest predation rate, we conducted a meta-analysis using MetaWin 2.0 (Rosenberg et al. 2000). The following data were extracted from each study found according to the search methods: (1) author and year of publication, (2) study species, (3) number of depredated and successful nests under the high perturbation treatment, (4) number of depredated and successful nests under the low perturbation treatment, and (5) method of study. We chose a two x two contingency tables meta-analysis as data collected from each article could be presented as such a table. We used rate difference (RD) as association measure and a random effects model to calculate the effect sizes. Random effects models in meta-analysis are more realistics than fixed effects models since they use both within and between studies variability (Hedges and Vevea 1998). We also explored the effect of avian order using a categorical meta-analytical model because each order has particular features that could modificate the influence of investigator effects (i.e. passeriforms are mainly territorial and nest in trees while charadriforms are principally colonial and ground nesters). Equally, and using a categorical meta-analysis too, we tested for differences in research disturbance due to the methodological approaches of each study. We also analysed if there was a temporal trend with a continuous model using year of publication as the methods to study avian biology have improved/changed over the years (i.e. use of GPS devices to locate nests instead of other more conspicuous nest markers). Effect size was considered significant if its confidence interval did not include zero (Rosenberg et al. 2000).

Results

In total, 20 studies involving 26 different bird species were considered fitting the search criterion and included in the analyses (Table 5). In general, investigator activities do not significantly affect nest predation of birds (mean effect sizes = -0.0355, 95% CI -0.0928 to 0.0217; Fig. 30). However, when analyzed by avian order (Fig. 30), *Passeriformes* showed a significant effect of researcher perturbance (effect size = -0.1048, 95% CI -0.1844 to -0.0251). The negative value of the effect size indicates that there were less predated nests in the treatment group (high investigator disturbance) than in the control (low investigator disturbance), therefore suggesting a positive effect of researchers in breeding success.

None of the other orders showed significant effects (Fig. 30). There was a significant total heterogeneity among effect sizes of studies (Q_T = 56.4, df = 39, p=0.04). This total heterogeneity can be partitioned into heterogeneity between (Q_B = 11.1, df = 4, p=0.03) and within avian orders (Q_W = 45.2, df = 35, p=0.16). The fact that the first was significant but not the second indicates that the heterogeneity in effect sizes detected is due to the differences among avian orders while the data within them is consistent.

***Table 5**. Information on avian order, sample size and effect size (calculated as Rate Difference) for each species considered for the meta-analysis. A negative effect size value indicates that nest predation is lower when research activities are higher (noted that this is refered as a positive effect within the text).*

Species	Avian order	Sample size	Effect size (RD)	Sources
Aphelocoma coerulescens	*Passeriformes*	112	-0,0223	Schaub et al. (1992)
Calcarius ornatus	*Passeriformes*	52	-0,1993	O'Grady et al. (1996)
Calidris alpina	*Charadriformes*	262	-0,0916	Thorup et al. (1995)
Carduelis cannabina	*Passeriformes*	72	0,0913	Mayer-Gross et al. (1997)
Carduelis chloris	*Passeriformes*	94	0,1083	Mayer-Gross et al. (1997)
Charadrius alexandrinus	*Charadriformes*	60	-0,1333	Domínguez and Vidal (2003)
Charadrius melodus	*Charadriformes*	37	-0,4545	MacIvor et al. (1990)
Chatarus guttatus	*Passeriformes*	54	-0,1106	Martin and Roper (1988)
Chen caerulescens atlantica	*Anseriformes*	713	0,0214	Bêty and Gauthier (2001)
Eremopterix sp.	*Passeriformes*	228	-0,0375	Lloyd et al. (2000)
Fratercula arctica	*Charadriformes*	597	0,1611	Rodway et al. (1996)
Fringilia coelebs	*Passeriformes*	61	-0,1887	Mayer-Gross et al. (1997)
Lagopus mutus	*Galliformes*	48	-0,0167	Cotter and Gratto (1995)
Larus delawarensis	*Charadriformes*	229	0,0123	Brown & Morris (1994)
Prunella modularis	*Passeriformes*	142	-0,2231	Mayer-Gross et al. (1997)
Pygoscelis adeliae	*Esfenisciformes*	290	0,1197	Giese (1996)
Pyrrhula pyrrhula	*Passeriformes*	40	0,3712	Mayer-Gross et al. (1997)
Somateria mollisima	*Anseriformes*	744	0,0507	Choate (1967) Götmark and Åhlund (1984)
Sylvia sp.	*Passeriformes*	53	0,0935	Mayer-Gross et al. (1997)
Troglodytes troglodytes	*Passeriformes*	82	0,0171	Mayer-Gross et al. (1997)
Turdus merula	*Passeriformes*	490	-0,1462	Ibáñez-Álamo and Soler (in press) Mayer-Gross et al. (1997)
Turdus migratorius	*Passeriformes*	244	-0,3566	Ortega et al. (1997)
Turdus philomelos	*Passeriformes*	376	-0,0800	Mayer-Gross et al. (1997)
Vanellus vanellus	*Charadriformes*	321	0,0199	Galbraith (1987)
Zenaida macroura	*Columbiformes*	344	0,0743	Nichols et al. (1984) Westmoreland and Best (1985)

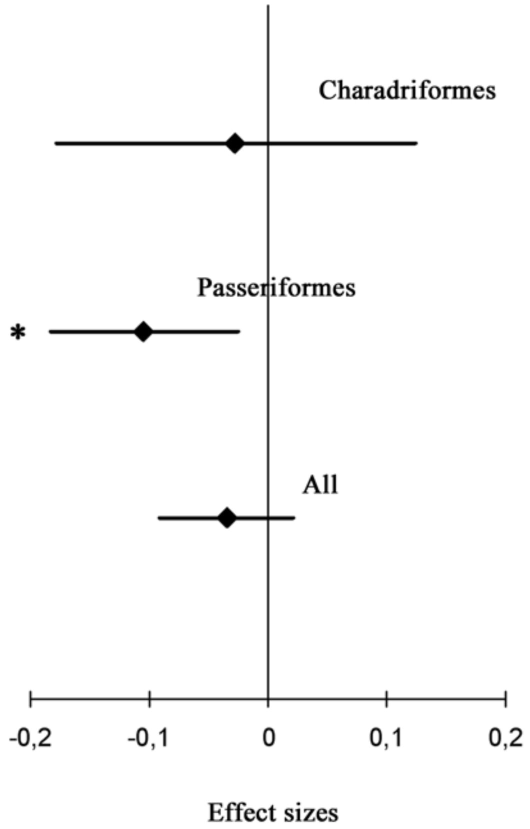

Figure 30. *Forest plot showing mean effect sizes (± 95% CI) for each avian order and the sum of all orders. Those orders counting less than two species has not been represented. * corresponds with a p<0.02. A negative effect size value indicates that nest predation is lower when research activities are higher. (Done with MS Excel and Photoshop).*

We found no significant differences between the two main methodologies used to test for research activities on avian nest predation: frequency of visits and with/without visits (Fig.31).

The results for the continuous meta-analytical model involving year of publication showed that there was a significant temporal trend in effect sizes (b_1 = -0.008, SE = 0.004, p=0,029). The negative value of the slope indicates that negative effect sizes (reflecting positive effects of investigator disturbance on breeding success) were more common in recent years.

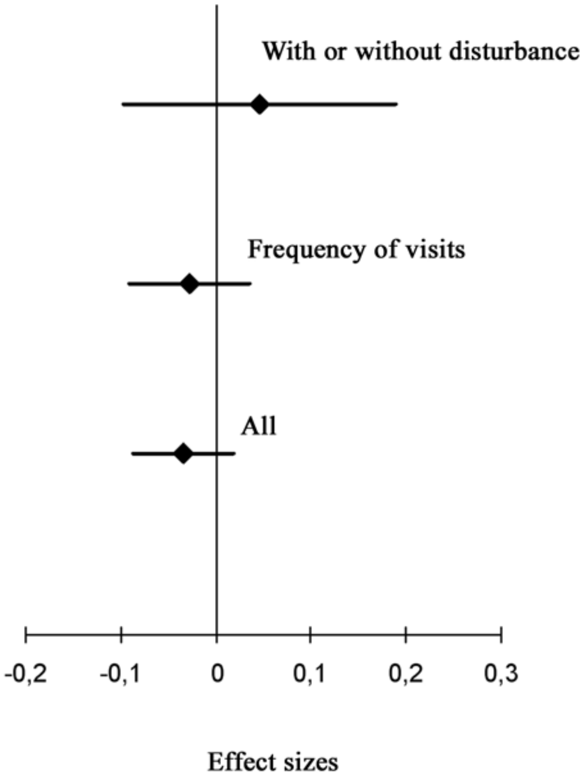

Figure 31. *Forest plot showing mean effect sizes (± 95% CI) for each methodology utilized to study investigator perturbance on avian nest predation and the sum of all methods. Different manipulation is not represented as there is only one value for this methodology included in the meta-analysis. A negative effect size value indicates that nest predation is lower when research activities are higher. (Done with MS Excel and Photoshop).*

Discussion

Traditionally, research activities have been considered to have an overall negative effect on avian nesting success, as claimed by the last major review on the subject (Götmark 1992). This effect was attributed mainly to nest predation. Several causes have been suggested to be responsible for the increase of nest predation rates in studied nests. The most important are: (a) predators following field workers (Strang 1980), (b) easy detection of nests due to objects such as flags (Picozzi 1975; Reynolds 1985), and (3) predators simply taking advantage of the absence of the parents at the nest (Westmoreland and Best 1985). However, contrary to the general opinion, our results clearly show that there is not a general effect of investigator disturbance on nest predation of birds studied (Fig. 30). Then, how is it possible that for almost 40 years scientists have had the idea that there is a negative impact of their activities on avian breeding success? This could be explained because negative effects were more frequently found in the first studies analyzing the impact of researcher perturbance (see results). These initial works, therefore, could have influenced the interpretations of the following articles on the subject. Three factors could have helped to finally establish this mistaken idea into the scientific community. First, the fact that many previous articles with anecdotal or not experimentally data found negative effects (e.g. Kadlec and Drury 1968; Ollason and Dunnet 1980). Second, that some initial papers found significant negative effects (e.g. Choate 1967) and others published later in time found mainly no significant results although with positive trends (e.g. Schaub et al. 1992; Cotter and Gratto 1995; O'Grady et al. 1996; Lloyd et al. 2000). Third, a conservational fervor mediated by an anthropocentric point of view stressing the negative influence of human activities in natural processes.

The methods used to test investigator effects did not influence the results as there were not significant differences between them (Fig. 31). Although we have differenciated between two main methodologies (frequency of visits and with/without disturbance), in fact both could be considered the same, frequency of visits. Papers comparing the effect with and without nest disturbance are not but the comparison of an extreme regime of visits. These findings give strong support to our results as they do not depend on the methodological approach used, a consideration that could also be useful for wildlife managers. However, more subtle methodological changes developed through the years could partially explain the significant temporal trend found. Field methods for the study of birds have been greatly improved over the last 25 years. In many cases intrusive methods used by investigators had significant impacts on reproductive performance of birds (e.g. Tremblay and Ellison 1979; Boellstorff et al. 1988; Rodway et al. 1996). Nest monitoring techniques have been demonstrated to influence levels of perturbation on birds (Fetterolf 1983; Brown and Morris 1995). Therefore, many papers have given methodological recommendations to minimize these perturbations (Götmark 1992; Armstrong 1993; Rodway et al. 1996; Mayer-Gross et al. 1997; reviewed for colonial waterbirds in Carney and Sydeman 1999; Nisbet 2000). Due to these recommendations, recent papers include a more cautious methodological approach reducing the impact of scientists on breeding success. A good example of this improvement is the use of nest markers. Until recently, the use of physical nest markers (e.g. flags) in the field was widespread despite that predators seemed to use these makers as a clue to locate and predate nests (Picozzi 1975; Reynolds 1985; but see Hannon et al. 1993). New technological advances such as GPS led to a progressive substitution of physical nest markers for other virtual ones with no or little impact on birds.

Other possible explanation for this temporal change in disturbance findings could be the lack of a theoretical background to explain positive results. Until recently, the possibility that predators can be more sensitive to disturbance than the species under study has been largely ignored (Sutherland 2007). This becomes even more relevant because birds as predators could increase nest predation after scientific manipulations, while mammals or reptiles do not have this behaviour (Götmark 1992; Skagen et al. 1999; Miller and Hobbs 2000; Keedwell and Sanders 2002), indicating differential sensitivity to investigator perturbation. These so far unconsidered facts reopen the interpretation of results as the focus species is not the only one that could be negativelly affected by the presence of scientists. Thus, in this theorethical framework a positive effect of researcher impact could be easily explained. This point of view facilitates the appearance of works in which the effect on the species studied is positive, mediated by the higher sensitivity to disturbance of their main predators (Sandvik and Barret 2001; Weidinger 2008; Ibáñez-Alamo and Soler in press).

Götmark's (1992) review not only underlined an overall negative effect of investigators on breeding success, but also highlighted differences among avian orders. According to this, most papers on *Charadriformes* reported a negative effect on nesting success, while only few did so for *Passeriformes*. In contrast to the findings of Götmark's review, our results show that *Charadriformes* do not have a significant increase of nest predation due to research perturbation. Moreover, *Passeriformes* are not only less negatively affected, but even beneficiated by the investigator's presence (Fig. 30). As we have explained above, these results could be due to the different sensitivity to research activities of predators. It has been suggested that *Charadriformes* are usually predated by other birds (e.g. Burger 1981) which are the least affected by investigator perturbance (see references above). However, paserines have a broader community of nest predators, and a partial reduction in predation (i.e. due to mammals) could be enough to increase the breeding success of species of this order (e.g. Ibáñez-Álamo & Soler in press). These results concerning avian order variability, and especially the significant positive effect for *Passeriformes*, has important implications in research. Paserines have been greatly utilized to test general hypothesis in several fields such as Behaviour, Ecology or Evolution (i.e.

life-history theory; e.g. Martin 2004). Therefore, their conclusions should be taken cautiously given the possibility that they do not reflect the natural situation. The fact that this effect is positive suggests that significant results involving nest predation would be more difficult to detect as nest predation pressure is relaxed when studied, thus, reducing this concern and supporting previous findings. However, investigators should be aware that the predator community in their study sites could modulate, even impede, to obtain significant results.

Our findings have conservation and wildlife management implications as well, especially in groups such as *Charadriformes*. Researchers investigating this order have always been worried about their possible negative effects (reviewed in Götmark 1992 and Carney and Sydeman 1999). Because investigator procedures are considered the most intense of human disturbance effects (Carney and Sydeman 1999), scientists have given many recommendations to manage areas where these species live, usually avian sanctuaries (reviewed in Carney and Sydeman 1999). Nevertherless, based on empirical evidence, investigator disturbance seems not to have an overall negative impact on breeding success for this taxon (Fig. 30). Thus, managers of such sites might reconsider the prohibition of research activities in protected areas. This consideration, however, should be taken with caution and local studies are recommended in each case since the effect of human perturbation could vary among different populations of the same species (Fetterolf 1983; Armstrong 1993; Brown and Morris 1995; Bêty and Gauthier 2001).

A related issue to investigator disturbance is the process of urbanisation. The particular characteristics of urban areas (e.g. low predator pressures) may facilitate the colonization by certain avian groups (Shochat et al. 2006). Proximity to humans could be problematic for species with low thresholds for disturbance (Møller 2008), but beneficial for those less affected by perturbation than their own predators (Noske 1998). Investigator disturbance is the most intense of the three main categories of human disturbance, the other two being ecotourism and recreation (Carney and Sydeman 1999). Therefore, it is conservative to consider avian responses to research activities as a reflection of the effect of human perturbation in general. According to this scenario, *Passeriformes* seem more prone to become adapted in the urbanisation process as they are the group that benefits the most from the effect of scientists. This is exactly what we find when we consider the most abundant taxa in urban and suburban areas (Fernández-Juricic 2000; van Heezik et al. 2008).

Conclusions and future directions

It seems clear that investigator disturbance does not necessarily involve a negative effect on breeding success, mediated by nest predation, as considered traditionally. Moreover, research activities could be positive in certain avian taxa, like *Passeriformes*. Thus, to be sure of the validity of their findings, we suggest investigators to carry out studies on the effect of their own activities in every site used to test hypothesis involving nest predation.

There is a large body of literature on the effect of research activities on avian reproductive performance. However, information on certain avian taxa (i.e. columbiforms) and on cavity nesters is scarce, and completely non-existent for nest box populations, although they are widely used in scientific studies. Thus, studies on the impact of investigator disturbance on breeding biology in these taxa and especially in cavity nesting species would be of special interest. In addition, there seems to be a large inter-annual variation in the response of study species on disturbance (Armstrong 1993; Sandvik and Barret 2001), but only a few studies address this issue. Finally, the effect of research activities on nest predator species has been overlooked, despite that nest predators seem to be more sensitive to investigator disturbance than the studied species. Thus, it will be crucial for future studies to investigate the interaction among scientific perturbance, nesting success and nest predator deterrence.

Acknowledgements

M. Griesser, O. Sanllorente and specially A. P. Møller made useful suggestions to previous drafts. This study was partially supported by the Spanish Ministry of Environment (Project 87/2003) to M. Soler and by a FPU predoctoral grant to J.D. Ibáñez-Álamo.

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DISCUSIÓN GENERAL INTEGRADORA

La presente tesis ha abordado el estudio de la depredación de nidos desde el punto de vista de los adultos y los pollos, manteniendo un enfoque evolutivo y multidisciplinar.

Ya que es imprescindible tener unas nociones básicas de la biología reproductora de la especie objeto de estudio para poder profundizar en otros aspectos como la depredación de nidos, uno de los objetivos del **capítulo 1** ha sido precisamente recabar dicha información en nuestras poblaciones de estudio. Los resultados de este trabajo nos muestran la existencia de diferencias significativas entre las poblaciones investigadas para algunos parámetros reproductivos (p.e. tamaño de puesta, actividad en el nido o tiempo de estancia de los pollos en el nido), especialmente entre la población de Granada y Güejar-Sierra. Esta variación podría ser explicada por la diferente importancia que tienen las presiones selectivas (limitación por alimento o depredación de nidos) en cada población y la mayor adaptación de sus individuos a las mismas. Debido a que las tres poblaciones estudiadas difieren en influencia humana (zona urbana, rural y forestal), la distinta importancia hallada con respecto a las presiones selectivas y algunos caracteres reproductivos indicarían el posible efecto de la urbanización en las modificaciones observadas. De esta forma, los resultados de este capítulo sugieren que las zonas urbanas presentan una mayor limitación de alimento que la situación originaria de la especie (hábitat boscoso), pero también, una reducción de la presión por depredación de nidos apoyando otros estudios previos (Gering y Blair 1999; Shawkey et al. 2004; Chamberlain et al. 2009). Esto indicaría, además, la capacidad que tienen algunas especies de aves de modificar sus estrategias vitales según la presión selectiva dominante en la población.

Otro resultado importante del capítulo, en este caso desde el punto de vista metodológico, consiste en el hecho de que el estudio de rasgos de estrategias vitales considerados independientemente puede llevar a equívoco aunque sean partícipes de gran parte de la variación en la inversión en reproducción (p.e. tamaño de puesta). Se apoyaría, por el contrario, el uso de síndromes de estrategias vitales (grupos de rasgos) en este tipo de estudios, algo defendido previamente por otros artículos (p.e. Martin 2004). Consecuentemente recomendamos el uso de estos síndromes en todas aquellas investigaciones centradas en las estrategias vitales, algo aún poco extendido.

Nuestros datos también podrían explicar la aparente incongruencia de resultados cuando se intentan determinar patrones generales sobre urbanización (p.e. Chamberlain et al. 2009). En el **capítulo 1** hemos hallado muy poca variación en los parámetros reproductivos de la población rural con respecto a la urbana (sólo para la tasa de crecimiento), mientras que ésta variación ha sido bastante importante entre la población forestal y urbana (p.e. tamaño de puesta, actividad del nido, tasa de crecimiento…). Esto sugiere que no se deben hacer comparaciones usando meramente la distinción entre hábitats urbanos y no urbanos (zonas forestales y rurales), sino que la diferenciación debe ser más fina evitando mezclar estos dos tipos de hábitats.

Los **capítulos 2** y **3** han ido encaminados al estudio de las respuestas utilizadas por los adultos ante un riesgo alto de depredación del nido, prestando especial atención a la hipótesis de Skutch (Skutch 1949). Se ha procurado obtener una visión lo más completa posible de estas respuestas antidepredatorias con respecto al periodo de dependencia de las crías (estancia en el nido), investigándolas tanto durante la fase de incubación (**capítulo 2**) como durante la fase de pollos (**capítulo 3**). Así pues, el **capítulo 2** confirma de manera directa que las hembras también pueden seguir una estrategia de reducción del número de visitas con objeto de dar menos pistas a los potenciales depredadores presentes en las proximidades del nido. Por tanto, se puede decir que las hembras de mirlo común, y posiblemente de otras especies de aves, cumplen la hipótesis de Skutch, de igual manera que ocurre con los machos como ya se había demostrado previamente (p.e. Ghalambor and Martin 2000, 2002). Sin embargo, no se ha podido descartar que las hembras de mirlo común puedan utilizar otras estrategias con objeto de aumentar la ocultación del nido como sugieren otros trabajos (p.e. Montgomerie y Weatherhead 1988; Weathers y Sullivan 1989).

Continuando con el estudio de las diferencias sexuales en cuanto a las respuestas antidepredatorias de las aves, el **capítulo 3** explora simultáneamente las estrategias de ambos sexos durante la fase de pollos. Como indican los resultados del capítulo previo, machos y hembras responden de manera similar ante el riesgo de depredación del nido, aunque no para todos los rasgos estudiados (p.e. duración media de las visitas). Los resultados de los **capítulos 2** y **3** sugieren también la influencia del riesgo de depredación ambiental específico de la población en la modificación de los comportamientos antidepredatorios inducidos por los depredadores. De esta manera, el comportamiento de los adultos de mirlo se ve modificado según el riesgo del hábitat sea alto o bajo en ambos periodos de desarrollo. Así pues, en la fase de incubación este factor parece regir la estrategia seguida (reducción de visitas o aumento de la ocultación). Por otro lado, el riesgo de depredación ambiental también parece ser responsable de que se reduzca o no la tasa de visitas en presencia de un depredador durante la fase de estancia de los pollos en el nido, afectando a ambos sexos de manera similar. Estos resultados sugieren la existencia de un comportamiento modificado a dos niveles (riesgo inmediato y riesgo a largo plazo) de acuerdo con lo que ocurre en el arrendajo funesto (*Perisoneus infaustus*) durante la fase de pollos (Eggers et al. 2005).

También en el **capítulo 3** se han investigado los costes asociados a la reducción de actividad de los padres. En este sentido se ha observado que esta respuesta antidepredatoria no tiene porqué conllevar un coste directo en alimentación para los pollos. Este resultado es importante, ya que sugiere un replanteamiento de la idea tradicional, basada principalmente en pruebas indirectas, como la reducción del tamaño de puesta, que apoyan la existencia de este coste (p.e. Eggers et al. 2005, 2006; Zanette et al. 2006; Olsen et al. 2008).

Pero, como se ha comentado en la introducción, los depredadores no sólo usan las pistas de los adultos para localizar el nido, también puede utilizar directamente la actividad de los pollos con ese fin. La petición de alimento es la principal actividad que realizan los pollos durante su estancia en el nido, por lo que en el **capítulo 4,** hemos intentado confirmar la existencia del coste en depredación del nido asociado a dicho comportamiento de petición de alimento. La utilización de una nueva metodología nos ha permitido evitar los problemas metodológicos previos existentes en este tipo de trabajos, por los que los resultados a favor de la existencia de este coste habían sido criticados (Haskell 2001). En consonancia con estos estudios, nuestros datos apoyan la existencia de este coste en depredación de nidos. Esto implica que esta fuerza selectiva impone importantes restricciones a la evolución de la petición, como predicen algunos modelos teóricos sobre el tema (revisado en Wright and Leonard 2001; Moreno-Rueda 2007). Sería, por tanto, lógico pensar que el comportamiento de los pollos, igual que con respecto a los padres, se va a ver modificado por el riesgo de depredación, lo que apoyan varios estudios con evidencias directas e indirectas (p.e. Davies et al. 2004, Platzen y Magrath 2004, 2005, Magrath et al. 2007).

El **capítulo 5** ha abordado el estudio de las modificaciones comportamentales de los pollos desde un punto de vista fisiológico, en concreto investigando las alteraciones hormonales asociadas al riesgo de depredación. Nuestros resultados confirman la existencia de modificaciones hormonales a consecuencia de la presencia de un potencial depredador de nidos, contrastando con otros estudios que muestran que el riesgo de depredación del nido no parece modificar los niveles hormonales de los adultos (Butler et al. 2009). Igualmente los resultados de este capítulo indican la existencia de diferentes mecanismos para responder ante el riesgo de depredación a corto y largo plazo. Con respecto al primero parece que las modificaciones en corticosterona (reducción) y testosterona (aumento) van encaminadas a disminuir la actividad de petición de los pollos. Sin embargo, en relación al segundo, la testosterona parece no verse alterada, mientras que el aumento de corticosterona encontrado en la población con mayor riesgo de depredación podría explicarse (i) bien como una estrategia por parte de los pollos para crecer más rápidamente en aquella población con mayor riesgo de que el nido sea depredado, (ii) bien como la respuesta de los pollos a la diferente actividad de los padres en cada población como consecuencia de las diferencias en presión por depredación. Ambas explicaciones son apoyadas por los resultados del **capítulo 1**.

Los dos últimos capítulos han explorado la influencia de la actividad investigadora en el éxito reproductor de las aves, desde una perspectiva local (**capítulo 6**) y general (**capítulo 7**). Así pues, los resultados del **capítulo 6**, sorprendentemente, nos muestran una reducción de la tasa de depredación de nidos ante la presencia de los investigadores en nuestras zonas de estudio, independientemente de las diferencias entre las comunidades de depredadores. Esto no quita importancia a los resultados del resto de capítulos en general, ya que implica que estos son conservativos, al reducir y no aumentar la depredación por nuestra actividad. Aunque sí que habría que tomar con cierta precaución las conclusiones basadas en las diferencias en la presión de depredación entre poblaciones, ya que la composición de la comunidad de depredadores (aves o mamíferos) de cada zona puede afectar de manera diferencial a la magnitud de esa reducción de la depredación de nidos. Este hecho unido a otros estudios que muestran una influencia negativa de la actividad investigadora (p.e. Anderson y Keith 1980; Cairns 1980; Pierce y Simons 1986; Boellstorff et al. 1988) sugiere que este tipo de estudios se deberían extender a todos los sistemas utilizados para llevar a cabo investigaciones sobre la depredación de nidos con objeto de ver la posible influencia del observador en sus propias conclusiones. Enlazando con los resultados del **capítulo 7** que explora la generalidad de estas influencias en las aves por medio de la metodología de meta-análisis, y por tanto, de manera cuantitativa a diferencia de revisiones previas (Götmark 1992), no se ha hallado ningún efecto significativo a nivel general. Sin embargo, ese efecto es positivo cuando nos restringimos a los Paseriformes, apoyando los resultados obtenidos en el **capítulo 6**. También hemos detectado una tendencia temporal que muestra que los estudios con efectos positivos de los científicos en las aves son más frecuentes cuanto más recientes sean. Esto parece ser explicado por un cambio en el uso de las metodologías de campo con los años (p.e. sustitución de marcadores físicos por virtuales) y la aparición de un marco conceptual que sugiere que los depredadores pueden ser también afectados por las actividades científicas (Sutherland 2007). Las conclusiones de estos dos últimos capítulos, por tanto, apoyan la reciente tendencia de que las actividades de los investigadores no son tan perniciosas como se ha creído tradicionalmente (Nisbet 2000). Es más, ésta puede ser beneficiosa para ciertas aves como los Paseriformes, lo que tiene importantes implicaciones en Biología de la Conservación.

CONCLUSIONES

1. La limitación por alimento parece ser la presión selectiva más importante para la población de mirlos de la ciudad de Granada (situación altamente influida por humanos), mientras que la depredación de nidos lo es para la del bosque de Güejar-Sierra (situación natural), siendo ambas presiones intermedias en el caso de los naranjales de Melegís (situación seminatural). Estos resultados sugieren el posible efecto de la urbanización en el cambio de las presiones selectivas de las aves.

2. Los mirlos comunes han adaptado sus estrategias vitales a la presión selectiva dominante en cada población, sugiriendo que algunas especies de aves pueden responder adaptativamente a la alteración humana del ambiente.

3. Los mirlos comunes muestran un incremento de su eficacia biológica con el aumento de la influencia humana, indicando que esta especie parece beneficiarse de las actividades humanas. Esta podría ser la causa de la continua expansión de los mirlos hacía zonas urbanas.

4. En los estudios evolutivos es importante trabajar utilizando síndromes de estrategia vital en lugar de rasgos individuales como el tamaño de puesta.

5. Las hembras de mirlo común reducen su actividad en el nido (p.e.: disminuyendo el número de visitas) ante un riesgo elevado de depredación con lo que evitan revelar la localización del mismo al potencial depredador. Además, este cambio de comportamiento parece estar modulado por la presión de depredación ambiental, siendo más exagerado en el hábitat de mayor riesgo.

6. Aunque las hembras de mirlo común parecen cumplir la hipótesis de Skutch, los resultados en el tiempo de latencia (tiempo que tardan las hembras en volver al nido) impiden rechazar la hipótesis de la ocultación, y sugieren que la estrategia utilizada depende del riesgo de depredación específico de cada población.

7. Ambos sexos modifican de igual manera sus comportamientos de cuidados parentales según el riesgo de depredación del nido, aunque no en todos los rasgos considerados (p.e.: duración media de las visitas). Estas modificaciones comportamentales parecen estar influidas, en general, por el riesgo de depredación específico del hábitat.

8. Las respuestas parentales al riesgo de depredación del nido no tienen porqué implicar costes en alimentación para los pollos, lo que sugiere que hay que replantearse las ideas que se asumen habitualmente.

9. La evolución de la petición de alimento por parte de los pollos está constreñida por el coste que supone la atracción de depredadores provocada por las exageradas señales acústicas propias de dicha petición.

10. Los pollos de mirlo común modifican sus niveles hormonales de corticosterona y testosterona según el riesgo inmediato de depredación del nido, posiblemente

para reducir la petición de alimento y evitar ser detectados acústicamente por el potencial depredador.

11. Los pollos de mirlo común modifican sus niveles hormonales de corticosterona, pero no de testosterona, según el riesgo crónico de depredación del nido. Estas alteraciones endocrinas podrían aumentar la tasa de crecimiento de los pollos de la población con mayor presión de depredación o ser la respuesta de los pollos a cambios en la actividad de los padres.

12. Las diferentes respuestas de los pollos al riesgo inmediato y crónico sugieren la existencia de diferentes mecanismos fisiológicos para hacer frente a cada situación.

13. El incremento de la tasa de visitas del investigador aumenta el éxito reproductivo de los mirlos puesto que provoca una reducción de la depredación de nidos. Este efecto positivo se mantiene entre poblaciones a pesar de las diferencias en cuanto a las comunidades de depredadores que existen en ellas.

14. Contrariamente a la idea tradicional, la actividad de los investigadores no parece tener ningún efecto sobre la depredación de nidos en las aves en general. Esta relación varía según el orden considerado, llegando a reducir significativamente la depredación en los Paseriformes.

15. El cambio en métodos de estudio y la falta de un marco teórico claro que apoyara los efectos positivos en el éxito reproductor de las aves de la influencia investigadora parecen ser los factores responsables de que los estudios más recientes muestren significativamente más efectos positivos que los de hace 30 años.

CONCLUSIONS

1. Food limitation seems to be the most important selective pressure for blackbirds inhabiting the city of Granada (a situation highly influenced by humans), while it is nest predation for those from the Güejar-Sierra's forest (a natural situation). Melegís' orange groves (a seminatural situation) are characterized by an intermediate influence of these two factors. These results suggest that avian selective pressures are possibly affected by urbanization.

2. Common blackbirds are adapted to the main selective pressure of each population suggesting that, at least, some bird species could adaptively respond to the environmental changes imposed by humans.

3. Blackbirds show a significantly higher fitness with increasing human impact, indicating that this species benefits from human activities. This could be the ultimate explanation for its expansion into urban areas.

4. The use of life-history syndromes instead of single traits (i.e. clutch size) is vital in evolutionary studies.

5. Female blackbirds reduce their activities at the nest (i.e. nest visits) in a high predation risk situation to avoid giving away the nest location to potential predators. Moreover, environmental predation pressure seems to modulate this behavioral change with antipredator responses being more intense in the higher risk habitat.

6. Female blackbirds seem to follow Skutch's hypothesis, however, results in time of latency (female's time of return to the nest) avoid the rejection of the nest concealment hypothesis and suggest that the strategy used depends on the population specific nest predation risk.

7. Both sexes altered their parental care behaviour depending on the risk of nest predation in a similar way, although not for all traits analysed (i.e. mean visit duration). These behavioral changes seem to be influenced by the level of environmental nest predation risk of each habitat.

8. Parents can respond to the risk of nest predation without compromising the food delivered (without feeding costs) to their nestlings, thus, suggesting the re-consideration of established theory in this field.

9. The evolution of begging is thought to be restricted by a nest predation cost due to the attraction of predators with the acoustic signal associated to this behaviour.

10. Blackbird nestlings altered their corticosterone and testosterone hormonal levels depending on the short-term (impending) nest predation risk, possibly to reduce begging and avoid being detected acoustically by the potential predator.

11. Blackbird nestlings altered their corticosterone, but not testosterone, hormonal levels depending on the long-term (chronic) nest predation risk. These endocrine

modifications suggest whether a hormonal strategy to favor a fast growth in chicks of the population with the higher predation pressure or the offspring indirect response to nest predation risk mediated by parental activities.

12. Different nestling's hormonal responses to short and long-term nest predation risk seem to point out the existence of different mechanistic pathways to cope with each situation.

13. The increase in researcher's nest-visiting rates favours blackbird's breeding success through a reduction of nest predation, possibly because predators avoid those places highly visited by investigators. This positive effect is maintained between populations despite the difference in the community of nest predators.

14. Research activities in general did not affect avian nest predation, contrary to the traditional idea. This relationship is heterogeneous among avian orders and significantly positive for passerines.

15. There is a significant trend in time showing that recent studies on this subject found more easily that investigation activities increase avian breeding success. This trend could be explained mainly by the change in methodologies through the years and the lack of a theoretical background to support positive human effects.

AGRADECIMIENTOS

Aunque mi nombre sea el único escrito en la portada de la tesis, muchas otras personas han ayudado de una u otra forma a la realización y finalización de la misma. Estas líneas están dedicadas a ellas, espero no olvidarme a ninguna.

En primer lugar me gustaría destacar muy especialmente a dos de ellas: Manuel Soler Cruz, mi director, y Olivia Sanllorente Bolinches, mi mujer.

Manolo Soler me dio la oportunidad de iniciar mi carrera profesional y hacer realidad mis sueños de la infancia: ser científico. Su guía, orientación y apoyo han hecho posible que en estos momentos estéis leyendo estas líneas. Gracias por confiar en mí, por ayudarme en los momentos difíciles y por enseñarme a buscar las respuestas que subyacen a la realidad. Su mente aguda e ingeniosa ha sido siempre un acicate para esforzarme más en un intento vano de alcanzarle.

Olivia Sanllorente ha sido el otro pilar fundamental, habiéndome ayudado en todas las fases de la tesis, desde acompañarme al campo en numerosas ocasiones (a veces a horas intempestivas) hasta darme rigurosos y útiles comentarios sobre los trabajos (de fondo y de forma), pasando por enriquecedoras charlas sobre Ciencia. Y más allá de los aspectos profesionales, por los que le estoy enormemente agradecido, están los aspectos personales. Gracias por darme estabilidad emocional, por facilitarme siempre el trabajo, haciendo un gran esfuerzo para hacerme más llevaderos los enormes sacrificios que he tenido que hacer durante la realización de la tesis. ¡Muchas gracias Oli!

Pero como he dicho antes, son muchas las personas que han ayudado en la tesis.

La gran cantidad de datos presentados aquí no se habrían podido recopilar sin la ayuda de cuatro magníficos “ayudantes de campo”. Juanjo Iglesias me transmitió su ilusión por las aves y me hizo darme cuenta de lo poco que sabía. David Villanueva y Juan “Gavilán” pusieron todo su empeño en realizar una estupenda labor profesional, ¡y lo consiguieron! Laura Arco me sorprendió con su enorme capacidad de trabajo y profesionalidad, siempre dispuesta a superar sus propios límites y a hacer grandes sacrificios. Pero quizás lo que más le agradezca a Laura sea su amistad, inquebrantable y duradera, que me ha demostrado una y otra vez. ¡Gracias!

Otras personas, aún no siendo profesionales de la Ciencia, han hecho mis jornadas de muestreo mucho más llevaderas. El pastor de Güejar siempre cordial y

agradable. Los jardineros de Granada, en especial Román Barros Vílchez, simpático y forofo de las aves. Bonifacio Sánchez Conde y Cecilio Cobo Aguilar, que me facilitaron los accesos a los parques de la ciudad y que compartían su ilusión y preocupación por la Naturaleza conmigo. Los lugareños de Melegís, que me dejaron investigar en sus fincas (aunque no entendían muy bien lo que hacía), siempre me trataron con afecto y cariño, sobre todo Cristóbal, Carmen y su familia (Alejandro, los sueños se cumplen). Gracias a todas esas personas que me conocían como “el loco los mirlos”.

Olivier Chastel me permitió realizar los análisis de hormonas en su laboratorio del Centre d’Etudes Biologiques de Chizé y conocer la investigación fuera de España, por lo que le estoy muy agradecido. Los miembros del laboratorio de Bioquímica me trataron como a uno más de la familia e hicieron que me ilusionara y apasionara por el trabajo de laboratorio. Mis más sinceros agradecimientos a André Lacroix, Colette Trouvé, Stephanié Dano y Raphaelle Dhennin. Aunque muchas otras personas han hecho que mis estancias en Chizé fueran experiencias maravillosas e inolvidables no sólo en lo profesional sino también en lo personal. Muchas gracias a todos ellos: Alban Thomas, Amine Khalil, Audrey Sternalski, Aurélie Goutte, Catherine Michel, Céline Clément-Chastel, Clara Péron, Fred Angelier, Fred Barraquant, Mael, Maite Louzao, Morgane Viviant, Pablo Inchausti, Vincent Lacomte…

También tienen un lugar especial en mi corazón los que considero mis “tres hermanos mayores”: Liesbeth de Neve, Adela González y Manuel Martín-Vivaldi. Siempre dispuestos a escuchar mis dudas y preocupaciones (no siempre científicas), y aconsejándome sabiamente. Gran parte de lo que he aprendido se lo debo a ellos. Gracias por estar ahí, gracias por ser pacientes conmigo (que lo sé, a veces hablo mucho).

Juan Gabriel Martínez, Carmen Zamora, Juan Soler, Alberto Tinaut, Paqui Ruano, David Martín y Rafael Barrientos me han brindado no sólo estupendos consejos y ayuda profesional sino también su magnífica amistad. ¡Muchas gracias amigos!

Los miembros del Departamento de Biología Animal que con su cordialidad y amabilidad han hecho de la primera planta del edificio de Biología un estupendo lugar de trabajo. En especial a mis compañeros y amigos del laboratorio de Etología (María Roldán, Merche Molina, Magdalena Ruíz y Elena Macías) y a los esporádicos alumnos internos (Jorge, Alfredo, José Miguel…). También a los “energúmenos” del laboratorio de Entomología Terrestre (Carlos Jerez, Oscar Mira, Alejandra Fernández, Nacho,

Angel, Jota, Quique…) por alegrarme los días y soportar mis constantes intromisiones en sus dominios (aunque no siempre iba buscando a Oli).

Michael Griesser, Anders Møller, Juanga Martínez, Juan Soler, José Martín y Olivia Sanllorente me ayudaron a mejorar los manuscritos. ¡Muchas gracias por vuestros enriquecedores y útiles consejos!

El ministerio de Educación me concedió una beca-contrato FPU (2004-5269), que me permitió dedicarme profesionalmente a la investigación y conseguir mi independencia económica. Los gastos necesarios para la realización de la tesis fueron cubiertos gracias a dos proyectos (Parques Nacionales 87/2003 y CGL2007-61940/BOS). El ministerio de Educación también me concedió las ayudas económicas que me permitieron realizar mis estancias en Chizé (Francia). La Universidad de Granada con una ayuda de asistencia a congresos permitió que pudiera acudir al ECBB en Dijon (Francia) y el grupo “Comportamiento y Ecología Animal” hizo lo propio para que pudiera asistir a dos congresos de la SEE en Canarias y Valencia.

No querría terminar sin mencionar a mis amigos no vinculados directamente con el trabajo de mi tesis: Daniel Menéndez, Magdalena Caballero, Fran Bullejos, Alberto Ollero, Celes Sánchez, Alejándro Morales, Miguel Angel Alejo, Oihane Fernández, María Cabello, Lucía, Carlos y Sergio. Ellos me han mantenido con los pies en el suelo, haciéndome ver que hay vida más allá de la investigación. Especialmente me gustaría agradecer a Dani, por su especial cariño y amistad, el único que repitió (a parte de Oli) acompañándome en mis salidas a Güejar-Sierra. Los miembros del grupo de baloncesto “Biología” (también conocido como “Los Mataos”) que me ha mantenido en forma durante la última parte (bastante sedentaria) de esta tesis.

Finalmente, y con un cariño particular, me gustaría agradecer a mis padres, Blas Ibáñez Carrillo y Manoli Álamo Pulido que me han ayudado en todo lo que ha estado en sus manos, y más. Gracias papá. Gracias mamá. Y a ti también, Bea, aunque nos encontramos de vez en cuando, sé que siempre has estado ahí apoyándome. Lo mismo es extensible al resto de mi familia (sí, a los “adoptados” también Rubén).

¡Muchas gracias a todos!

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