Ecología y evolución de las interacciones parásito de cría-hospedador: un estudio longitudinal con individuos marcados

Mercedes Molina Morales

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Memoria presentada por Mercedes Molina Morales para optar al Grado de Doctor en Biología por la Universidad de Granada.

Tesis realizada bajo la dirección de los doctores Juan Gabriel Martínez Suárez y Jesús M. Avilés Regodón

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CERTIFICAN

Que los trabajos de investigación desarrollados en la Memoria de Tesis Doctoral: "Ecología y evolución de las interacciones parásito de cría-hospedador: un estudio longitudinal con animales marcados" son aptos para ser presentados por la Lda. Mercedes Molina Morales ante el tribunal que en su día se designe, para aspirar al Grado de Doctor por la Universidad de Granada.

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A Julia

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Resumen

Uno de los principales objetivos de la teoría coevolutiva es entender la dinámica espacial y temporal en los procesos de infección y en las defensas y contra-defensas que se producen entre parásitos y sus hospedadores. Multitud de factores abióticos y bióticos que varían espacio-temporalmente pueden directa o indirectamente afectar a esta dinámica. Las interacciones entre los parásitos de cría aviar y sus hospedadores proporcionan un sistema ideal para el estudio de la coevolución, y en concreto el sistema que forman el críalo europeo (*Clamator glandarius*) y su principal hospedador en la Península Ibérica, la urraca (*Pica pica*), es un sistema idóneo para abordar estas cuestiones. Este sistema ha proporcionado evidencias robustas sobre el efecto del parasitismo de cría en (1) en el éxito reproductivo del hospedador y (2) en la evolución de defensas que contrarrestan estos efectos. Sin embargo, son muy escasos los estudios a largo plazo que aborden aspectos relacionados con los patrones de uso de los hospedadores por los parásitos, y los mecanismos de defensa desde la perspectiva individual, y de hecho no existe ningún estudio de este tipo con urracas.

Esta tesis se ha desarrollado con el objetivo de analizar en un primer bloque, mediante estudios desde el punto de vista poblacional y longitudinales (basado en el remuestreo de individuos conocidos en distintas temporadas), los patrones temporales de parasitismo en la población, y en un segundo bloque, a través de estudios longitudinales, cuestiones relacionadas con los mecanismos de defensa de los hospedadores a nivel individual y a lo largo de su vida en respuesta al parasitismo de cría.

Los resultados de esta tesis sugieren que el patrón de parasitismo, estudiado a nivel poblacional, respondería a un proceso probabilístico basado en la disponibilidad espacio-temporal de nidos de urraca y la abundancia de críalos en la población. Además, se ha registrado que el ajuste fenológico en la población entre el críalo y la urraca cambia entre temporadas en función de factores climáticos, proporcionando una explicación adicional a la variación anual en la probabilidad de parasitismo. Asimismo, se observó una estructuración moderada del parasitismo a nivel individual dentro de la población basada en una combinación particular de rasgos (tamaño de nido, fecha de puesta y características del hábitat) que diferenciaron a las hembras que evitaron el parasitismo de las que sufrieron sus costes.

Los mecanismos de defensa que han evolucionado en las urracas para contrarrestar los efectos del parasitismo por el críalo son, principalmente, la defensa de nidos frente a los parásitos antes de que éstos pongan sus huevos y el reconocimiento y rechazo de huevos extraños. Los resultados de esta tesis muestran diferencias entre las parejas de urraca en relación a la intensidad en la defensa de su nido y la propensión a acudir al mismo en presencia de un intruso potencial, así como evidencia de una relación entre el comportamiento de rechazo de huevos y la defensa del nido, que podría indicar la evolución de un síndrome comportamental defensivo en respuesta al parasitismo por el críalo.

El estudio del comportamiento de rechazo en urracas a lo largo de la vida del individuo muestra que hay hembras en la población que siempre aceptaron, otras que siempre rechazaron y otras que modificaron su respuesta a los huevos miméticos, y siempre lo hicieron de aceptar a rechazar. Además, las hembras en su primer intento de cría siempre aceptaron, incluso aquellas cuyas madres fueron rechazadoras de huevos. El análisis longitudinal mostró que la probabilidad de rechazo se incrementó con la edad relativa de las hembras y no en respuesta al riesgo de parasitismo en la población. Todo esto indicaría que la transición hacia el reconocimiento de huevos estaría relacionada con la edad, siendo hembras más viejas las que habrían desarrollado las habilidades cognitivas o mecánicas necesarias para el reconocimiento y rechazo.

Los resultados de esta tesis muestran además, que el efecto del parasitismo sobre la distancia de dispersión reproductiva de la urraca estuvo mediado por la densidad de hospedadores, y fue sólo evidente para uno de los sexos, los machos, sugiriendo que no hay una clara evidencia de que los hospedadores intenten minimizar los costes asociados al parasitismo a través de la dispersión reproductiva.

De manera general esta tesis muestra la importancia del estudio de los patrones de parasitismo, expresión de los rasgos fenotípicos y de los comportamientos defensivos a lo largo de los años y de la vida del individuo. En el contexto de las dinámicas coevolutivas de las interacciones parásito-hospedador, los hallazgos encontrados sugieren la necesidad de considerar la estructuración del parasitismo dentro de las poblaciones y la influencia de factores abióticos ajenos a la interacción como aspectos que podrían determinar su dinámica. Asimismo, los resultados sugieren que la ontogenia puede jugar un papel muy relevante y previamente ignorado en la expresión de las defensas, en particular para especies de larga vida. La consideración de estos aspectos (i.e. estructuración del parasitismo, influencia climática y ontogenia), que sólo pueden ser estudiados y calibrados mediante aproximaciones basadas en el individuo y estudios a largo plazo, ofrecen novedosas perspectivas para el entendimiento de las dinámicas ecológicas y evolutivas que se dan entre los parásitos de cría aviar y sus hospedadores.

Abstract

One of the major challenges of the coevolutionary theory is to understand the spatio-temporal dynamic of the infection processes and the evolution of defenses and counter-defenses between parasites and their hosts. A number of abiotic and biotic factors that can vary spatiotemporally may indirectly or directly affect the dynamic of the interaction. Avian brood parasites and their hosts provide an ideal system for the study of coevolution and in particular, the great spotted cuckoo (*Clamator glandarius*) and its main host in the Iberian Peninsula, the magpie (Pica pica), constitutes a suitable system for studying these issues. This system has provided strong support for an effect of brood parasitism on (1) the reproductive success of the host and (2) on the evolution of host defensive mechanisms counteracting the negative effects of parasitism. However long-term studies with marked animals addressing issues related to patterns of host use by parasites, and defense mechanisms, are very scarce in general, and indeed nonexistent for this system.

This thesis analyzes in a first block, through cross-sectional and longitudinal studies (based on resampling known individuals in different breeding seasons), the patterns of parasitism in the population. In a second block, through longitudinal studies, questions related to the evolution of defensive mechanisms against parasitism along the life of individual hosts are addressed.

The results of this thesis suggest that the pattern of parasitism, at the population level, respond to a probabilistic process based on the spatial-temporal availability of magpie nests and the abundance of cuckoos in the population. In addition, it has been found that the population phenological mismatch between great spotted cuckoos and magpies changes between seasons depending on climatic factors, thus providing an additional explanation to the found annual variation in the probability of parasitism. Also, a longitudinal study reveals a pattern of structured parasitism at the individual level within the population. Indeed, females with a particular combination of traits (nest size, laying date and habitat characteristics) consistently escaped from cuckoo parasitism.

The main defensive mechanisms evolved in magpies to counteract the effects of great spotted cuckoo parasitism are nest defense against the parasite before laying (mobbing) and parasite egg discrimination and rejection. The results of this thesis show that magpie pairs differ on their baseline levels of nest defense and their propensity to approach the nest after detecting a potential intruder. We have also found evidence for a direct relationship between egg rejection and nest defense behavior, that may suggest the evolution of a general defensive behavioral syndrome in response to great spotted cuckoo parasitism.

The study of the rejection behaviour of magpies throughout their lives show that some females always accept, others always reject and some others modify their response to model eggs, in all cases switching from acceptance to rejection. Also females tested in their first breeding attempt always accepted the model eggs, even those individuals whose mothers were egg rejecters. A longitudinal analysis showed that the probability of rejecting eggs increased with the relative age of the female, but was not related to the risk of parasitism in the population. All this suggests that the transition toward egg recognition is related to age, being more likely for older females to develop the cognitive and/ or mechanical skills necessary for recognition and rejection.

In addition, the results of this thesis show that the effect of parasitism on breeding dispersal was mediated by host density, and it was only evident for males. Globally suggest that there is no clear evidence that magpies minimized the costs associated with great spotted cuckoo parasitism through dispersing further away.

In general, this thesis shows the importance of performing long-term studies through the life of an individual for studying patterns of parasitism and the expression of phenotypic traits and defensive behavior at the population. In the context of coevolutionary dynamics of parasite-host interactions, the findings of this thesis urge for considering that brood parasitism can be structured within host populations, and the possible influence of abiotic factors beyond the interaction as potential aspects determining the evolution of the interaction. Also, these results suggest that ontogeny may play a fundamental role in the expression of host defenses, particularly for long life host species.

Summing up, consideration of these novel aspects (i.e. structured parasitism, climatic influence and ontogeny), whose importance can only be qualified by performing individual-based and long-term studies, provides intriguing and deeper perspectives for the understanding of the ecology and evolution of avian brood parasites and their hosts.

Capítulo 1

Introducción general

Introducción general

El parasitismo es un tipo de interacción biológica entre dos organismos, en la cual uno de ellos, el parásito, obtiene beneficios del otro, el hospedador, que a su vez se ve perjudicado. En la mayoría de los casos el beneficio que obtiene el parásito del hospedador es una fuente de alimentación, o bien cuidados parentales para su descendencia, lo que se denomina, específicamente, parasitismo de cría. El parasitismo trae consigo una reducción del crecimiento, la superviviencia y el éxito reproductivo de los hospedadores (Clayton y Moore 1997, Davies 2000, Payne 2005), y, por tanto, es un potente agente selectivo sobre los rasgos del hospedador. De hecho, los rasgos de historia vital de muchas especies están a menudo mediados por la acción de los parásitos y su expresión es el resultado evolutivo de una variedad de respuestas para reducir sus efectos negativos (Clayton y Moore 1997, Feeny *et al.* 2013).

El parasitismo de cría ha evolucionado en varios grupos de animales, tanto en el Subfilo Vertebrata (Clase Aves: Payne 2005, Davies 2000; Clase Actinopterigii: Taborsky 2001, Ota *et al.* 2010), como en el filo Artrópodos (Clase Insecta: Libersat *et al.* 2009, González-Megías y Sánchez Piñero 2003), siendo más frecuente en la Clase Aves y en el Orden Himenóptera dentro de los insectos (principalmente en hormigas, Wilson 1971, y abejas del gén. *Psythirus,* Williams 1998, 2008, Kilner y Langmore 2011).

El parasitismo de cría aviar es una estrategia reproductiva que se da en algunas especies de aves en la cual el parásito pone sus huevos en el nido de otro individuo, el hospedador, que lleva a cabo el cuidado parental desde la incubación a la alimentación de los pollos. El parasitismo de cría aviar puede ser intra- o inter-específico dependiendo de si los huevos del parásito son puestos en nidos de individuos de la misma (e.j. Yom-Tov 2001, Eady y Lyon 2011), o distinta especie (Davies 2000, Payne 2005). Tanto en un caso como en otro, el parasitismo de cría puede ser facultativo, cuando el parásito además de criar su propia descendencia parasita a otros individuos de su especie u otra (Amat 1991, Amat 1993, Payne 2005), u obligado, cuando los parásitos no hacen nidos y ponen sus huevos siempre en nidos de otros individuos de su misma o distinta especie (Payne 2005). El parasitismo de cría inter-específico obligado, cuyo estudio es el objeto de esta tesis, se da casi exclusivamente en aves nidícolas o altriciales, aunque también aparece esporádicamente entre algunas especies de aves nidífugas como el pato rinconero, *Heteronetta atricapilla*, que parasita a otras anátidas (Eady *et al.* 1988).

El parasitismo de cría inter-especifico ha evolucionado en linajes de aves que hacen nido y proporcionan cuidados parentales, y su origen evolutivo ha sido explicado fundamentalmente mediante dos hipótesis (revisado en Davies 2000). En primer lugar se ha sugerido que podría originarse a partir del parasitismo intra-específico en situaciones en las que la falta de nidos de conespecíficos que parasitar favorecería el uso de nidos de otras especies para poner huevos (Hamilton and Orians 1965, Payne 1977a). Alternativamente, puesto que muchas especies de aves usan nidos viejos de otras especies o crían en cavidades con poca luz, podría darse el caso que un parásito primigenio pusiese por error sus huevos en nidos en los que aún estuviesen los huevos del propietario. Si el propietario expulsara a la hembra parásita los huevos de ésta podrían ser incubados con los huevos del propietario, originándose el parasitismo de cría inter-específico (Davies 2000). En este sentido, se ha sugerido que el parasitismo de puesta inter-especifico podría haber evolucionado directamente a partir de especies de mayor tamaño y con periodos de incubación más cortos, que comenzarían a explotar por error a otras especies de menor tamaño y con tiempos de incubación más prolongados, de modo que los pollos parásitos se beneficiarían de su mayor tamaño, ya que los padres hospedadores los alimentarían preferentemente (Slagsvold 1998).

El parasitismo de cría inter-específico ha evolucionado al menos siete veces distintas en varios clados dentro de la clase aves: una vez en los indicadores de la miel (Fam. Indicatoridae, 17 especies), en una especie de anátida (Fam. Anatidae, *Heteronetta atricapilla* de Sur América); tres orígenes independientes en cucos del Viejo y Nuevo Mundo, (Fam. Cuculinae, 57 especies) y dos orígenes independientes en Paseriformes, incluyendo los tordos americanos (Fam. Icteridae, 5 especies) y los pinzones africanos (Gén. *Vidua* y Gén. *Anomalospiza*, 20 especies) (Sorenson y Payne 2002).

Los parásitos de cría pueden reducir el éxito reproductivo de sus hospedadores de tres maneras principales. En primer lugar, durante la fase de la puesta, se ha descrito que en muchas especies parásitas la hembra suele retirar un huevo del hospedador al poner el suyo, como en *Cuculus canorus*, o incluso en visitas previas a poner su huevo, como es el caso de *Molothrus ater* (Sealy 1992, Payne 2005, Davies 2000). En aquellas especies parásitas donde la hembra no retira huevos, también se puede producir una reducción del tamaño de puesta del hospedador debido a que la hembra parásita rompe los huevos hospedadores, bien debido a la caída de los huevos parásitos sobre los del hospedador, (*Clamator* sp. Soler *et al.* 1997b, Soler y Martínez 2000, Hoover 2003, (*Indicator indicator*) Spottiswoode y Colebrook- Rodjent 2007) o bien picándolos para conocer el estado embrionario de los mismos y ajustar el momento de parasitismo (Massoni y Reboreda 1999, 2002). En segundo lugar, el parasitismo a menudo reduce el éxito de eclosión del hospedador (Rothstein 1990), ya que el periodo de incubación de los huevos parásitos es más corto y la hembra hospedadora deja de incubar para alimentar a los pollos eclosionados (Payne 2005). Por último, tras la eclosión los parásitos también infligen costes al hospedador al acaparar sus cuidados parentales. Los pollos del cuco europeo en cuanto eclosionan expulsan a los pollos hospedadores del nido evitando la competencia por el alimento con ellos (Davies 2000, Payne 2005), e infringiendo un coste muy elevado al hospedador que tiene que alimentar a un pollo que no tiene su información genética. Los hospedadores de los indicadores de la miel, Gén. *Indicator*, sufren también costos muy elevados ya que el pollo parásito a las pocas horas de haber eclosionado lacera con su pico y mata a los pollos del hospedador, los cuales son sacados del nido por los padres (Spottiswoode y Koorebaar 2012). En otras especies parásitas como el críalo (*Clamator glandarius*) o el tordo americano de cabeza marrón o tordo negro (*Molothrus ater*), sin embargo, los pollos parásitos y los hospedadores comparten el nido durante un período variable de tiempo. No obstante, los pollos parásitos son preferentemente alimentados por sus padres putativos debido a que, al eclosionar antes, son de mayor tamaño que los pollos hospedadores (Soler y Soler 1991), y además porque normalmente presentan una mayor intensidad petitoria que los pollos hospedadores (Redondo y Zúñiga

2002, Soler *et al.* 1999e, Kilner y Davies 1999, Hauber y Ramsey 2003, Rivers 2007). Debido a ello, en algunas especies los pollos parásitos acaban por monopolizar el alimento y matar de hambre a los pollos hospedadores, quedando solos en el nido (por ejemplo Soler 1990, Soler *et al.* 1995c, Davies 2000).

El parasitismo de cría aviar es un sistema ideal para el estudio de los procesos coevolutivos porque involucra, en la mayoría de los casos, la interacción de dos especies en simpatría y porque muchas de las adaptaciones relacionadas con la interacción se manifiestan en el nido, y por tanto, pueden ser fácilmente estudiadas por los investigadores. Estas razones hacen que el parasitismo de cría inter-específico sea un sistema idóneo donde estudiar la evolución (Rothstein 1990).

Interacción parásito de cría - hospedador: Carrera de armamentos coevolutiva

En general los parásitos de cría infligen grandes costes reproductivos a sus hospedadores, por lo que existe una fuerte presión selectiva en los mismos para el desarrollo de estrategias de defensa contra los parásitos. A su vez, el éxito reproductivo de los parásitos de cría obligados depende de los hospedadores, lo que selecciona contra-defensas adaptativas, dando lugar, por tanto, a una carrera de armamentos coevolutiva (Davies 2000). Las interacciones entre parásitos de cría y hospedadores proporcionan algunos de los más claros ejemplos de coevolución. En concreto, la evolución de adaptaciones y contra-adaptaciones en los mecanismos de defensa y contra-defensa se han demostrado en diferentes estadios del periodo reproductivo de algunas especies de parásitos de cría y sus hospedadores (Krüger 2007, Soler 2013).

Consideramos el primer estadio como el periodo de tiempo en el que la pareja de hospedadores escogen el territorio, construyen y defienden el nido antes de la puesta. La búsqueda o selección de nidos por parte del parásito se realiza antes de la puesta de los huevos, por el hospedador. La hembra parásita dedica un considerable tiempo observando nidos desde un emplazamiento oculto, y la respuesta adaptativa por parte del hospedador sería pues, criar en ciertos lugares alejados de las perchas de los parásitos, lo que reduciría la probabilidad de ser parasitados (Álvarez 1993, Øien *et al.* 1996, Clotfelter 1998, Moskat y Honza 2000, Hauber 2001), así como el ocultamiento de los nidos (Moskat y Honza 2000), un comportamiento sigiloso (Banks y Martin 2001) y un momento de la puesta impredecible, que también puede ayudar a minimizar el riesgo de parasitismo (ver revisión en Davies 2011). Una vez el parásito se acerca al nido, la primera respuesta del hospedador es la defensa directa del nido, atacando a los parásitos (Mokness *et al.* 1991, Røskaft *et al.* 2002). Estudios experimentales han mostrado que los hospedadores son capaces de discriminar entre depredadores y parásitos de cría (Welbergen y Davies 2008) y que ese comportamiento defensivo es efectivo disminuyendo la probabilidad de parasitismo (Welbergen y Davies 2009). Criar en zonas densas o cerca de vecinos también puede reducir la probabilidad de un individuo de ser parasitado por un efecto dilución o través de defensa colectiva (Martínez *et al.* 1996, 1998b, Welbergen y Davies 2009, Jélinek *et al.* 2014). Todos estos factores, a su vez, actuarían como una presión selectiva sobre el parásito que podría evolucionar hacía un comportamiento más reservado y sigiloso para evitar ser visto mientras pone sus huevos, o bien, haber evolucionado un plumaje que confundiera a los hospedadores, como por ejemplo el parecido en el plumaje entre cucos y gavilanes que reduciría el hostigamiento hacia los parásitos al confundirlos con un depredador (Davies y Welbergen 2008, Davies 2011).

Tradicionalmente, los estudios sobre la evolución de las adaptaciones y contra-adaptaciones en el sistema parasito de cría-hospedador se han centrado en el rechazo de los huevos parásitos por parte de los hospedadores, ya que se trata del momento en el que esta interacción es más acusada. El hospedador, ya sea mediante aprendizaje o discordancia (basándose en la comparación de características como tamaño, coloración, y/o patrón de motas, entre sus propios huevos y los huevos parásitos), reconoce y expulsa el huevo parásito del nido (Victoria 1972, Rothstein 1975, Lotem *et al.* 1992, 1995, Davies y Brooke 1988, Marchetti 2000). Se ha sugerido que el rechazo lo lleva a cabo el sexo responsable de la incubación, frecuentemente las hembras, pero en algunos casos también los machos (Soler *et al.* 2002). El comportamiento de rechazo a veces consiste en el abandono de las puestas parasitadas o en la construcción de un nuevo nido sobre el anterior, como consecuencia de limitaciones en la capacidad de los hospedadores para retirar los huevos parásitos del interior del nido (Hill y Sealy 1994). El rechazo de huevos, a su vez, ha generado la evolución de contra-defensas en algunos parásitos, como son el mimetismo en la apariencia de los huevos (Davies y Brooke 1988, Langmore *et al.* 2003, Avilés 2008) y cáscaras de los huevos parásitos más fuertes y gruesas (Brooker y Brooker 1991, Davies 2000, Payne 2005, Antonov *et al.* 2009) que tenderían a minimizar los efectos del rechazo. A su vez la apariencia de los huevos de los hospedadores estaría evolucionando para favorecer el reconocimiento de huevos parásitos. Especies y poblaciones hospedadoras parasitadas muestran menor variación intrapuesta en la apariencia de sus huevos, y como consecuencia mayor variación entre puestas de diferentes hembras que especies y/o poblaciones no parasitadas (Øien *et al.* 1995, Soler y Møller 1996a, Stokke *et al.* 2002, Avilés y Møller 2003), y dentro de algunas poblaciones hospedadoras se ha comprobado que los rechazadores tienen menor variación intra-puesta en la apariencia de sus huevos (e.g. Stokke *et al.* 1999; Moskat *et al.* 2008), si bien existirían numerosas excepciones a la regla (e.g. Avilés *et al.* 2004).

Los pollos parásitos presentan diversas adaptaciones que facilitan su éxito en el nido de los hospedadores, tal y como son un rápido desarrollo embrionario, un comportamiento petitorio exagerado, o comportamientos de expulsión o eliminación de huevos o pollos parásitos (ver revisión en Krüger 2007 y Soler 2009). Durante la fase en que los pollos están en el nido, se han descrito mecanismos de defensa del hospedador como son el reconocimiento y rechazo de pollos mediante abandono del pollo en el nido sin alimentarlo o picándolo y tirándolo del nido (Langmore *et al.* 2003, Sato *et al.* 2010, Tokue y Ueda, 2010, Delhey *et al.* 2011). El reconocimiento de pollos ha generado la evolución de mimetismo visual en los pollos parásitos de algunos cucos australianos (Langmore *et al.* 2011). Además, los pollos de cuco presentan en algunas especies mimetismo vocal que podría ser usado para combatir el rechazo (Payne y Payne 1998, Langmore *et al.* 2008, Anderson *et al.* 2009, ver revisión en Soler 2009 y Davies 2011).

En este contexto en el que los beneficios de presentar adaptaciones y contra-adaptaciones parecen tan evidentes, uno de los mayores enigmas a los que se enfrentan los ecólogos evolutivos es entender por qué muchas especies (tanto hospedadoras como parásitas) no han evolucionado defensas antiparásitas o contra-defensas cuando éstas parecen, *a priori,* tan ventajosas. En el caso del comportamiento de expulsión de huevos parásitos, por ejemplo, la ausencia de rechazo ha sido explicada mediante tres hipótesis evolutivas. La hipótesis del retraso evolutivo atribuye la ausencia de rechazo a un retardo en la evolución de los mecanismos de defensa en el hospedador debido a la ausencia de variantes genéticas o del tiempo suficiente para que esas variantes se expandan (Rothstein 1975, Davies y Brooke 1988, Hoover 2003). La hipótesis del equilibrio evolutivo sugiere que dado que existen restricciones cognitivas y fisiólogicas que podrían afectar a la expresión de la defensa, la ventaja de expresarla sería dependiente del contexto. Así, los hospedadores aceptarían el parasitismo cuando los costes de evitarlo excedieran los beneficios de aceptarlo (Rohwer y Spaw 1988, Lotem *et al.* 1992, Avilés *et al.* 2005, Krüger 2011). Finalmente, la hipótesis de la transmisión horizontal limitada sugiere que la existencia de parasitismo estructurado espacialmente en una población y/o entre varias poblaciones puede también conducir a una ausencia de defensas aparentemente maladaptativa desde un punto de vista poblacional (Hauber *et al.* 2004, Hoover *et al.* 2006). En este sentido, el hecho de que un individuo tenga significativamente más probabilidad de ser parasitado a lo largo de su vida que otros de la población, se ha considerado como un caso particular de transmisión horizontal (Galvani 2003). Se ha sugerido que la transmisión horizontal limitada en el parasitismo enlentece la evolución de la resistencia en el hospedador, en las comparaciones intrapoblacionales, donde algunos individuos hospedadores son parasitados diferencialmente a lo largo de su vida (Hauber 2001).

Una laguna fundamental para la evaluación exhaustiva de estas hipótesis evolutivas es la escasez de estudios a largo plazo y a nivel individual lo que impide conocer el papel de la ontogenia en la evolución de las defensa, los efectos del papel de cada sexo en las defensas contra el parasitismo y cómo la estructura de edades en la población puede afectar tanto a la probabilidad de parasitismo como al desarrollo de las estrategias de defensa que se dan en las poblaciones de hospedadores potenciales.

Sistema de estudio Críalo (*Clamator glandarius*) - Urraca (*Pica pica*) como modelo de estudio en coevolución animal

La urraca

La urraca es un córvido de tamaño medio (43-50cm, 180-236gr, Birkhead 1991), cuyo rango de distribución es la región Holártica. Las urracas son aves territoriales, sedentarias y de vida relativamente larga para ser paseriformes; en diferentes poblaciones la esperanza de vida media varía entre 2 y 3,5 años, aunque algunos individuos pueden llegar a alcanzar 15 años de edad (Birkhead 1991). En nuestra población de estudio hemos registrado un individuo reproductor con 11 años. En esta especie solo hay dimorfismo sexual en tamaño, siendo las hembras algo más pequeñas que los machos (Birkhead 1991). Se trata de aves fundamentalmente monógamas aunque se han descrito cópulas extra-pareja (Birkhead 1991). Las urracas construyen en cada intento

Figura 1. Aspecto de los nidos de urraca construidos en almendros dentro de la zona de estudio.

reproductor nidos elaborados que consisten en una taza de barro, rellena de raíces, pelos y otros materiales suaves, y una cúpula de ramas, lo que hace que el nido presente forma ovalada (Martinez *et al.* 1997; Figura 1).

Ambos miembros de la pareja participan en la construcción del nido, pero cada uno tiene un rol distinto. Los machos generalmente colectan más ramitas que las hembras, que en cambio pasan más tiempo en el nido colocando el material traído por el macho o acercando al nido raíces (Buitron 1988). Normalmente ponen una puesta de entre dos y diez huevos entre los meses de Abril y Junio, y la mayoría pueden tener una puesta de reposición si ésta falla. El tamaño de puesta típico en nuestra zona de estudio es de entre 6 y 8 huevos (ver Soler *et al.* 2013). El periodo de incubación es de unos 21 días, y solo incuban las hembras (Birkhead 1991).

Figura 2. Diferencia de tamaño de pollos de Urraca de la misma nidada.

Las urracas presentan eclosión asincrónica, las hembras empiezan a incubar con el cuarto o quinto huevo, de forma que primero eclosionan 4 o 5 pollos y en días sucesivos el resto, creando una jerarquía de tamaño en la pollada (Birkhead 1991, Martinez *et al.* 1997, Figura 2). Ambos padres alimentan a los pollos en el nido y los defienden mediante llamadas y acoso de potenciales depredadores o intrusos en general. Los volantones abandonan el nido aproximadamente a los 27 días, y los padres continúan alimentándolos durante varias semanas (Birkhead 1991, Martinez *et al.* 1997).

El críalo

El críalo es un parásito de cría obligado de la Familia Cuculidae. Es un ave migratoria de tamaño medio (38-40cm, 138-169 gr), que llega a la Península Ibérica desde África, donde pasa el invierno, a finales de Febrero- principios de Marzo (Soler *et al.* 1997a). Su distribución en Europa se restringe a la Península Ibérica, el sur de Francia e Italia (Cramp 1985). El principal hospedador europeo de esta especie parásita es la urraca, aunque también parasita a otras especies de córvidos como la corneja (*Corvus corone*, Soler 1990a, Soler *et al.* 1997a). La hembra de críalo puede poner más de 15 huevos en 44 días en nuestra zona de estudio (Martinez *et al.* 1998b) y pueden poner más de un huevo en el mismo nido hospedador (Martinez *et al.* 1998a). Además más de una hembra de críalo puede parasitar el mismo nido (Martinez *et al.* 1998a, 1998b). Los huevos son elípticos, lisos y bastante brillantes, de color verde-azulado pálido, con motas de color marrón o marrón rojizas (Cramp 1985). Tanto el tamaño como la coloración varían entre hembras. La cáscara del huevo es gruesa y el periodo de incubación es corto en relación al tamaño del cuerpo del adulto, de unos 14 o 15 días (Soler 1990). Durante los primeros días los pollos crecen muy rápidamente y dejan el nido entre 17 y 20 días de media después de la eclosión (Soler y Soler 1991; Figura 3).

En esta especie no se da el desalojo de huevos o pollos hospedadores por parte del pollo parásito. Sin embargo, los pollos de críalo son muy eficaces pidiendo y recibiendo comida de parte de las urracas, de forma que son alimentados preferentemente a los pollos de urraca (Soler *et al.* 1995c) y con frecuencia acaban ocasionando la muerte por inanición de éstos, más pequeños y menos capaces de conseguir cebas (Soler 1990, Soler *et al.* 1996b). En la mayoría de los nidos parasitados no vuela ningún pollo de urraca (Soler *et al.* 1996b). Los críalos adultos visitan, esporádicamente, los nidos parasitados y cuando los pollos se marchan del nido pueden mantener contacto con los volantones (Soler y Soler 1999). Una vez abandonado el nido, los pollos parásitos siguen siendo alimentados por los padres adoptivos durante más de un mes (Soler *et al.* 1994a) y forman grupos con otros pollos parásitos salidos de otros nidos.

Figura 3. Pollo de críalo en nido de urraca esperando la ceba.

Adaptaciones y contra-adaptaciones descritas en este sistema

El sistema críalo-urraca es uno de los sistemas donde mejor se han estudiado los efectos del parasitismo de cría en la evolución de defensas y donde se han descrito una serie de adaptaciones y contra-adaptaciones que en algunos casos sugieren la existencia de un proceso coevolutivo entre ambas especies (Soler y Soler 2000, Møller y Soler 2012).

El proceso de parasitismo comienza con la observación, por parte de las hembras de críalo, de la actividad de construcción de nidos en la urraca. Las urracas, como aves territoriales, defienden su nido de intrusos y de depredadores (Birkhead 1991), pero además, identifican al críalo como una amenaza y cuando una hembra de críalo se acerca al nido, sus ocupantes intentan expulsarla (Álvarez y Arias de Reyna 1974). Este sería el primer mecanismo de defensa que presenta la urraca contra el parasitismo durante el ciclo reproductivo. De hecho, se ha descrito lo que se conoce como estrategia de distracción en los críalos, consistente en que el macho distrae mediante un ruidoso y conspicuo vuelo a las urracas, que frecuentemente lo persiguen, mientras que la hembra de críalo accede al nido vacío para poner su huevo (Alvarez y Arias de Reyna 1974). Se ha sugerido que esta estrategia de distracción en pareja sería una respuesta adaptativa para evitar el acoso por parte de las urracas (Soler *et al.* 1999c, Soler y Soler 2000). Sin embargo no todas las parejas de urracas muestran el mismo grado de defensa activa del nido, y se ha propuesto que esta diferencia entre parejas podría venir dada porque hay individuos en los que habría evolucionado otro mecanismo de defensa para compensar el parasitismo. Este mecanismo sería el de reconocimiento y rechazo de huevos (ver más abajo). De modo que habría parejas de urracas que invertirían más en la defensa activa del nido y que aceptarían los huevos de críalo, mientras que las parejas que defienden el nido en menor grado, rechazarían los huevos parásitos, lo que sugiere una expresión antagonista de estos dos mecanismos de defensa (Soler *et al.* 1999c).

El parasitismo se puede llevar a cabo mediante varias estrategias: parasitando al azar los nidos disponibles o mediante selección de rasgos del hospedador que indiquen una ventaja para el parásito. La selección natural favorecería a aquellos individuos parásitos que maximizaran su éxito reproductivo y una manera de asegurar ese éxito es la selección de hospedadores de alta calidad. Se ha mostrado que los críalos seleccionaban a las parejas de urraca que tenían nidos más grandes, que son además los nidos donde los pollos de críalo tenían más posibilidades de volar (Soler *et al.* 1995a). En este sentido, se ha sugerido que el menor tamaño del nido de urraca en poblaciones simpátricas con el críalo es la respuesta adaptativa a esta presión selectiva del parásito (Soler *et al.* 1999a).

El parasitismo del críalo supone importantes costos reproductivos en las urracas, como se ha mencionado antes. Estos costes se dan a varios niveles. La hembra de críalo no retira huevos del hospedador pero los daña cuando pone el suyo ya que lo deja caer desde el borde del nido, abollando y agrietando los huevos de urraca (Soler *et al.* 1997b). Como ya hemos dicho anteriormente, el periodo de incubación de los huevos de críalo es más corto que en la urraca por lo que el pollo de críalo eclosiona antes deteniendo la incubación de algunos huevos de urraca y haciendo que no eclosionen (Soler *et al.* 1997a). Se ha sugerido que una manera de contrarrestar la pérdida o rotura de los huevos es incrementar el tamaño de puesta (Soler *et al.* 2001). Pero la principal respuesta adaptativa que ha evolucionado para contrarrestar los efectos del parasitismo es el reconocimiento y rechazo de huevos parásitos (Soler y Møller 1990). Una vez las urracas reconocen un huevo extraño en su nido, lo cogen con el pico y lo sacan de éste. Se sabe que el reconocimiento y rechazo de huevos extraños en la urraca es una respuesta adaptativa al parasitismo que depende de la presión de parasitismo y que varía entre poblaciones en relación, entre otras cosas, con el tiempo de simpatría entre críalo y urraca (Soler y Møller 1990), con las distancias geográficas y genéticas entre poblaciones (Soler *et al.* 1999d, Soler *et al.* 2001), y con la variación en el aspecto de los huevos del hospedador (Avilés *et al.* 2004). Una condición indispensable para que se considere que un comportamiento haya evolucionado como respuesta adaptativa es que tiene que ser heredable y por tanto, tener una base genética. Martín-Gálvez *et al.* (2006, 2007) evidenciaron diferencias en las frecuencias alélicas para el marcador microsateliteAse64 entre volantones hijos de aceptores y volantones hijos de rechazadores pudiéndose considerar ese microsatélite como un marcador asociado al comportamiento de rechazo de huevos parásitos (Martín-Gálvez *et al.* 2006, 2007). La principal contra-adaptación frente al rechazo de huevos parásitos, que está muy bien documentada en el cuco común, es el mimetismo de huevos (Davies y Brooke 1989). Sin embargo, los huevos de críalo no mimetizan a los huevos de sus hospedadores debido a que no existe variación entre los huevos del parásito puestos en nidos de distintas especies (Soler *et al.* 2003). Una posible contra-adaptación frente al rechazo podría ser el comportamiento "vengativo" o "mafioso" descrito por Soler *et al.* (1995b). Los críalos revisitan los nidos parasitados y destruyen la puesta del hospedador si éstos rechazan el huevo parásito con el fin de provocar una puesta de reposición en un nuevo nido y de esta manera incrementar la posibilidad de parasitismo. La hipótesis original y el nombre de “hipótesis de la mafia” se deben a Zahavi (1979) y fue comprobado experimentalmente por primera vez en urracas por Soler *et al.* (1995b). Además, este comportamiento parece inducir en los hospedadores, un cambio en la respuesta al huevo parásito, aceptándolo en la segunda puesta (Soler *et al.* 1999).

Los pollos de críalo cuentan con una serie de características que pueden considerarse adaptaciones a la forma de vida parásita, como ocurre en general en todos los parásitos de cría (Krüger 2007, Soler 2009). Se pueden destacar algunos rasgos fenotípicos como el comportamiento petitorio exagerado y las papilas de la cavidad bucal que propician una alimentación preferente por parte de los adultos de urraca (Soler *et al.* 1995c). La posible existencia de mecanismos de defensa durante la fase de pollo en el nido en este sistema no ha sido verificado (ver por ejemplo Soler *et al.* 1995d, Soler 2009, pero ver también Soler 2008). Finalmente, estudios recientes sugieren que el olor fétido de los excrementos de los pollos de críalo podría ser una adaptación para evitar la depredación, que favoreciera la aceptación del parasitismo cuando el críalo comparte nido con pollos a los que no es capaz de aventajar en tamaño durante el desarrollo (Canestrari *et al.* 2014).

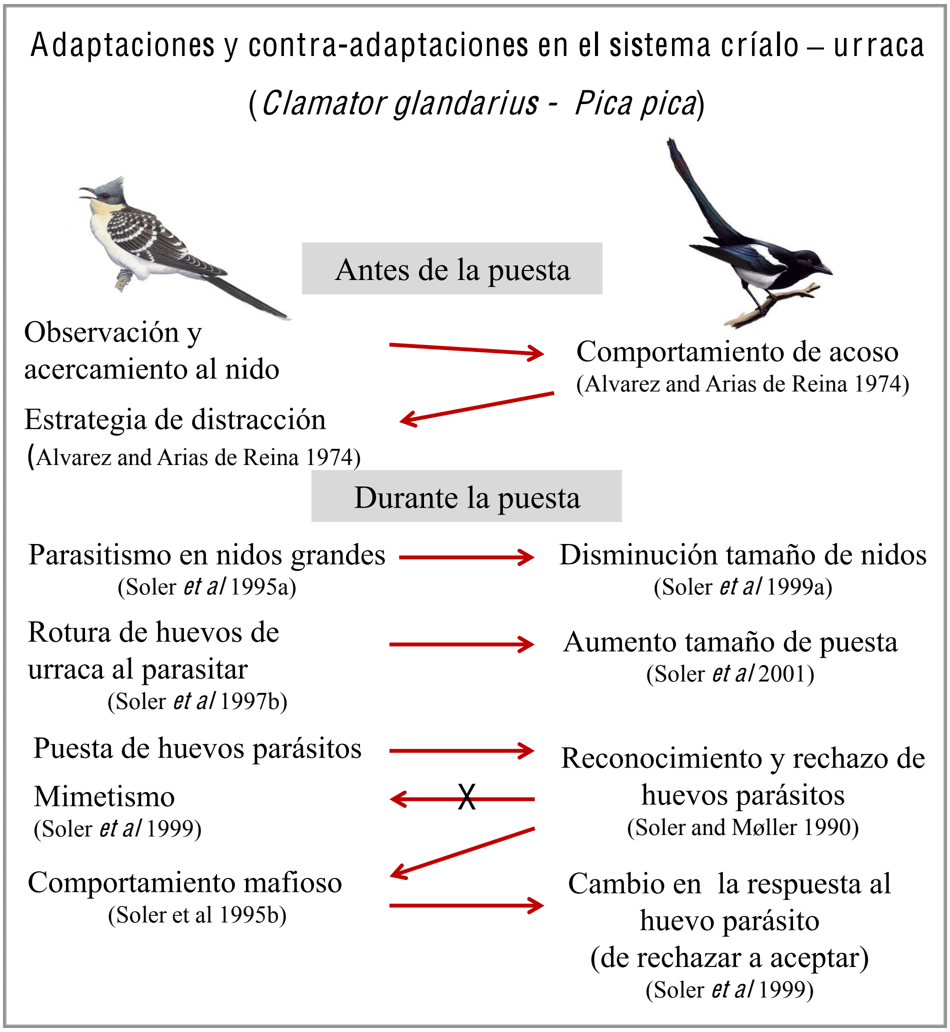

Figura 4. Resumen de las principales adaptaciones y contra-adaptaciones descritas en el sistema críalo- urraca, antes y durante la puesta, que sugieren una carrera de armamentos coevolutiva entre ambas especies.

Justificación del enfoque de la tesis. Estudios a corto versus largo plazo y estudios poblacionales versus estudios individuales.

La mayoría de los trabajos sobre interacciones parásito de cría - hospedador se han hecho desde un punto de vista poblacional, involucrando una o varias poblaciones y frecuentemente a corto plazo, con datos tomados en una o pocas temporadas de cría. Así, se han estudiado las dinámicas evolutivas espaciales implicadas, por ejemplo, en el reconocimiento de huevos, pero también en muchos otros aspectos de esta interacción (selección de hospedador, efectos del parasitismo, evolución de defensas y mimetismo) (ver por ejemplo Rothstein 1990, Davies 2000, Krüger 2007, Stokke *et al.* 2008, Avilés *et al.* 2011, Avilés *et al.* 2012, Soler 2013). En el sistema críalo-urraca se mantiene esta tónica y la mayoría de los estudios se han centrado en trabajos llevados a cabo en una sola o a lo sumo unas pocas temporadas de cría (por ejemplo Soler *et al.* 1995b, Martinez *et al.* 1996, Soler *et al.* 1999a, Soler *et al.* 1999c), aunque hay algunas excepciones en las que el marco temporal incluido es más amplio, por ejemplo Soler *et al.* (1994). Sin embargo, incluso aunque en algunos casos pudiéramos hablar de estudios a medio-largo plazo, en el sistema críalo-urraca existe una carencia de estudios en los que se conozca la identidad de los hospedadores que permita estudiar la interacción con el parásito y sus efectos a lo largo de la vida. Esta carencia es característica también de los estudios de parasitismo de cría, en los que los trabajos hechos con animales marcados y a lo largo de su vida son escasos (aunque hay algunas excepciones notables, como por ejemplo Smith 1981, Soler *et al.* 1999, Hauber 2001, Hauber *et al.* 2004, Hoover *et al.* 2006, 2007). Los estudios longitudinales, es decir, estudios sobre características o comportamientos concretos a lo largo de la vida de individuos marcados para poder reconocerlos, representan una aproximación alternativa a los estudios poblacionales en ecología y biología evolutiva (Clutton-Brock y Sheldon 2010). Tienen al menos seis ventajas; el análisis de la estructura de edad, la posibilidad de relacionar distintos estadios de las historias vitales, la cuantificación de la estructura social, la cuantificación del éxito reproductivo a lo largo de la vida, la posibilidad de replicar estimas de selección y la posibilidad de relacionar distintas generaciones (Clutton-Brock y Sheldon 2010). En el marco de los trabajos de parasitismo de cría, el estudio con hospedadores marcados individualmente, permitiría discriminar si los patrones de parasitismo y comportamentales que se encuentran a nivel poblacional, vienen determinados por diferencias entre individuos o porque los individuos modifican esos comportamientos a lo largo de su vida. El seguimiento continuado de individuos permitiría acometer cuestiones hasta ahora poco estudiadas en este sistema como el rol de los sexos en los distintos mecanismos de defensa, la consistencia de los distintos comportamientos a lo largo de la vida de esos individuos, el papel de la ontogenia sobre los comportamientos defensivos y es un escenario ideal para estimar el grado de plasticidad fenotípica (Clutton-Brock y Sheldon 2010). En este sentido, es fundamental poder determinar la consistencia de una determinada conducta en los individuos porque ésta marcará el potencial para que se produzca la evolución (Boake 1989).

La presente tesis pretende por tanto contribuir al conocimiento de la ecología y evolución de las interacciones parásito de cría-hospedador mediante un enfoque basado en individuos y usando el sistema críalo-urraca como modelo de estudio. Además, se combinará el enfoque individual con una aproximación longitudinal mediante el seguimiento continuado de una población hospedadora durante nueve años.

Los objetivos concretos que se abordan en esta tesis son:

1. Determinar qué factores están involucrados en la probabilidad de sufrir parasitismo dentro de una población hospedadora a lo largo de un período de tiempo largo en el que varía la presión de parasitismo, lo que nos permitirá estudiar si los patrones de parasitismo resultante son dependientes del contexto o si los parásitos usan una estrategia fija de selección (Capítulo 3).
2. Investigar el papel de factores abióticos sobre la dinámica temporal de la interacción urraca-críalo mediante el análisis de la relaciones entre condiciones climáticas y fenología. Específicamente se estudiará, a) si la fenología reproductiva del hospedador y el ajuste fenológico parásito-hospedador ha cambiado en la población durante los años de estudio, b) si esos cambios han estado asociados a cambios en las condiciones climáticas locales o regionales y, c) si la respuesta del hospedador (desde la perspectiva del individuo) puede contribuir a explicar patrones poblacionales de desajuste fenológico (Capítulo 4).
3. Identificar los patrones de parasitismo individual a lo largo de la vida de las hembras de urraca y las características fenotípicas y/o de los territorios que se relacionan con la probabilidad de sufrir parasitismo repetido (Capítulo 5).
4. Estudiar la existencia de correlaciones inter-individuales y las diferencias sexuales en comportamientos defensivos en el marco de la hipótesis de personalidad del hospedador trabajando con individuos marcados (Capítulo 6).
5. Identificar los patrones del comportamiento del rechazo de huevos a lo largo de la vida de hembras de urraca y determinar los factores que determinan la expulsión de huevos parásitos considerando factores como la percepción del riesgo de parasitismo (el parasitismo de la puesta y la presión de parasitismo en la población) y la edad relativa de la hembra (Capítulo 7).
6. Identificar el papel del parasitismo sobre los movimientos dispersivos (tanto natales como reproductivos) de la urraca (Capítulo 8).

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Capítulo 2

Metodología General

Metodología

Población de estudio

Este estudio se ha llevado a cabo durante los años 2005-2013 en La Calahorra, una población de la Hoya de Guadíx, provincia de Granada (37º10´N, 3º03´W). El área de estudio tiene una superficie de unos 12 km^2, y es un hábitat antropizado donde predomina el cultivo de cereal y de almendro, árbol en el que principalmente nidifican las urracas, aunque pueden usar otros árboles, como moreras, álamos y olivos, y arbustos como el rosal silvestre y la retama. El número de parejas reproductoras ha cambiado a lo largo de los años de estudio, pero de forma general suelen criar entre 60 y 90 parejas.

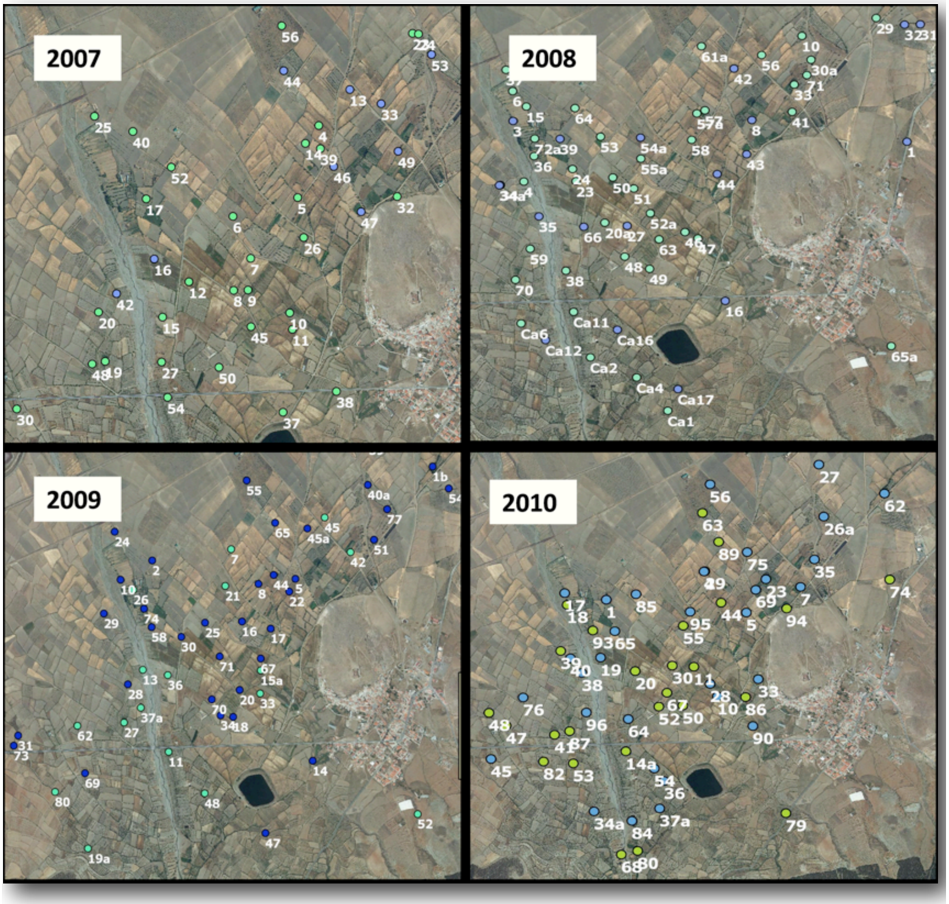

Figura 1. Distribución del parasitismo en la población de estudio. Los puntos azules corresponden a nidos parasitiados y los puntos verdes a nidos no parasitados.

La densidad de nidos de urraca en nuestra población es en promedio de 6.25 parejas/Km^2, distribuidas de forma no uniforme por el territorio. El porcentaje de nidos parasitados en la población de estudio ha variado durante los años de estudio (22.6% en 2005, 30.7% en 2006,15.9% en 2007, 25.4% en 2008, 65.6% en 2009, 50.7% en 2010,55.77% en 2011, 36.2% en 2012 y 18.7% en 2013).

La metodología general seguida para realizar el estudio se puede dividir en dos partes, la primera correspondiente al trabajo de campo y una segunda, al trabajo de laboratorio. En este capítulo se describe la metodología general seguida involucrada en todos los objetivos. Los aspectos metodológicos específicos necesarios para resolver cada uno de los objetivos se describen de forma detallada en cada capítulo.

Marcaje de los individuos

Los individuos reproductores fueron capturados durante el periodo de construcción de los nidos, mediante trampas en las que introducíamos una urraca viva en el interior como señuelo (Figura 2). Durante el periodo de estancia de pollo en el nido, usábamos redes japonesas ubicadas cerca de los nidos. Los individuos capturados fueron marcados con una combinación individual de anillas de colores. Al tiempo de anillarlos tomamos una muestra de sangre de cada individuo de la vena braquial que conservábamos en 1ml de etanol absoluto. Las muestras de sangre fueron usadas para la extracción del ADN para posteriormente sexar y genotipar a los individuos (ver análisis de parentesco). Para cada individuo tomamos también el peso con una pesola (con una precisión de 0.5g), la longitud del tarso con un calibre (precisión 0.01mm) y la longitud del ala y de la cola con una regla (precisión 1mm).

Seguimiento de los nidos

Los nidos de urraca fueron controlados desde principios de Marzo hasta principios de Julio cada año de estudio. Tras búsquedas exhaustivas, los nidos eran detectados y registrados con un GPS. Cada nido era observado con telescopio o prismáticos desde unos 100m desde un escondite o el coche, durante la fase de construcción del nido para detectar si los individuos de la pareja estaban marcados. Se visitaba el nido antes de la puesta cada 5 días y durante la puesta y la eclosión de los huevos, reducíamos el tiempo de visita a cada 2 o 3 días para determinar si el nido estaba parasitado y determinar la fecha de eclosión. Se consideró que el nido estaba parasitado si había al menos un huevo de críalo en el nido. Para cada nido registrábamos la fecha de puesta, el número de huevos de urraca y críalo, la fecha de eclosión y una foto de la puesta completa que nos permitiría conocer la apariencia de los huevos de cada hembra. Las fotografías fueron realizadas con una cámara digital Canon 350D colocando los huevos siempre sobre la misma cartulina gris con una carta de color en el borde y en condiciones de sombra. Todos los pollos fueron marcados en el nido. A los 15-18 días de la eclosión de los pollos de urraca, los marcamos con una combinación de anillas de colores y les tomamos una muestra de sangre siguiendo el mismo procedimiento que con los adultos. En los nidos parasitados, ya que los críalos eclosionan antes que las urracas y que la hembra de urraca deja de incubar a los dos o tres días de la eclosión del pollo de críalo, retirábamos los huevos de urraca con el objetivo de poder obtener muestras de los embriones y así poder realizar análisis de parentesco. Estos huevos se marcaban y congelaban hasta su posterior análisis.

Figura 2. Trampa usada para la captura de individuos adultos de urraca. El marcaje individual consistió en una combinación de cuatro anillas de colores.

Experimento de reconocimiento de huevos

El experimento de discriminación y rechazo de huevos por las urracas se llevó a cabo usando huevos modelo de escayola mezclada con pegamento blanco, realizados a partir de moldes de látex obtenidos de huevos reales de críalo y pintados con pintura acrílica, siguiendo la metodología clásica en este tipo de estudios (Soler y Møller 1990, Soler *et al.* 1995, Soler *et al.* 1999, Avilés *et al.* 2004). Los modelos fueron hechos para imitar los huevos de críalo tanto en apariencia, como en tamaño y masa, y, aunque al ojo humano el grado de parecido entre modelos y huevos de críalo es notable, los análisis espectrofotométricos revelan que los modelos no coinciden perfectamente con la coloración de los huevos reales de críalo (Figura 3).

El huevo modelo se introdujo cuando las urracas ya habían empezado a poner y se revisitó el nido cuando la puesta de la urraca había finalizado. Estudios previos en la población han mostrado que en el 75% de los nidos el rechazo de modelos ocurrió en las primeras 24 horas después de su introducción, y que después de 72 horas todas las urracas rechazadoras habían expulsado ya el modelo (Avilés *et al.* 2004). Si el modelo había desaparecido del nido, la respuesta al huevo modelo fue considerada como un rechazo pero si el modelo permaneció en el nido siendo incubado con la puesta del hospedador cuando revisitamos el nido se consideró que esos individuos habían aceptado el modelo. Ese procedimiento experimental se realizó de manera consistente durante todos los años y en la mayoría de los nidos. Sólo en los nidos donde se encontró la puesta ya completa de urraca y algunos que fueron intencionadamente elegidos como parte de otro experimento para mostrar el efecto del contacto con el huevo parásito durante la primera reproducción (ver capítulo 5), no fue introducido el huevo modelo.

Trabajo de laboratorio

Análisis genéticos

Extracción y amplificación de ADN

Las muestras usadas para la extracción de ADN provienen de sangre (adultos y pollos) y de los embriones de los huevos recogidos. En total se usaron 1084 muestras. La extracción de ADN se realiza mediante el método de precipitación con acetato amónico (adaptado de Bruford *et al.* 1998). Para el genotipeo de los individuos se amplificaron 29 loci microsatelite polimórficos por medio de la reacción en cadena de la polimerasa (PCR). Dos de ellos, Ppi1 y Ppi2, habían sido aislados previamente en urracas (Martinez *et al.* 1999); dos (Ase 18 y

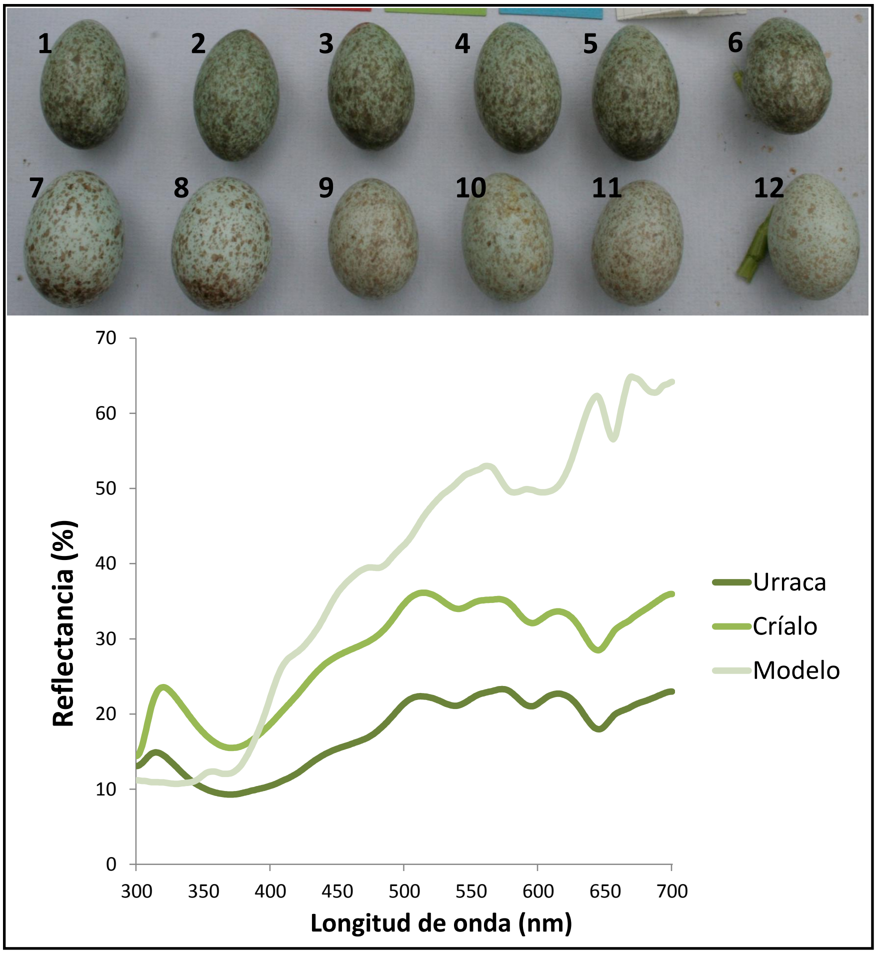

Figura 3. Puesta de urraca parasitada por críalo y con un huevo modelo de críalo. Los huevos del 1 al 6 son de urraca, del 7 al 11 son de críalo y el huevo número 12 es un huevo modelo. (T-test de diferencia de medias en longitud y anchura entre huevos de críalo y huevos modelo: Longitud: t = 1.79, df= 147, p= 0.074; anchura: t = 1.43, df= 147, p= 0.15). Las curvas representan el espectro de reflectancia de los tres tipos de huevos medidos con un espectrofotómetro.

Ase64) en las currucas de las Seychelles, *Acrocephalus sechellensis*, (Richardson *et al.* 2000); y Pocc1 fue aislado en *Phylloscopus occipitalis* (Bensch *et al.* 1997). Los detalles del resto de marcadores microsatélite (DKiD12, TG01-040, TG04- 004, TG13-017, Ppi4, MSLP4, Tgu05, Cpi7, Ppi11, Ppi12, Aar4, TG01-147, ZF09-005, ApCo46, PmaTGAn42, Ppi18, Ppi8, Sjr4, Tgu06, Tgu07, Cum02, TG01-000, TG04-061, Pij15) pueden ser encontrados en Martín-Gálvez *et al.* (2009) y Dawson *et al.* (2010) (Tabla 1). Las PCRs se realizaron con uno de los cebadores (primers) para cada marcador marcado con una tinción fluorescente. Para la amplificación se combinaron todos los marcadores en cuatro paquetes para PCRs multiplex, compuestos de 11 marcadores (set I), diez marcadores (set II), nueve marcadores (set III) y dos marcadores (set IV). Nueve de los marcadores fueron excluidos de los análisis porque no se encontraban en equilibrio Hardy-Weinberg y presentaron una alta frecuencia de alelos nulos (estimado siguiendo a Amos *et al.* 2001).

Genotipado

Los productos de amplificación de la PCR fueron procesados mediante electroforesis usando un secuenciador ABi Prism 377 DNA (Applied Biosystems Inc., Foster City, CA, U.S.A.). El análisis de fragmentos fue analizado con ABI Genemapper Software versión 3.7.

Sexado de los individuos

La urraca solo presenta un leve dimorfismo sexual en tamaño, que hace que la determinación del sexo basada en medidas corporales tenga cierto nivel de error (Birkhead 1991), por lo que determinamos con exactitud el sexo de cada individuo usando los marcadores específicos para el sexo P2/P8 (Griffiths *et al.* 1998) y Z-043B (D.A.Dawson, datos no publicados).

Análisis de parentesco

Al principio del estudio solo unos pocos animales estaban marcados individualmente, y aunque esa fracción fue creciendo año tras año conforme marcábamos nuevos individuos, para llevar a cabo el seguimiento individual de hembras no marcadas utilizamos un método indirecto basado en los análisis de parentesco y paternidad. Para estimar el grado de parentesco entre parejas de pollos de urraca usamos el programa ML-Relate (Kalinowski *et al.* 2006). ML-Relate calcula la estima de parentesco en base a máxima probabilidad para pares de individuos, permitiéndonos comparar relaciones putativas con alternativas (ej. hermanos completos versus medio hermanos o no relacionados). El procedimiento que seguimos fue usar los datos en bloques de dos años; por ejemplo, primero calculamos el parentesco de todos los pollos de 2006 y 2005, y después de eso el de todos los pollos de 2007 y 2005, y así con todos los pollos en todos los años. En cada caso, y para todos los nidos de cada año (cada nido o familia), todos los pollos de cada nido/familia fueron hermanos completos (con unas pocas excepciones de parejas de medio hermanos causados por paternidad extrapareja). Al comparar los pollos (familia) de cada nido concreto con los pollos de todos los nidos de otros años encontramos que en cada año solo los pollos de una familia como máximo se relacionaban con éstos como hermanos completos, considerando por lo tanto que aquellas dos familias o nidos correspondían a dos intentos de cría de la misma pareja de urracas, es decir, esos pollos de esos dos nidos en diferentes años, compartían los mismos padres (Figura 4). Por repetición de este procedimiento, comparamos todas las familias en pares de años y así pudimos asignar a un número de parejas de urraca sus intentos de cría en un número de años en el periodo de estudio. En algunos casos, un grupo de pollos o familia dada podía aparecer como medio hermano de otro grupo de pollos en otro año. Esto fue así cuando todos los pollos del nido focal presentaban mayor probabilidad de ser medio hermanos que hermanos completos o individuos no relacionados con los pollos de otro nido. En este caso consideramos que ambos nidos correspondían a intentos de cría de un individuo (macho o hembra) y dos parejas diferentes en los dos años. Estos casos no fueron usados en nuestros análisis excepto que tuviéramos identificado (mediante observación o análisis de paternidad, ver abajo) a uno de los adultos implicados.

Tabla 1. Detalles de la variabilidad de los loci usados en el estudio.

Locus	Referencias de los primer usados	N	N_{A}	H_{O}	H_{E}	P_{E}
Cpi7	(ZF09-012)*	78	9	0.782	0.829	0.526
MSLP4	Dawson DA unpublished data (MLSP4-ZEST)*	78	8	0.731	0.738	0.663
Ase18	Richardson *et al.* 2000*	78	15	0.795	0.883	0.394
TG01-040	Dawson *et al.* 2010*	78	4	0.410	0.482	0.879
TG04-004	Dawson *et al.* 2010*	78	13	0.859	0.897	0.362
TG13-017	Dawson *et al.* 2010*	78	7	0.667	0.684	0.736
DV946651 (Tgu05)	Slate *et al.* 2007*	78	4	0.436	0.447	0.899
ZF09-005	Dawson DA unpublished data, for primer sequences see Ball *et al.*, 2010*	78	5	0.397	0.481	0.883
ApCo46	Dawson DA unpublished data (ApCo46-ZEST)*	78	4	0.487	0.539	0.855
PmaTGAn42	Saladín *et al.* 2003	78	17	0.923	0.910	0.318
DV948303 (Tgu07)	Slate *et al.* 2007*	78	5	0.538	0.551	0.846
CK307697 (Tgu06)	Slate *et al.* 2007*	78	5	0.641	0.568	0.827
TG01-147	Dawson *et al.* 2010*	78	3	0.526	0.552	0.850
Ppi1	Martínez *et al.* 1999	78	10	0.769	0.811	0.550
Ppi008	Martín-Gálvez *et al.* 2009	78	5	0.551	0.594	0.816
Sjr4 (DQ179381)[16]	McDonald & Potts unpublished, See footnote§, Hansson B, *et al.* (2000)*	78	6	0.718	0.669	0.747
Ppi012	Martín-Gálvez *et al.* 2009	78	12	0.885	0.885	0.392
Ppi011	Martín-Gálvez *et al.* 2009	78	28	0.769	0.950	0.203
Ppi2	Martínez *et al.* 1999	78	16	0.910	0.897	0.358
Ase64	Richardson *et al.* 2000*	78	14	0.795	0.906	0.337
Total			9.5		0.714	<0.001

(*) Para los detalles de las secuencias de los cebadores y la evaluación de la caracterización de los loci en urraca ver Martín-Gálvez et al (2009). La tabla muestra el número de alelos encontrados en las urracas adultas (N_{A}), la heterocigosidad observada (H_{O}) y esperada (H_{E}) y la probabilidad media de no exclusión (P_{E}) para cada locus (calculado usando CERVUS). § El locus *Sjr4* fue aislado de *Aphelocoma coerulescens* por McDonald y Potts en 1994, pero no fue publicado. Usamos la secuencia disponible para este locus, el cual fue obtenido de *Acrocephalus arundinaceus* (Dawson *et al.* 2007) y la secuencias del cebador como se detalla en Hansson *et al.* (2000).

Análisis de paternidad

Para los análisis de paternidad y maternidad usamos el programa CERVUS 3.0.3 (Kalinowski *et al.* 2007) con dos objetivos: el primero era comprobar si los adultos observados en el los nidos eran realmente los padres de los pollos de esos nidos, y segundo, asignar adultos capturados en un año dado a intentos de cría en otros años. Seguimos ese proceso porque las urracas capturadas en un año dado pueden haber estado criando previamente en la zona y por lo tanto sus nidos anteriores pueden haber sido encontrados y estudiados.

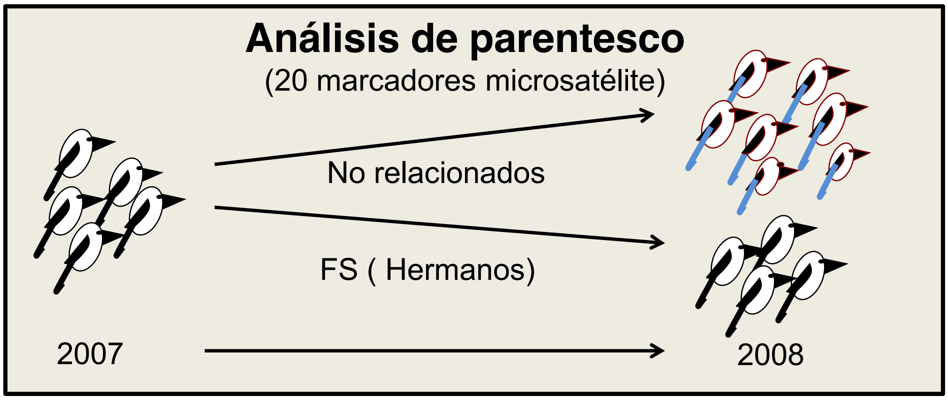

Figura 4. Un ejemplo de nido/familia coincidente siguiendo los métodos explicados en el texto. Todos los pollos del nido 2007 fueron encontrados hermanos completos de los pollos de un nido en 2008, lo que indica que comparten a la misma pareja de urracas.

Los análisis de paternidad se llevaron a cabo con los genotipos de los adultos marcados y observados en la población como padres potenciales de los pollos de cada año. CERVUS asignó un par de padres más probables a cada pollo pero sólo consideramos la asignación cuando los LOD (el logaritmo natural de la relación total de probabilidad) fueron positivos, ya que esto significa que el padre candidato tiene mayor probabilidad de ser el padre verdadero que de no serlo, y Delta (definida como la diferencia entre el LOD del candidato más probable y el segundo más probable) fue significativo (Kalinowski *et al.* 2007).

Una vez obtenidos los padres más probables a través de la asignación genética, comparamos estos datos con las observaciones en campo en aquellos nidos donde al menos un individuo estaba marcado. Todas las hembras que fueron observadas criando en un nido dado (construyendo, poniendo, incubando o alimentando a los pollos) fueron asignadas como madres de todos los pollos en el nido. De modo similar, todos los machos observados criando en un nido, fueron asignados como padres en esos nidos, aunque a veces no de todos los pollos de ese nido, probablemente debido a paternidad extra pareja. En estos casos (10%-20% de los nidos dependiendo de los años) al menos la mitad de los pollos fueron asignados al padre social. Cuando no hubo observaciones de campo para los padres o madres asignados a un nido dado usamos los mismos criterios: consideramos una hembra como madre en ese nido cuando fue asignada como la madre de todos los pollos en ese nido con un LOD positivo y significativo, y consideramos a un macho como padre de un nido particular cuando fue asignado al menos como padre de la mitad de los pollos en ese nido. Cuando un macho fue asignado por CERVUS a unos pocos, pero no la mayoría de los pollos de un nido, consideramos que podría ser un caso de paternidad extra pareja y por tanto no consideramos ese nido como un intento de cría para ese macho en cuestión.

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Capítulo 3

Habitat features and nest availability explain the probability of brood parasitism in magpies (*Pica pica*)

Habitat features and nest availability explain the probability of brood parasitism in Magpies (*Pica pica*)

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Abstract

Most studies that have investigated the patterns of host´s nest use by avian brood parasites have suggested non-random patterns of parasitism. These patterns may arise from active parasite selection of individual host`s traits enhancing parasite fitness or when the probability of parasitism was spatially and/or temporally clumped within host populations. Our main goal in this study is to ascertain which are the factors related with probability of brood parasitism across the years in a long-term study with oscillating pressures of parasitism. We have found that probability of parasitism of a given magpie nest was related to the level of parasitism in the population, the proportion of parasitized nests around it, its laying date and characteristics of the habitat surrounding the nest. On the other hand, the intensity of parasitism, characterized as the number of cuckoo eggs laid in a given parasitized nest, was high in years with high parasitism rate in the population, and when number of available nests around the focal nest was low. These results would suggest the existence of a non-random pattern of nest use by great spotted cuckoos in our magpie population, but do not clearly suggest active host selection. Results do not support that cuckoos select magpies based on their phenotypic traits revealing parental quality, such as nest size or early laying dates, however, they show some preference to parasitize magpie nests in habitats with particular features. Globally, our results would suggest that the patterns of magpie parasitism by great spotted cuckoos may fit a probabilistic process based on host nest availability and parasite abundance.

Introduction

Interactions between brood parasites and their hosts provide a good example of coevolution (review in Krüger 2007, Soler 2013). Obligate brood parasites have to search for host nests where to lay their eggs. When several host nests are available, parasites may select among them (Rothstein 1990). Because obligate brood parasites entirely depend on their hosts to breed, their fitness must be related to their efficiency in finding and/or selecting the best possible host nests (Parejo & Avilés 2007).

Temporal and spatial patterns of parasitism may reveal important information about ecological and evolutionary dynamics of the interaction between parasites and its hosts, including the occurrence and extent of nest searching and selection strategies by cuckoo parasites (ex: Thorogood & Davies 2013). Most studies that have investigated the patterns of hosts' nest use by brood parasites have found evidences of non-random host use. Non-random host use may arise from active parasite selection of individual hosts traits revealing parental quality (Parejo & Avilés 2007). For instance, great spotted cuckoos *Clamator glandarius* in one Spanish magpie population laid more eggs in relatively bigger magpie nests among the available ones at the same date (Soler *et al.* 1995). Cuckoo nestlings also had a higher fledging success in naturally parasitized nests than in non-parasitized nests in which cuckoo eggs were introduced, which may globally suggest that cuckoos were searching for good parents in that population (Soler *et al.* 1995, see however Soler *et al.* 2014). Also several sources of evidence suggests that common cuckoos (*Cuculus canorus*) may actively select nests within a host population to match the egg appearance of a particular host clutch and to reduce the chance of being refused (Avilés *et al.* 2006, Honza *et al.* 2014). Other studies show non-random patterns of parasitism with regard to host age or experience (Lotem *et al.* 1992, Brooker & Brooker 1996; Smith & Arcese 1984; Sedgwick & Iko 1997, Langmore & Kilner 2007). Even recent studies in which individual hosts were monitored in different breeding seasons have shown that some individuals are more likely to be parasitized through their lives than others (Hauber *et al.* 2004, Hoover *et al.* 2006, Hoover & Hauber 2007, Molina-Morales *et al.* 2013).

Non-random host use may also arise if the probability of cuckoo parasitism was somehow spatially and/or temporally clumped within host populations. For instance a number of studies have shown that the main factor affecting parasitism rates in some common cuckoo and cowbird hosts is the availability of perch sites close to host nests (Øien *et al.* 1996, Moskat & Honza 2000, Hauber 2001, Antonov 2007, Begum 2011). Indeed, the spatial habitat structure hypothesis posed by Roskaft *et al.* (2002) argues that the dynamic of the interaction between cuckoos and their host may speed up for host populations and or species breeding near trees in woodland or its edges. Probability of parasitism may also change over time, and, indeed in some host species females with delayed laying dates experience a higher probability of parasitism (Øien *et al.* 1996, Martínez *et al.* 1996, Strausburger 1998, Fiorini *et al.* 2009, Begum 2011); however, there are also examples where no such relationship has been detected (Hauber 2001, Hoover *et al.* 2006, Antonov *et al.* 2007, Welbergen & Davies 2009, Jélinek *et al.* 2013). Laying date may reflect host quality, but it is also a variable that relates to the temporal availability of host nests, since typically the number of nests available for parasitism change over the course of the season. In fact laying date may also relate with other attributes of the host population such as density and synchrony of host nests that may have an influence in the probability of parasitism through their effect in the availability of host nests in the adequate moment to be parasitized, that is, during host egg laying. Host density has been described to be one of the main factors positively related to probability of cuckoo parasitism (Barber & Martin 1977, Stokke *et al.* 2007). Most natural populations experience some degree of social and/or spatial structure (Thompson 2006), and therefore, it is expected that one host will be more likely parasitized if its close neighbors or individuals within its social group are so (Débarre *et al.* 2012). Recently, it has been shown that magpie nests close to each other experienced a similar probability of parasitism (Soler *et al.* 2013). However it has also been described that hosts breeding synchronously and/or in dense plots may experience an indirect advantage against brood parasitism through a dilution effect (Øien *et al.* 1996, Martínez *et al.* 1996, Soler *et al.* 1998, Jélinek *et al.* 2013; Begum 2011). In purity, the pattern of parasitism in relation to spatial and/or temporal proximity described in these studies can be considered to be independent of individual hosts' parental qualities, and thus to rather revealing a probabilistic process related to the availability of host nests. An example of a probabilistic pattern of parasitism has been described in shiny cowbirds (*Molothrus bonariensis*) following a so-called shotgun strategy: all potential host nests that are located are used by female parasites irrespective of host quality (Kattan 1997, Rothstein & Robinson 1998). *Vidua* finches seem to use some host nests more likely than expected by chance, although their laying strategy was considered as "scatter-laying" (Payne 1977), and in the brown-headed cowbird (*Molothurs ater*) the laying strategy has been considered random (Orians *et al.* 1989).

Some brood parasites such as cowbirds (*Molothrus* sp.), viduine finches (*Vidua* sp), and some cuckoos (*Clamator* sp) may lay more than one egg per host nest, what is called multiparasitism (Rothstein 1990). When it happens multiparasitism can be due to a female laying several eggs per nest or several females laying one or more eggs in the same nest (see for example Martínez *et al.* 1998). Host selection by parasites may be also studied from the point of view of multiparasitism, since the preferred attributes in a nest or host pair should lead to multiparasitism of that nest, and, on the other hand, if temporal and/or spatial availability of host nests explains the likelihood of parasitism, it should also explain multiparasitism.

So, most published studies suggest non-random patterns of parasitism, but this can reflect either strategic choices by parasites (that is, choosing hosts providing high fitness to parasites) or it can be a consequence of hosts and/or nests characteristics that make them more exposed to parasitism in a given place and/or time. Both parasites and hosts populations vary in relative density, maybe as a result of ecological or environmental heterogeneity (Thompson 2005). This leads to heterogeneity in parasitism risk in space and time, which in turn, may generate different selection strategies or patterns of host nests use by brood parasites. When the density of hosts is high so that there is a large availability of host nests, an active selection strategy by parasites could become an important adaptive aspect of parasitic laying strategies because there are nests of different quality to choose among (Soler *et al.* 1995, Hauber 2001, Parejo & Avilés 2007). However, in habitats with a relative low nest availability due to low host density, high temporal synchrony and/or high number of parasites that lead to intraspecific competition for host nests, the best strategy may be parasitize all potential host nests that are located, which would lead to a probabilistic pattern of host nest use, likely to be random regarding host's quality.

The differences found in previous studies regarding the factors explaining host use by brood parasites may be due to differences between host-parasite systems or populations, or to inconsistencies in the use of host nests by parasites under changing conditions. In any case, previous papers have neglected the influence of heterogeneity in environmental conditions on the pattern of host use by parasites. Therefore our main goal in this study is to ascertain which are the factors related with probability of brood parasitism across the years in a long-term study with oscillating pressures of parasitism. We have used as study system the great spotted cuckoo (*Clamator glandarius*) and its main host in Europe, the magpie (*Pica pica*), in a population in southern Spain (La Calahorra, Granada). In this population host´s density has been very similar during the years but parasitism rate increased during the study, reaching even up to 65%, thus providing us an ideal scenario for testing for the first time if patterns of host use might depend on level of cuckoo parasitism at the population.

The factors proposed to affect patterns of parasitism by great spotted cuckoo on magpies include the following variables: nest volume, laying date, nest availability, habitat characteristics and parasite abundance (see for example Soler *et al.* 1995, Martínez *et al.* 1996, Soler & Soler 2000, Molina-Morales *et al.* 2013). We make the following predictions regarding patterns of host nest use:

(a) if active host selection occurs we expect that the probability of parasitism and the intensity of parasitism should be related with host traits independently of parasitism pressure, specifically that parasites should select phenotypic traits indicating host quality and therefore, larger nests (see Soler *et al.* 1995) and early breeders (time of breeding in magpie is negatively related with territory quality Birkhead 1991, Goodburn 1991),

(b) if host use by parasites was a probabilistic process consequence of habitat features and spatio-temporal availability of host nests we may expect an association between some habitat features and variables reflecting temporal and spatial distribution of nests and nest host use and intensity of parasitism. In particular we expect that nests in wooded areas suffer less from parasitism that nests in open areas and nests located further away from feeding parasites sites may escape or avoid parasitism (see Molina-Morales *et al.* 2013). If parasites use some patches better than others we also expect a contagious distribution of parasitism, so that, a larger proportion of the nests surrounding a particular or focal nest should be parasitized and multiply parasitized if the focal nest is parasitized than if it is not parasitized.

Methods

Study area and system

The study was conducted in La Calahorra (37º 10´ N, 3º 03´ W, Hoya de Guadix, Granada, Southern Spain) during the years 2005-2012. This is a patchy area where groves of almond trees (*Prunus dulcis*), in which magpies preferentially build their nests, are very common. Magpies are territorial, sedentary, and socially monogamous long-lived passerines (Birkhead 1991).

In our study area magpies lay one clutch during April-May, and are the main host of the great spotted cuckoo. Cuckoo parasitism severely reduces magpie reproductive success through early hatching and effective competition for parental food delivery of cuckoo nestlings (see for example Soler *et al.* 1996, Soler *et al.* 1997), and it has selected for host recognition and rejection of cuckoo eggs (Soler & Soler 2000). The percentage of parasitized nests in our population (i.e. parasitism rate) varied between years (22.58% in 2005, 30.77% in 2006, 15.9% in 2007, 25.4% in 2008, 65.6% in 2009, 50.7% in 2010, 55.77% in 2011, 35.6% in 2012).

Nest monitoring and individual characteristics of female magpie hosts and of the territories

Magpie nests were monitored from 1 March to the beginning of July each breeding season. Nests were found by careful inspection of all trees in the area, and GPS positioned. Nests were visited at 5 days intervals, although during egg laying and hatching the nests were visited every 2-3 days. For each nest we recorded the following data:

(a) Laying date, estimated as the number of days from the first of April. We used this variable as a continuos predictor.

(b) Nest volume. The size of nests was estimated using a measuring tape (precision 1 cm); we measured height and width and calculated nest volume using the ellipsoid formula $4/3(\Pi \text{ x } a \text{ x } (b/2)^2) / 1000$ (in litres), where a is the height of the ellipsoid nest and b is half of the nest width.

(c) Host density. Number of nests available for parasitism within 500 meters of each nest. We consider available nests those whose laying date is between seven days before and after the laying date of each nest (first egg laid) because the average size of the clutch is 6.56 ± 1.21(SD).

(d) Proportion of parasitized nests around 500m from each nest, calculated as the proportion of those available nests following the same criteria used for host density, that were actually parasitized by great spotted cuckoos.

(e) Spatial information. We used GIS software ArcGIS 9.3 version (ESRI 2008) to obtain environmental data based on aerial photographs and 2003 Vegetation Cover and Land Use Databases for the Province of Granada that were freely available from Junta de Andalucía (VV.AA. 2003, Junta de Andalucía 2006). We recorded the following information regarding the spatial situation of each nest: (1) distance in meters to the closest pine forests as an estimate of distance to great spotted cuckoo`s feeding site as great spotted cuckoos feed almost exclusively on Pine Processionary, *Thaumetopoea pityocampa* (Soler 2012), (2) distance to the closest track, (3) distance to the motorway, (4) distance to the nearest village, (5) percentage of wooded surface within 100 m around the nest, and (6) percentage of herbaceous crop within 100 m around the nest.

Statistical analyses

All analyses were conducted in SAS, version 9.2. and Statistica.

We have used Principal Components Analysis (PCA) in order to transform several correlated habitat variables into a few orthogonal variables (the principal components). We obtained three PCA factors with eigenvalues >1. The first principal component (PC1) explained 31.8% of the habitat characteristics and had negative loadings for woody surface (factor loading: -0.773), distance to the motorway (factor loading: -0.774) and the nearest village (factor loading: -0.772). That represents a gradation in the area surrounding the nest from clear to wooded spaces (Table 1). PC2 component explained 20.2% of the variance and was negatively related with distance to the pine forests (factor loading:-0.738) and closed tracks (-0.621). And PC3 component explained 16.5% of the variance explained was negatively related with percentage of herbs crops (factor loading:-0.849) (Table 1).

We constructed a factorial generalized linear mixed model (GLMM hereafter) to determine which factors explained probability of parasitism in a nest. Therefore, parasitism was entered as a binary dependent variable, and the variables host density, proportion of parasitized nests, the scores from the three principal components derived from the PCA on spatial variables, laying date, nest volume, and parasitism rate in the population each year were entered as fixed predictors. Year of study was considered as a random intercept to account for the fact that different years could be not statistically independent. We also included the interaction between parasitism rate and the rest of the variables as fixed predictor to test the hypothesis that the predictors are conditioned by parasitism rates and therefore the effect of predictors may change under different parasitism pressures. We first fitted the model with all possible predictors, the interactions between parasitism rate and all the predictors and the random term. The variable year of study as random intercept was estimated to be zero and therefore was removed from the model. We thus rerun the model excluding year of study. Model simplification was performed following backward stepwise elimination of nonsignificant terms from the initial model. All continuous predictors were centred by substracting their mean value to each value. This analysis provides a direct estimate of which are the variables that explain probability of parasitism and also whether the association between probability of parasitism and the predictors depends on the parasitism rate in the population (interaction between variables and parasitism rate).

Table 1. Results of the PCA on spatial variables

Variable	PC1	PC2	PC3
Wooded surface	**-0.77**	-0.02	0.19
Distance to closest track	0.23	**-0.62**	0.11
Distance to motorway	**-0.77**	-0.42	-0.19
Distance to the village	**-0.77**	0.09	-0.20
Distance to the closest pine forest	0.23	**-0.74**	-0.38
Percentage of herb growing	0.10	0.31	**-0.85**
% of variance	31.8	20.2	16.5

Factor loadings for the three first axes of a principal component analysis on spatial variables. Loadings in bold indicate the most important factors (score > |0.60|). Percentage of variance explained by each axis is also shown.

In a second analysis we studied whether intensity of parasitism, estimated as number of cuckoo eggs per parasitized nests, was determined by the same variables explaining probability of parasitism. We performed a model (GLMM) in which number of cuckoo eggs per nest was the dependent variable, with a poisson error distribution and log link function. Year of study was considered as a random effect to account for the fact that nests in different years could be not statistically independent. The same variables involved in the analysis of probability of parasitism were introduced as fixed predictors and we followed the same backward stepwise procedure. In the view that year did not explain significantly differences between years in the number of cuckoo eggs per nest, year was removed from the analysis.

Results

Factors influencing probability of parasitism

The probability of a nest of being parasitized by great spotted cuckoos increased with parasitism rate in the population and over the course of the breeding season, with later breeders suffering higher probability of parasitism (Table 2, Appendix Table 1, Fig. 1).

Table 2. Results of final models testing for the effect of density measured as number of nests surrounded in 500m, proportion of parasitized nests around 500m (PF), laying date, spatial variables (PC1) and parasitism rate in the population (PR) on probability of parasitism as a binary dependent variable (logit link function, n= 324 nests) and on number of cuckoo eggs in magpie nests (poisson distribution and log link function, n= 129 parasitized nests). Random effect was zero and removed from the analyses. Non-significant terms were removed following a backward procedure.

Probability of parasitism					
Fixed effects	*β* (95% CI)	SE	*F*	df	*P*
Intercept	**-1.76(-2.41 - -1.11)**	**0.330**			
PF (500m)	**1.17(0.51- 1.83)**	**0.34**	**12.06**	**1,368**	**0.0006**
Laying date	**0.03(0.01-0.05)**	**0.01**	**7.11**	**1,368**	**0.0080**
PR	**0.03(0.02-0.05)**	**0.017**	**20.04**	**1,368**	**<0.0001**
PC1	**0.27(0.04-0.49)**	**0.12**	**5.46**	**1,368**	**0.0200**
Intensity of parasitism					
Fixed effects	*β* (95% CI)	SE	*F*	df	*P*
Intercept	**0.15 (-0.22 – 0.52)**	**0.19**			
PR	**0.01 (0.001 – 0.02)**	**0.003**	**6.12**	**1,152**	**0.014**
Density (500m)	**-0.081 (-0.14 – -0.02)**	**0.03**	**7.83**	**1,152**	**0.005**

Moreover, the proportion of parasitized nests among those surrounding a focal nest was significantly higher for parasitized nest than for non-parasitized nests (Table 2, Fig. 1). Parasitized nests were located in areas with larger percentage of wooded surface, and farther away from the village and motorway than non-parasitized ones (Table 2, Fig. 1). No such relationship was found for the other two other components of habitat or spatial features. Parasitized and non-parasitized nests did not differ in their volume nor in density of available nest surrounding them (Appendix Table 1). Emerging patterns of host use in our population did not differ with the level of parasitism as the interaction between parasitism rate and any of the considered predictors was not significant (Appendix Table 1).

Regarding intensity of parasitism, we found that parasitized nests were more frequently multiply parasitized in years with high parasitism rate in the population, and when number of available nests around the focal nest was low (Table 1, Fig. 2). However, the interaction between parasitism rate and any of the predictors was not significant (Appendix Table 1).

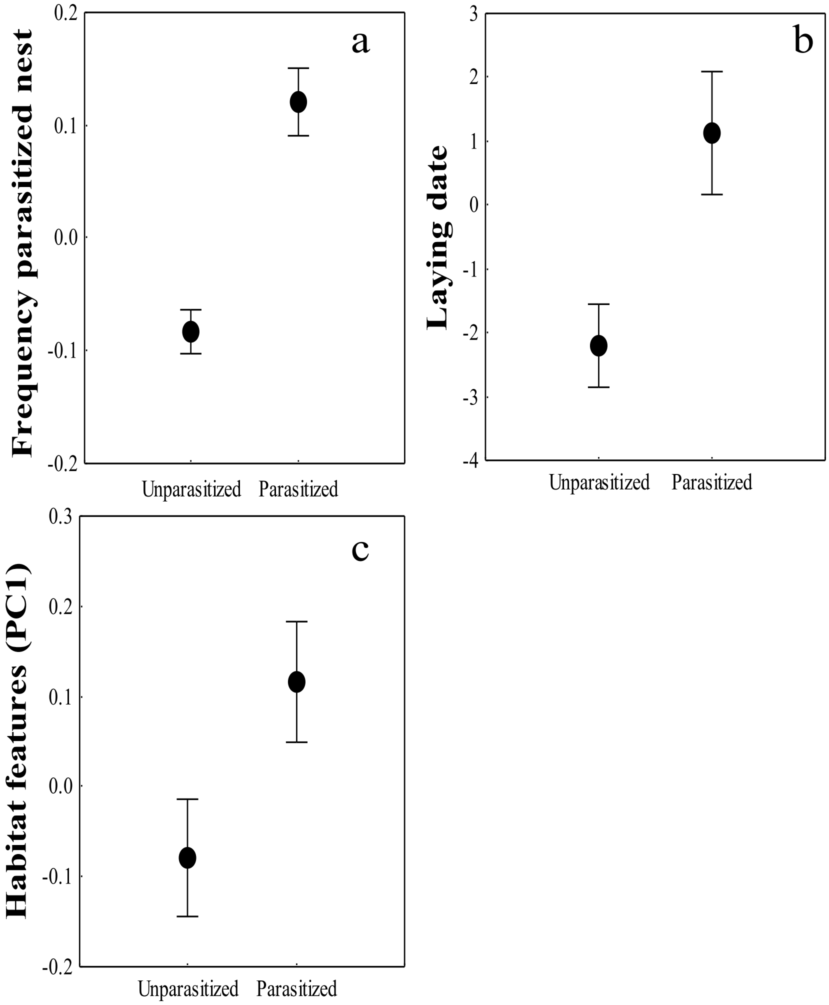

Figure 1. Relationship between probability of parasitism and frequency of parasitized nests (centred)(a), laying date (centred)(b) and habitat characteristics (PC1) (c) in magpies. Mean (SE).

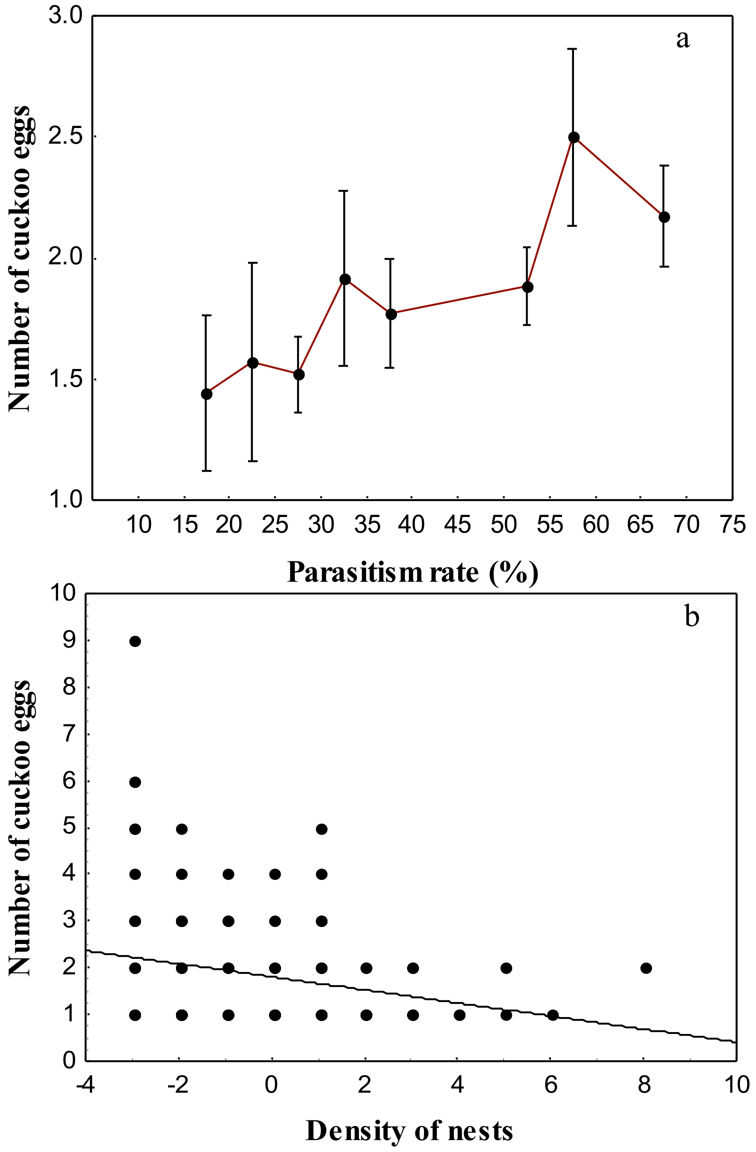

Figure 2. Relationship between intensity of parasitism (number of cuckoo eggs) and (a) parasitism rate in the population and (b) number of nests surrounding the focal nest.

Discussion

In our long term study we have found that the probability of parasitism of a given magpie nest was related to the parasitism pressure in the population that year, the proportion of parasitized nests around it, its laying date and characteristics of the habitat surrounding it. On the other hand, the intensity of parasitism, characterized as the number of cuckoo eggs laid in a given parasitized nest, was determined by the local density of magpies, that is, the number of magpie nest available for parasitism in the surroundings of the focal nest, and parasitism rate in the population that year. These results would suggest the existence of a non-random pattern of nest use by great spotted cuckoos in our magpie population, but do not clearly suggest active host selection. On one side, results do not support that cuckoos select magpies based on their phenotypic traits revealing parental quality, such as nest size or early laying dates; on the other side they show some preference to parasitize magpie nests in habitats with particular features. Our results also suggest in several ways that the patterns of magpie parasitism by great spotted cuckoos may fit a probabilistic process based on host availability, since laying date and parasitism rate in the population are also factors determining the likelihood of a nest of being parasitized.

Nest likelihood of being parasitized and intensity of parasitism increased with the parasitism rate in the population. Previous studies have shown that parasitism rate is closely related to parasite`s density in our population (see Soler *et al.* 1998). So, an increase in the number of cuckoo females laying eggs would translate into a higher parasitism rate in the population, and an increase in the probability of being parasitized and multiply parasitized for any particular nest irrespective of their characteristics. Although we cannot definitively rule out that cuckoo female may be actively selecting magpie nests, this relationship is clearly compatible with a probabilistic process in which the higher the number of females laying, the higher the probability of getting parasitized. In agreement with this view we also found a clear seasonal increase in probability of parasitism, which contradicts the prediction of selection of higher quality magpie pairs, which breed at the beginning of the season (Birkhead 1991). So, the effect of laying date on probability and intensity of parasitism is likely related with changes in host availability throughout the season rather than due to active host selection by cuckoos. Egg laying peaks at the middle of the season and the number of active or available nests decreases gradually until the end of the season (Molina-Morales *et al.*

2013), so that if the number of cuckoo females laying remains the same, and so parasitism pressure, the likelihood of being parasitized should increase. Again, this result may be interpreted as compatible with host selection, because cuckoo females might be selecting those traits they prefer, such as larger nests, among those available, which in fact is what previous papers suggest (Soler *et al.*. 1995). Our analyses, however, take into account the effect of laying date and thus nest availability and do not support host selection based on nest size. A plausible scenario would be that at the beginning of the season cuckoos could select among a large number of available nests but, and the end of the season the few remaining nests are more likely to be parasitized independently of their characteristics.

We found evidence for clumped probability of parasitism in our population because those magpie nests that were parasitized had a significantly higher proportion of nests around them also parasitized than non parasitized ones. This suggests that either cuckoo females prefer some areas to others, and all the nests in those areas have higher probability of being parasitized, or that magpie nests with the features preferred by cuckoos clump together in particular areas. This second possibility, however, seems unlikely because nest size was not related to probability and/or intensity of parasitism, thus suggesting that nest size was not clumped as it is cuckoo parasitism in our population. We can discard the heterospecific attraction hypothesis (Banks and Martin 2001) that states that cuckoos would be attracted to areas with high magpie density or simultaneous breeding because these areas would be more easily detected by prospecting cuckoos, since the density of nests surrounding each nest does not explain its probability of parasitism. Indeed, what we have found is that intensity of cuckoo parasitism was high when density of host nests around a parasitized nest was low; again suggesting that the emerging patterns of host use are merely due to a probabilistic process based on host nest availability.

The structure of the habitat is another factor that may explain probability and intensity of parasitism (Roskaft *et al.* 2002). Our results show significant differences in the characteristics of the habitat surrounding parasitized and non-parasitized nests. Non-parasitized nests were in areas with larger percentage of wooded surface and smaller percentage of herbaceous crops whereas parasitized magpies bred in clearer habitats with larger percentage of cereal crops. A recent study at the individual level in the same magpie population showed the same trend (Molina-Morales 2013). This result could be explained in terms of nest concealment because nests in more forested areas are more difficult to be detected than nests in open areas, or because cuckoo females actively selected open areas because finding nests was easier, which could be interpreted as some form of active choice.

Despite our long data set we did not find any relationship between probability of parasitism and nest size. Nest volume has been described as a factor explaining the probability of parasitism of magpies both at the population (Soler *et al.* 1995) and individual level (Molina-Morales *et al.* 2013), although a recent experimental study in which nest size was manipulated to study cuckoo` responses in nest choice has failed to find a relationship between nest size and probability of parasitism (Soler *et al.* 2014). There are several possible explanations for this. One of them is that nest size affects the probability of parasitism only if there is enough variance in nest size, so that differences in size reflect differences in magpie pair quality. If nests are very similar in size cuckoos may be unable to select between different nests based on this criterium. Because nest size varies between nearby populations and years, this variation might explain discrepancies between studies. Another possibility is that nest size explains probability of parasitism only in interaction with other variables such as laying date. In an individual-based study in our population, females that were never parasitized built larger nests than parasitized females at the beginning of the season but smaller nests than those of parasitized females later in the season (Molina-Morales *et al.* 2013). The use of large nests in the middle of the season can be explained in terms of conspicuity (large nests are easier to find when there are more nests available and tree leaves are fully grown, see Molina-Morales *et al.* 2013). So, all join together, claiming evidence of host choice based in nest size revealing parental abilities still requires confirmation in this system.

Intensity of parasitism was positively related with the level of parasitism in the population and negatively with magpie nest density around each parasitized nest. This suggest a probabilistic pattern where, for a given number of cuckoo females laying eggs, the smaller the number of host nests available, the higher the probability that a nest receives more than one cuckoo egg. In the only published study where the identity of laying females was known (Martinez *et al.* 1998), multiple parasitism due to several females was due to a shortage of nest availability, whereas multiple parasitism due to a single female was not related to nest availability, suggesting that some females seem to prefer to use twice (or more) the same nest despite being others available, and thus some form of host selection. At this point we would like also to point out that our approach neglects the role of heterogeneity in the behavior of female parasites as we cannot ascribe cuckoo eggs to cuckoo females in this study. Some females may be more willing to select good quality host pairs and others more likely to use nests independently of their quality and that may vary with environmental conditions such as host nest availability. That would make more difficult to interpret population patterns of host nest use.

In conclusion, the results of this study suggest the existence of a consistent pattern of magpie nest use by great spotted cuckoos irrespective of the level of parasitism at the population. Our results suggest that the pattern of nest use is not random but is related more to some characteristic of the habitat and nest availability than to selection by cuckoos of hosts of particular traits reflecting high parenting ability. Despite this we believe that the term “host selection” should not be used in these cases, but left to those where parasites do actively select particular hosts of preferred characteristics because that choice enhances their fitness.

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Appendix

Table 1. Results of models testing for the effect of density measured as number of nests surrounded in 500m, frequency of parasitized nests surrounded in 500m, laying date, egg volume, spatial variables (PC1, PC2 and PC3), nest volume on probability of parasitism as a binary dependent variable (GLMM) and on intensity of parasitism as number of cuckoo eggs in magpie nests (poisson distribution and log link function, n= 129). Random effect was cero and removed from the analyses. Non-significant terms were removed following a backward procedure. Terms included in final models are highlighted in bold.

Probability of parasitism *Fixed effects*	β (95% CI)	SE	*F*	df	*P*
Intercept	**-1.7570(-2.407 - -1.106)**	**0.330**			
PF (500m)	**1.172(0.508- 1.836)**	**0.337**	**12.06**	**1,368**	**0.0006**
Laying date	**0.028(0.0075-0.0498)**	**0.010**	**7.11**	**1,368**	**0.0080**
PR	**0.034(0.019-0.049)**	**0.007**	**20.04**	**1,368**	**<0.0001**
PC1	**0.269(0.042-0.496)**	**0.115**	**5.46**	**1,368**	**0.0200**
Density (500m)	-0.055(-0.154 – 0.0437)	0.050	1.21	1,367	0.272
Nest volume	0.0011(-0.0018- 0.0041)	0.0015	0.55	1,317	0.457
PC2	-0.030(-0.285- 0.224)	0.129	0.05	1,316	0.814
PC3	-0.023(-0.281-0.235)	0.131	0.03	1,315	0.859
Nest volume*PR	-0.0001(-0.0003-0.00004)	0.00009	2.28	1,314	0.132
PC3*PR	0.019(-0.010 – 0.048)	0.015	1.59	1,313	0.207
PC2*PR	-0.013(-0.034-0.007)	0.01	1.66	1,312	0.198
PC1*PR	-0.0012(-0.030-0.0056)	0.009	1.84	1,311	0.176
Laying date*PR	-0.0009(-0.0024-0.0005)	0.0007	1.41	1,310	0.236
Density (500m)*PR	0.0037(-0.004-0.011)	0.004	0.82	1,309	0.365
PF (500m)*PR	-0.0078(-0.057-0.042)	0.025	0.09	1,308	0.76
Cuckoo eggs *Fixed effects*					
Term	β (95% CI)	SE	*F*	df	*P*
Intercept	**0.147 (-0.222 – 0.516)**	**0.187**			
PR	**0.009 (0.001 – 0.016)**	**0.003**	**6.12**	**1,152**	**0.014**
Density (500m)	**-0.081 (-0.138 – -0.023)**	**0.029**	**7.83**	**1,152**	**0.005**
PC1	0.079 (-0.051 – 0.209)	0.066	1.44	1,147	0.231
PF (500m)	-0.235 (-0.588 – 0.087)	0.163	2.08	1,142	0.151

PC2	0.067 (-0.048 – 0.183)	0.058	1,33	1,141	0.249
Laying date	0.002 (-0.007 – 0.013)	0.005	0.29	1,140	0.589
Nest volume	-0.0008 (-0.002 – 0.0008)	0.000	0.96	1,121	0.329
PC3	0.036 (-0.109 – 0.182)	0.073	0.24	1,120	0.621
PC3*PR	0.007 (-0.006 – 0.021)	0.007	1.02	1,119	0.313
PC2*PR	-0.001 (-0.010 – 0.006)	0.004	0.18	1,118	0.672
Nest volume*PR	0.00002 (-0.0001 – 0.00009)	0.000	0.08	1,117	0.782
PF (500m)*PR	-0.002 (-0.027 – 0.023)	0.012	0.02	1,116	0.876
Density (500m)*PR	0.0003 (-0.004 – 0.004)	0.002	0.03	1,115	0.866
Laying date*PR	0.00003 (-0.0007 – 0.0008)	0.000	0.01	1,114	0.936
PC1*PR	-0.0002 (-0.012 – 0.011)	0.006	0.00	1,113	0.093

Capítulo 4

Climatic effects and phenological mismatch in cuckoo-host interactions: a role for host phenotypic plasticity in laying date?

Este capítulo reproduce íntegramente el artículo

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Climatic effects and phenological mismatch in cuckoo-host interactions: a role for host phenotypic plasticity in laying date?

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Abstract

Climatic effects on breeding phenology vary across organisms and therefore might promote a phenological mismatch in ecologically interacting species, including those engaged in coevolutionary interactions such as brood parasites and their hosts. Recent studies suggest that climatic induced changes in migration phenology may have mismatched cuckoos and their hosts in Europe. However, it is currently unknown whether cuckoo-host phenological mismatch results from different degrees of phenotypic plasticity or to different speeds of microevolutionary processes affecting hosts and parasites. Here we performed (i) cross-sectional correlations between climate conditions and population level of phenological mismatch between the migratory brood parasite great spotted cuckoo *Clamator glandarius* and its main resident host in Europe, the magpie *Pica pica*; and (ii) a longitudinal analysis to study within-individual variation in breeding phenology for individual hosts experiencing different climate conditions over a period of nine years (2005-2013). Cross-sectional analyses revealed independent and contrary effects of winter and spring temperature on magpie phenology: magpie hosts tend to breed earlier those years with lower February temperatures, however, high temperature in the first half of April spur individuals to lay eggs. Breeding phenology of cuckoos was tuned to that of their magpie host in time and duration. However, annual phenological mismatch between cuckoos and magpie hosts increased with NAO index and January temperature. Longitudinal analyses revealed high individual consistency in magpie host phenology, but a low influence of climate, suggesting that the climatic-driven phenological mismatch between cuckoos and magpies at the population-level cannot be explained by a host plastic response to climatic conditions.

Keywords: cuckoo-host coevolution, climatic effects, phenological mismatch

Introduction

Organisms adapt to spatio-temporal environmental variation by modifying their breeding phenology (sensu Parmesan and Yohe 2003; Nussey *et al.* 2007; Charmantier *et al.* 2008; Porlier *et al.* 2012). Phenological responses to environmental variation, however, are not consistent across species (Visser and Both 2005), and, thereby, a fundamental challenge of current evolutionary biologists is to understand how differences among species in their phenological responses to environmental conditions might affect species interactions (Visser *et al.* 1998), and the direction and strength of local coevolutionary processes (Brooks and Hoberg 2007; Dobson *et al.* 2008; Møller 2010; Toju *et al.* 2011).

An ideal system for studying how climate may promote phenological mismatch involves the specialized interaction between some obligate avian brood parasites (hereafter cuckoos) and some of their favourite hosts (Saino *et al.* 2009; Møller *et al.* 2011). Cuckoo females lay their eggs in the nests of host species, and leave parental care of their offspring to unrelated foster parents (Davies 2000). Brood parasites usually reduce (often drastically) their host's breeding success (Rothstein 1990, Davies 2000, Payne 2005) resulting in strong selection pressures on the hosts favouring the evolution of defences against parasitism (Rothstein 1990, Davies 2000) which in response have selected for further counter-defences in the parasite side, thus giving rise to a coevolutionary arms race. In this highly specialized interaction cuckoos must finely adjust their phenology to that of their favourite hosts, because otherwise the cuckoo will not be able to reproduce in the absence of that host.

So far, evidence of climatic effects promoting trait mismatch in cuckoo-host interactions came from studies showing that the degree of egg mimicry between European cuckoos *Cuculus canorus* and their favourite reed warbler *Acrocephalus scirpaceus* hosts was affected by climatic conditions both at local

(Avilés *et al.* 2007), and wider continental spatial scales (Avilés *et al.* 2012). Also a recent study has shown that annual variation in climatic conditions might result in asymmetric effects on great spotted cuckoos *Clamator glandarius* and magpie *Pica pica* hosts with respect to probability of ectoparasitism, immunity and growth in a single population (Soler *et al.* 2014). Climate may also affect migration phenology of the European cuckoo and/or their hosts promoting phenological mismatches (Saino *et al.* 2009; Møller *et al.* 2011). Indeed, a recent study revealed that short-distance, but not long-distance, migratory European cuckoo hosts have advanced their arrival to spring quarters in response to climate change more than cuckoos (Saino *et al.* 2009), which has lead to an increase of the frequency of cuckoo parasitism of long-distance migratory hosts at a continental scale (Møller *et al.* 2011). Climatic induced effects on time of breeding of dunnocks *Prunella modularis* and reed warblers hosts also leaded to changes in the availability of host nests with eggs for cuckoos, although it did not result in changes in cuckoo abundance in the UK (Douglas *et al.* 2010).

Changes in phenological matching between cuckoos and their hosts may result from a number of mechanisms that may act in conjunction or in isolation and that have not been previously investigated. Phenological mismatch might reflect a micro-evolutionary process due to changes in allele frequency of hosts and/or cuckoos due to selection within the population, and/or due to gene flow by dispersal of individuals (either cuckoos or hosts) adapted to breed relatively earlier or later in the year (e.g. Przybylo *et al.* 2000). In addition, cuckoo-host phenological mismatch may arise if either individual hosts and/or cuckoos show variable levels of phenotypic plasticity and/or if plastic responses of cuckoos and their hosts rely on different environmental cues (Charmantier *et al.* 2008; Brommer *et al.* 2008; Porlier *et al.* 2012). Previous studies reporting changes in the time of breeding of the European cuckoo and/or its hosts relied on cross-sectional data reported at a regional and/or continental spatial scale (Saino *et al.* 2009; Douglas *et al.* 2010; Møller *et al.* 2011), which cannot discriminate among these mechanisms.

Here, we combine cross-sectional and longitudinal analyses on individuals to investigate possible climatic effects on the phenology of a non-migratory magpie host population parasitized by the great spotted cuckoo in the south of Spain over a period of nine years (2005-2013). Previous studies have reported adaptations and counter-adaptations of both magpies and cuckoos at different stages of the reproductive cycle suggesting that these two species have been engaged for a long time in a coevolutionary arms race (reviewed in Soler and Soler 2000), but have neglected the possibility that abiotic factors, such as climate, may affect the dynamic of the interaction (see however Soler *et al.* 2013). Specifically, we were interested in the following points: 1) whether host breeding phenology and cuckoo-host phenological mismatch had changed in that population over that period; 2) whether those changes were associated with changes in local and regional climatic conditions; 3) whether host responses to climatic conditions at the individual level may contribute to explain population patterns of phenological mismatch. If the later was the case, it would constitute a strong evidence for a previously neglected role of host phenotypic plasticity in determining cuckoo-host phenological mismatches.

Materials and Methods

Study area and system

The study was conducted in La Calahorra (37º 10′ N, 3º 03′ W, Granada, Southern Spain) during the years 2005-2013 in the frame of a long-term project on the interaction between great spotted cuckoos and magpies (Molina-Morales *et al.* 2012; Molina-Morales *et al.* 2013). Landscape is a patchy mosaic of groves of almond trees (*Prunus dulcis*), cereal crops and meadows. Magpies preferentially built their nests in almond trees. Magpies are territorial, sedentary and socially monogamous long-lived passerines (Birkhead 1991). This population was separated from other nearby populations in the area by arable lands with few or no potential nest sites for magpies (Martín-Gálvez *et al.* 2007). Furthermore, previous work has shown that our population differed from nearby populations in ecological conditions (i.e. phenology, breeding synchrony, density of nests and parasitism level, Martínez *et al.* 1996), which suggests it can be treated as a distinct unit for this study.

In our study area magpies lay one clutch during April-May, and are the main host of the great spotted cuckoo. Cuckoo parasitism reduces magpie reproductive success by 80% through early hatching and effective competition for parental food delivery of cuckoo nestlings (Soler *et al.* 1996, 1997). This fact has selected for host recognition and rejection of cuckoo eggs by magpies (Soler & Moller 1990), which in turn has selected for punitive cuckoo behaviours promoting parasitism acceptance (Soler *et al.* 1995). In fact, the interaction between magpies and great spotted cuckoos can be regarded a classical example of coevolution (Soler & Soler 2000). Unlike magpies, great spotted cuckoos are migratory birds wintering in tropical Africa (i.e. trans-Saharan) and arriving to the breeding grounds in Guadix in late February or early March (Soler & Soler 2000).

Individual marking and monitoring

Some of the adult magpies were individually marked by unique combinations of colour rings, and subsequently monitored (see Molina-Morales *et al.* 2012 for further details). Monitoring of non-ringed females was based in parentage analyses (Molina-Morales *et al.* 2012). We assigned particular breeding attempts in different years to the same female when the nestlings in those broods were full siblings to each other. Also, we could assign early breeding attempts to later marked females using paternity analyses (Molina-Morales *et al.* 2012). Most sampled individuals were caught with adult plumage according to Birkhead (1991), and we did not know their exact age, thus we assigned them a two-level relative minimum age: females were coded as 1, if they were breeding at the first sighting and in the following year and 2, if they were breeding two years or more after the first sighting.

Nest monitoring

Magpie nests were monitored from 1 March to the beginning of July each breeding season between 2005 and 2013. We found 675 nests across the nine years of study, but could estimate laying date only in 424 nests (63%) because some nests were abandoned during the early stage of building or predated before host clutch was completed. The average number of sampled nests with laying date information was 52.44 nests per year (range 31-70), and the average number of parasitized nests was 20.11 nests per year (range 7-37). Nests were found by careful inspection of all trees in the breeding area, and GPS devise. Each nest was observed with telescope or binoculars from a hide around 100 meters away during the nest building stage in order to identify marked birds involved in each nesting attempt. All nests were monitored at 5 days intervals during the breeding season. During egg laying nests were monitored at shorter (2-3 day) intervals which allowed estimating the date of laying of the first magpie egg, as magpies lay one egg per day (Cramp 1998), and knowing whether the nest was parasitized by great spotted cuckoos. Nests were categorized as parasitized if at least one great spotted cuckoo egg was detected in the nest.

Every year we calculated average and range (i.e. max-min) of laying dates of all magpie nests and of the subset of parasitized nests to estimate temporal variation in the phenology of magpie hosts and cuckoos at the population level. Annual differences in median laying date of cuckoos and magpies were then used as an index of phenological mismatch. Years with a high phenological mismatch were thus years in which clutches parasitized with great spotted cuckoo eggs were more delayed compared to the average laying date of magpie nests.

Between 2007 and 2011 we also monitored the nests till fledging, which allowed us to test for an effect on laying date on probability of magpie nest failure (in all nests, and in the subset of parasitized nests) and on magpie fledgling success (calculated as the percentage of fledglings relative to the number of host eggs laid in the nest). In addition, in 2009 and 2010 we took body mass measurements of all great spotted cuckoo nestlings in the population at day 14 with a Pesola spring balance with a precision of 0.1g. Analyses on this subset of nests and nestlings thus allowed estimating seasonal effects on magpie (i.e. host nest failure and fledgling success) and cuckoo (i.e. nest failure of cuckoo parasitized nests and body mass at fledging) fitness surrogates.

Climatic variables

We used the winter (December to March) North Atlantic oscillation index

(NAO) as a natural large-scale proxy of the climatic condition that may affect laying date in the Mediterranean region. Negative winter NAO values are associated with increased precipitation in the Mediterranean region (Stenseth *et al.* 2003), including the Iberian Peninsula (Gordo *et al.* 2011). A number of studies have reported a relationship between the NAO-index and timing of breeding in birds including long-distance migrants (Przybylo *et al.* 2000; Møller *et al.* 2006). We obtained the winter NAO index from the web site (http://www.cgd.ucar.edu/cas/jhurrell/indices.html).

We also used total daily rainfall and average temperature calculated on a daily basis (hereafter rainfall and temperature) during the previous winter months (i.e. January and February) collected at a nearby meteorological station (Supplementary material Appendix 1 Table A1) as a proxy of the winter climatic conditions generally affecting the studied population. We target on January and February climate conditions because at that time, but not before, most of magpie pairs are already in their breeding territories in La Calahorra (Molina-Morales unpublished data). Therefore, climatic conditions in January and February are likely to influence the ability of female magpies to accumulate resources for egg laying. Rainfall and temperature records were retrieved from the web http://www.juntadeandalucia.es managed by the regional governments of Andalucía. Preliminary correlation analyses revealed that the NAO index was strongly correlated with rainfall (January rainfall vs NAO index: r_p=-0.69, P =0.039; February rainfall vs NAO index: r_p= - 0.84, P =0.009), but not with temperature (January temperature vs NAO index: r_p=0.41, P =0.26; February temperature vs NAO index: r_p=-0.07, P =0.85) in our study area. Therefore, we disregarded using rainfall information in subsequent analyses about winter climatic condition influence on phenology because most of its variation is retrieved by the NAO index.

In addition, because magpie and cuckoo phenology might also be affected by short-term weather effects we also investigated spring immediate temperature effects on phenology. The effect of spring temperature on breeding phenology has been demonstrated in many birds species included the magpie in UK (Crick and Sparks 1999). Most of magpies in our population start laying in the second half of April (Supplementary material Appendix Fig. A1), therefore we used total daily average temperature calculated on a daily basis during the first half of April as a surrogate of short-term weather effects likely affecting cuckoo and magpie individual decisions to lay eggs. Spring temperature was not related to the winter NAO index (April temperature vs NAO index: r_{p}=-0.38, P =0.312) nor to winter temperature (April temperature vs January temperature: r_{p}=-0.40, P =0.281; April temperature vs February temperature: r_{p}=0.02, P =0.954) in our study area, suggesting that both winter and spring temperature effects may independently affect magpie and cuckoo phenology.

Statistical analyses

Population-level analyses

In a first step, we used a linear mixed model (LMM hereafter) for studying variation in magpie laying date in relation to study year (i.e. 2005-2013) and parasitism status (i.e. parasitized versus non-parasitized) as random and fixed categorical effects, respectively. The interaction between study year and parasitism status was entered as a random factor in the model aiming to describe whether differences in the breeding phenology of parasitized and non-parasitized magpie nests changed between years.

Aiming to study the relationship between magpie and cuckoo fitness surrogates and laying date we first modeled probability of nest failure for all magpie nests studied between 2007 and 2011 as a binary dependent variable. We constructed a generalized linear mixed model (GLMM hereafter) where the fact that the nest was parasitized (cuckoo parasitized versus non-parasitized) was treated as fixed effect, and the year was treated as a random effect. Laying date and clutch size were included as covariates. In addition, we entered the interaction between laying date and parasitism which allowed us to test whether the effect of cuckoo parasitism on magpie host nest failure was mediated by laying date. Secondly we used a LMM to test the influence of the same factors (i.e. parasitism as fixed effect, and year as a random effect) and covariates (i.e. laying date and clutch size) on fledging success of magpie hosts. This analysis was performed on the set of magpie nests in which at least one nestling fledged.

These models thus responded the specific questions of whether breeding late increased host nest failure and decreased host productivity in our population.

In a second GLMM we modeled nest failure probability of cuckoo parasitized nests in relation to laying date and clutch size while entering year as random factor. In addition, we used a LMM to test the influence of laying date on cuckoo body mass at fledging while accounting for the non-independence of nestling raised in the same year and nest (i.e. year and nest as random effects). These models thus respond the question of whether being late increases nest failure of magpie nests holding a cuckoo egg and affects cuckoo nestling development. Temporal trends in magpie phenology and climatic conditions over the 2005-2013 period were analyzed with linear regression models weighted by number of nests using year as a continuous predictor. Similarly, we performed cross-sectional analyses to test changes in annual laying date of magpies and degree of cuckoo-host phenological mismatch in relation to regional and local climatic conditions using linear regression models. As the existence of temporal autocorrelation in time series analyses may artificially inflate the estimates of regression coefficients and their significance levels we checked for the presence of temporal autocorrelation in all the climatic variables and in laying date and in the residuals of linear models of these variable on year as a continuous predictor using the Box & Ljung *Q* test as described in Statistica (StatSoft Inc. 1995). Neither climatic indices nor magpie laying date or residuals were temporally autocorrelated at time lags of 1, 2, 3 and 4 years (Supplementary material Appendix 2, Table A2), suggesting that temporal autocorrelation would have a negligible effect in our cross-sectional analyses.

Longitudinal analyses

To assess whether magpie population responses to climatic conditions were due to individual variation in laying date, that is, plasticity we adopted the linear reaction norm approach (sensu Nussey *et al.* 2007) using those individuals that bred two or more years over the course of the study (we used 157 records from 58 females that bred at least twice during the period 2005-2013). We built different LMMs: one for each of the climatic variable affecting population-level variation in laying date (i.e. February and April Temperature) and cuckoo-host mismatch (i.e. NAO index and January temperature). Each model included laying date as the dependent variable, and age and one of the climatic variables as fixed effects. As random terms we included year, female identity (to test for variation between individuals in the average climatic conditions, i.e. elevations), and the interaction between female identity and the corresponding climatic variable (to test for between-individual variation in plasticity, i.e. slopes).

All models were fit using SAS 9.3. (PROC GLIMMIX procedure with link functions: identity and logit for LMMs and GLMMs, respectively), with type III tests of fixed effects. Degrees of freedom for fixed effects were estimated using the Kenward-Roger approximation and significance of covariance parameters was tested with Wald Z tests (SAS Institute Inc. 2013).

Results

Population level variation in host and parasite phenology and cuckoo-host mismatch

Laying date of magpies varied between years (Random effect of year: Z=1.57, P= 0.05) and in relation with cuckoo parasitism (Fixed effect of parasitism: $F_{1,421.9}$=10.78, P= 0.001), being late breeding magpies more likely cuckoo parasitized than earlier ones (mean (SE) = 26.3 of April (0.90 days) in 165 parasitized *versus* 22.5 of April (0.61 days) in 259 non-parasitized nests) (Supplementary material Appendix 1 Fig. A1). Differences in laying date between parasitized and non-parasitized magpie nests did not differ between years (Interaction year X parasitism: Z=0.55, p = 0.29). No significant temporal trend was detected for the laying date of magpies (slope= 0.004±0.02, t_7=0.18, p=0.85) and cuckoos (slope= 0.01±0.02, t_7=0.51, p =0.62) over the nine years of study. However, breeding phenology of cuckoos was finely tuned to the breeding phenology of their magpie host: cuckoos tend to breed earlier those years in which their magpie hosts did it earlier (slope= 1.06±0.26, t_7=3.96, p =0.005, r^2=0.69), also cuckoos tend to extend their breeding more time those years where magpie hosts extended more time their breeding (slope= 0.96±0.09,

t_7=10.52, p=0.00001, r^2=0.94).

Breeding success of magpies and cuckoos in relation to laying date

Nest failure probability of magpie host nests showed a year to year consistent pattern to increase seasonally (Fig. 1, Table 1). Among the nests with fledglings, however, host fledgling success decreased in parasitized nests, but did not change seasonally (Table 1). On the parasite side, nest failure probability of parasitized nests did not change seasonally (Table 1). However, cuckoo fledglings raised in late host nests were lighter than those raised in early host nests (Fig. 1, Table 1).

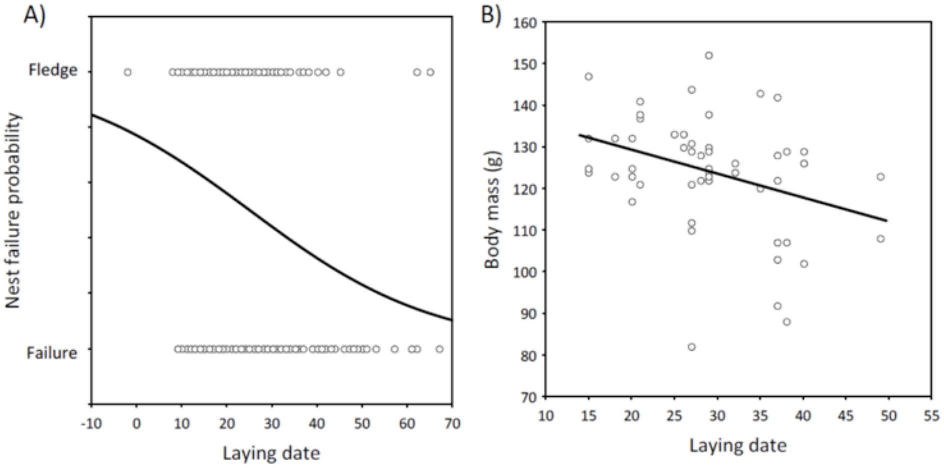

Figure 1. Magpie and cuckoo fitness surrogate in relation to laying date. (A) Magpie host nest failure probability in relation to laying date (N=238 nests). (B) Cuckoo nestling body mass in relation to laying date (N=55 nestlings raised in 34 nests.

Climatic conditions and population level magpie host phenology and cuckoo-host mismatch

The NAO index was not related to the time of breeding of magpie (slope= -0.28±0.53, t_7=0.54, P=0.61). January temperature (slope= 0.58±1.15, t_7=0.50, p =0.63) was not significantly related with the laying date of magpies. However, magpies tends to breed earlier those years with low February temperatures (slope= 1.41±0.57, t_7=2.55, p =0.038, r^2=0.47). April temperature alone did not

Table 1. Results of linear and generalized mixed-effects models of fitness correlates of magpie hosts and cuckoo parasites in relation to laying date.

Magpie host nest failure (n=238 nests)					
		Random effects			
Term	Covariance parameter	SE	*Z*	*P*	
Year	0.45	0.45	1.00	0.15	
		Fixed effects			
Term	Coefficient	SE	*F*	df	*P*
Clutch size	**0.41**	**0.15**	**7.46**	**1,233**	**0.006**
Laying date	**-0.04**	**0.02**	**4.13**	**1,233**	**0.04**
Parasitism	1.48	0.90	2.74	1,233	0.09
Laying date*Parasitism	0.02	0.03	0.47	1,233	0.49
Magpie host fledging success (n=118 nests)					
		Random effects			
Term	Covariance parameter	SE	*Z*	*P*	
Year	5.25	23.10	0.23	0.41	
		Fixed effects			
Term	Coefficient	SE	*F*	df	*P*
Clutch size	2.69	2.42	1.24	1,92.66	0.27
Laying date	-0.07	0.26	0.09	1,104.2	0.77
Parasitism	**-49.51**	**17.74**	**7.79**	**1,112.7**	**0.006**
Laying date*Parasitism	1.24	0.79	2.49	1,112.3	0.11
Cuckoo nest failure (n=97 nests)					
		Random effects			
Term	Covariance parameter	SE	*Z*	*P*	
Year	0.38	0.58	0.67	0.25	
		Fixed effects			
Term	Coefficient	SE	*F*	df	*P*
Clutch size	-0.14	0.20	0.46	1,94	0.50
Laying date	-0.02	0.02	0.58	1,94	0.44
Fledging cuckoo body mass (n=55 nestlings)					
		Random effects			
Term	Covariance parameter	SE	*Z*	*P*	
Nest	3.51	28.85	0.12	0.45	
Year	7.71	22.65	0.34	0.36	
		Fixed effects			
Term	Coefficient	SE	*F*	df	*P*
Laying date	**-0.48**	**0.28**	**2.93**	**1,25.33**	**0.05**

Significant terms are highlighted in bold. Degrees of freedom for fixed effects were estimated using the Kenward-Roger approximation.

significantly explain variation in laying date of magpies (slope= -0.71±0.44, t_7=1.60, *P*=0.15). However, a multiple regression analysis in which we simultaneously assessed the effects of February and April temperature on magpie laying date revealed independent and contrary effects of winter and spring temperature on magpie phenology ($F_{2,6}$=9.16, p =0.01 r^2=0.75): magpie hosts tend to breed earlier those years with lower February temperatures (β = -0.69±0.20, t_6=3.43, P=0.01; Fig 2); however, high temperature in the first half of April induced early laying in magpies (β= -0.53±0.20, t_6=2.63, P=0.04; Fig 2). Annual degree of phenological mismatching between great spotted cuckoos and magpies was related with regional and local winter climatic conditions: cuckoos delayed more their breeding time relative to magpies those years with a high NAO index (slope= 1.10±0.36, t_7=3.04, p=0.018, r^2=0.57) and high January temperature (slope= 2.53±0.71, t_7=3.54, p=0.009, r^2=0.64). February temperature (slope= 0.97±0.70, t_7=1.37, p=0.21) and spring temperature (slope= -0.66±0.46, t_7=1.42, p=0.19) were not related with the degree of phenological mismatch between cuckoos and magpies. In addition, when we reassessed the associations between winter NAO index and degree of phenological mismatch, and between January temperature and degree of phenological mismatch, by including spring temperature in multiple regression models results remained qualitatively identical and did not reveal any effect of spring temperature on cuckoo-host mismatch (multiple regression with NAO index and spring temperature as predictors: laying date vs NAO index: β = 0.67±0.27, t_6=2.43, p=0.05; laying date vs April temperature: β = -0.22±0.27, t_6=0.79, p=0.45; Multiple regression with January temperature and spring temperature as predictors: laying date vs January temperature: β = 0.72±0.25, t_6=2.83, p=0.03; laying date vs April temperature: β = -0.18±0.25, t_6=0.70, p=0.51).

Individual-level analyses of host phenology in relation to climatic conditions

Linear mixed-effects models revealed significant differences in laying date between different females (see the climate variables in the fixed effects sections Table 2), but not evidence of an average plastic response to regional and local climatic conditions amongst magpie individuals (i.e. flat reaction norms; see interaction terms in the random effects sections of Table 2). In addition, individual female magpies did not exhibit plastic variation in laying date in response to the NAO index and winter or spring temperature (i.e. slopes) (Table

2). Hence, all results were concordant and suggested high variation in the laying schedule between females but low individual plasticity in laying date in response to the climatic conditions affecting our magpie population.

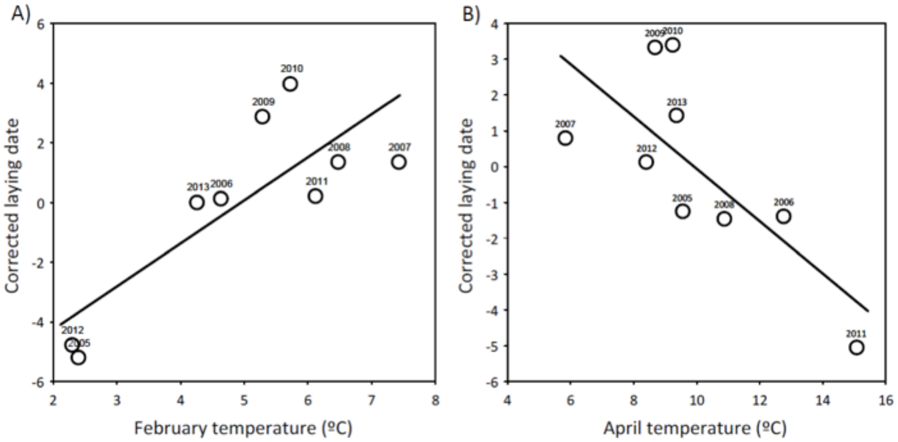

Figure 2. Relationship between the annual mean laying date of magpie hosts in each of the nine years and February (A) and April (B) temperature. Laying date is expressed as the residual of laying date on April (A) and February (B) temperature aiming to illustrate pure effects of winter and spring temperature in magpie population phenology.

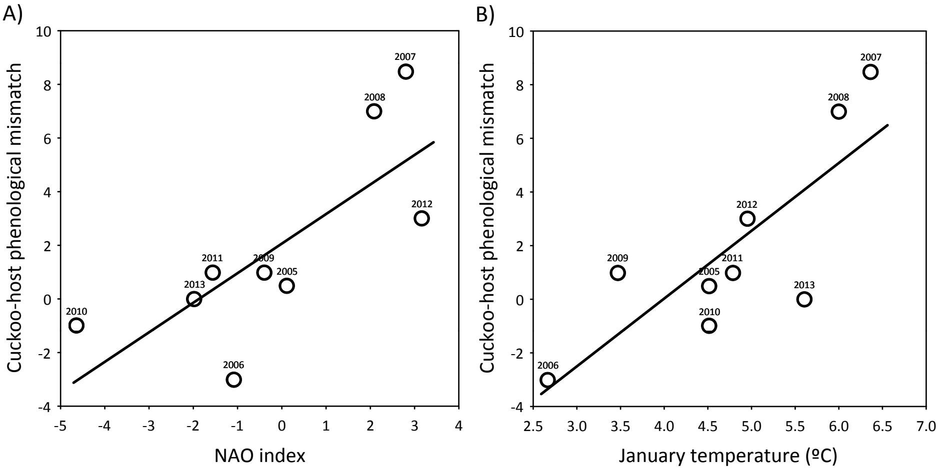

Figure 3. Relationship between the annual degree of phenological mismatch (i.e. differences in median laying date of cuckoos and magpie hosts) and NAO index (A) and temperature in January (B).

Table 2. Results of linear mixed-effects models of plasticity in magpie egg-laying date in response to regional (i.e. NAO index) and local winter climatic (January and February temperature) and spring (first half of April temperature) conditions.

NAO index		*Random effects*			
Term	Covariance Parameter	SE	*Z*	*P*	
Female	**27.09**	**8.55**	**3.17**	**0.0008**	
Year	11.27	8.43	1.34	0.09	
NAO index*Female	0.02	0.45	0.06	0.47	
		Fixed effects			
Term	Coefficient	SE	*F*	df	*P*
Age	**7.59**	**2.91**	**6.78**	**1,126**	**0.01**
NAO index	-0.22	0.53	0.17	1,5.9	0.69
January temp		*Random effects*			
Term	Covariance Parameter	SE	*Z*	*P*	
Female	**27.14**	**8.41**	**3.23**	**0.0006**	
Year	11.91	8.61	1.38	0.08	
January temp*Female	1.09	3.24	0.34	0.36	
		Fixed effects			
Term	Coefficient	SE	*F*	df	*P*
Age	**7.57**	**2.93**	**6.68**	**1, 122.1**	**0.01**
January temp	0.02	1.20	0.00	1,6.33	0.98
February temp		*Random effects*			
Term	Covariance Parameter	SE	*Z*	*P*	
Female	**24.98**	**7.85**	**3.18**	**0.0007**	
Year	5.93	5.40	1.10	0.13	
February temp*Female	3.37	2.89	1.16	0.12	
		Fixed effects			
Term	Coefficient	SE	*F*	df	*P*
Age	**7.18**	**2.80**	**6.54**	**1,1112.7**	**0.01**
February temp	1.16	0.73	2.49	1,10.15	0.14
April temp		*Random effects*			
Term	Covariance Parameter	SE	*Z*	*P*	
Female	**26.75**	**8.34**	**3.21**	**0.0007**	
Year	9.14	7.29	1.25	0.10	
April temp*Female	$2.28\ 10^{-17}$		0.00	1.00	
		Fixed effects			
Term	Coefficient	SE	*F*	df	*P*
Age	**7.57**	**2.89**	**6.83**	**1,130.8**	**0.01**
April temp	-0.52	0.47	1.25	1,5.90	0.30

Analyses were done using 157 records from 58 females that bred at least twice during the period 2005-2013. Climatic variables were mean centered. Significant terms are highlighted in bold. Degrees of freedom for fixed effects were estimated using the Kenward-Roger approximation.

Discussion

Population-level analyses

The cross-sectional analyses performed in this study revealed that annual phenology of sedentary magpie hosts and the degree of phenological mismatch between migratory great spotted cuckoos and magpies is influenced by large-scale and local climatic conditions. Specifically, we found contrary and independent effects of winter and spring temperature on magpie phenology: magpies tend to breed earlier those years with low February temperatures, whereas high temperature in the first half of April favoured early laying. In addition, we found that cuckoos breed later relative to magpie hosts those years with a high NAO index and high January temperature. Previous studies have provided support for the existence of phenological mismatch between the European cuckoo and some of their main hosts at a continental scale due to changes in cuckoo and host migration phenology (Saino *et al.* 2009; Møller *et al.* 2011). Our results based on long-term monitoring of a single population extend previous findings and suggest that changes in breeding phenology due to climatic conditions may also apply to sedentary hosts, and may ultimately result in phenological mismatch between parasitic cuckoos and their hosts.

There was no evidence of changes in local climatic conditions in our study site during the period 2005-2013 and not surprisingly magpie host population has not shown any shift in its time of laying during that time. This may simply reflect the short time period considered in these analyses. However, February and April temperature explained 75% of annual variation in magpie laying date, which still would suggest that temperature has a fundamental influence on breeding phenology of magpies in this population. We cannot discard, however, that the absence of any clear temporal trend in magpie phenology was due to the conflicting effects of winter (i.e. delayed) and spring (i.e. immediate) temperature on magpie laying dates.

Previous studies in other bird species (Dunn 2004; Both *et al.* 2004), included magpies in UK (Birkhead 1991), had found that high spring temperatures induced early laying. Our results confirmed these findings as high April temperature triggered laying of magpies in our population once we control for winter temperature. However, the simultaneous consideration of winter and spring temperature also revealed a high influence of winter temperature on magpie phenology. Indeed, we found that, irrespective of the influence of spring temperature, magpies breed earlier those years with low winter temperature. This pattern was not confounded by concomitant temporal changes in climate and host phenology, and is contrary to the general pattern reported in birds (Crick and Sparks 1999; Dunn 2004), and also in magpies (Birkhead 1991; Crick and Sparks 1999), of advancing egg production time in response to the increase in spring temperature. Previous studies have shown that the effect of temperature on laying date may greatly vary both between (Dunn 2004; Torti and Dunn 2005) and within bird species (e.g. Visser *et al.* 2003; Husby *et al.* 2010) due to geographical and ecological differences. However, the simultaneous effect of winter and spring temperature on bird phenology has been rarely investigated.

The positive effect of winter temperature on magpie phenology is intriguing, and could be explained by immigration of breeding individuals from colder populations. If magpies migrate into our population from colder areas, magpie population phenology may advance because migrants would experience earlier environmental cues for reproduction than in their origin populations. As mentioned above, our magpie population is close to others (see methods) differing in phenology (Martín-Gálvez *et al.* 2007), and previous genetic studies have shown that they all form a metapopulation in which gene flow is frequent (Martin-Galvez *et al.* 2007). However, it remains unstudied whether gene flow can or cannot be mediated by climate-dependent dispersal in magpies. Alternatively, this pattern might result from changes in the age pyramid of our population due to an effect of winter conditions on survival probability of first-year magpies. A similar mechanism was proposed to explain male shortening of tail length under favorable conditions in a Danish barn swallow *Hirundo rustica* population (Møller and Szép 2005). Magpie′ highest mortality occurs during the first winter (Birkhead 1991; Molina-Morales & Martínez unpublished data), and it might be particularly high in cold winters. Furthermore, it is known that young magpies breed later than old ones (Birkhead 1991). Thus, in harsh conditions (i.e. in cold winters) selective mortality might eliminate young magpies and adults may have earlier or better access (i.e. lower competence) to resources and/or territories resulting in the advance of laying date. Winter temperature effects might thus induce changes in magpie phenology through their possible effects on migration and/or selective mortality which would ultimately determine the pull of magpies that will have a chance to breed in spring. In that time, individual decisions to lay would be more likely influenced by spring immediate temperature.

A critical point is to know how changes in climatic conditions may impact on the dynamic of the interaction between great spotted cuckoos and magpies in our population. Unfortunately, we cannot compare the breeding outcome of magpies and great spotted cuckoos at the level of individual as we could not ascribe cuckoo eggs to females. However, the analyses of the relationships between laying date and breeding success of magpies and cuckoos provide some insight about the potential fitness consequences of breeding late for magpies and great spotted cuckoos. Being late penalizes magpies by increasing nest failure, but not fledging success, irrespective of parasitism, while late cuckoo fledglings were significantly lighter than those raised in early nests. Therefore, cuckoos laying late relative to magpies would face the double cost of nest failure of their hosts besides a lower fitness prospect due to reduction of body weight at fledging of their offspring.

Individual-level analyses of host phenology

Our longitudinal analyses performed on magpie individual hosts experiencing different value of climatic conditions over several breeding seasons allowed us to disentangle the potential role of plasticity and microevolution on the observed annual changes at population level. Magpie plasticity at the level of population was not due to individual plasticity in phenology in response to climate as magpies were relatively invariant to climatic conditions (see also Charmantier *et al.* 2008). Previous theoretical studies had suggested a higher potential for selection of phenotypic plasticity in more predictable environments (Gavrilets and Scheiner 1993). Indeed, a recent empirical study has shown that individual great tit plasticity was relatively lower in less food predictable populations (Porlier *et al.* 2012). We reported a low temporal autocorrelation for all climatic cues analysed in la Calahorra (Supplementary material Appendix 2, Table A2), which would suggest a low potential for selection on individual plasticity in our population.

Our study revealed a disagreement between cross-sectional and longitudinal analyses. We found population-level plasticity in host phenology in response to climatic conditions, but very little between-individual plasticity in response to climate. A previous study had documented a similar pattern in the colonial breeder common guillemot *Uria aalge* where selection imposed by a need of breeding synchronously might have limited selection on individual plasticity to climate (Reed *et al.* 2006). Knowing how magpies may show an annual tracking of local climatic conditions without phenotypic plasticity is intriguing. Magpie have a relatively short generation time (life expectancy of magpies in U.K. was 2.0 years, Birkhead 1991), an although animals may show evolutionary responses over short time scales (Grant and Grant 1995), it still seems unlikely that selection due to fluctuating climatic conditions caused the population to track the local climatic conditions every year. Alternatively, population tracking of local climatic conditions may arise if climatic conditions affected dispersal movements of magpies in our study area. Our magpie population is connected by gene flow and migration to other nearby populations (Martin-Gálvez *et al.* 2007). In this vein, marked gene drift due to departure or entrance of individuals selected to breed at different times of the year may induce the average population matching to climatic conditions and limited the potential of selection on individual plasticity.

Conclusion

Despite the fact our results should be considered with caution given the short study period considered, our study is the first providing support for the idea that both regional and local climatic conditions may impact on cuckoo-host interaction through their influence on cuckoo and host breeding phenology at the population scale. Although the mechanisms behind the link between temperature and phenology of cuckoos and magpies clearly deserve further investigation, if magpies and cuckoos differ in their sensitivities to temperature magpie hosts could be exposed to great spotted cuckoos earlier or later in the season as climate changes with potential consequences for the dynamic of the interaction. Our longitudinal analysis based on a set of marked individuals that experienced different values of environmental conditions over several breeding seasons revealed that magpie host population plasticity in breeding phenology did not arise from individual plasticity in response to local climatic conditions. This finding might be related to the high genetic and migration connectivity of our magpie population with nearby ecologically different populations and the low predictability of climatic conditions that may favour selection to act on short dispersal rather than on plastic laying in response to climatic change. Our results, thus, emphasize the need of including climatic variables as factors potentially explaining the dynamic of interaction between parasites and its hosts, and illustrate the importance of considering studies at the level of individual in order to deepen in the knowledge of the evolutionary mechanisms shaping population responses to climatic variation.

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Supplementary material Appendix 1, Table A1.

Details of the local weather station and values of the NAO index and temporal variation in winter and spring climatic variables during the study. Total rainfall and temperature during winter (January-February) and spring temperature (first half of April) in the weather station nearby to the study population. Details of the station; Name Iznalloz. Location: 37° 25' 03" N, 3° 33' 00" W. Altitude: 935 m. Regional and local winter climatic conditions did not experience any obvious linear trend over the nine years of study (linear regressions of NAO index, temperature and rainfall on year; NAO index: slope= -0.18±0.34, t_7=-0.53, P=0.61; January temperature: slope= 0.11±0.15, t_7=0.69, P=0.51; February temperature: slope= -0.04±0.24, t_7=-0.19, P=0.84; January rainfall: slope= 4.09±3.55, t_7=1.14, P=0.28; February rainfall: slope= 0.96±5.13, t_7=0.18, P=0.85; April temperature: slope= 0.05±0.37, t_7=0.14, P=0.89).

	Rainfall (mm)		**Temperature (°C)**			
Year	**January**	**February**	**January**	**February**	**April**	**NAO index**
2005	**0.0**	**30.2**	**4.5**	**2.3**	**9.5**	0.12
2006	**32.6**	**50.2**	**2.7**	**4.6**	**12.8**	-1.09
2007	**18.6**	**20.4**	**6.3**	**7.4**	**5.8**	2.79
2008	**27.8**	**25.0**	**5.9**	**6.4**	**10.9**	2.10
2009	**31.2**	**39.8**	**3.4**	**5.2**	**8.7**	-0.41
2010	**98.8**	**134.8**	**4.5**	**5.7**	**9.2**	-4.64
2011	**16.2**	**28.4**	**4.7**	**6.1**	**15.1**	-1.57
2012	**24.8**	**5.8**	**4.9**	**2.2**	**8.4**	3.17
2013	**50.6**	**46.4**	**5.6**	**4.2**	**9.4**	-1.97
Total Mean±SD	**33.4 ± 28.1**	**42.3 ± 37.2**	**4.8 ± 1.2**	**4.9 ± 1.8**	**9.9 ± 2.7**	**-0.16 ± 2.5**

Supplementary material Appendix 2, Table A2.

Temporal autocorrelation in regional and local climatic conditions and laying date of magpies over the nine years of study and of the residuals of linear models of these variables on study year.

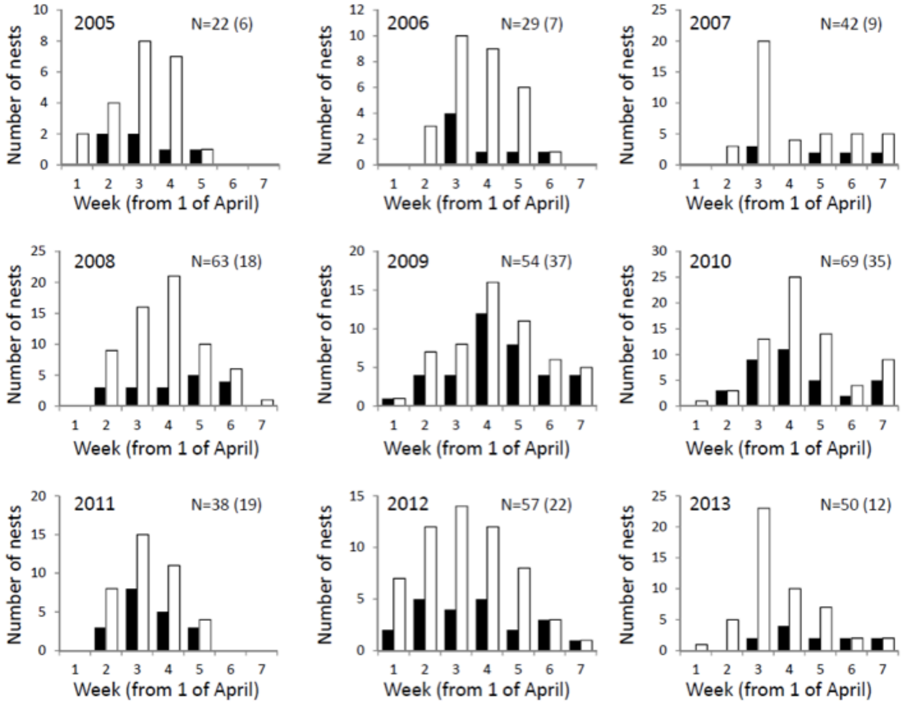
NAO index					**January rainfall**				
Lag	*r*	*S.E.*	*Q statistic*	*P*	Lag	*r*	*S.E.*	*Q statistic*	*P*
1	-0.003	0.28	0.00	0.99	1	-0.180	0.28	0.40	0.52
2	-0.471	0.27	3.14	0.20	2	-0.104	0.26	0.55	0.76
3	-0.162	0.24	3.57	0.31	3	0.073	0.24	0.64	0.88
4	0.154	0.22	4.05	0.39	4	0.073	0.24	0.64	0.88
January temperature					**February rainfall**				
Lag	*r*	*S.E.*	*Q statistic*	*P*	Lag	*r*	*S.E.*	*Q statistic*	*P*
1	-0.180	0.28	0.40	0.52	1	-0.091	0.28	0.10	0.74
2	-0.491	0.26	3.81	0.14	2	-0.434	0.26	2.77	0.25
3	0.144	0.24	4.16	0.24	3	-0.102	0.24	2.94	0.40
4	-0.000	0.22	4.16	0.38	4	0.152	0.22	3.40	0.49
February temperature					**Laying date**				
Lag	*r*	*S.E.*	*Q statistic*	*P*	Lag	*r*	*S.E.*	*Q statistic*	*P*
1	0.168	0.28	0.35	0.55	1	0.185	0.28	0.42	0.51
2	-0.297	0.26	1.60	0.45	2	-0.317	0.26	1.84	0.39
3	-0.070	0.24	1.68	0.64	3	-0.052	0.24	1.88	0.59
4	-0.100	0.22	1.88	0.75	4	-0.399	0.22	5.04	0.28
Residuals NAO index					Residuals January rainfall				
Lag	r	S.E.	Q statistic	P	Lag	R	S.E.	Q statistic	P
1	-0.020	0.28	0.00	0.94	1	-0.271	0.28	0.91	0.34
2	-0.592	0.27	4.96	0.08	2	-0.231	0.26	1.66	0.43
3	-0.207	0.25	5.67	0.13	3	-0.051	0.25	1.79	0.63
4	0.246	0.22	6.87	0.14	4	0.177	0.22	2.32	0.67
Residuals January temperature					Residuals February rainfall				
Lag	r	S.E.	Q statistic	P	Lag	R	S.E.	Q statistic	P
1	-0.227	0.28	0.64	0.42	1	-0.091	0.28	0.10	0.74
2	-0.450	0.26	3.50	0.17	2	-0.455	0.26	304	0.22
3	0.162	0.25	3.93	0.27	3	-0.122	0.24	3.28	0.35
4	-0.068	0.22	4.02	0.40	4	0.178	0.22	3.91	0.41

Residuals February temperature					Residuals Laying date				
Lag	r	S.E.	Q statistic	P	Lag	r	S.E.	Q statistic	P
1	0.144	0.28	0.26	0.61	1	0.216	0.28	0.58	0.44
2	-0.306	0.26	1.58	0.45	2	-0.311	0.26	1.95	0.37
3	-0.067	0.25	1.66	0.64	3	-0.085	0.24	207	0.55
4	-0.088	0.22	1.81	0.77	4	-0.400	0.22	5.24	0.26
April temperature					Residuals April TªC				
Lag	r	S.E.	Q statistic	P	Lag	r	S.E.	Q statistic	P
1	-0.483	0.28	2.89	0.08	1	-0.489	0.28	2.96	0.08
2	0.009	0.26	2.89	0.24	2	-0.016	0.26	2.96	0.22
3	0.107	0.25	3.08	0.38	3	+0.119	0.25	3.20	0.36
4	-0.408	0.22	6.38	0.17	4	-0.401	0.22	6.38	0.17

Supplementary material Appendix 3, Figure A1.

Number of magpie nests (white bars) in relation to laying date in the period 2005-2013. Number of parasitized nests is represented by black bars. N=424 nests of which 165 were cuckoo parasitized. Number of magpie nests and cuckoo parasitized nests (in brackets) for each year are provided on top right of each panel.

Median laying dates (in days from 1 of April) for parasitized magpie nests were 19.5, 19.0, 30.0, 30.0, 27.0, 25.0, 19.0, 21.0, and 29.0 from 2005 to 2013, respectively, and 19.0, 22.0, 21.5, 23.0, 26.0, 26.0,18.0, 18.0, and 20.0 from 2005 to 2013 for all magpie nests.



Capítulo 5

Evidence of long-term structured cuckoo parasitism on individual magpie hosts

Este capítulo reproduce íntegramente el artículo:

Molina-Morales, M., Martínez, J.G., Martín-Gálvez, D., Dawson, D.A., Rodríguez-Ruiz, J., Burke, T., & Aviles, J. M. (2013). Evidence of long-term structured cuckoo parasitism on individual magpie hosts. *Journal of Animal Ecology*, *82*(2), 389-398.

Evidence of long-term structured cuckoo parasitism on individual magpie hosts

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Summary

1. Brood parasites usually reduce their host's breeding success resulting in strong selection for the evolution of host defenses. Intriguingly, some host individuals/populations show no defense against parasitism which has been explained within the frame of three different evolutionary hypotheses. One of these hypotheses posits that intermediate levels of defense at the population level may result from nonrandom distribution of parasitism among host individuals (i.e. structured parasitism). Empirical evidence for structured brood parasitism is, however, lacking for hosts of European cuckoos due to the absence of long term studies.

2. Here we seek to identify patterns of structured parasitism by studying great spotted cuckoo parasitism on individual magpie hosts over five breeding seasons. We also aim to identify whether individual characteristics of female magpie and/or their territories related to the status of repeated parasitism.

3. We found that 28.3% of the females of our population consistently escaped from cuckoo parasitism. Only 11.3% of females were always parasitized, and the remaining 60.4% changed their parasitism status. The percentage of females that maintained their status of parasitism (i.e. either parasitized or non-parasitized) between consecutive years varied over the study. Females that never suffered cuckoo parasitism built bigger nests than parasitized females at

the beginning of the breeding season and smaller nests than those of parasitized females later in the season. Non-parasitized females also moved little from year to year, and preferred areas of different characteristics over the course of the breeding season than parasitized females. Overall, females escaping from cuckoo parasitism reared twice as many chicks per year than those that were parasitized.

4. In conclusion, our study reveals for first time the existence of a structured pattern of cuckoo parasitism based on phenotypic characteristics of individual hosts and of their territories.

Keywords: Great spotted cuckoo, host phenotype, long-term studies, Magpie, nest size, structured parasitism.

Introduction

Parasites exert major selective pressures on their hosts. Therefore, natural selection is expected to favour individual hosts that effectively counteract the effect of parasitism. Understanding the spatial and temporal dynamic of parasite-host interactions and identifying the factors affecting the evolution of host defences and parasite virulence are major challenges in current evolutionary ecology (Thompson 2006). Theoretical models aiming to investigate coevolution of parasites and their hosts have largely assumed that all host individuals in a population have the same chance of being infected (e.g. van Baalen 1998; Gandon, Agnew & Michalakis 2002; Best, White & Boots 2009). Most natural populations, however, experience some degree of social and/or spatial structure (Thompson 2006) and, therefore, it is expected that one host will be more likely infected if its close neighbours or individuals within its social group are infected. Only recently space and population viscosity were incorporated into theoretical models to approach the question of how host within-population spatial and social structure may affect the evolution of host resistance (e.g. Best *et al*. 2011; Débarre *et al*. 2012). Yet empirical evidence supporting structured parasitism and its effect on parasite-host coevolution in natural populations is scant (see however Kerr *et al*. 2006; Boots & Mealor 2007; Martínez-Padilla *et al*. 2012).

Interspecific avian brood parasitism is a particular form of parasitism in which a species, the parasite, lays its eggs in the nest of another species, the host, which carries out all the parental care, from incubating parasite eggs to feeding parasite chicks. Brood parasites usually reduce their host's breeding success (Davies 2000; Payne 2005). For instance, parasite eggs can hatch considerably earlier than hosts ones, because of their shorter incubation period, and parasite hatchlings may either remove all host eggs and nestlings from the nest (Davies 2000), or outcompete host nest siblings in their competition for food (Soler & Soler 2000). There is a large body of evidence showing that hosts can evolve behavioural mechanisms to respond to these selective pressures (Rothstein 1990; Davies 2000). These are nest defence against adult parasites (e.g. Røskaft *et al.* 2002; Davies *et al.* 2003; Welbergen & Davies 2009) and/or discrimination and removal of parasitic eggs (e.g. Brooke & Davies 1988; Soler & Møller 1990; Avilés *et al.* 2010; Spottiswoode & Stevens 2010) and/or nestlings (e.g. Langmore, Hunt & Kilner 2003; Grim 2007; Sato *et al.* 2010). On the other hand, the evolution of host defences can select for further counter-defences in the parasite, such as highly mimetic parasitic eggs to evade host detection (Brooke & Davies 1988), causing a coevolutionary arms race (Davies 2000).

Although brood parasites exert strong selection for the evolution of host defences many hosts display a striking lack of anti-parasite defences. For instance, British dunnocks (*Prunella modularis*) do not discriminate common cuckoo (*Cuculus canorus*) eggs despite their very different appearance and that dunnocks were affected by cuckoo parasitism for the last 600 years (Davies & Brooke 1989). Many other hosts of different cuckoos and cowbird species exhibit a noticeable absence of defensive behaviours (Davies 2000; Payne 2005). Understanding why some hosts accept avian brood parasitism despite its costs remains a challenge in this coevolutionary scenario (Rothstein & Robinson 1998; Payne 2005).

Three main evolutionary hypotheses can explain the apparent paradox of absence of anti-parasite defences in the face of costly parasitism. The *evolutionary lag hypothesis* attributes the absence of defence to an evolutionary lag in the development of the defensive mechanisms by hosts due to either an absence of the genetic variants needed to evolve the defence, or because there has been not enough time for the defence to spread out (Rothstein 1975). The *evolutionary equilibrium hypothesis* suggests that, given cognitive and physiological constraints on defence, the advantage of bearing it would be context-dependent. Accordingly, hosts would accept parasitism when the costs of avoiding it will exceed the costs of accepting it (Rohwer & Spaw 1988; Lotem, Nakamura & Zahavi 1992; Avilés, Rutila & Møller 2005; Krüger 2011). Finally *limited transmission of genetic variants* due to spatially structured parasitism between and within populations may also lead to apparent maladaptive absence of defences at the population level (Soler *et al.* 1999; Røskaft *et al.* 2002; Hauber, Yeh & Roberts 2004; Hoover, Yasukawa & Hauber 2006). Indeed, theoretical models predicted that accepter and rejecter phenotypes may coexist within a population as a consequence of nonrandom distribution of parasitism among host individuals ("repeated parasitism" *sensu* Hauber, Yeh & Roberts 2004). Although empirical evidence in support of the evolutionary lag and the equilibrium hypotheses has been reported for a wide variety of brood parasitic systems (reviewed in Winfrie 1999; Krüger 2007), empirical evidence for limited transmission of parasitism only exists for hosts of the brown-headed cowbird (*Molothrus ater*). These studies showed a higher than expected probability of parasitism for previously parasitized individuals between first and second breeding attempts within the same year and in consecutive breeding years (Hauber, Yeh & Roberts 2004; Hoover, Yasukawa & Hauber 2006; Hoover & Hauber 2007). Brown-headed cowbird parasitism inflicts comparatively lower costs on its hosts than the parasitic cuckoo species in Europe (Payne 2005). Therefore, more studies with different brood parasite-host systems in which parasites exert strong selection on their hosts are needed before general trends about the occurrence of repeated parasitism and its role on the evolution of host defences can be disclosed.

Here we first seek to identify long term patterns of repeated parasitism by great spotted cuckoos (*Clamator glandarius*) on female magpies (*Pica pica*) from 2007 to 2011 in a scenario of increased parasitism pressure. Hitherto, repeated parasitism was identified by studying individual host and/or territory exposure to brown-headed parasitism between pairs of consecutive years (Hauber, Yeh & Roberts 2004; Hoover, Yasukawa & Hauber 2006). Identifying individual hosts' exposure to parasitism over long term data frames will allow ascertaining to what extent the parasitism status of individuals and the emerging patterns of repeated parasitism at the population are affected by parasitism levels.

Secondly, we aim to identify what individual characteristics of female magpies and/or of the territories they hold across their life relate to the probability of suffering repeated parasitism. There is overwhelming empirical evidence that a variety of ecological and host phenotypic traits influence the exposure to brood parasites over the course of a breeding season (reviewed in Parejo & Avilés 2007; Cherry, Bennet & Moskat 2007). However, previous work has failed to identify phenotypic differences between individuals suffering repeated parasitism and those escaping from brown-headed cowbirds (Hoover, Yasukawa & Hauber 2006; Hoover & Hauber 2007). These studies targeted on morphological characteristics and age of hosts but did not study differences in explicit aspects known to reveal host quality (e.g. Parejo & Avilés 2007). Here, we will focus on studying repeated parasitism in relation to phenotypic and reproductive magpie traits for which empirical and experimental evidence supports a link with individual quality (Birkhead 1991). In addition, we also examine differences in nest size in magpie hosts in relation to repeated parasitism as experimental evidence supports that nest size is a post-mating sexually selected signal revealing parental abilities in magpies (de Neve *et al.* 2004) and may be used as a cue to choose profitable hosts by cuckoos (Soler *et al.* 1995a).

Thirdly we examine the relationship between repeated parasitism and level of defence in magpie hosts. Theoretical and empirical work suggests that limited transmission of parasitism may greatly impact the spatial dynamics of parasite-host interactions (Lenormand 2002), and in a scenario of brood parasitism, theoretical models have shown that structured parasitism may affect the dynamic of cuckoo-host interaction at the population level by retarding the evolution of host defences (Grim 2002; Hauber, Yeh & Roberts 2004; Røskaft *et al.* 2006; Avilés & Parejo 2011). Limited horizontal transmission of brood parasitism is expected to diminish the fitness benefits of egg rejection and to shape the ontogeny of hosts′ recognition systems (Hauber, Yeh & Roberts 2004; Hoover, Yasukawa & Hauber 2006). An empirical premise remaining to be tested in this theoretical scenario is that host phenotypes differing in their exposure to parasitism also differed in their baseline levels of defences against brood parasites. Here we provide a first empirical test of this assumption by studying the relationship between long-term exposure to great spotted cuckoo parasitism and level of defences in individual magpies. We will specifically target on two known key components of magpie defence against cuckoo parasitism, namely egg discrimination (Soler & Møller 1990; Soler *et al.* 1999) and intra-clutch variation in egg appearance (Soler, Soler & Møller 2000; Avilés *et al.* 2004).

Finally, we will examine for the first time the effect of repeated parasitism on fitness and dispersal of magpie hosts. So far empirical studies have demonstrated deleterious effects of cuckoo parasites on host reproduction (reviewed in Davies 2000; Payne 2005), and found some support for a link between host dispersal and brood parasitism (e.g. Hoover 2003; Sedgwick 2004; Molina-Morales, Martínez & Avilés 2012), over the course of a breeding season. However, previous studies disregarded analyzing individual long-term exposure to cuckoo parasitism in relation to host productivity and dispersal which may hamper a realistic assessment of the strength of cuckoo selection on host defences.

Methods

Study area and system

The study was conducted in La Calahorra (37º 10′ N, 3º 03′ W, Hoya de Guadix, Southern Spain) during the years 2007-2011. This is a patchy area where groves of almond trees (*Prunus dulcis*), in which magpies preferentially build their nests, are very common. Magpies are territorial, sedentary, and socially monogamous long-lived passerines (Birkhead 1991).

In our study area magpies lay one clutch during April-May, and are the main host of the great spotted cuckoo. Cuckoo parasitism severely reduces magpie reproductive success through early hatching and effective competition for parental food delivery of cuckoo nestlings (e.g. Soler, Martínez & Soler 1996; Soler, Soler & Martínez 1997), and it has selected for host recognition and rejection of cuckoo eggs, which in turn has selected for punitive cuckoo behaviours promoting parasitism acceptance (Soler *et al.* 1995b). Thus, the interaction between magpies and great spotted cuckoos can be regarded as an example of coevolution (Soler & Soler 2000).

Temporal variation in prevalence of great spotted cuckoo parasitism

The percentage of parasitized nests in our population (i.e. parasitism rate) varied between years (15.9% in 2007, 25.4% in 2998, 65.6% in 2009, 50.7% in 2010, 55.77% in 2011) and increased through the breeding season affecting in average almost 70 % of magpie nest in the last ten-days of the laying season (Spearman correlation, r_{p}=0.88, P = 0.01, N =6, Figure 1).

Individual marking and monitoring

Some of the adult magpies were captured and colour ringed and thus monitored by observation. Monitoring of non-ringed females was based in parentage analyses (details of captures, molecular methods and parentage analyses can be found in Molina-Morales *et al.* 2012).

Briefly, we assigned particular breeding attempts in different years to the same female when the nestlings in those broods were found to be all full siblings to each other. Also, we could assign breeding attempts to marked females using paternity analyses, so that females could be matched to their nest in a given year even if they had not been marked in that year but later on and thus observations were not available (Molina-Morales *et al.* 2012).

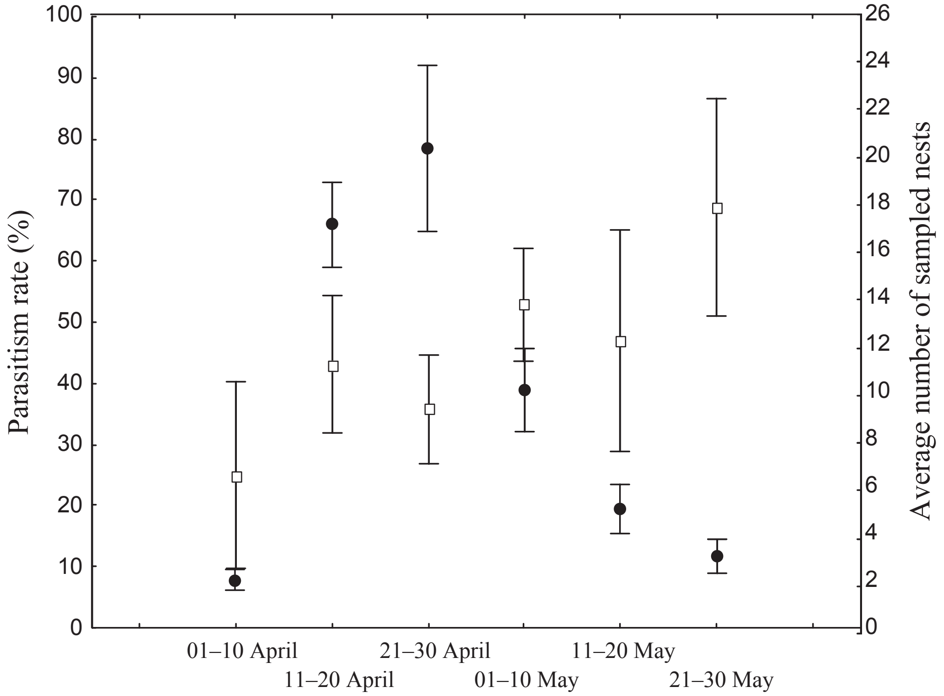

Figure 1. Distribution of great spotted cuckoo parasitism during the breeding season. Each interval corresponds to 10 days. The left Y axe corresponds to the average percentage (± SE) of parasitism calculated for each ten day interval since the start of parasitism in the five years of study (white dots). The right Y axe corresponds with the average (± SE) number of sampled nests in each interval in the five years of study (black dots).

Nest monitoring and individual characteristics of female magpie hosts and of the territories

Magpie nests were monitored from 1 March to the beginning of July each breeding season. Nests were found by careful inspection of all trees in the area, and GPS positioned. Each nest was observed with telescope from a hide around 100 meters away during nest building in order to assign marked birds to each nesting attempt. Nests were visited at 5 days intervals, although during egg laying and hatching the nests were visited every 2-3 days to check whether the nest was parasitized by great spotted cuckoos and to record all required data (see below). Nests were categorized as parasitized if at least one cuckoo egg was detected in the nest. Replacement clutches were not included in this study in order to obtain unbiased estimates for individual characteristics of female magpies known to decline with season (for example clutch size, nest productivity, nest size). For each breeding event we recorded the following data:

(a) Laying date, estimated as the number of days from the first of April, clutch size and number of fledglings.

(b) Average egg volume, as the mean value of the volume of all magpie eggs in a clutch. The volume of each individual egg was estimated as $4/3(\Pi \text{ x } a \text{ x } b^2) / 1000$ (in litres), where a is the largest radius of the ellipsoid egg and b is half of the egg width measured with a calliper (precision 1 mm).

(c) Nest volume. The size of nests was estimated using a measuring tape (precision 1 cm); we measured height and width and calculated nest volume using the same ellipsoid formula used to estimate egg volume.

(d) Intra-clutch variation in egg appearance. We took a picture of each clutch using a CANON 350D digital camera. All photographs were taken under standardized light conditions on a Kodak grey card. Intra-clutch variation was estimated on these photographs following the scale of Øien, Moksnes & Røskaft (1995). Briefly, this method attributes increasing levels of variation in egg appearance within a clutch on an ordinal scale (1, no variation, to 5, all the eggs were different from one another) based on human perception. Seven experienced observers scored intra-clutch variation for all clutches (103 clutches). The different assessments of a clutch were moderately consistent (repeatability = 0.428, $F_{103,624} = 6.23$, $P < 0.001$), thus justifying the use of the mean values attained for the seven observers as an estimate of the degree of intra-clutch variation in all further analyses.

(e) Response to mimetic model eggs. We tested magpie responses to mimetic model eggs to classify females as acceptors or rejecters (e.g. Soler & Møller 1990; Soler *et al.* 1999). We introduced one mimetic model egg during magpie egg laying and revisited the nest after 6-7 days. Previous work in our magpie population has shown that 75 % of all rejection of artificial models occurs in the first 24 hour after parasitism and that after 72 hours all eggs have been rejected

(Avilés et al. 2004). The response was regarded as a rejection if the model egg disappeared from the nest or acceptance if the model egg was incubated with the host's clutch.

(f) Host density. For each nest we measured the distance to the two nearer conspecific nests (nearest neighbour distance), and used the average of both distances as an estimate of magpie density in the area surrounding each nest.

(g) Breeding dispersal distance. Following Molina-Morales *et al.* (2012) this was calculated as the shortest distance in meters between two nests occupied by the same bird in consecutive years.

(h) Spatial information. We used GIS software ArcGIS 9.3 version (ESRI 2008) to obtain environmental data based on aerial photographs and 2003 Vegetation Cover and Land Use Databases for the Province of Granada that were freely available from Junta de Andalucía (VV.AA. 2003, Junta de Andalucía 2006). We recorded the following information regarding the spatial situation of each nest: (1) distance in meters to the closest pine forests as an estimate of distance to great spotted cuckoo`s feeding site as great spotted cuckoos feed almost exclusively on Pine Processionary, *Thaumetopoea pityocampa* (Soler 2003), (2) distance to the closest track, (3) distance to dry riverbeds, open habitats (4) distance to the motorway, (5) distance to the nearest village, (6) percentage of wooded surface within 100 m around the nest, (7) percentage of herbaceous crop within 100 m around the nest, and (8) percentage of surface occupied by almond trees within 100 m around the nest.

Statistical analyses

Analyses were performed using Statistica 7.0 (Statsoft Inc. Tulsa, OK, USA). We characterized each magpie female regarding its parasitism status along all her known breeding attempts as never parasitized, sometimes parasitized or always parasitized. Firstly, we tested for differences in number of years that a magpie female was sampled in relation to status of parasitism as a categorical predictor using a Poisson Generalized Linear Model which allowed us to know whether the probability of classifying a given female in relation to parasitism status depended on the number of years it was monitored.

In addition we ran a Montecarlo simulation based on the null model that, within each year, parasitism would occur randomly among the individuals. We wrote a program in R to perform the following algorithm:

1.- Set a counter (“count”) to 0.

2.- Within each year, randomly permute the parasitic status (non-parasitized=0, parasitized=1) among the magpies.

3.- Once step 2 is done for each year, recalculate the random frequencies of birds that are “always”, “sometimes” or “never” parasitized.

4.- Compare the randomly calculated frequencies of “always” and “never” parasitized females with the observed ones. If both random frequencies are identical as, or smaller than the observed frequencies add one unit to the counter: $count_{i+1}=count_i+1$.

5.- Repeat 1000 times steps 2 through 4.

6.- Calculate the final p-value as: p=count (1/1000). If $p < 0.05$ the observed frequencies will be significantly different from that that would be expected by chance alone.

Because the parasitic status among females is reshuffled within each year, the above algorithm is neither affected by the number of years that a bird was observed nor by the inter-annual variability in parasitic frequencies.

Secondly, for each identified female we determined whether it maintained its parasitism status (i.e. either if it was parasitized or non-parasitized) or if its status was reversed (either if it passed from parasitized to non-parasitized or from non-parasitized to parasitized) in two consecutive years. This yielded a dichotomous variable revealing parasitism status (changed/ maintained). We then determined whether the frequency of females repeating parasitism status in consecutive years differed from that in other two consecutive years by using contingency analyses (Zar 1996). Given that parasitism rate steadily increased over the course of the study, this approach allowed us to detect how fluctuations in prevalence of parasitism may affect female status of parasitism by comparing the frequency of changes in the status between consecutive years with similarly low parasitism (i.e. 2007-2008), with that in consecutive years with similarly high parasitism (i.e. 2009-2010), or with that in consecutive years after a sudden increase of parasitism (i.e. 2008-2009).

Aiming to relate long term parasitism status with the phenotypic and spatial variables previously described, and because we have several breeding attempts (2-5) for each female, we calculated the average value of each phenotypic and spatial variable for each female. This is justified because repeatability analysis revealed that all these variables were repeatable ($r > 0.25$, $F_{53,74} > 2.16$, $P > 0.0001$), except intra-clutch variation in egg appearance ($F_{1,53} = 1.48$, $P = 0.09$). When calculating mean clutch size and number of fledglings of females sometimes parasitized we excluded those years when females were parasitized, because it is well established that parasitized magpie nests have smaller clutch and brood sizes than unparasitized ones (Soler, Martínez & Soler 1996). In this way we can test for the first time whether the breeding outcome of never parasitized females differed from that of females that are parasitized but eventually escaped parasitism. However, because we were also interested in the long term fitness consequences of repeated parasitism in a second analysis we also averaged the number of fledglings a female produced during her life including years that it was parasitized for females that sometimes were parasitized. In this way we can contrast long term female productivity in relation to repeated parasitism. Finally, since we tested each female several times for model egg rejection, females were classified as rejecters if they rejected the model egg at least one time, or acceptors if they always accepted the model egg.

Mean laying date, clutch size, number of fledglings and volume of eggs of each female followed a normal distribution (Shapiro-Wilk tests: $W > 0.95$, $P > 0.06$). Body condition, tarsus length and bill length were approximately normally distributed (Shapiro-Wilk tests: $W > 0.94$, $P > 0.29$). Mean nest volume and mean breeding dispersal distance were log transformed to fit a normal distribution (Shapiro-Wilk tests: $W > 0.96$, $P > 0.13$). Regarding spatial information, we have first calculated the mean value of each variable for the different nests of each female. After that, we have used Principal Components Analysis (PCA) in order to transform several correlated variables into a few orthogonal variables (the principal components). We obtained three PCA factors with eigenvalues >1. The first principal component (PC1) was negatively related with percentage of herbaceous crop and positively with percentage of woody surface and percentage of surface occupied by almond trees, and thus represents a gradation in the area surrounding the nest from clear to wooded spaces (Table 1). PC2 was positively related with distance to tracks and negatively with distance to villages and to dry riverbeds. PC3 was positively related with distance to parasites feeding places (Table 1).

All the females were divided in two groups. The first group included nonparasitized females, and the second group was compound by always and sometimes parasitized females. We did it so because of the low number of cases for always parasitized females, (only 6 cases). and because our aim was to identify factors that may explain why some females systematically escaped from cuckoo parasitism and others did not. General linear models were used to look for differences among individuals parasitized or not in phenotypic and

Table 1. Results of the PCA on spatial variables

Variable	PC1	PC2	PC3
Distance to the closest pine forests	0.248	0.314	**0.818**
Wooded surface	**0.815**	-0.117	-0.116
Distance to closest track	0.281	**0.657**	0.266
Distance to motorway	**0.735**	-0.359	0.503
Distance to nearest village	0.303	**-0.811**	0.162
Distance to dry riverbed	-0.494	**-0.682**	0.169
Percentage of herb growing	**-0.822**	-0.222	0.346
Percentage of wooden growing	**0.838**	-0.211	-0.278
% of variance	38.2	23.6	15.7

Factor loadings for the three first axes of a principal component analysis on spatial variables. Loadings in bold indicate the most important factors (score > |0.60|). Percentage of variance explained by each axis is also shown.

spatial variables. Previous work has shown that laying date is a correlate of individual quality in magpies (Soler *et al.* 1995a). Therefore, aiming to account for individual variation in quality in our analyses we entered laying date and its interaction with parasitism status in all the models except in the analysis of breeding dispersal distances, because dispersal movements occur after the breeding season. Finally we used a logistic regression model to test whether rejection of parasite eggs in magpies (i.e., rejection *vs* acceptance) was explained by parasitism status while accounting for laying date.

Results

Parasitism status of females in different breeding attempts

We assessed parasitism status for 53 females that were monitored on average 2.41 years (range 2-5 years; standard deviation: 0.718). The number of years that a female was monitored did not significantly differ between females always parasitized, sometimes parasitized and never parasitized ($\chi^2_2 = 0.193$, $P = 0.908$), suggesting that differences in parasitism status were not due to the number of years a given female was monitored.

Interestingly, 15 out of 53 females of our population (28.3 %) were never parasitized. Of the remaining 38 females only 6 (i.e. 11.3 %) were always parasitized, and 32 (i.e. 60.4 %) switched their parasitism status. The Monte-Carlo simulation analysis showed that in 90% of cases the observed frequency of always, never and sometimes parasitized females was different from that expected by chance ($P = 0.10$).

The percentage of females that maintained their status of parasitism (i.e. either parasitized or non-parasitized) between consecutive years varied over the course of the study. Up to 77.8 % of females maintained their parasitism status from 2007 to 2008, when parasitism rate was low in the population. This percentage doubled that of females maintaining parasitism status between 2008 and 2009 (37.5%; $\chi^2_1 = 5.67$, $P = 0.017$), when cuckoo parasitism markedly increased in the population. However, the proportion of magpie females maintaining their parasitism status between pair of years with similarly low (i.e. 77.8%, 2007-2008) and high (i.e. 56.3%, 2009-2010) parasitism did not significantly differ ($\chi^2_1 = 1.79$, $P = 0.18$). Similarly, non-significant differences existed in the proportion of females maintaining parasitism status between 2008 and 2009 and between 2009 and 2010 ($\chi^2_1 = 1.13$, $P = 0.28$).

Parasitism status and phenotypic and reproductive traits of magpie hosts.

Among the analysed traits only the relationship between nest size and laying date was different for parasitized and never parasitized females (Table 2, Appendix S1). The size of the nests modestly decreased across the season, although non-significantly, for females escaping from cuckoo parasitism ($R = 0.345$, $F_{13} = 1.754$, $P = 0.207$), whereas among the parasitized females those breeding later in the season had larger nests than those breeding at the beginning of the season ($R = 0.357$, $F_{36} = 5.259$, $P = 0.028$, Fig. 2).

Parasitism status and host defensive traits

Parasitized and non-parasitized females did not differ in intra-clutch variation in egg appearance (Table 2) nor in their capacity to reject model eggs (Table 2).

Parasitism status and host dispersal and density

Never parasitized and parasitized females differed in their average breeding dispersal distances (Table 2). Magpies escaping from cuckoo parasitism moved less than those suffering cuckoo parasitism (Never parasitized: mean =151.60, SD =143.70, n=15; Parasitized: mean = 327.60, SD = 521.96, n = 38).

Long term conspecific density experienced by females did not differ between parasitized and never parasitized magpies (Table 2).

Parasitism status and long term host breeding productivity

Long term host productivity was affected by repeated parasitism (Table 2). Indeed, never parasitized females produced on average 4.49 (SD=1.57) fledglings per breeding attempt across their life whereas females that were occasionally or always parasitized produced 2.45 (SD=1.69) and 0.83 (SD=1.16) fledglings, respectively.

Table 2. Relationships between long-term parasitism status (i.e. either parasitized sometimes or always *versus* never parasitized) and reproductive and defensive traits of magpie hosts.

Dependent variable	**Predictor**	**df**	***F*/ *Wald stat***	***P***
Body condition	Parasitism	1, 17	1.44	0.246
Tarsus length	Parasitism	1,17	1.80	0.197
Bill length	Parasitism	1,16	4.04	0.061
Laying date	Parasitism	1, 51	1.77	0.188
Clutch size	Parasitism	1, 40	1.915	0.174
	Laying date		0.506	0.480
	Laying date x Parasitism		3.013	0.090
Number of fledglings	Parasitism	1, 43	0.687	0.412
	Laying date		2.467	0.123
	Laying date x Parasitism		1.320	0.257
Egg Volume	Parasitism	1, 49	0.234	0.630
	Laying date		0.044	0.834
	Laying date x Parasitism		0.262	0.610
Nest size	Parasitism	1, 49	2.525	0.118
	Laying date		0.653	0.422
	Laying date x Parasitism		5.837	0.019
Intraclutch variation	Parasitism	1, 46	2.185	0.146
	Laying date		0.032	0.857
	Laying date x Parasitism		1.792	0.187
Rejector/Aceptor	Parasitism	1	1.106	0.293
	Laying date		2.390	0.122
	Laying date x Parasitism		1.662	0.197
Dispersal movements	Parasitism	1,51	4.67	0.035
Conspecific density	Parasitism	1, 49	0.968	0.329
	Laying date		6.245	0.016
	Laying date x Parasitism		0.0001	0.993
Breeding success	Parasitism(never,sometimes,always)	1, 47	12.314	<0.0001
	Number of breeding attempts		0.890	0.350
PC1 habitat score	Parasitism	1, 49	3.378	0.072
	Laying date		7.666	0.007
	Laying date x Parasitism		4.164	0.046

Analyses are general linear models and logistic regressions with reproductive and defensive variables of magpies as dependent variables. Laying date (1=1 april) was introduced as a covariate (see methods).

Parasitism status and long term host habitat preference

We found that long term habitat preference of magpie hosts was related to parasitism status in interaction with laying date (Table 2). Magpies escaping from cuckoo parasitism bred in areas with a larger percentage of wooded surface (i.e. high positive PC1 scores) at the beginning of the breeding season, whereas those breeding late in the season preferred to breed in areas with a smaller percentage of wooded surface and larger percentage of herbaceous crops (Figure 3).

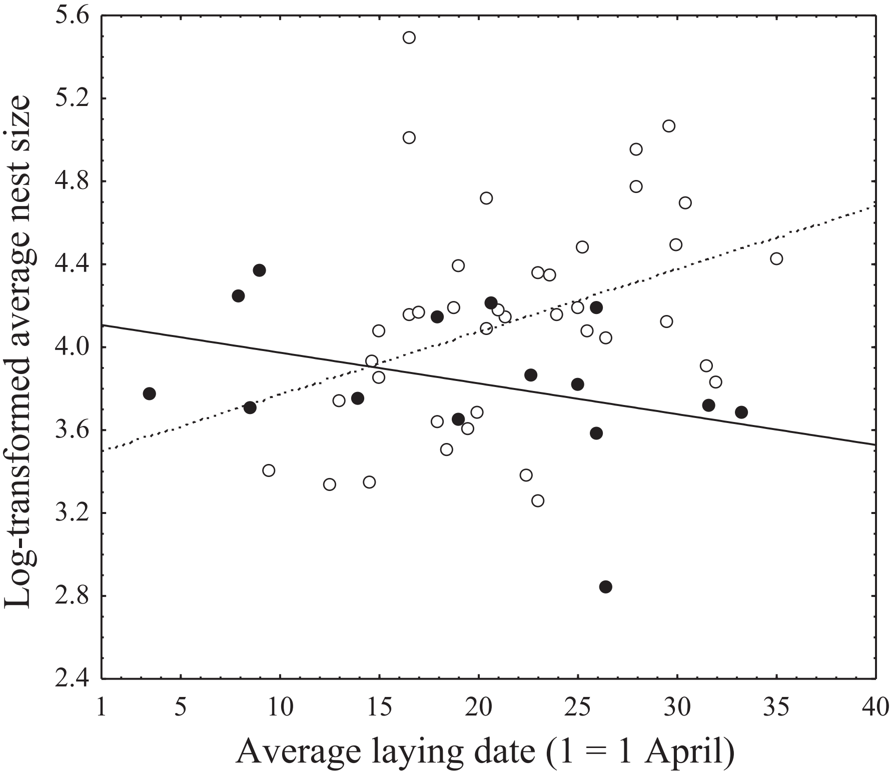

Figure 2. Relationship between log-transformed nest size and laying date for parasitized (open marks, dashed line) and never-parasitized (filled marks, continuous line) magpie host females.

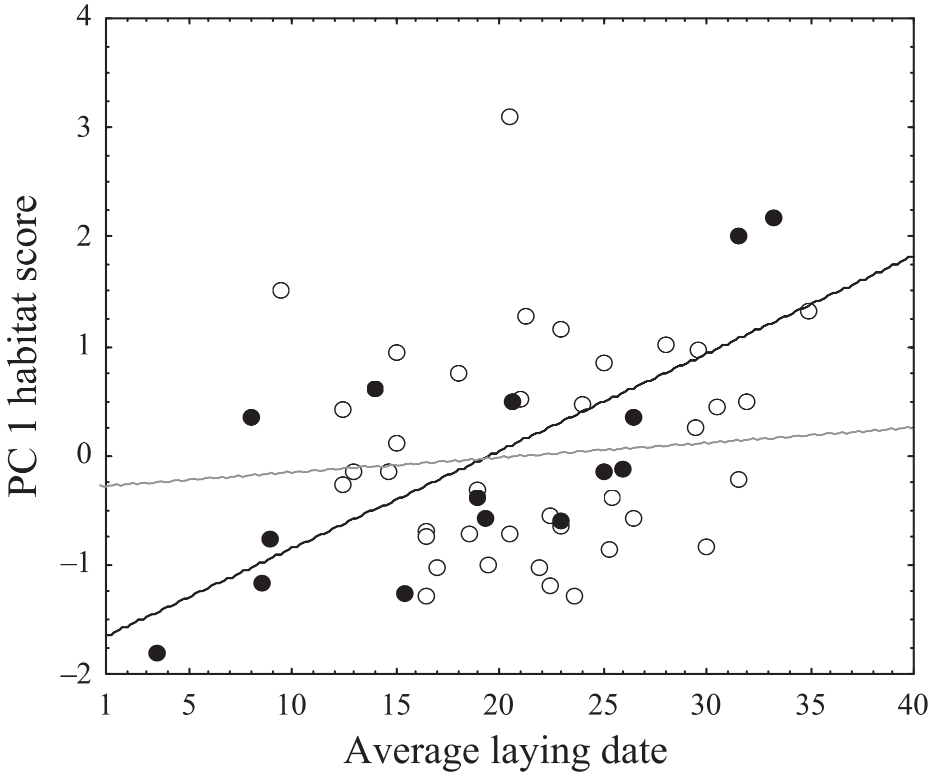

Figure 3. Relationship between long-term habitat use (i.e. PC1 habitat score) and laying date for parasitized (open marks, dashed line) and never parasitized (filled marks, continuous line) magpie host females. Parasitized: R=0.059*,* *F*=0.125, df=1, *P*=0.726. Never-parasitized: R=0.682, *F*=11.30, df=1, *P*=0.005.

Discussion

Theoretical models predict that spatial and temporally structured patterns of parasitism may influence co-evolutionary dynamics of parasite-host interactions (Hoover, Yasukawa & Hauber 2006; Røskaft *et al.* 2002; Best *et al.* 2011; Débarre *et al.* 2012). An obvious prerequisite of these models is that most natural host populations are somehow socially and/or spatially structured and therefore that not all host phenotypes have the same chance of being infected across their life. So far studies of avian brood parasitism aiming to detect structured parasitism have considered a short temporal scale, usually of two consecutive years, and targeted on hosts of the brown headed cowbird (e.g.

Hauber, Yeh & Roberts 2004; Hoover, Yasukawa & Hauber 2006). However, hosts of brood parasites often breed more than two years, and parasitism level may vary greatly from one year to another at the population scale (see methods). Thus, it is only by performing long term studies that we will be able to ascertain the true occurrence of repeated parasitism (sensu Hauber, Yeh & Roberts 2004), and to establish the causes and consequences of a long term status of parasitism in individual females. Here we monitored female magpies during several years, detecting repeated parasitism over several breeding seasons tested for consistency of the emerging patterns over a scenario of variable parasitism pressure. We also looked for differences in individual characteristics of females and of the territories they hold across their life and in long term nestling productivity, as a correlate of fitness, between females suffering parasitism and those that have never been parasitized.

We found that almost 30% of the sampled females systematically escaped from cuckoo parasitism, even when parasitism level steadily increased in the population, and neighbour conspecific nests were parasitized to some extent over the five study years. In addition, we report that 11.3% of sampled females were always parasitized, and that the probability of characterizing the status of parasitism of individual magpies as never parasitized or sometimes/ always parasitized was not due to differences in sampling effort. Furthermore, our simulation analysis revealed that random expectation had a low chance (10%) of producing the observed pattern of repeated parasitism. Altogether, this can be interpreted as an evidence of moderate limited horizontal transmission of parasitism at the within population scale (sensu Hoover, Yasukawa & Hauber 2006), suggesting that selection for the evolution of host defences is not uniform within our magpie population, and therefore, setting the scenario for the study of phenotypic and reproductive differences between females that are able to avoid parasitism and those that are parasitized. Probability of repeating parasitism status (either parasitized or not parasitized) in consecutive years varied across the study. Almost 80% of females repeated their parasitism status between 2007 and 2008, when great spotted cuckoo parasitism affected about 25% of the host population. Previous studies with cowbird hosts have found evidence of a non-random pattern of parasitism across breeding seasons irrespectively of parasitism rates (Hauber, Yeh & Roberts 2004; Hoover, Yasukawa & Hauber 2006). Indeed, these studies show parasitism rates of cowbirds that are around 40% in Eastern phoebes (Hauber, Yeh & Roberts 2004), and around 60% in Prothonotary warblers (Hoover, Yasukawa & Hauber 2006). In the same vein, here we reported that the proportion of females maintaining their parasitism status between two years with low level of parasitism (i.e. 2007-2008) did not differ from that between years with high level of parasitism (i.e. 2009-2010), which suggests that cuckoo parasitism may be temporally structured within host populations provided parasitism levels, either high or low, were homogeneous over time.

In a scenario of changing parasitism pressure, however, our results show a significant lower consistency in the pattern of repeated status of parasitism between consecutive years at the level of individual. Indeed, the proportion of females that repeated their parasitism status suffered a drastic decrease between 2008 and 2009 after a marked increase of parasitism level in 2009. Therefore, our findings constitute the first empirical support for the contention that patterns of repeated status of parasitism within host populations are not consistent over time and can be influenced by parasitism level oscillations at the population scale.

Our results show that there are no differences between magpies consistently escaping parasitism and those parasitized (either sometime or always) in terms of most phenotypic (i.e. body condition, body size, bill length or egg volume) or reproductive traits (i.e. clutch size, laying date and number of fledglings). Interestingly, however, we found that among the earlier breeding magpies, those building bigger nests had a lower chance of being parasitized. The trend was the opposite for the late breeders, as those having in average larger nests across their life had the highest chance of suffering parasitism (Fig. 2). This pattern emerging from long-term monitoring of status of parasitism and of phenotypic and reproductive magpie host traits contrasts with previous findings showing that great spotted cuckoos selected larger magpie nests to lay their eggs (Soler *et al.* 1995a). Contrary, our results show that some females breeding early in the season escape from cuckoo parasitism even those with large nests. This nest-size mediated change in status of parasitism over the course of the season may be explained in terms of seasonal changes in nest conspicuity. At the beginning of the season the number of magpie nests available (that is laying eggs) for cuckoos is small (see Fig 1), and because trees are yet leafless (M.M-M pers. observ.) all nests may be easily located by cuckoos. At this time, cuckoos may simply parasitize smaller nests because they are more accessible, since they have fewer sticks in the roof and the cup may have several entrances. However, later in the season the amount of nests available increases (Fig. 1) as trees grow leaves and, therefore, nests become less conspicuous. The highest availability of host's nests corresponds with the higher level of parasitism, and then cuckoos may parasitize more conspicuous (large) nests. Alternatively, assuming active selection by nest size revealing parental quality (sensu Soler *et al.* 1995a), there may be not enough differences in quality between pairs with different nest size at the beginning of the season to select for "choosing" cuckoos, whereas later on larger nests correspond to better magpie pairs. Indeed, variation in nest size among earlier breeding magpies was lower than among late breeding magpies (see Fig. 2). In any case, irrespective of the host selection mechanism behind our results do point out to nest size as one of the traits explaining differences in long term status of parasitism of magpie hosts.

Our results provide support for the existence of a pattern of structured parasitism related to habitat characteristics. Indeed, we have found that never parasitized females chose wooded areas at the beginning of the season whereas later they bred in clearer places. There are no differences between parasitized and unparasitized females in other characteristics of the habitat, such as distance to cuckoo feeding areas, to the village or roads. The spatial habitat structure hypothesis (Røskaft *et al.* 2002) predicts that different habitat characteristics such as the presence of perches (trees or other vantage points) will influence the probability of nests of being parasitized. Our results support this hypothesis, because individual hosts escaping from cuckoo parasitism across their life breed in areas with different characteristics along the season than those suffering some degree of cuckoo parasitism. Why more wooded areas should be better than clearer areas at the beginning of the season to escape parasitism (and the opposite) remains to be explained, but may be related to changes in detectability and availability of host nests across the season (see above).

Limited horizontal transmission of brood parasitism has been suggested to slow down the evolution of host resistance (Hauber, Yeh & Roberts 2004)). The theoretical model proposed in Hauber, Yeh & Roberts (2004) predicts that both the selective consequences and the time-frame for the evolution of host resistance strategies depend on the costs of brood parasitism and the magnitude of repeated parasitism. Since the costs of parasitism in cowbird are not too high and there are consistent patterns of repeated parasitism at the individual level, the model predicted a low chance for the evolution of host resistance that was empirically confirmed with field data in two cowbird hosts (Hoover, Yasukawa & Hauber 2006). In the great spotted cuckoo-magpie system the costs of parasitism are higher than in cowbirds (Davies 2000; Payne 2005) and the magnitude of repeated parasitism is low or moderate, as more than half the females studied changed their parasitism status along their life. Thus the benefits of rejection must be large enough to spread out this character in the populations, as previous work shows (Soler & Moller 1990; Soler & Soler 2000). Accordingly, we have not found evidence that long term parasitism status was related with host defences at the analyzed spatial scale. Indeed, rejection behaviour and intra-clutch variation in egg appearance, two traits related to the defensive capacity of magpie hosts (Soler *et al.* 1999; Avilés *et al.* 2004), did not differ between magpies never parasitized and those parasitized. This may suggest that the detected degree of structured parasitism is not high enough to select for a structured pattern of host defence at the within-population level. This is not surprising given the close spatial proximity, and thus probably high gene flow, between parasitized and non-parasitized host phenotypes within the population, that would impede genetic or phenotypic differentiation (Lenormand 2002; Slatkin 1985). Alternatively, it cannot be discarded that structured parasitism was only a recent phenomenon in the population, and, therefore, that the absence of a structured level of defence was due to an evolutionary lag sensu Rothstein (1975).

In conclusion, females systematically escaping from cuckoo parasitism in our population presented a breeding strategy that allow them to evade parasitism, characterized by building a nest of different size and settling in areas of different characteristics along the breeding season in comparison with parasitized females. In addition, we reported that unparasitized females move little between years as compared to females experiencing parasitism. Although our sample size are small and thus be regarded with caution, this might suggest that a combination of nest size/laying date/characteristics of the habitat make unparasitized magpies successful at avoiding parasitism, and this might explain why they disperse less than parasitized females. That they are very successful is clear in that these females were able to rear doubled the number of chicks than those that were parasitized once or more.

A final theoretical inference emerging from our study is that more long-term individual-based studies are needed to establish the variation in spatial and temporal structure of brood parasitism in different systems, and thus to understand the long term dynamics of avian brood-host parasite interactions. Critically, long term individual-based studies will provide a more accurate picture of individual host success, allowing the possibility of adding up offspring production over the whole life span (Brooker & Brooker 1996; Krüger & Lindström 2001) and so of estimating the real costs of brood parasitism, which is the relevant parameter to understand the evolution of defensive mechanisms.

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Capítulo 6

The multidimensionality of behavioural defences against brood parasites: evidence for a behavioural syndrome in magpies

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The multidimensionality of behavioural defences against brood parasites: evidence for a behavioural syndrome in magpies

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Abstract

Studies of anti-parasite defences against cuckoo parasites have largely neglected the possibility that behavioural components of host defence may correlate giving rise to a behavioural syndrome. Furthermore, the different contribution of host´s sex in nest defence has traditionally been disregarded. Here, we studied magpie (*Pica pica*) mobbing behaviour towards dummies of great spotted cuckoo (*Clamator glandarius*) and non-harmful hoopoes (*Upupa epops*) and egg rejection of parasite eggs in a population of colour-banded magpies. We predicted a positive correlation between the intensity of nest defence and egg rejection within each sex and that females respond more intensely than males to the threat of brood parasitism as they undertake incubation. Magpie males, but not females, defended their nests more intensely in those nests in which cuckoo model eggs were rejected. Individual magpies did significantly differ in their baseline level of nest attentiveness; however, there were no individual differences once pair identity was considered Males and females defended their nests more intensely when it was exposed to the presence of a great spotted cuckoo dummy. Males, but not females, were more prone to appear at their nests, and females, but not males, were more prone to defend more intensely when their nests were challenged by a parasite threat. Our results thus agree with the view that mobbing behaviour and parasite egg rejection may actually constitute a pseudosyndrome and highlight the necessity to integrate interindividual variation and the sex of the host in studies of the evolution of host defences.

Key-words: behavioural syndrome, cuckoo parasitism, host defence, mobbing behaviour, host personality, sexual differences.

Introduction

Interspecific brood parasitism is a reproductive strategy of some birds in which the parasite species lays its eggs in the nest of another species, the host, which carries out the parental duties, from the incubation of eggs to chick feeding (Rothstein 1990; Davies 2000). It is often the case that, in parasitized nests, parasitic chicks survive but host´s offspring dies. This can be due to egg breakage during egg laying by parasite females, rejection of eggs and/or chicks from the nest by the parasite chick, and starvation of host's chicks due to parasite chick monopolization of parental feeds (Payne 1977; Rothstein 1990). The consequence of this interaction is the dramatic reduction of the host´s reproductive success, resulting in natural selection favouring certain features or behaviours that represent defences against parasitism (Davies 2000). Hosts of brood parasites have evolved a set of specific defences against parasites including mobbing of parasites before laying (e.g. Røskaft et al. 2002; Welbergen and Davies 2009), parasite egg discrimination and rejection (e.g. Davies and Brooke 1988; Soler and Møller 1990; Moksnes et al. 1991; Avilés et al. 2010; Spottiswoode and Stevens 2010), and nestling discrimination (Langmore et al. 2003; Grim 2007; Sato et al. 2010; Tokue and Ueda 2010).

Very often, it has been reported that several of these behavioural defences may simultaneously occur in the same host population. For instance, reed warblers *Acrocephalus scirpaceus,* hosts of the common cuckoo *Cuculus canorus,* mob cuckoos to avoid parasitism as a front line defence (Welbergen and Davies 2009) but can also reject cuckoo eggs from their nests once that the first defensive barrier is broken by cuckoos (Davies and Brooke 1988). The same applies to other cuckoo hosts (Moksnes et al. 1991; Røskaft et al. 2002) and magpie *Pica pica* populations exposed to great spotted cuckoo *Clamator glandarius* parasitism in the south of Spain (Soler and Soler 2000). Also, although many cowbird hosts in North America are egg acceptors and defend their nests intensely against brood parasites (revised in Sealy et al 1998), still several cowbird hosts can simultaneously reject cowbird eggs and defend their nests against the threat of parasites (MacLean and Rhodes 199; Briskie et al. 1992; Gill et al. 1997).

Occurrence of different behavioural defences against brood parasitism within a host population may arise because either: 1) different subsets of individuals display different behavioural defences in the population or 2) a subset of individuals in the population display all the behavioural defences and another subset of individuals do not display defences at all because behavioural defences are correlated across individuals forming a defensive syndrome (sensu Sih and Bell 2008) or because individuals showed similar condition-dependence in the expression of nest defence and egg rejection rendering that the two behaviours were correlated at the same point in time (Dingemanse and Dochtermann 2013). Discriminating between these possibilities is critical to achieve a better understating of the dynamics of host-brood parasite interactions because the linkage between different behaviours expressed by cuckoo hosts may greatly impact on the evolution of hosts defences at the population level (Avilés and Parejo 2011). Although overwhelming empirical evidence has been rapidly joined on the occurrence, and evolutionary consequences of behavioural syndromes (i.e. behavioural correlations within or across contexts) in several species and domains other than brood parasitism over the last two decades (reviewed in Sih et al. 2004; Reale et al. 2007; Sih and Bell 2008; Garamszegi et al. 2012), the possible interplay between different behavioural components of host defences within host populations has only recently been hypothesized in a theoretical study (see Avilés and Parejo 2011), and seldom considered in empirical studies (see however Guigueno and Sealy 2011).

In the case of the magpie *P. pica* hosts parasitized by the great spotted cuckoo *C. glandarius* in the south of Spain, field observations and experimental work have shown that some individuals mob adult great spotted cuckoos, while other individuals do not (Soler et al. 1999a). Interestingly, nests where magpies recognized and rejected cuckoo eggs defended their nests against great spotted cuckoos at a lower level than non-recognizers did, which was interpreted as an evidence of an antagonistic expression of these two kinds of defences due to their inherent costs at the nest level (Soler et al. 1999a). Given that magpies were not banded in that study, however, the role of sexes in nest defence remains to be elucidated, and it is unknown whether a behavioural syndrome structure may exist or not within each sex.

The aim of this study is threefold. First, we aimed to explore interindividual correlations between defensive behaviours in the frame of the host personality hypothesis (Avilés and Parejo 2011) by working with individually marked magpie hosts. As great spotted cuckoo male and female often cooperate during laying in a distraction strategy (Arias de Reyna 1998), we may expect magpie hosts to have evolved the tendency to use both nest defence and egg rejection, as mobbing only may not be sufficient to prevent the female cuckoo to lay her egg. Therefore, this would predict a positive correlation between the intensity of nest defence of individual magpies in a nest and egg rejection behaviour in the same nest.

Our second aim was exploring whether average nest defensive behaviour against brood parasites differ between sexes in magpie hosts. The role of sexes in mobbing against cuckoo parasites has traditionally been disregarded despite empirical evidence suggesting that males and females may differ in the expression of their defences (e.g. Gill and Sealy 1996; Palomino et al. 1998; Soler et al. 2002; Požgayová et al. 2009), and theoretical models predicting that if both sexes expressed a defensive trait in a population, it may speed up the spread of defensive variants as compared to a population where only one sex expressed it (e.g. Rothstein 1975; Liang et al. 2012). Magpie males are more aggressive against intruders and predators than females during laying and incubation (Buitron 1988; Birkhead 1991), but sex differences in defences against brood parasites have not been studied. In parasitized magpie populations the two sexes are expected to participate in nest defence, as parasitism impairs fitness of both mates. However, females undertake incubation (Birkhead 1991), and, meanwhile, males can be courting other females and be engaged in extra pair paternity. As a consequence, if parasitism occurs, the female magpie loses all, whereas the male may have still gained some fitness in other nests. Thus we expected females to respond more intensely than males to the threat of brood parasitism because parasitism entails larger costs to them during that period.

Finally, we studied the costs of nest defence in terms of probability of cuckoo parasitism. There is compelling evidence that conspicuous nest defence might attract predators (Krama & Krams 2005), and even serves as cue for locating nests to parasitize by searching brood parasites (the nesting-cue hypothesis; Smith et al. 1984; Gill et al 1997). Given that multiple parasitism by different cuckoo females is frequent in this population (Martínez et al. 1998), the nesting-cue hypothesis specifically predicted that nests whose owners exhibited more intense nest defence were more likely parasitized. Testing this prediction is clearly relevant to our first aim of exploring the existence of a syndrome structure as if costs of nest defence existed; it may favour antagonistic expression of defences (Soler et al. 2009a) rather than the existence of a correlation between defensive behaviours in magpie hosts.

Material and Methods

Study area

Field work was conducted during the breeding season of 2012 (March – May) in La Calahorra (37º 10′ N, 3º 03′ W, Hoya de Guadix, Granada), at approximately 1000m above sea level (see Molina-Morales et al. 2012 for further details). The location is characterized by open areas with sparse vegetation, cultivated cereals, and numerous almond tree (*Prunus dulcis*) groves, in which magpies preferentially build their nests (Molina-Morales et al. 2012, 2013).

Study system

The magpie is a medium-sized, sedentary, territorial, socially monogamous, and relatively long-lived corvid (Birkhead 1991), and it is the main host of the great spotted cuckoo in the south of Spain (Soler 1990). It builds a domed spherical nest over trees and lays a clutch from March to May that ranges from five to ten eggs. The great spotted cuckoo parasitism drastically impairs magpie reproductive success through early hatching and effective competition for parental food delivery of cuckoo nestlings (e.g. Soler et al. 1996,1997). Costly parasitism has selected for magpie recognition and rejection of cuckoo eggs (Soler and Møller 1990), which on the other hand has been selected for mafia tactics of great spotted cuckoos to ensure the acceptance of parasitism (Soler et al. 1995). This escalated suite of interactions between cuckoos and magpies in the south of Spain has been considered to be an example of coevolution (Soler and Soler 2000).

Individual monitoring

In this study, at least one of the members of the monitored magpie pairs was marked with a unique combination of colour rings. Magpies were marked in previous years and sexed using molecular markers in the frame of a long-term project on magpie life history strategies (see Molina-Morales et al. 2012, 2013 for details). Previous studies in several bird species have found that older individuals are more likely to defend their nests against predators (Caro 2005) and brood parasites (e.g. Smith et al. 1984; Hobson and Sealy 1989) than naive individuals. However, most tested magpies in this study were old (i.e. older than 2 years old), and do not show any obvious difference in nest defence from naive ones (see Electronic Supplementary Material), which suggests a negligible effect of age on our results.

Nest Defence Experiment

At the beginning of the breeding season (mid-March) we searched for magpie nests and marked their position with a GPS. We also made observations during nest building in order to determine whether any of the pair members were marked and to identify them. Ninety nests were found, but we were only able to conduct 58 experiments as in some nests, both magpies were unmarked and a few nests were not accessible. The experiments were conducted at the beginning of the laying period if possible (i.e. when magpies had laid between one and five magpie eggs) in order to i) decrease the level of disturbance to females (magpie females start incubating with the fourth or fifth egg, Birkhead

1991) and ii) decrease the likelihood that the nests were parasitized by the great spotted cuckoo before the experiment (although this could not be avoided in some cases [N=11 nests out of 58 nests]). Thus, 18.97% of magpie nests were already parasitized when we performed the experiment. Our results for probability of appearance are qualitatively similar and remain significant if we limit our sample to non-parasitized nests, and the results for intensity show the same trends reported in Table 1 for all nests. Therefore, it is unlikely that early parasitism has unduly influenced our results.

In our experiment, we exposed each nest to a great spotted cuckoo dummy and a hoopoe (*Upupa epops*) dummy as a non-harmful control and recorded magpie mobbing responses. The dummies (two great spotted cuckoos and two hoopoes) were handmade out of plaster and hand painted by a specialist (http://www.replica-animal.com/) to resemble real great spotted cuckoos and hoopoes in a standing position. Following Dochtermann (2010), the order of the presentation of the two dummies at the nests was randomly assigned. They were both presented on the same day with at least 2 hours between expositions (e.g. Welbergen and Davies 2008) to lower stress on the magpies and to prevent carry-over aggression (eight experiments were performed across consecutive days due to bad weather conditions or problems with the equipment). This time interval between two trials is sufficient to prevent carry-over aggression because we noted that magpies readily returned to their nests when a trial was finished (see also Avilés and Parejo 2006). The time when the experiments began and ended was annotated. We chose the hoopoe as a non-harmful control because it poses no threat to magpies and lives in sympatry with them; therefore, magpies are familiar with their presence in the study area. In addition, hoopoes are only slightly smaller than great spotted cuckoos (range of size: 26-32 cm in hoopoes vs 35-39 cm in great spotted cuckoos; del Hoyo et al. 1997, 2001).

Models were placed, while parents were away, on top of a 50 cm height camouflaged box placed on the ground at a maximum distance of 1.0 m from the vertical line of the nest to the ground, thus being clearly visible by any magpie approaching the nest. The box contained a speaker connected to an mp3 player that played the calls of either the great spotted cuckoo or hoopoe, depending on the dummy used. The recorded calls consisted of three different tracks of great spotted cuckoo calls and four hoopoe calls with two and three one-minute silence tracks, respectively, that were randomly selected and played continuously during the experiment. The use of great spotted cuckoo and hoopoe recordings produces a unique assortment of calls by their randomized presentation and combination with silence tracks for each nest and treatment, thus minimizing the risk of pseudoreplication by using only two dummies in each treatment (e.g. Eggers et al. 2006; Schmidt 2006; Bell et al. 2009; Parejo et al. 2012). The great spotted cuckoo male and female often cooperate during laying in a distraction strategy. Male cuckoos perch close to the host nests aiming to attract magpie attention by calling, while the female cuckoo makes a silent approach to the nest to lay (Arias de Reyna 1998). Therefore, the inclusion of great spotted cuckoo calls together with a parasite model close to a magpie nest mimics well a real parasitism challenge for magpies. Indeed, during the present study we reported a few instances in which magpies attacked and strongly produced alarm calls to the cuckoo dummy, suggesting that plaster dummies elicited reliable defensive responses in magpies. We did not analyze alarm calls by sex in this study because we were unable to determine the sex of the caller during the observations.

We observed the behaviour of magpies during half an hour after detecting the arrival of the first magpie. We used a telescope and binoculars and observed the magpies from a car or from a hide 50-100m from the nest, and recorded their behaviour on an audio recorder. We considered the following defence variables: 1) presence in the field of view of the observer close to the tree holding the nest of the male and female separately during the length of observation; 2) latency of approach to the nest in minutes. We consider an approach if a magpie flew within the field of view of the observer; 3) minimum distance of approach in meters to the dummy; 4) number of times that they approached at 10m or less to the dummy. The numerical variables (i.e. latency of approach, distance to the dummy and number of times at a distance ≤10m) likely represent facets of the same trait and therefore were included in one principal component analyses (PCA). The first principal component (PC1) explained 52.0% (eigenvalue=1.55) of variance in defensive behaviour and had high positive loadings for number of times the host approach at a distance ≤10m (factor loading: 0.79) and high negative ones for the distance to the dummy (factor loading -0.79) and latency to approach (factor loading -0.54). Thus, high positive PC1 scores from this PCA identified individuals that approached more times, closer and sooner to the dummy, and thereby individuals that exhibited a more intense defensive response. PCA factor scores have been subsequently used as a response variable for analyses of mobbing behaviour.

Egg rejection experiment

The nest defence experiment was conducted simultaneously with an egg rejection experiment. Before placing the dummies near the nest, we checked each nest in order to know the number of magpie eggs (and great spotted cuckoo eggs, if any) at the time of conducting the experiments and marked them with an indelible pen. Then, a plaster model egg, mimetic to the great spotted cuckoo eggs (see Molina-Morales et al. 2012), was introduced in the nest just before setting the great spotted cuckoo dummy for the nest defence experiment. The experiment was set in such a way that magpies would detect the dummy before the model egg when returning to their nest (Soler et al. 1999a). The magpies´ response to the mimetic model egg was checked 5 to 7 days later, and we classified pairs as acceptors (if the model egg remained in the nest) or rejecters (if the model egg had disappeared or the nest had been abandoned). Previous work in our magpie population has shown that 75% of all rejection of artificial models occurs in the first 24 hours after parasitism and that after 72 hours all eggs have been rejected (Avilés et al. 2004). We also checked whether more magpie or cuckoo eggs had been laid in the meantime,, which gives us the possibility of testing whether mobbing behaviour is related to probability of being parasitized by cuckoos. Because magpie rejection behaviour may change in replacement clutches (e.g. Soler at al. 1999b), we did not use magpie replacement clutches for the experiments.

Repeatability estimates

We are aware that our study design, where individual hosts were not assayed several times for a same behaviour, does not allow testing for individual repeatability in behaviours, which is critical to disentangle the mechanisms behind between-individual correlations in behaviour (Dingemanse and Dochtermann 2013). However, obtaining host response takes 5-7 days, and thus, a second assay would have been done once incubation had started, when selection for rejection is expected to be low. This might have rendered unrealistic low repeatabilities in egg rejection. In the same vein, previous studies have shown that host mobbing intensity decreased with probability of successful parasitism (i.e. from host laying to incubation) (e.g. Gill and Sealy 1996). Therefore, given temporal constraints to obtain repeated measures of truly identical stimulus during the same breeding season, we opted for critically discussing evidence for a behavioural syndrome in host defense while acknowledging this limitation.

Statistical analysis

All analyses were conducted in SAS, version 9.2. Aiming to test for the presence of a behavioural syndrome structure in magpie defences we used a mixed-effect modelling approach (Dingemanse and Dochtermann 2013). Specifically, in a first step, we modelled variation in probability of appearance (i.e. nest attentiveness (individuals show up near the nest after the dummy had been placed)) and mobbing intensity (i.e. PC1 scores of the PCA on nest defence variables) in relation to rejection behaviour (i.e. acceptor versus rejecter), level of threat (i.e. great spotted cuckoo versus hoopoe control dummy) and their interaction as fixed effects for female and male magpies in separate models. The individual was treated as a random intercept. The probability of appearance and mobbing intensity were respectively modelled with a binomial (link function: logit; PROC GLIMMIX procedure) and Gaussian (LMM hereafter, PROC MIXED procedure) error structures. Adjusted repeatabilities were calculated using between-individual and residual variances following the methods of Nakagawa and Schielzeth (2010). Briefly, adjusted repeatability for mobbing intensity was calculated as the proportion of total variance in mobbing behaviour accounted for by differences within individuals:

$r = \sigma^2{}_\alpha / \sigma^2{}_\alpha + \sigma^2{}_\varepsilon$

where $\sigma^2{}_\alpha$ is the between-individual variance and $\sigma^2{}_\varepsilon$ is the within- individual variance (Nakagawa and Schielzeth 2010). Given that GLMMs do not provide a direct estimate of residual variance for binomial models, which is necessary to estimate repeatability, we fixed it to one and calculated latent-scale repeatability for proportiondata (Nakagawa and Schielzeth 2010):

$r = \sigma^2{}_\alpha / (\sigma^2{}_\alpha + \omega(\pi^2/3))$

where $\sigma^2{}_\alpha$ is the between-individual variance and ω is the dispersion parameter for the model fixed to 1 for binary data.Ninety-five per cent confidence intervals (95% CI) for adjusted repeatabilities were calculated using parametric boostrapping (1000 simulation iterations) (Nakagawa and Schielzeth 2010). These analyses will allow responding 1) whether nest defence is associated to rejection behaviour within each sex (rejection effect); 2) whether the association between nest defence and egg rejection depends on level of threat at the nest within each sex (interaction rejection x threat) and 3) what the level of within-sex adjusted repeatability is in our data (i.e. whether different magpie individuals had different proneness to attend and defend their nest (individual random intercept)).

We performed a second set of mixed-effect models where sex-specific analyses are combined into a single model, thus allowing a control for the dependence of data within the same pair Specifically, the response variable (probability of appearance and mobbing intensity) was modelled as a function of level of threat, rejection behavior, sex, and appropriate interactions. In this model pair (i.e. nest) identity and individual identity (nested within pair) were entered as random effects. This analysis thereby enables testing for sex-specific relationships between nest defense and egg rejection (interaction rejection x sex), and for sex-specific plasticity in nest defense (interaction threat x sex) while accounting for non-independence of male and female responses in the same nest. Also, this analysis provides a direct estimate of where the variation in nest defense comes from: between nests versus between individuals within nests, thereby providing information on the level of within-nest adjusted repeatability in our data (i.e. whether different magpie pairs had different proneness to attend and defend towards an object presentation at the nest). Given that sample sizes are unequal between factors we used the Satterthwaite method to calculate degrees of freedom (Quinn and Keough 2002). The alpha threshold was set at 5%.

It must be highlighted that is not our intention to establish any cause-causative relationship between nest defense and rejection when we use the former as dependent variable and the second as a predictor in models. However, given that one of the aims of our study was determining the role of sex and level of threat in nest defense, we opted to use nest defense variables as dependent variables and to enter rejection as a predictor fixed term in the models, as this allows testing in the same model whether a behavioral correlation between defense variables may exist while studying variation in nest defense in relation to relevant predictors.

Finally we tested in two separate logistic regression models (GENMOD procedure in SAS) whether the probability of a nest of being parasitized by cuckoos after it was exposed to the cuckoo dummy was related with female and male intensity of mobbing behaviour (i.e. PC1 scores of the PCA on nest defence variables).

Results

We exposed 58 magpie nests to the presence of a great spotted cuckoo and a hoopoe dummy. We also carried out the egg rejection experiment in all of these nests; although 8 out of 58 nests were predated before we could report the response to the model egg. The rejection rate of mimetic model eggs was 32% (16 out of 50).

Sources of variation in magpie nest defence within each sex

The probability that an individual to arrive in a trial was not related with egg rejection in either sex (Table 1). Both males and females appeared more frequently at their nests when it was exposed to the presence of a great spotted cuckoo dummy than when exposed to a hoopoe dummy (Fig. 1, Table 1).

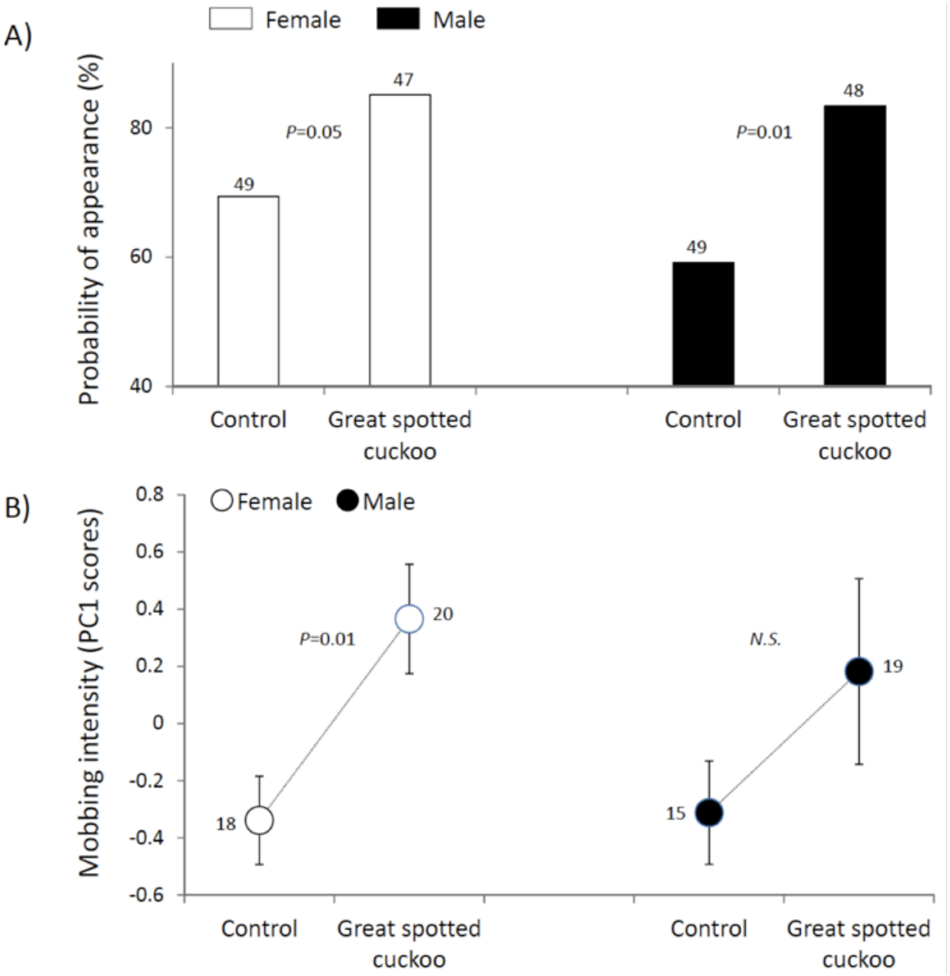

Figure 1. (A) Probability of individual magpie appearance (percentage) in a trial, and (B) mobbing intensity (Mean ± SE) (i.e. PC1 scores of a PCA on nest defence variables) in relation to sex and level of threat. Sample sizes are shown above bars and close to the mean. Differences within each sex are marked with their associated probabilities as reported in Table 1, and *P* values over 0.05 are denoted as *N.S.*

Magpie males defended their nests more intensely (i.e. high PC1 scores) in those nests in which cuckoo model eggs were rejected (Table 1, Fig. 2). Differences in mobbing intensity of females between nests that rejected and accepted cuckoo model eggs were not significant (Table 1, Fig. 2). Females' mobbing intensity also differed with the level of threat at the nest: females defended more intensely their nests when they were exposed to the great spotted cuckoo than to the hoopoe (Table 1, Fig. 1). However the relationship between mobbing and rejection behaviour did not differ in relation to level of threat in either sex (Table 1). Within both sexes, individuals differed significantly in their baseline level of nest attentiveness, but do not in aggressiveness (individual random intercepts; Table 1). However, there were no individual differences once pair identity was considered (Table 2). Source of variation in magpie nest defence within each nest.

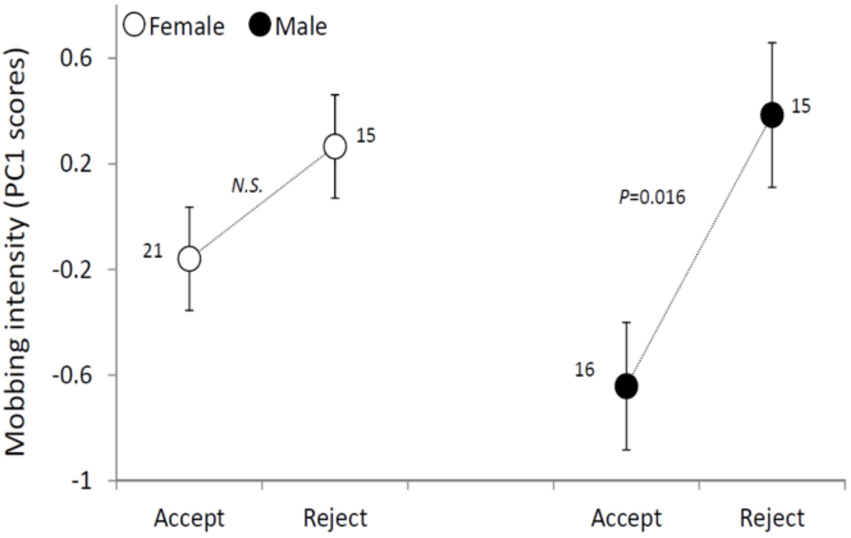

Figure 2. Mobbing intensity (Mean ± SE) (i.e. PC1 scores of a PCA on nest defence variables) in relation to sex and discrimination behaviour against cuckoo models. Sample sizes are shown close to the mean. Differences within each sex are marked with their associated probabilities as reported in Table 1, and *P* values over 0.05 are denoted as *N.S.*

Probability of individual host's appearance in a trial and intensity of mobbing against the dummy (either cuckoo or control) varied between different nests (nest as random intercept, Table 2). Indeed, our variance partitioning analyses revealed that most of variation in nest defence occurred between nests rather than between individual within nests (Table 2), suggesting that magpie pairs instead of magpie individuals differ in their level of nest attentiveness and aggressiveness in our population. Probability of individual host's appearance in a trial and intensity of mobbing varied with the level of threat at the nests (Table 2): individuals appeared more frequently at their nests and defended it more intensely when the nest was exposed to the presence of a great spotted cuckoo dummy than when exposed to a hoopoe dummy (Fig. 1, Table 2). Nest defence, however, does not differ between sexes (Table 2), and the interaction between sex and level of threat was not significant either (Table 2). Corroborating the analysis with each sex, there was a nearly significant interaction between sex and rejection on mobbing intensity (Table 2): males showing larger differences than females in mobbing intensity between nest that accepted and rejected the model egg (Fig. 2).

Mobbing behaviour and probability of cuckoo parasitism

Ten out of 47 nests (21.3 %) that were not parasitized at the time when we measured intensity of nest defence were later parasitized, thus allowing us to check whether parasitism after the nest defence experiment was influenced by intensity of nest defence. Nest probability of being cuckoo parasitized was unrelated to either female ($\chi^2 = 0.50$, d.f.=1, $P = 0.47$) or male ($\chi^2 = 0.35$, d.f.=1, $P = 0.55$) intensity of mobbing against the cuckoo dummy.

Table 1. Sources of variation in magpie nest defence within each sex.

	Probability of appearance (female)				Probability of appearance (male)				Mobbing intensity (female)				Mobbing intensity (male)			
Fixed effects	β (95% CI)	F	df	P	β (95% CI)	F	df	P	β (95% CI)	F	df	P	β (95% CI)	F	df	P
intercept	272 (1.50, 3.95)				2.13 (1.04, 3.21)				0.14 (-0.32, 0.61)				-0.66 (-1.32, 0.0006)			
threat	-1.41 (-2.63, -0.19)	3.87	1, 47.8	0.05	-1.66 (-2.83, -0.48)	6.91	1, 42.6	0.01	-0.65 (-1.29, -0.008)	8.68	1, 10.7	0.01	-0.02 (-0.99, 0.94)	2.78	1,17.8	0.11
rejection	-1.15 (-3.11, 0.80)	0.87	1, 42.7	0.35	-0.65 (-2.42, 1.11)	0.18	1, 42.7	0.67	0.43 (-0.31, 0.18)	2.13	1, 18.5	0.16	1.47 (0.51, 2.43)	6.74	1,21	0.016
threat* rejection	0.86 (-1.13, 2.87)	0.76	1, 47.9	0.38	0.75 (-1.21, 2.72)	0.59	1, 42.6	0.44	-0.02 (-1.02, 0.96)	0.00	1,10.7	0.95	-1.00 (-2.33, 0.32)	2.51	1,17.8	0.13
Random effects	σ^2 (95% CI)	Z	P		σ^2 (95% CI)	Z	P		σ^2 (95% CI)	Z	P		σ^2 (95% CI)	Z	P	
individual	3.27 (1.68, 8.90)	2.45	0.007		2.05 (0.88, 9.06)	1.81	0.03		0.26 (0.06, 14.38)	0.95	0.17		0.37 (0.11, 11.46)	1.04	0.14	
residual	0.49 (0.34, 0.80)	4.60	<0.001		0.64 (0.43, 1.08)	4.30	<0.001		0.35 (0.13, 2.26)	1.54	0.06		0.57 (0.24, 2.77)	1.74	0.04	
repeatability	r(95% CI) 0.46 (0.30, 0.88)				r (95% CI) 0.34 (0.11, 0.69)				r(95% CI) 0.43 (0.01, 0.79)				r (95% CI) 0.39 (0.02, 0.93)			

Models test for the effect of threat, pair rejection behaviour and individual identity on probability of magpie appearance in a trial as a binary dependent variable (GLMM) and on magpie mobbing intensity (i.e. PC1 scores of the PCA on nest defense variables, LMM) in the two sex separately (see methods for details). We provide with estimates for each fixed (β; mean) and random (σ^2; variance) parameters and adjusted repeatabilities with their 95% Credible Intervals.

Table 2. Sources of variation in magpie nest defence within each nest**.** Results of models testing for the effect of sex, pair rejection, threat and appropriate interactions on probability of magpie appearance in a trial as a binary dependent variable (GLMM) and on magpie mobbing intensity (i.e. PC1 scores of the PCA on nest defense variables, LMM). In this models pair (i.e. nest) identity and individual identity (nested within pair) were entered as random effects. We provide with estimates for each fixed (β; mean) and random (σ^2; variance) parameters and adjusted repeatabilities with their 95% Credible Intervals.

	Probability of appearance				Mobbing intensity			
Fixed effects	β (95% CI)	*F*	df	*P*	β (95% CI)	*F*	df	*P*
intercept	2.12 (0.34, 3.90)				0.79 (0.21, 1.38)			
threat	-1.56 (-3.23, 0.10)	16.19	1, 28.4	<0.001	-1.05 (-1.75, -0.36)	8.99	1,51.0	0.004
rejection	0.68 (-1.53, 2.89)	0.57	1, 36.2	0.45	-1.49 (-2.30, -0.69)	4.84	1,25.9	0.037
sex	-0.13 (-1.93, 1.66)	1.80	1, 116.8	0.18	-0.31 (-0.97, 0.35)	1.36	1,37.5	0.25
threat*rejection	-0.67 (-2.74, 1.39)	0.76	1, 128.4	0.38	1.15 (0.13, 2.16)	2.81	1,51.0	0.09
sex*rejection	0.59 (-1.66, 2.86)	0.64	1, 116.8	0.42	1.14 (0.25, 2.02)	3.60	1,37.5	0.065
sex*threat	0.66 (-1.65, 2.96)	0.78	1, 116.5	0.38	0.39 (-0.54, 1.33)	0.19	1,37.7	0.66
sex*threat*reject	0.63 (-1.10, 2.37)	0.00	1, 116.5	0.98	-1.07 (-2.36, 0.22)	2.80	1,37.7	0.10
Random effects	σ^2 (95% CI)	*Z*	*P*		σ^2 (95% CI)	*Z*	*P*	
nest	5.16 (2.99, 11.00)	3.09	0.001		0.36 (0.18, 1.02)	2.38	0.008	
individual (nest)	0.00*				0.00*			
residual	0.50 (0.39, 0.66)	7.60	<0.001		0.38 (0.25, 0.66)	4.17	<0.001	
repeatability*	r (95% CI)				*r* (95% CI)			
	0.57 (0.12, 0.89)				0.48 (0.04, 1.00)			

* given that most of variance occurred between nests rather than between individuals, adjusted repeatabilities in this table quantify whether different magpie pairs had different proneness to attend and defend towards an object presentation at the nest.

Discussion

Inter-individual correlations between defensive behaviours in male and female magpie hosts

Our results show empirical evidence for a link between egg rejection and a mobbing behaviour in a host of an avian brood parasite. Interestingly, we found that in nests where individual male magpies showed a more general aggressive response to intruders (either a cuckoo or a control hoopoe) cuckoo eggs were more likely rejected. Based on the fact that egg rejection might be somehow learned (Victoria 1972; Rothstein 1974, 1978), and given a growing evidence for a link between learning-based discriminatory tasks and personality traits in birds (Verbeek et al. 1994; Boogert et al. 2006; Guillette et al. 2009, 2011; Brust et al. 2013), it has been recently suggested the possibility that parasite egg rejection was a carryover from other personality traits in cuckoo hosts (Avilés and Parejo 2011). Indeed, a previous empirical study had reported a positive correlation between two defensive behaviours against brown-headed cowbirds in yellow warbler *Dendroica petechia* hosts (Guigueno and Sealy 2011). Our results would suggest that egg rejection may correlate with a general tendency to be more or less aggressive in magpies, with individuals accepting cuckoo parasitism being in general less aggressive.

We found evidence of a correlation between mobbing behaviour and egg rejection in males but not in female magpies. Egg rejection is a trait that varies at the level of pairs as it was measured in this study. Given that syndromes are defined as between-individual correlations between behaviours not as between-pair correlations, the potential of our approach to reveal an actual syndrome structure will greatly depend on which sex was responsible for egg rejection in magpies. It has been suggested that male recognition of parasite eggs is unlikely in those host species in which males played a minor role in egg incubation (Soler et al. 2002; Požgayová et al. 2009). Exception to this rule, however, has been reported in the Northern Oriole (*Icterus galbula*) host of the brown-headed cowbird where males eject parasite eggs even though they do not incubate(Sealy and Neudorf 1995). Although females play the key role in incubation (Birkhead 1991), it is currently unknown which is the sex responsible for rejection of parasite eggs in magpies. If rejection in magpies was exclusively female-based, our results may come from non-random mating (i.e. rejecter females preferred mating with aggressive males). Alternatively, whether male magpies rejected eggs, our results might correspond with a sex-specific syndrome in defence behaviour. A logical next step is, thus, to precisely determine the sex responsible for egg rejection in magpie hosts.

Our study design did not allow estimating whether individual magpies were repeatable in their behaviour towards specific objects, which is a key pre-requisite for detecting a behavioural syndrome (see Dingemanse and Dochtermann 2013). Therefore, we cannot discard that individuals might show full condition-dependence in both nest defence and egg rejection behaviour from one day (month, or year) to the next, such that the two behaviours were correlated at the same point in time (as in our study). Indeed, individual identity did not explain significant variation in nest defence once pair identity effect was fitted (Table 2). There are a number of explanations for this pair effect. First it is possible that males were not responding to the threat per se, but female reacting to that threat, hence producing a pair-level correlation due to plasticity not personality. Alternatively, this pair effect could also be achieved if different personality types were assortatively paired. Finally, it is possible that both males and females are plastically responding to some unmeasured environmental variable and do so in the same fashion, and because this environmental bariable is stable at the nest level, there is the appearance of individual consistency that simply reflects “pseudopersonality” (see Westneat et al. 2011; Dingemanse and Dochtermann 2012). Nonetheless, future studies aiming to study whether behavioral defenses in cuckoo hosts constitute a behavioral syndrome should ideally assay a same defensive behavior multiple times in order to achieve a better understanding of mechanisms promoting defensive syndromes.

Previous work in two unbanded magpie Spanish populations had reported that magpies that rejected cuckoo eggs defended their nests against great spotted cuckoos at a lower level than did non-rejecters (Soler et al. 1999a). Interestingly, it was also found that individuals in nests in which cuckoo eggs were rejected showed lower nest defence against great spotted cuckoos than against a nest predator (carrion crow Corvus corone) which, all together, was interpreted as an evidence of antagonistic expression of magpie antiparasite defences (Soler et al. 1999a). Our analyses based on banded individuals with known sex revealed the opposite pattern. Several non-exclusive explanations are possible for the disagreement between these results. One of the differences in the level of defence of acceptor and rejecter pairs found in Soler et al. (1999a) is the number of magpies that defended the nest, an average of 1.3 in acceptors and 0.7 in rejecters. This could imply that in most of the nests of rejecter pairs there was only one individual or none defending the nest. Thus, it is possible that the differences between acceptor and rejecter pairs showed by Soler et al. (1999a) could be due to a female's distinct contribution to nest defence depending on its egg rejection behaviour which could not have been noticed due to non-individual recognition. This possibility, however, seems unlikely because females should have defended with less intensity in those nests in which the cuckoo eggs were rejected, and our results show that this is not the case. In addition, our study was performed in a single population and year, whereas their study was carried out in two populations and in two different years. Several studies have shown that host defence might be plastically expressed depending on the risk of parasitism among other factors (Soler et al. 1999b; Welbergen and Davies 2009, 2012, this study). Therefore, it is possible that mobbing behaviour in that study was affected by host perception of risk of great spotted cuckoo parasitism in different years and populations (e.g. the parasitism rate in Guadix was much higher than in Doñana, Soler et al. 1999a). Finally, our experiment also differed in the kind of stimulus used to be compared with the response to the great spotted cuckoo. Soler et al. (1999a) used live carrion crows to simulate a generalist nest predator and here we utilized hoopoes as non-harmful controls. The selection of the controls may greatly influence results in nest defence experiments (Grim 2005), but is unlikely to be the cause of the contrasting patterns discussed here, because differences between studies persisted even after excluding experiments performed with carrion crows and hoopoes. Indeed, carrion crows are a main predator of magpie nests in Guadix. Finally, it is possible that contrasting covariation between nest mobbing and rejection resulted from changes over time in the selective regimes promoting the association between these two behaviours in the population. Indeed, comparative studies have shown that behavioural syndromes should evolve only in those populations where natural selection has favoured such trait covariance, and they should therefore exist only in particular types of populations (Dingemanse et al. 2007; Bell and Sih 2007). Mobbing behaviour is likely selected for by predators and brood parasites in magpies while egg discrimination is exclusively selected for by great spotted cuckoos, therefore changes in predation and parasitism level over time may account for differences between studies. Unfortunately we do not have any data to test this possibility.

Nonetheless, our results do not suggest antagonistic expression of mobbing against cuckoos and egg rejection in magpies. A key factor promoting antagonistic expression of host defensive behaviours in cuckoo hosts would be the existence of costs and constraints associated to the expression of these behaviours (Soler et al. 1999a). In our egg recognition experiment, rejecter magpies made no recognition errors when it came to rejecting the parasitic egg rather than their own eggs. Moreover, we found no evidence of costs of nest defence in terms of great spotted cuckoo parasitism, as more aggressive pairs showed no difference in parasitism rate with less aggressive ones. Therefore, our results would suggest that there exist no obvious costs and constraints for the existence of a behavioural syndrome between aggressive behaviours to hoopoe and great spotted cuckoo dummies and egg rejection of great spotted cuckoo eggs in magpies.

The role of sex and plastic nest defence against brood parasites

We confirmed that both female and male magpie hosts showed a greater level of nest defence towards the brood parasite than towards the control (individuals appeared more often during the presentation of the great spotted cuckoo and approached it closer and more times than they did with the control hoopoe). These findings would suggest that magpies perceive the great spotted cuckoo as a threat confirming previous results in this and other brood parasite-host systems (e.g. Davies and Brooke 1988; Sealy et al. 1998; MacLean and Rhodes 1991; Duckworth 1991; Moksnes et al. 1991; Briskie et al. 1992; Gill et al. 1997; Grim 2005; Avilés and Parejo 2006; Welbergen and Davies 2008, 2009) and thus that mobbing by the two pair members in magpies is phenotypically plastic with respect to the level of threat a great spotted cuckoo poses at the nest. Although plasticity in nest defence was not previously tested in magpies, experimental evidence has shown that they display plastic rejection of cuckoo eggs in relation with nest predation (Soler et al. 1999b). Plastic nest defence in relation to level of threat would be beneficial for a host given the costs of nest defence (Smith et al. 1994; Krama and Krams 2005) and spatio-temporal variation in risk of parasitism (Welbergen and Davies 2009; Davies and Welbergen 2009; Campobello and Sealy 2011). We have not found evidence that nest defence was costly in terms of risk of cuckoo parasitism in the study year. However, parasitism level may greatly vary from one year to another in our population (Molina-Morales et al. 2013), and these costs might only be evident at a certain parasitism threshold (e.g. Davies and Welbergen 2009). Alternatively, costs of nest defence might have been undetected in this study as we only targeted a very specific aspect of them.

Previous studies of nest defence in cuckoo-free magpie populations (e.g. Buitron 1988; Birkhead 1991) showed that males more actively expelled co-specific intruders and predators than females especially so during the female ′s fertile period. Here we had predicted females to respond more intensely than males to the threat of brood parasitism because we assume cuckoo parasitism would entail larger costs to females during that period (see “Introduction”). Our results based in one magpie population exposed to high levels of cuckoo parasitism revealed that males and females showed similar higher levels of nest defence against cuckoos than hoopoes, suggesting that sex differences in average nest defence behaviour against cuckoos were minor in our population. Perhaps, costs of cuckoo parasitism are high for males despite the fact they do not incubate. After all, males also feed parasitic cuckoos till fledging which is costly and may have selected for enemy recognition.

Analyses within sex, however, revealed subtle differences in nest defence between males and females. Males, but not females, were more prone to appear at their nests, and, females, but not males, to defend more intensely when their nests were challenged by a parasite threat but not by a control hoopoe. The reason why females appeared at their nests irrespective of the level of threat may be due to the fact that female magpies are solely responsible for laying and incubation and as a result spend more time close to their nest than males (see also Požgayová et al. 2009). Once females are at their nests, however, they would be able to adjust the intensity of their defence to the level of threat whereas males would exhibit lower sensitivity to threat variation.

We can conclude that female and male magpie hosts can plastically modify the intensity of mobbing behaviour as a function of the threat at their nests. In addition, we have found support for the view that egg rejection may correlate with a general tendency to be aggressive in male magpies, with individuals accepting cuckoo parasitism being in general less aggressive. These findings suggest that under variable risk of parasitism individual magpies might adjust their defensive behaviour against cuckoos while remaining consistently different in aggressiveness from each other and highlight the importance of considering inter-individual variation of the host and sex in our comprehension of the evolution of host defences.

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Ethical Standards

Exposure of magpie nests to cuckoo and hoopoe dummies had no effect on magpie reproduction as all tested nests resumed incubation and we did not detect abandon after one week of the experiment (N=58 nests). Eight of 58 (13.8 %) experimental nests were depredated after performing the experiment. This percentage was within the range of predated nests reported in our population in the last five years (range: 8.33% – 36.7%, Molina-Morales et al. Unpublished data), and was sensibly lower than the average predation rate reported in that period (25.67%, N =275 nests), suggesting that disturbance at the nests due to our experiment had a minor impact on attracting predators. Our experiment comply with the current laws of Spain and was carried out under license from the Consejería de Medio Ambiente de la Junta de Andalucía (SGYB/FOA/AFR/CFS), and was framed within the project CGL2011-27561 funded by the Spanish Ministry of Science and Economy of Spain.

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Electronic Supplementary Appendix 1. Effect of age on nest defence.

In our study we can differentiate between two age categories in magpie hosts: group 1 (composed by individuals ringed as nestlings in our study site in the two previous years and by individuals not ringed as nestlings but showing plumage characteristics of first breeders according to Birkhead (1991) when trapped at the nest); group 2 (composed by individuals older than two years given ringing date and plumage characteristics) (see also Molina-Morales et al. 2012, 2013). This allowed us to consider whether magpies in the two groups (i.e. age classes) differed in their intensity of mobbing behavior against a parasite threat.

We did not find significant differences in intensity of mobbing (i.e. see below) between these two age groups (One-way ANOVA; $F_{1,22}=0.06$, $P=0.80$; age 1 class mean (SD): 0.13 (0.79), N=5; versus age 2 class mean (SD): 0.06 (1.08), N=19), which may suggest that nest defensive behavior did not differ between magpies aged 1 or 2 and older ones. Therefore, although further studies of age and anti-parasite defenses would clearly be needed to confirm the absence of an age-related pattern in defense in this species, we can discard that an age effect might have influenced the reported patterns of defence in this study.

Capítulo 7

Cuckoo hosts shift from accepting to rejecting parasitic eggs across their lifetime

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Cuckoo hosts shift from accepting to rejecting parasitic eggs across their lifetime

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Summary

One of the best known outcomes of coevolution between species is the rejection of mimetic parasite eggs by avian hosts, which has evolved to reduce costly cuckoo parasitism. How this behavioral adaptation varies along the life of individual hosts remains poorly understood. Here we identify for the first time, lifetime patterns of egg rejection in a parasitized long-lived bird, the magpie *Pica pica* and show that, during the years they were studied, some females accept, others reject, and some others modify their response to model eggs, in all cases switching from acceptance to rejection. Females tested in their first breeding attempt always accepted the model egg, even those individuals whose mothers were egg rejecters. A longitudinal analysis showed that the probability of egg rejection increased with the relative age of the female, but was not related to the risk of parasitism in the population. We conclude that ontogeny plays a fundamental role in the process leading to egg rejection in magpies.

Keywords: Brood parasitism, Coevolution, Egg rejection, Longitudinal analysis, Ontogeny.

Introduction

Obligate avian brood parasites lay their eggs in the nest of another species, the host, which carries out all the parental care, from incubating the parasitic eggs to feeding parasitic chicks (Rothstein 1990; Davies 2000; Payne 2005; Krüger 2007; Kilner and Langmore 2011). In some brood parasite-host systems high fitness costs due to parasitism have favored the evolution of anti-parasite host defensive mechanism like the recognition and rejection of parasite eggs by hosts (Brooke and Davies 1988; Davies and Brooke 1988; Rothstein 1990; Davies 2000; Langmore et al. 2003). Given that discrimination and rejection of parasitic eggs by hosts can be feasibly measured in the wild (Rothstein 1990), the study of variation in egg recognition by hosts constitutes an ideal system to investigate the evolution of flexibility in animal recognition systems (Sherman et al. 1997; Lyon 2003; Thorogood & Davies 2013).

Most theoretical and empirical studies about the evolution of host egg recognition are based on the assumption that individual hosts are either rejecters (always reject) or acceptors (always accept) of parasite eggs (Davies and Brooke 1988; Rothstein 1990; Servedio and Lande 2003; Hauber et al. 2004), and that ability to recognise and reject eggs is a reliable predictor of an heritable defensive capacity of individual hosts (Takasu et al. 1993; Servedio and Lande 2003; Martín-Gálvez et al. 2006; Martín-Gálvez et al. 2007). However, factors such as learning and/or phenotypic plasticity may also play a role in egg rejection. For instance, cross-sectional studies have suggested that the acceptance of parasite eggs was more common among naïve breeders (Rothstein 1978; Davies and Brooke 1988; Lotem et al. 1992; Lotem et al. 1995; Hauber et al. 2004). Also, abundant empirical and experimental evidence has shown that individual hosts may modify egg rejection based on perceived risk of parasitism and/or previous experience (e.g. Davies and Brooke 1988; Moksness et al. 1991; Briskie et al 1992; Soler et al 2012; Thorogood & Davies 2013). In these cases, individuals may act as acceptors sometimes but rejecters on other occasions. In order to achieve a full understanding of the evolution of egg rejection it seems critical to understand how often and in which ecological circumstances individual hosts may shift their expression of egg rejection behavior along their life, or in other words, studying the patterns of egg rejection behavior of individual hosts across their lives.

So far only a few studies have tested for individual consistency in rejection behavior with mimetic models (Lotem et al. 1995; Alvarez 1996; Honza et al. 2007; Peer and Rothstein 2010; Samas et al. 2011). Most of these studies tested consistency within the same breeding attempt (Honza et al. 2007; Peer and Rothstein 2010; Samas et al. 2011), or between different breeding attempts in the same season (Lotem et al. 1995; Alvarez 1996), and mostly found support for high individual consistency in egg rejection over that short time window (however see also Alvarez 1996). Further indirect support for consistency in egg rejection came from studies carried out at the population level in New World passerines that are current or potential hosts of parasitic cowbirds (*Molothrus ater*), as they show that these species show an acceptance or rejection rate of non-mimetic eggs of nearly 100% (Winfree 1999; Davies 2000). Individual consistency in rejection behavior between consecutive breeding seasons has only been examined in the rufous bush robin (*Cercotrichas galactotes*) parasitized by the common cuckoo in Spain (Palomino et al. 1998; Soler et al. 2000). This species showed a lack of consistency in the response to model eggs between two consecutive years. Despite the fact that many avian-brood parasite hosts are long-lived birds, no study to date has quantified individual consistency of egg recognition for a time period longer than two years, nor has this been studied between adult hosts and their offspring, which has left the question unresolved of how egg recognition and rejection by hosts is actually transmitted over generations.

Our aim here is to identify the patterns of egg rejection behavior in a longitudinal empirical study of individual magpie (*Pica pica*) hosts over eight years. The magpie is the main host of the great spotted cuckoo (*Clamator glandarius*) in southern Europe (Soler 1990). In this system, cuckoo parasitism impairs magpie reproduction and has resulted in selection for recognition and rejection of cuckoo eggs by magpies (Soler and Møller 1990). The magpie is a long-lived host species in which only females incubate (Birkhead 1991) and thus females are assumed to be the sex that rejects cuckoo eggs (Soler et al.

2002), which makes this an ideal species to study the individual consistency of host defense. We initially study the consistency of egg discrimination throughout the lives of individual magpies and determine which factors may influence the probability of rejecting parasite eggs, including factors such as the parasitism status of the clutch, the relative age of the female, and the pressure of parasitism in the population. Second, because several naïve females were recruited in the population, we were able to test their egg rejection behavior in their first breeding attempt as well as to compare it with that of their mothers. In this context, if learning played a role in egg recognition and rejection we would expect that females modified their response to model eggs across their lifetime and did it always by shifting from accepting to rejecting parasitic eggs. In addition, if rejection was plastically expressed in relation with risk of cuckoo parasitism we would expect that individual magpies were prone to reject or to accept according to the risk of parasitism in the population.

Methods

Study area and system. The study was conducted in La Calahorra (37º 10´ N, 3º 03´ W, Hoya de Guadix, Granada, Southern Spain) during April – May of the years 2005 – 2012. The study area contains groves of almond trees (*Prunus dulcis*), in which magpies prefer to build their nests, mixed with cereal fields (Molina-Morales et al. 2013). Magpies are territorial, sedentary, and socially monogamous (Birkhead 1991). The percentage of parasitized nests in our population (i.e. parasitism rate) varied between years (15.9% in 2007, 25.4% in 2008, 65.6% in 2009, 50.7% in 2010, 55.8% in 2011, 36.2% in 2012), and naïve and adult magpies are equally likely to be parasitized by cuckoos (4 out of 11 naïve breeders (36.4 %) were parasitized *versus* 15 out of 62 adults in 2008, χ^2=0.23, d.f.=1, P =0.63, 38 out of 59 in 2009, χ^2=1.98, d.f.=1, P =0.16 and 35 out of 67 in 2010, χ^2=0.42, d.f.=1, P =0.41). Previous work has shown that parasitism rate is closely correlated with cuckoo abundance in our study area (Soler et al. 1998). Furthermore, magpies in our study area only reject about 5% of real cuckoo eggs (see Soler et al. 1995), and so the risk of not detecting parasitized nests (because magpies rejected the cuckoo eggs quickly) is very low. Therefore, variation between years in the parasitism rate provides a suitable scenario to study the role of the perceived risk of parasitism on the consistency of rejection at the individual level.

Individual marking and monitoring. Adult magpies were captured during nest building using square traps with a live magpie inside to attract other magpies. Trapped adults were marked with a unique combination of colour rings. We also marked all fledglings at each nest with a unique combination of colour rings just before they fledged the nest (16 – 18 days after hatching). At the time of ringing, we took a (ca. 50µl) blood sample from each individual by puncturing the brachial vein with a sterile small gauge needle. Blood samples were placed in microfuge tubes containing 1ml of absolute ethanol, sealed with rubber-sealed screw-tops and stored at room temperature. Genomic DNA was extracted and used for sexing and genotyping individuals. In addition we were able to assign broods to unmarked host females by performing genetic parentage analyses (details of molecular methods, sex-typing and parentage analyses can be found in Molina-Morales et al. 2012). Briefly, we assigned particular broods in different years to the same female when all offspring shared an allele with the mother and all the nestlings in those broods were found to be full siblings based on the genetic analyses.

We knew the age of those individuals marked at the nest that were later recruited into the breeding population, and for a subset of them (11 females) we could monitor their first breeding attempt when they were one year old. We refer to these as first breeders or naïve breeders. Most individuals, however, were caught with adult plumage according to Birkhead (1991) (41 out of 45 females for which repeated egg recognition experiments were performed). For those individuals not marked at the nest, we did not know their exact age, but we assigned them a relative age for the analyses, categorized as 1, 2, and so on from the first year the individual was studied.

Nest and individual monitoring. Magpie nests were monitored from the 1st of March until the beginning of July during each breeding season. Nests were found by careful inspection of all trees in the area. To determine the identity of marked birds associated with each nests and each nesting attempt we observed nests during nest building with a telescope or binoculars from a hide or a car approximately 100 meters from the nest. Nests were visited at 5 days intervals, although during egg laying and hatching the nests were visited every 2–3 days.

Egg discrimination experiment. We tested magpie responses to model eggs made of plaster of Paris mixed with white glue and painted with acrylic paints. Model eggs were made to resemble the cuckoo eggs in appearance, size and mass, and although they do not perfectly match the colour of real great spotted cuckoo eggs (see Figure 1), and their rejection is higher than that of real cuckoo eggs (Soler et al. 1995), previous studies have consistently shown that they would provide a reliable estimate of magpie discrimination abilities selected by cuckoo parasitism (Soler & Møller 1990; Soler et al. 1998; Soler et al. 1999a). Although swapping real great spotted cuckoo eggs between nests would, in principle, help avoiding pitfalls of using model eggs (Martín-Vivaldi et al. 2002), this is not possible in our system. First, swapping real cuckoo eggs could prove problematic because the same female might be exposed by chance to cuckoo eggs differing in mimicry with their ones in different years. Second, cuckoo eggs are so rarely rejected in our study area (about 5 %, Soler et al. 1995), that gathering sufficient samples to detect shifts from acceptance to rejection of real cuckoo eggs would be exceptionally difficult. Finally, by using models we avoid affecting the reproductive success of cuckoos during the study, and we are not limited by the number of real cuckoo eggs available._

We introduced the model egg at the same time period when the magpies were laying their own eggs and revisited the nest after 6–7 days. Previous studies of our magpie population have shown that 75 % of all rejection of model eggs occurs within the first 24 hours after experimental parasitism and that after 72 hours all rejecter magpies had rejected the model egg (Avilés et al. 2004). Therefore the response was regarded as a rejection if the model egg disappeared from the nest or acceptance if the model egg was incubated with the host's clutch when we revisited the nest. Replacement clutches were not included in this study because nest failure may induce changes in rejection behaviour of magpies (Soler et al. 1999). A pilot study revealed that coloration of magpie eggs as measured by spectrophotometry do not obviously differ between accepter and rejecter females within the same breeding season (Supporting Information 1), suggesting that the eggs of 'accepter' females were not more similar in appearance to experimental eggs, than were the eggs of 'rejecter' females.

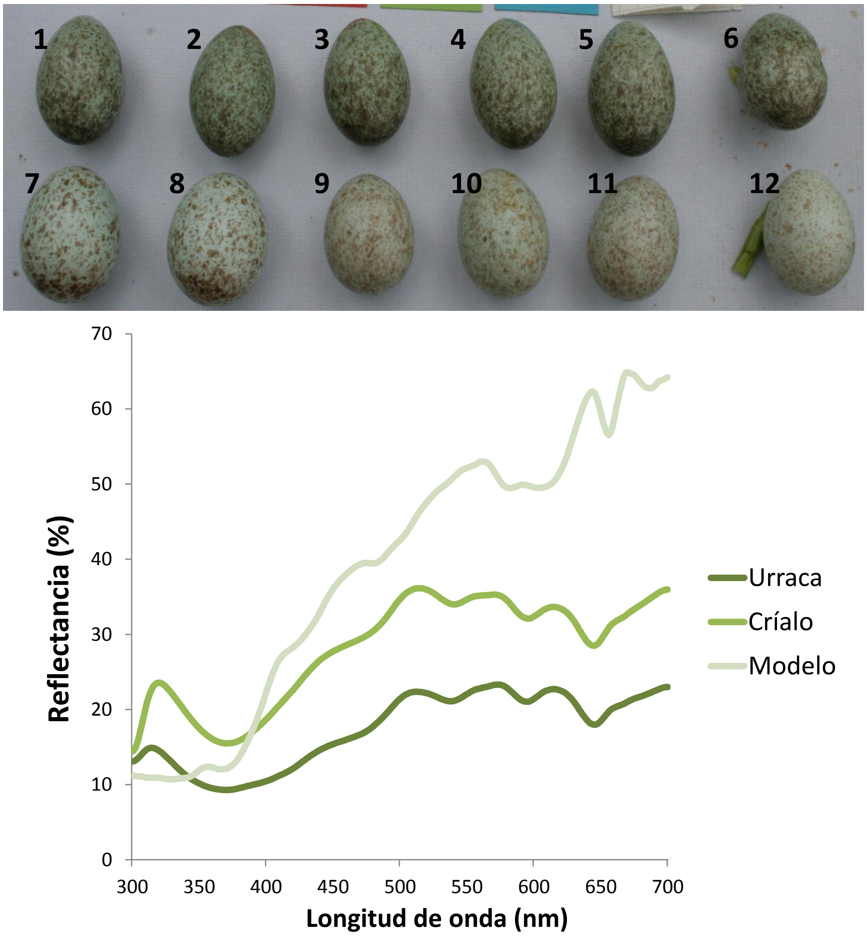

Figure 1. Representative eggs of a magpie clutch parasitized by the great spotted cuckoo and an example of a model cuckoo egg. Eggs 1 to 6 are magpie eggs, 7 to 11 are parasitic great spotted cuckoo eggs, and egg number 12 is a model egg. (T-test for mean differences in length and width between great spotted cuckoo eggs and model eggs: Length: t = 1.79, df= 147, p= 0.074; width: t = 1.43, df=147, p = 0.15). Curves represent the reflectance spectrum for the three eggs.

Given that we parasitized all magpies in our population with model eggs, it is possible that acceptor females were individuals that misimprinted on model eggs the first time they were exposed to them. To test this possibility we did not experimentally parasitize 21 naïve females in our study. If misimprinting occurs, and given that naïve birds are not in contact with the model egg and therefore they would form the “correct” template, we would expect that they would reject the model egg in their following breeding attempt.

Statistical analyses. Analyses were carried out using Statistica 7.1 (Statsoft Inc. 2005, Tulsa, OK, USA) and SAS (SAS Institute, 1996, Cary, NC, USA). We conducted longitudinal analyses of the effect of the risk of parasitism and age on egg rejection behavior that allowed us to determine the relative importance of these factors in causing any changes in individual responses to cuckoo parasitism. Briefly, we modeled probability of rejection in a trial (i.e. one discriminatory challenge per year) of individual magpies as a binary dependent variable (rejection versus acceptance; link function: logit; PROC GLIMMIX procedure in SAS). We constructed a factorial generalized linear mixed model (GLMM hereafter) where the relative age of the female was fitted as a continuous term (1 for the first year they were in the study, 2 for the next and so on, except for known-age females for which we used their real age). The number of years that a female was monitored was significantly larger for those females that changed their response to the model egg during the study (Kruskal-Wallis test: $H_{2,45} = 6.48$, $P = 0.039$; females who change mean= 3.25, SE=0.33, acceptors mean=2.44, SE=0.18, rejecters mean= 2.5, SE=0.37), suggesting that our ability to detect changes in the response to model eggs may depend on the number of years a female was sampled.

In addition, for females who were of unknown age when first observed, we may have underestimated their ages. Thus, we also entered and retained the number of years a female was sampled, as well as a categorical factor for the estimated age of each individual (known versus estimated age), in the models to study the independent effect of relative age on egg rejection. We also fitted status of parasitism in each trial (as a two-level factor: parasitized by great spotted cuckoos versus non-parasitized) and the parasitism rate (as an estimate of risk of parasitism) in the population as fixed terms. In addition, we fitted the interactions of relative age with individual and population status of parasitism to the model. Clutch size and its interaction with age were also entered in the model to account for a possible role of discordancy on rejection. Finally, the female identity was treated as a random term to account for the fact that different discriminatory trials in the same female are not statistically independent.

During the eight years of our study we could test rejection of 11 first-time breeders. Seven of these 11 females are not included in the Figure 2 because we have only one breeding attempt recorded (they could not be subsequently found again in the study area, i.e. they either left the area or were depredated). Egg rejection behaviour of these 11 females allowed us to check whether probability of rejecting eggs in naïve birds was different from that of the population, after excluding the 11 known first breeders, with a Fisher exact test.

Results

Individual variation in rejection behavior

We tested the egg discrimination of 45 females over eight years, ranging from 2 to 5 tests per female (a total of 125 egg-rejection tests, Figure 2). We found that there was significant among-female variation in egg rejection behavior (Figure 2; Table 1). Indeed, of the multiply sampled females, 25 always accepted the model egg (55.6%), 8 females always rejected (17.8%) and the remaining 12 changed their response to the model egg (26.6%) (Figure 2). The probability of egg rejection was only affected by the relative age of females, with relatively older females being more likely to be egg rejecters (Table 1, Figure 2). Interestingly, all 12 females that changed their response to model eggs between different years changed from acceptance to rejection and never from rejection to acceptance (Figure 2). Variation in the probability of egg rejection did not depend on either individual status of parasitism nor parasitism rate in the population in a given year, either as main effects or in interaction with the relative age of females (Table 1).

Egg rejection of naïve magpie hosts. All of the eleven females in which egg rejection was tested in their first breeding attempt accepted the model egg. Acceptance of eggs among naïve magpies was higher than the acceptance of parasitic eggs at the population level (not considering known first-time breeders) (range for all years 82.3–91.3% of nests; all years combined; Fisher´s exact *P* = 0.04; Figure 3). We did not record any evidence that naïve or older magpies (either in trials where the models was accepted or rejected) made any rejection errors, such as rejecting or breaking their own eggs.

Table 1. Determinants of rejection behavior in magpie females.

Probability of rejection (GLMM)		*Random effects*			
Term	Covariance parameter	SE	*Z*	*P*	
Female ID	**3.82**	**1.65**	**2.30**	**0.01**	
		Fixed effects			
Term	Coefficient	SE	*F*	df	*P*
Relative age	**1.34**	**0.33**	**16.25**	**1,116**	**<0.0001**
Number of sampled years	**-0.62**	**0.40**	**2.41**	**1,43.6**	**0.12**
Relative age estimation	**3.46**	**1.93**	**3.21**	**1,116**	**0.07**
Parasitism	-0.27	0.70	0.15	1,114	0.70
Parasitism rate	-0.02	0.02	1.81	1,115	0.18
Clutch size	0.06	0.29	0.05	1,112	0.81
Relative age*Clutch size	0.18	0.21	0.71	1,111	0.40
Relative age*Parasitism	-0.50	0.65	0.59	1,110	0.44
Relative age*Parasitism rate	0.007	0.02	0.12	1,109	0.73
Relative age*Par*Par rate	0.03	0.03	0.89	1,108	0.34

Results of GLMM model testing for the effect of individual parasitism, parasitism rate in the population, relative age of the female, number of years individuals were studied, relative age estimation (known *vs* estimate age), clutch size and individual identity on probability of rejection of magpie females as a binary dependent variable. Non-significant terms were removed following a backward procedure. Terms included in final models are highlighted in bold. We used the Satterthwaite method to calculate degrees of freedom.

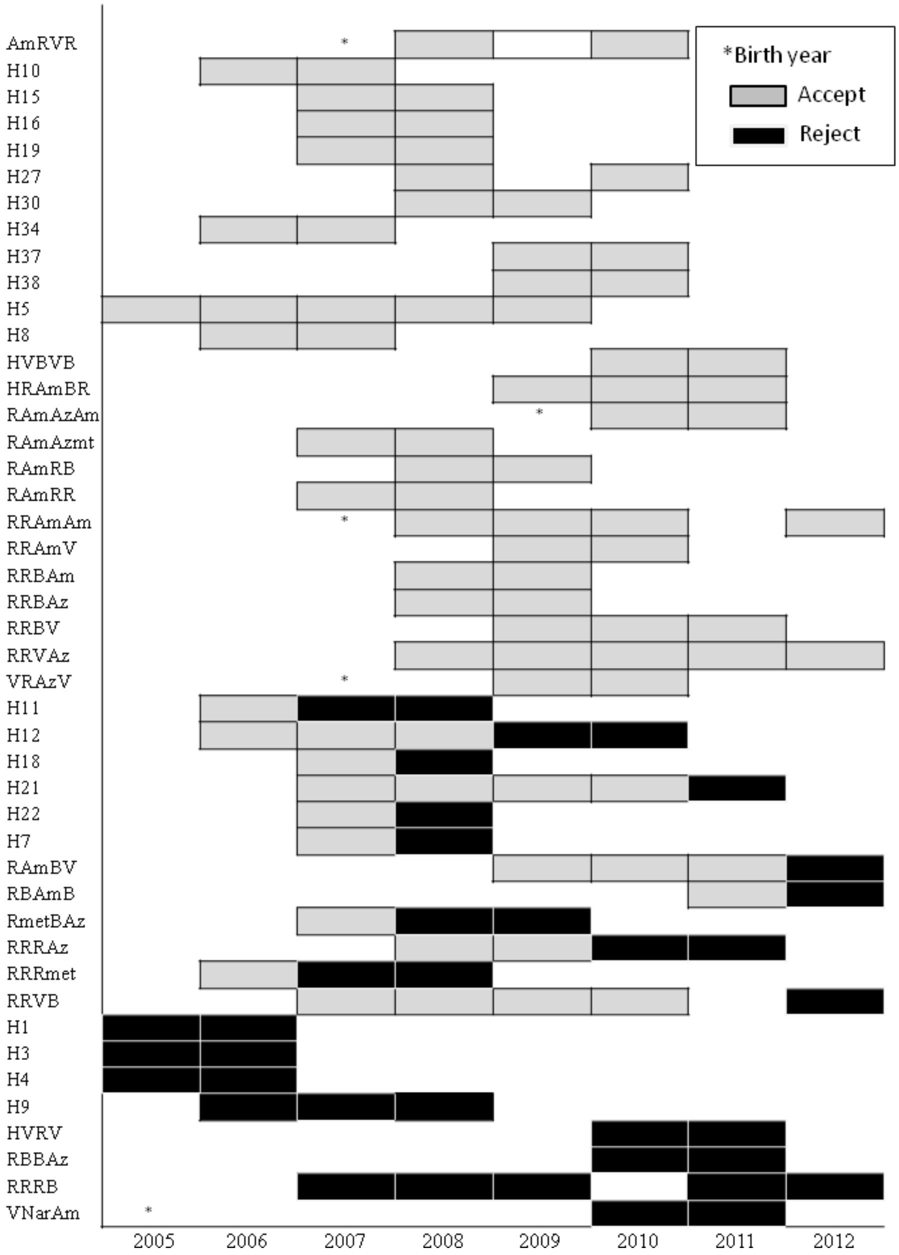

Figure 2. Results of egg discrimination experiments on individual females performed from 2005 to 2012. The grey and black squares represent discriminatory trials in which great spotted cuckoo model eggs were accepted and rejected, respectively. Birth year is indicated for those females that were ringed as fledglings and later found to breed in the population.

Six of the 21 females that had no experience of model eggs in their first breeding attempt were observed in the population in subsequent years. All of them accepted the model egg in their second breeding attempt. Four of them accepted in the rest of the tests (2–4 years) and two of them rejected in their fourth breeding attempt (when they were four years old).

Mother-daughter egg rejection behaviour. We tested egg rejection behavior of eight naïve magpies that were daughters of females known to be egg rejecters. A matched pair-comparison of the proportion of mothers and naïve daughters that accepted the parasite egg revealed that daughters accepted parasite eggs more often than their mothers (one-tailed sign test *P*=0.04; proportion of acceptance 100.0% for daughters, and 37.5% for their mothers).

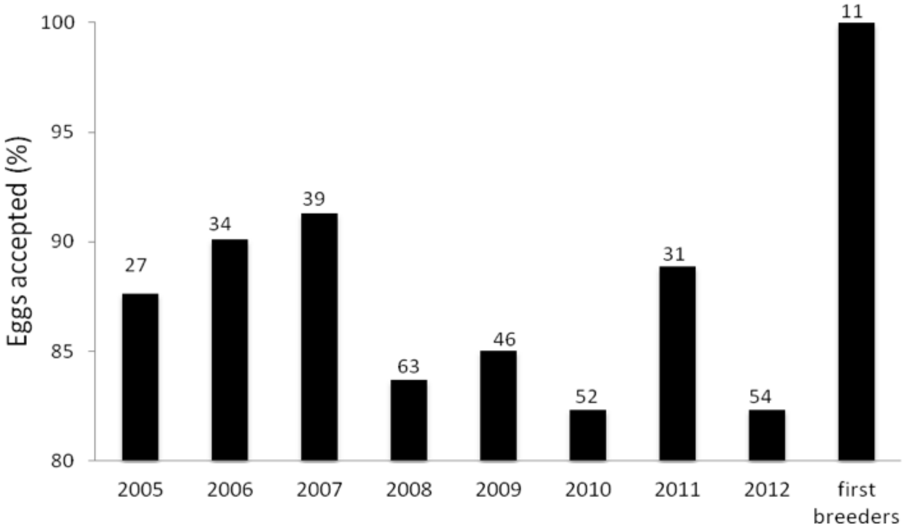

Figure 3. Percentage of acceptance of great spotted cuckoo model eggs in adult female magpies (i.e. individuals over one year old) during the eight years and for the female first-time breeders. Data from the first-time breeding females are pooled across years. The numbers above each bar indicate the number of nests in which egg discrimination was assessed.

Discussion

The study of the individual consistency of egg rejection offers important clues to understand the ecology, evolution and ontogeny of this adaptive component of host defense. Our study is unique in its consideration of several recognition challenges over several years for individual long-lived hosts. Here we found that almost 27% of magpie females sampled for more than one year changed their response to mimetic models eggs (from accept to reject). However, this percentage rose to 53% among the females sampled for three or more years suggesting that the number of females that change their behaviour may have been underestimated. Among the magpies that modified their response to model eggs across their lifetime, we reported a fixed pattern of always shifting from accepting to rejecting parasitic eggs in their nests.

Our results provide strong evidence for a role of aging in the acquisition of skills needed for parasite egg discrimination. We found that all of the naïve breeders sampled during the study accepted the mimetic model egg even when their mothers rejected it. In addition, we found that the probability of egg rejection increased with the relative age of females for the subset of females of unknown age at the first-time sighting. It can be argued that the effect of relative age on rejection might be confounded by immigration. If magpies breeding for the first time in our population were immigrants, they may exhibit lower rejection because they were exposed to a lower level of cuckoo parasitism in their source population but not because they are young. We cannot definitively rule out this possibility as we don’t know the exact age and population origin of most of magpies with repeated trials. However, previous work in our study area showed that our population presents lower rates of parasitism and rejection than most nearby magpie populations (Martín-Gálvez et al. 2007), suggesting that an effect of immigration on rejection would be unlikely. Age-dependent egg rejection in magpies may be due to learning although in our population naïve breeders have similar chance of being cuckoo parasitized than adults (see methods). Learning predicts that acceptance of parasitic eggs should be more common among naïve breeders (Davies and Brooke 1988), and indeed there is some empirical evidence for that contention in cowbird and common cuckoo hosts (Rothstein 1978; Lotem et al. 1992; Lotem et al. 1995; however, also see Sealy 1995; Soler et al. 2000b; Amundsen et al. 2002; Stokke et al. 2004). First-time breeder magpies cannot learn to recognize cuckoo parasitism and reject eggs (see however Soler 2011), unless they learn the appearance of their own eggs by imprinting on the first one or two eggs they lay in their first clutch (Rothstein 1974, 1978). The existence of a prolonged learning process based on observation of their own eggs in which recognition templates could also be updated at each subsequent breeding attempt (Lotem et al. 1995; Lahti and Lahti 2002; Moskat and Hauber 2007; Soler et al. 2013) may not explain changes from acceptance to rejection in magpies. If females had incorporated models eggs within their templates they would be less prone to modify rejection behavior. Alternatively, magpie perception of cuckoo-host differences may vary with age, and thus the patterns found may reflect the outcome of a decision rule to reject eggs that differ in appearance from the majority of the other eggs (rejection by discordancy *sensu* Rothstein 1974). However, we did not detect an effect of clutch size on rejection in agreement with previous studies showing a minor role of discordancy in egg recognition in birds (Lyon 2007; Moskat et al. 2010). Irrespective of the recognition mechanism behind the reported pattern, our results would confirm a key role of ontogenetic processes in magpie egg recognition.

When we simultaneously analyzed the effect of relative age and the risk of cuckoo parasitism that a female perceives at the nest and/or population, we found that only the relative age of females influenced the probability of rejection of parasite eggs. This result, therefore, would imply that plasticity in rejection is not in response to parasitism risk within the range of natural parasitism risk experienced during our study (15.9%–65.6% see methods) because when magpies changed and started to reject parasite eggs, rejection remained in the following breeding attempts irrespective of risk of parasitism. Previous studies have shown that European cuckoo host responses to parasitic eggs are affected by the female's perception of risk of cuckoo parasitism, even when it was socially transmitted from conspecifics (Lotem et al. 1995; Davies and Welbergen 2009). Also, a previous study in which magpies were experimentally parasitized and their nest depredated to simulate cuckoo predatory behaviour showed that magpies may change their behaviour from rejection to acceptance in a second breeding attempt in the same breeding season in study areas with high level of parasitism (Soler et al. 1999), suggesting that magpies might indeed express plastic rejection of parasitic eggs when exposed to a parasite threat higher than the one a female may naturally perceive at the nest and/or population.

It could be argued that our experiments may have influenced the learning process of magpies and thus affected patterns of rejection. It is possible that what appear to be lifelong acceptors were birds parasitized by cuckoos and/or exposed to models eggs in their first breeding attempt and who misimprinted on model eggs (see Strausberger and Rothstein 2009). If a female was naïve when she experienced a cuckoo or an experimental egg for the first time, this might cause her to learn the odd egg's appearance as part of the range of phenotypes reflecting her own and thus to accept in subsequent breeding attempts. Two sources of evidence would suggest that misimprinting on odd eggs is unlikely to explain the reported patterns. First, if females misimprinted on models, we would expect that females not tested with the model egg in their first breeding attempt, rejected it in their second and subsequent breeding attempts. However, the six naïve females who were deliberately not exposed to the model in their first breeding attempt accepted it in their second breeding attempt (first test with the model egg). Second, misimprinting on models cannot explain the patterns of rejection of those females changing to rejection after several years of model acceptance.

Although our conclusion about changes in rejection with age is robust to variation in number of years a female was sampled, we only have two years of data for many of sampled females (Figure 2), so it is likely that due to incomplete sampling we underestimated the number of females that changed from acceptance to rejection in our population. Indeed, first-time breeders always accepted the model egg, even those whose mothers were rejecters. This result would suggest that every long-term rejecter in our sample should have shown plasticity over their lifetime, and hence that there would not be pure rejecters that reject right from the first moment. Once females become rejecters, they continue to reject in subsequent years. Then females that always rejected in our experiments are likely to be early acceptors that have already made the transition from acceptance to rejection._

Summing up, our results show the importance of sampling host individuals several times across their lifetime to attain a reliable assessment of their defensive capacities against parasites, as female hosts may need several years to develop the skills for discrimination and rejection of foreign eggs. More broadly, longitudinal studies are clearly needed in long-lived hosts to assess the existence of (pure) life-long egg acceptors, rejecters or facultative rejecter strategies. In the context of the coevolutionary dynamics of cuckoo host interactions our findings suggest that ontogeny may play a fundamental role deserving further investigation in the expression of defense, in particular for long-lived hosts.

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Supporting Information. Differences in color appearance of eggs between rejecter and accepter females

In 2012 we measured with a Minolta spectrophotometer magpie eggs in 29 complete clutches in which we tested rejection of models in our population for a different purpose. This allowed us to test whether egg appearance of rejecter and accepter magpies do actually differ in our population. If they do not differ, this would constitute a proof that different magpies were exposed to similar mimicry challenges in our work as the appearance of models we used in the study was highly consistent (see methods). Principal component analysis (PCA) was performed on reflectance data (five measures for each magpie egg) to reduce the number of correlated variables (reflectance at 10 nm) into a few orthogonal variables summarizing color variation (see for instance Cuthill *et al.*, 1999; Avilés et al. 2006). PCA allowed us to distinguish between achromatic "brightness" variation represented by the first principal component (PC1) and chromatic variation represented by PC2 and PC3 (Endler and Théry 1996). Together these three first components explained 99.87 % of the total variance in spectra of magpie eggs (see figure below). PC1 was flat and described achromatic variation explaining 99.2 % of the overall variation. PC2 and PC3 were not spectrally flat and together they accounted for 83.36 % of the chromatic variance (see Figure S1). PC2 had high and positive loadings at short wavelengths and high negative ones at intermediate wavelengths and could therefore classify the sampled eggs along a gradient of long ultraviolet-blue-greenness. PC3, however, had high positive loadings approximately at the green (475-550 nm) wavelength and thus could be described as a greenness gradient. The average value of PC1, PC2 and PC3 scores attained for all host eggs in a clutch was used as an index of egg coloration.

In the table S1 are shown average values of PCs scores for accepter and rejecter females. A GLM in which we entered as dependent variables the scores of these three PCs revealed that eggs of magpies that rejected models (N=10 females) did not significantly differ in coloration from those of females that accepted them (N=19) (N=29, Wilks Lambda=2.60, d.f=3,25, P=0.074) suggesting that coloration of magpie eggs do not obviously differ between accepter and rejecter females.

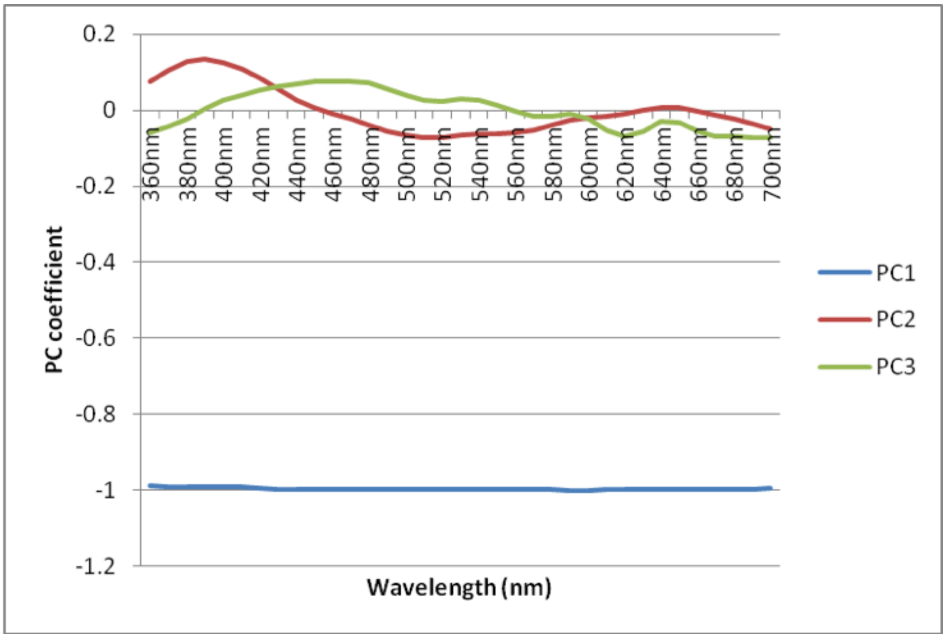

Figure S1. Principal components in relation to wavelength, derived from reflectance spectra from magpie eggs in each clutch. PC1 indicates principal component 1, PC2 principal component 2, and PC3 principal component 3.

Table S1. Average values of PC color scores for nests that accepted and rejected model eggs.

	PC1	**PC2**	**PC3**	
	Mean (SD)	Mean (SD)	Mean (SD)	N
Acceptor	-0.27 (1.59)	-0.10 (0.38)	0.07 (0.68)	19
Rejecter	0.19 (0.10)	0.19 (0.22)	-0.33 (0.67)	10

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Capítulo 8

Factors affecting natal and breeding magpie dispersal in a population parasitized by the great spotted cuckoo

Este capítulo reproduce íntegramente el artículo:

Molina-Morales, M., Martínez, J. G., Martín-Gálvez, D. & Avilés, J. M. (2012). Factors affecting natal and breeding magpie dispersal in a population parasitized by the great spotted cuckoo. *Animal Behaviour*, *83*(3), 671-680.

Factors affecting natal and breeding magpie dispersal in a population parasitized by the great spotted cuckoo

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Abstract

Understanding the causes and consequences of avian dispersal is important since dispersal movements may influence individual´s survival and reproductive success. Although obligate interspecific brood parasitism has obvious detrimental effects for its avian host, few empirical studies have addressed the question of how brood parasitism may influence host dispersal. We studied factors affecting, and consequences of natal and breeding dispersal movements in a population of magpies, *Pica pica,* parasitized by the great spotted cuckoo, *Clamator glandarius*, by monitoring dispersal of individuals and cuckoo parasitism over 6 years. Recruitment probability increased with fledging weight and was higher for individuals hatching early in the breeding season. Heaviest recruits at fledging settled closer to their natal nests and bred in more saturated sites within the study area. Natal dispersal distance did not predict magpie`s productivity or risk of cuckoo parasitism in its first reproductive attempt. Females and parasitized males decreased their breeding dispersal distance when breeding close to other pairs, whereas non-parasitized males breeding in close proximity to other pairs dispersed more in subsequent years. Females, but no males, breeding closer to their previous breeding places had larger clutch sizes than those breeding farther away. Dispersing longer or shorter distances between breeding attempts had no consequences in terms of cuckoo parasitism avoidance for magpies. Our results suggest a minor role of great spotted cuckoo parasitism on magpie dispersal movements at the spatial scale analysed. Instead, our study revealed density-dependent dispersal in our magpie parasitized population.

Keywords: *Breeding dispersal, brood parasitism, Clamator glandariurs, great spotted cuckoo, magpie, natal dispersal, nest density, Pica pica.*

Highlights: • Heaviest recruits settled closer to their natal nests and bred in saturated sites. • Females and parasitized males dispersed less when they breed close to other pairs. • Non-parasitized males breeding near to other pairs dispersed more the next year. • Results revealed density-dependent dispersal in a parasitized magpie population

Introduction

Animals generally move from their birth sites to the place where they reproduce (natal dispersal sensu Greenwood & Harvey 1982) or, once adult, between two consecutive reproductive attempts (i.e. breeding dispersal, Greenwood & Harvey 1982). Understanding the causes and consequences of these dispersal movements are central issues for a broad range of biological disciplines, ranging from conservation biology to research on population ecology, evolution of species, host–parasite interactions and communities of species (Clobert et al. 2001). Dispersal strategies affect the dynamics and the genetic and demographic structure of populations (Hanski & Gilpi 1997; Hanski & Gagglioto 2004), and may greatly influence the survival and reproductive success of individuals (e.g. Clobert et al. 2001; Forero et al. 2002; Williams & Rabenold 2005; Fjerdingstad et al. 2007; Pärn et al. 2009; Gienapp & Merilä 2011). In birds, a variety of social and environmental factors have been associated with natal dispersal (reviewed in Clobert et al. 2001). Dispersal may be an adaptive way to avoid or reduce kin competition or, more generally, intraspecific competition for resources (Strickland 1991; Ekman et al. 1999). Alternatively, remaining in a high-density conspecific population could be advantageous if social crowding provided individuals with benefits (e.g. in terms of antipredator behaviour and/or social foraging, reviewed in Matthysen

2005). On the other hand, inbreeding avoidance may also be a central determinant of dispersal (Pusey 1987; Wolff & Plissner 1998; Szulkin & Sheldon 2008). Finally, prenatal maternal effects (Tschirren et al. 2007) and the internal state of individuals (i.e. condition: Nilsson & Svensson 1996; Ellsworth & Belthoff 1999; Cichon & Dubiec 2005; Moreno et al. 2005) may affect natal dispersal. Social factors (i.e. density, parasitism and predation) also play a key role in explaining avian breeding dispersal. Fitness of territorial animals is often inversely related to population density (Rosenzweig 1981, 1985) because of competition for limited resources (Fretwell & Lucas 1970). Alternative models suggest some benefits from the presence of conspecific neighbours including increased efficiency in expelling intruders (Eason & Stamps 1993) or predators (Stamps 2001). Breeding dispersal may also be influenced by predation and parasitism, because prey and/or hosts may move away to lower their fitness consequences (Clobert 2001; Lima 2009). A large number of studies have reported a link between breeding dispersal and nest failure from predation (e.g. Greig-Smith 1982; Dow & Fredga 1985; reviewed in Lima 2009) or ectoparasites (e.g. Fitze et al. 2004).

Interspecific brood parasitism is a particular form of parasitism occurring in approximately 1% of birds in which a species, the parasite, lays its eggs in the nest of another, the foster or host species, which carries out all the parental care, from incubating parasite eggs to feeding parasite chicks. Brood parasites usually reduce (often drastically) their host’s breeding success (Rothstein 1990; Davies 2000; Payne 2005) resulting in strong selection pressures on the hosts favouring the evolution of defences against parasitism (Rothstein 1990; Davies 2000). Surprisingly, although reduced fitness potential by interspecific brood parasites has been widely demonstrated for a variety of brood–parasite host systems (reviewed in Davies 2000; Payne 2005), still very few empirical studies have addressed the question of how brood parasitism may impact host breeding dispersal. Evidence for a link between host dispersal and interspecific brood parasitism comes from studies performed in North America with hosts of the parasitic brown-headed cowbird, *Molothrus ater* (Hoover 2003; Sedgwick 2004). Brown-headed cowbird parasitism inflicts comparatively lower costs on its hosts than the parasitic cuckoo species in Europe (Davies 2000; Payne 2005). Therefore, more studies with different brood–parasite host systems in which parasites exert strong selection on their hosts are clearly needed before general trends about the possible role of avian brood parasites in host dispersal can be found.

In this study, we first aimed to identify factors affecting dispersal movements (both natal and breeding dispersal) in a population of magpies, *Pica pica,* parasitized by the great spotted cuckoo, *Clamator glandarius,* in the south of Spain by monitoring dispersal events of young and adult ringed host individuals and cuckoo parasitism over a period of 6 years. Concerning local recruitment and natal dispersal we can make the following specific predictions. If body condition is related to the ability to acquire resources and to compete with conspecifics for areas to settle, we expected that heavier and earlier hatching individuals would be more likely to recruit or settle close to their natal nest. In addition, we predicted that individuals from nests with more siblings of the opposite sex would move further if natal dispersal functioned as a mechanism of inbreeding avoidance. Parasitism by the great spotted cuckoo is the main cause of nest failure in magpies in the south of Spain (Soler et al. 1996); therefore we also aimed to test whether brood parasitism has an effect on the dispersal strategy of adult magpies (i.e. breeding dispersal distances) while considering other factors that may potentially influence dispersal such as conspecific nest density. Since cuckoo parasitism has a negative impact on magpie fitness, we predicted that parasitized individuals would tend to move further between years than individuals that were not parasitized by cuckoos. We also aimed to identify the fitness consequences of natal and breeding dispersal movements for magpie hosts in terms of breeding performance and great spotted cuckoo parasitism avoidance.

Methods

Study area and species

This research was conducted during the breeding seasons of 2005–2010 in La Calahorra (37º10´N, 3º03´W, Hoya de Guadix, southern Spain). The Hoya de

Guadix is a patchy area where groves of almond trees, *Prunus dulcis*, in which magpies preferentially build their nests, are very common. Magpies are territorial, sedentary and socially monogamous long-lived passerines (Birkhead 1991).

In our study area magpies lay one clutch during April–May, and they are the main host of the great spotted cuckoo. The interaction between magpies and great spotted cuckoos is considered an example of coevolution (Soler & Soler 2000). Cuckoo parasitism severely reduces magpie reproductive success because the cuckoo chick hatches early and competes with the magpie chicks for food (see for example Soler et al. 1996, 1997). This has selected for host defences against the parasite, such as recognition and rejection of cuckoo eggs (Soler & Soler 2000). The percentage of parasitized nests in our population (i.e. parasitism rate) changes between years (21.2% in 2005, 50.0% in 2006, 15.9% in 2007, 25.4% in 2008, 65.6% in 2009 and 50.7% in 2010).

Individual marking

Adult magpies were captured during the nestling period, using mist nets placed near the nests, or during nest building using square traps with a live decoy (magpie) inside. Trapped adults were marked with a unique combination of colour rings. We also marked all nestlings at the nests with a unique combination of colour rings just before fledging (16–18 days after hatching). At the time of ringing, we took a blood sample from each individual by puncturing the brachial vein. Blood samples were used for extracting DNA and later on sexing and genotyping individuals (see parentage methods). Body mass and other standard morphological measurements (tarsus length, wing length and tail length) were recorded for each ringed bird.

Monitoring unmarked individuals

A fraction of the breeding population was not marked, varying year to year as new animals were captured and marked, and individuals marked at the nest recruited into the population. So, at the beginning of the study very few animals were marked, and by 2010 we already had 82 breeding adults marked (38 females and 44 males). We were able to follow the breeding attempts of unmarked individuals in our population, however, by assigning nests of different years to the same adults using parentage analyses (see below).

Nest monitoring

Magpie nests were monitored from 1 March to the beginning of July each breeding season. Nests were found by careful inspection of all trees in the breeding area, and GPS positioned. Each nest was observed with telescope or binoculars from a hide around 100 m away during the nest-building stage to assign marked birds to each nesting attempt. Nests were monitored at 5-day intervals during the breeding season each year. During egg laying and hatching the nests were monitored at 2–3-day intervals to check whether the nest was parasitized by great spotted cuckoos and determine hatching date. Laying date, number of magpie and great spotted cuckoo eggs, hatching date and number of fledglings were recorded for each breeding attempt. Nests were categorized as parasitized if at least one great spotted cuckoo egg was detected in the nest. In all parasitized nests (except those depredated) at least one cuckoo fledged. Nests were categorized as nonparasitized if no cuckoo egg or chick was detected in the nest.

Density of nests

Population density may relate to natal and breeding avian dispersal movements in at least two ways. Higher density (i.e. short internest distances) may increase competitive interactions among neighbours thus favouring dispersal (Clobert 2001). Alternatively, high nest density may be caused by the concentration of magpie nests in high-quality territories, which would produce more fledglings than the relatively poorer low-density territories. We estimated population density as the average distance between the two nearest conspecific nests (nearest-neighbour distance, NND).

Sex ratio

Inbreeding risk may have an effect on both recruitment probability and natal dispersal distance (see Introduction). For a given individual the risk of inbreeding depends on the number of opposite-sex siblings in the area after leaving the nest. Therefore, we calculated sex ratio for each nestling as the proportion of individuals of the opposite sex in its nest, and we used this as an estimate of inbreeding risk.

Dispersal

Natal dispersal

We ringed all fledglings between 2005 and 2009 (N=446 from 107 nests) in our population. Natal dispersal was characterized by two variables: the recruitment probability of individuals born in our study area as a binary variable (recruit versus nonrecruit) and the natal dispersal distance (NDD) calculated as the distance between an individual's natal nest and its first breeding attempt. That distance was calculated for the 36 reported recruits. Individuals that were absent from the population in the year after fledging but that reappeared in the following year were included as recruits (17 of 36 recruits) because magpies breed in their first, or more usually, in their second year of life irrespective of their sex (Birkhead 1991).

Breeding dispersal distance

Breeding dispersal distance (BDD) was calculated as the shortest distance between two nests occupied by the same bird in consecutive years. Of those 90 individuals used in the analysis, 46% (41 individuals) were ringed individuals and 54% (49 individuals) were determined by genetic analysis (see below). For individuals with more than one recorded breeding dispersal event (28 of 90 individuals) we only considered the first one to avoid pseudoreplication. We decided not to use individual ID as a random factor controlling for intraindividual variation because of the large number of individuals with only one dispersal event, which led to statistical model collapse.

Laboratory work

Genotyping procedures

Genomic DNA was isolated from blood using the ammonium–acetate precipitation method (adapted from Bruford et al. 1998). Twenty-nine polymorphic microsatellite loci were used for genotyping. Two had been previously isolated from magpies, Ppi1 and Ppi2 (Martínez et al. 1999); two from Seychelles warblers, *Acrocephalus sechellensis*, Ase18 and Ase64 (Richardson et al. 2000); and one from western crowned warblers, *Phylloscopus occipitalis*, Pocc1 (Bensch et al. 1997). Details on the rest (DKiD12, TG01-040, TG04-004, TG13-017, Ppi4, MSLP4, Tgu05, Cpi7, Ppi11, Ppi12, Aar4, TG01-147, ZF09-005, ApCo46, PmaTGAn42, Ppi18, Ppi8, Sjr4, Tgu06, Tgu07, Cuμ02, TG01-000, TG04-061, Pij15) can be found in Martín-Gálvez et al. (2009) and Dawson et al. (2010).

Polymerase chain reactions (PCRs) were performed with the forward primer of each marker labelled with a fluorescent dye. The markers were combined in four multiplex PCRs systems composed of 11 primers (set I), 10 primers (set II), nine primers (set III) and two primers (set IV) and were used to genotype all the individuals. Nine markers were excluded from the analyses because they were not in Hardy–Weinberg equilibrium and were likely to have a high frequency of null alleles (estimated following Amos et al. 2001). A list containing the full set of markers we used for genetic analyses and details on their variability is shown in Appendix Table A1. PCR products were electrophoresed through an ABI Prism 377 DNA sequencer (Applied Biosystems Inc., Foster City, CA, U.S.A.). The outputs were analysed using ABI Genemapper Software version 3.7.

Sexing procedures

As the sexes are morphologically similar in magpies, we determined the sex of all individuals using sex-specific primers P2/P8 (Griffiths et al. 1998) and Z-043B (D. A. Dawson, unpublished data). Sex markers were included in multiplex PCRs.

Parentage analyses

We used the software ML-Relate (Kalinowswki et al. 2006) to calculate the more likely estimate of relatedness between pairs of nestlings. ML-Relate calculates maximum likelihood estimates of relatedness and relationships, allowing us to compare putative relationships with alternatives (i.e. full siblings versus half siblings or unrelated). We did this using the information in blocks of 2 years; for example, we first calculated the relatedness of all chicks of 2006 and 2005, and after that of 2007 and 2005, and so on. In each case, and for every nest in a given year (the focal nest or family), all chicks in that family were found to be full siblings of each other (with a few exceptions of pairs of half siblings caused by extrapair paternity, see below); we then looked for any other set of nestlings (family) in the other year that related to the nestlings in the focal nest as full siblings. In all cases, we found either none or only one set of nestlings (corresponding to one nest) that were full siblings of the nestlings in the focal nest, and so we can consider those two nests as two breeding attempts of the same magpie pair. By repeating this procedure, comparing all families in pairs of years we could assign a number of magpie pairs (*N*=39) to their breeding attempts in a number of years within the study period.

In some cases, a given set of nestlings may appear as half siblings of another set of nestlings in another year. This was so when all chicks in the focal nests appeared as half siblings of all the chicks in another nest and this relationship was more likely than being full siblings or unrelated. In this case we can consider that both nests correspond to breeding attempts of one individual (male or female) and two different mates in the two years. These cases cannot be used in our analyses unless we can identify (through observation or paternity analyses, see below) one of the adults implied. If the chicks of two nests appear as half siblings and we know from observations or paternity analyses that the same male (or female) bred in those two nests, we considered those two nests as the different breeding attempts of one individual (male or female) paired to different mates in the two nests.

Paternity analyses

We used paternity and maternity analyses as implemented in CERVUS 3.0.3 (Kalinowski et al. 2007) with two objectives: first, checking that the adults observed at the nests were really the parents of the chicks in those nests, and second, assigning parents captured in a given year to breeding attempts in previous years. This was necessary because magpies may live more than our 5-year study span and so any adult bird captured at the end of our study period may have been breeding in the area in previous years. Paternity/maternity was carried out with the genotypes of all adults marked and observed in the population as potential parents of the chicks for each year, with the exception of those individuals of known age that we could be sure were not yet alive in that year (i.e. recruitments, born in that year). CERVUS assigned a pair of most likely parents to each chick but we only considered the assignments when the LOD score (the natural log of the overall likelihood ratio) was positive and Delta score (defined as the difference in LOD scores between the most likely candidate parent and the second most likely candidate parent) between the (two) most likely parent(s) and the rest of the potential parents was significant.

We compared parentage assignments with field observations in those nests where at least one of the adults was marked. All the females observed breeding in a given nest (building, laying, incubating and/or feeding the young) were assigned as mothers of all the chicks in that nest. Similarly, all males observed breeding in a given nest (building, feeding the young) were assigned as fathers to that nest, but sometimes not to all the chicks in the nest, probably because of cases of extrapair paternity in those nests. In these cases (10–20% of nests depending on the year, unpublished data) at least half of the chicks in the nest were assigned to the social father.

When assigning paternity and maternity to nests where we had no field observations, or in the case of nests where adults were captured in later years and thus we had no observational data, we used the same criteria: we considered a female to be the female breeding in that nest when it was assigned as the mother of all the chicks in that nest with a positive and significant LOD score, and we considered a male to be the male of a particular nest when it was assigned as father of at least half the chicks in that nest with a positive and significant LOD score. When a male was assigned by CERVUS to a few, but not the majority of chicks in a nest we considered that to be a case of extrapair paternity and thus we did not consider that nest as a breeding attempt of that particular male (see Appendix Fig. A1 for further details on nest/family genetic matching).

Statistical analyses

Analyses were performed using Statistica and the R 2.12.0 Package (R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org). Owing to a lower effort of sampling in 2005 and 2006, we analysed factors affecting recruitment probability on the subset of 309 ringed fledglings marked in 2007–2009. We used a generalized linear mixed model (GLMM) for binary dependent variables (link function: logit) using the package 'nlme' in R 2.12.0 Package to test the effect of sex, body weight at fledging, laying date, NND and sex ratio as predictors of recruitment probability. Body weight at fledging and laying date were correlated (r_{244} =0.235, P= 0.0002, N= 246 nestlings weighed at fledging). However, the correlation was less than 0.70, which eliminates serious problems of collinearity (Green 1979). Nest and year were fitted as random factors to account for the fact that nestlings raised in the same nest and year were not statistically independent. Initially, we ran a model with all possible two-way interactions. However, no interaction was significant and this model did not differ significantly from the model with only single effects (log likelihood ratio for comparing models using the package 'nlme' in R: χ^2_4=1.95, P =0.74). Thus, here we only report the results for the reduced model.

Because of the low sample size we estimated factors affecting natal dispersal distance in two separate analyses and we could not test possible interactions between terms. First, we used a linear mixed model (LMM) to test the effect of individual and nest factors (i.e. sex, body weight at fledging, laying date, sex ratio) on natal dispersal distance. Year and sex were also entered as random and fixed factors in the model, respectively. In a second LMM we considered the effect of conspecific nest density in the natal nest (i.e. NND(t)) and in the first breeding attempt (i.e. NND(t+1)) as predictors of natal dispersal distance while accounting for the year and the sex as random and fixed factors, respectively. Similarly, we explored the effects of year as a random factor, parasitism as a fixed factor (parasitized versus nonparasitized) and NND(t) and NND(t+1) as covariates on log-transformed breeding dispersal distance using two separate LMMs for each sex.

We analysed the consequences of natal and breeding dispersal for female and male magpies separately, by performing Pearson correlations between log-transformed natal and breeding dispersal distances and laying date, clutch size and number of fledglings produced in year t+1. Probability of being parasitized in year t+1 in relation to natal and breeding dispersal distances was analysed with logistic regressions.

Ethical note

We had permits to capture and ring magpies and to perform this study from the conservation authorities of the regional government of Andalucía (Licence code: P06-RNM-01862). In a pilot study performed during the nest-building phase we realized that capturing the two adults at a nest led to nest abandonment (six of seven nests). Therefore, to minimize the probability of nest abandonment we only captured one adult per nest in this study. All captures were done at the nest-building stage and no nests were abandoned, confirming that capture of one adult per nest had a negligible effect on nest abandonment. For captures we used two adult magpie decoys per year (i.e. eight magpies) captured in a nearby population the autumn before each reproductive season. Magpie decoys were housed in an aviary measuring 12x8 m and 4 m high under ambient light and temperature regimes located at the University of Granada, and were released at the place of their capture after each field season. During housing they were fed at libitum with a mix of meat, crickets and mealworms and were given water for drinking. To minimize stress magpie decoys were used on alternate days. The trap consisted of a central compartment, in which the decoy was placed, and four compartments surrounding the central one. The decoy was always put in the central compartment and provided with some meat and water. After we set up the trap magpie decoys were normally calm. To minimize the time a magpie decoy spent in the trap we tried to capture magpies at only two nests per day lasting 1 h per nest. Trapped magpies could not make physical contact with the decoy. Once installed, we watched the trap from a hide so that we could remove the magpies as soon as they entered the trap, minimizing the stress for both the captured individual and the decoy. Subsequent visits to the nests and monitoring of adult individuals confirmed that collection of blood samples (ca. 10–20 µl per individual) of adults and nestlings (at the age of 15–18 days) also had negligible effects on magpies. Nest observation allowing individual identification was designed to minimize disturbance to the birds by locating hides at least 100 m away from the target nest. In all cases, parents were regularly visiting the nests during these observations suggesting that nest observations were not harmful for the magpies.

Results

Recruitment rate

Recruitment rate of fledglings in years 2007 to 2009 in our population was 11.65% (36 out of 309 ringed fledglings). The model of local recruitment showed that recruitment probability increased with nestling weight at fledging and was higher for individuals that hatched from earlier nests in the breeding season (Table 1, Figure 1).

Table 1. Factors affecting probability of local recruitment by magpies.

Effect	Statistic	*P*
Random		
Nest		0.692*
Year		0.998*
Fixed		
Sex	t= -1.195	0.209
NND (t)	t= -1.259	0.233
Laying date	t= -2.254	0.025
Weight	t= 4.060	0.0001
Sex ratio	t= 1.401	0.162

Results of GLMMs (binomial errors, logit link) to study nest and year were fitted as random factors. N=239 ringed individuals. **P* value analyzed using logLik test.

Natal dispersal distance

Factors affecting natal dispersal distances

In a first model we studied individual and nest factors affecting natal dispersal distance in magpies by testing for the association between sex, year, laying date, sex-ratio and body weight on natal dispersal distance. Females tended to move slightly farther than males (mean ± SD values were 1108.31 ± 421.25 m for females and 856.93 ± 529.72 m for males), although the difference was not statistically significant (Table 2). The only significant nest predictor of natal dispersal distance was body weight at fledging, with heaviest recruits at fledging settling closer to their natal nests (Table 2, Figure 2).

In a second model we tested for the effect of nest density of year t and t +1 on natal dispersal distance. We found that natal dispersal distance increased with NND(t+1) (Table 2), indicating that individuals that settled closer to their natal territories bred in more saturated sites of the study area (Figure 2).

Table 2. Factors affecting natal dispersal distance in magpies.

Effect		df	*F*	*P*
First model				
Sex	Fixed	1	0.18	0.67
Sex ratio	Fixed	1	0.92	0.35
Laying date	Fixed	1	0.15	0.70
Body mass	Fixed	1	5.59	0.026
Study Year	Random	2	0.33	0.72
Error		24		
Second model				
Sex	Fixed	1	2.57	0.12
NND(t)	Fixed	1	1.61	0.21
NND(t+1)	Fixed	1	4.63	0.039
Study year	Random	2	0.06	0.94
Error		29		

The first model shows results of a Linear Mixed Model to study nest factors affecting natal dispersal distance. The second model shows results of a Linear Mixed Model to study the influence of conspecific density in year t and t+1 on natal dispersal distance. Study year was fitted as a random factor in the two models.

Consequences of natal dispersal

Individuals that breed closer to their natal nests did not lay earlier (r_p=0.02, d.f. = 29, P=0.90, N=31), nor laid larger clutch sizes (r_p=0.04, d.f. = 28, P=0.82, N=30) or produced more fledglings (r_p=-0.23, d.f. = 30, P=0.19, N=32) in their first breeding attempt than those breeding farther away. Moreover, dispersing longer or shorter distances from their natal nests did not affect the probability of being parasitized for magpies in their first breeding attempt (mean ± SD (N) values were 804.02 ± 530.03 m (N=14) for parasitized and 991.41 ± 523.22 m (N=18) for unparasitized, (χ^2_1 =0.98, P=0.32).

Breeding dispersal distance

Factors affecting breeding dispersal

Females tended to move slightly farther than males (mean ± SD values were 289.23 ± 508.18 m for females and 230.32 ± 302.06 m for males). Breeding dispersal distance of female magpies was influenced by NND in year t (Table 3). Females that were breeding in high density territories in year t moved a shorter distance in year t+1 (Figure 3).

In males, breeding dispersal distance depended on NND in year t in interaction with great spotted cuckoo parasitism (Table 3). Males that were parasitized in year t, moved less in year t+1 when they bred in densely used areas in year t (Figure 4), but the opposite trend was found for males that were not parasitized in year t (Figure 4), those breeding in close proximity to other pairs in year t dispersed more in year t+1 than those breeding farther away from neighbours. In addition, we found that male breeding dispersal distance was significantly affected by NND in year t+1 (Table 3, Figure 4), suggesting that individuals that dispersed farther, moved to breed in more saturated territories.

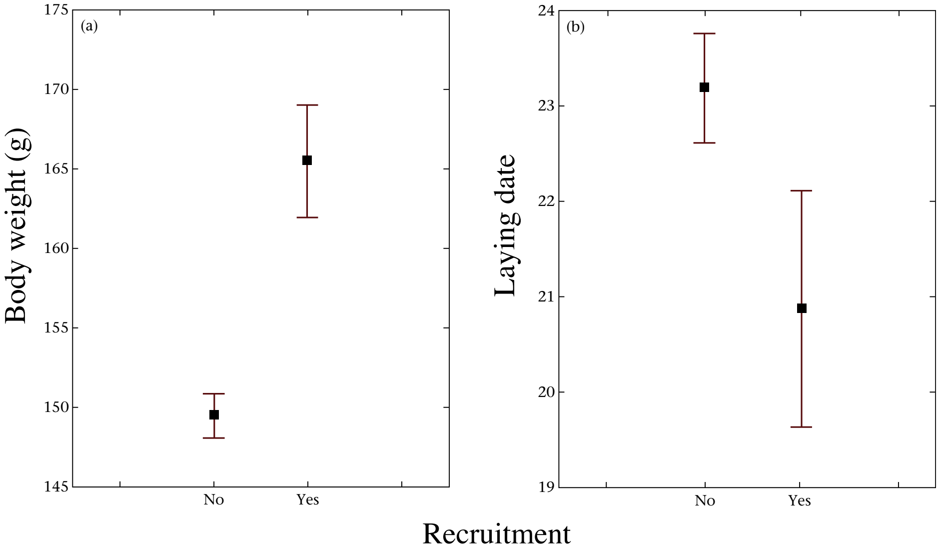

Figure 1. Relationship between magpie recruitment probability and (a) body weight (in grams) at 18 days from hatching and (b) laying date of the nests where individuals were born. *N*= 309 ringed birds.

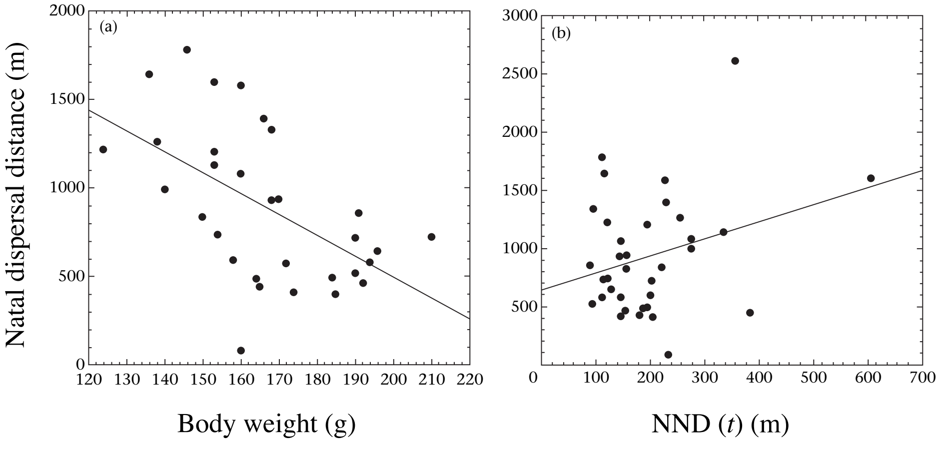

Figure 2. Relationship between natal dispersal distance (in meters) and (a) weight of fledglings at day 18 (in grams) and (b) nearest nest distance of the nest where the individual bred in the yeat t+1. *N*=36 birds.

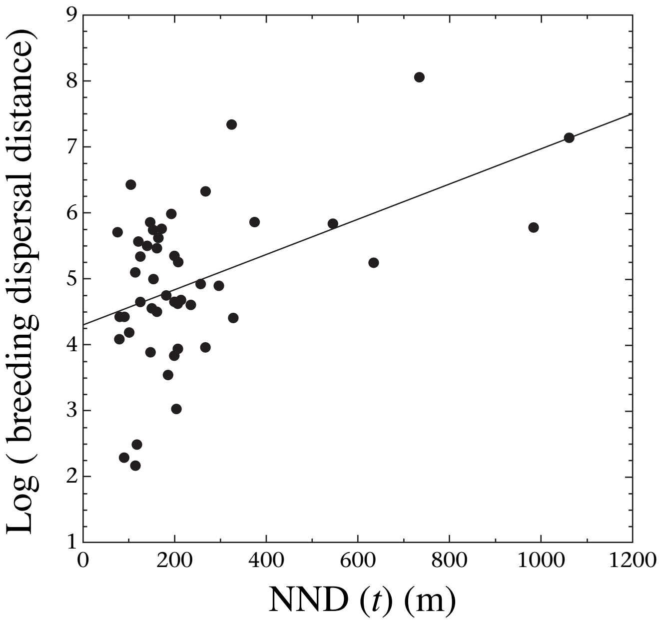

Figure 3. Relationship between female breeding dispersal distance (BDD, in log scale) and average distance to the two nearest neighbor magpie nests (NNDt, in meters). *N*=47 females.

Consequences of breeding dispersal

Female magpies that moved less between consecutive breeding attempts did not lay earlier in the season (r_p=0.09, d.f. = 42, *P*=0.53, *N*=44), nor produced more fledglings (r_p=-0.05, d.f. = 42, *P*=0.70, *N*=44). However, females that bred closer to their previous breeding attempt laid larger clutch sizes (r_p=-0.33, d.f. = 41, *P*=0.027, *N*=43, Figure 5) than those breeding farther away. In terms of cuckoo parasitism, dispersing longer or shorter distances between breeding attempts did not affect the probability of being parasitized for female magpies (χ^2_1=2.10, *P*=0.14).

In males breeding dispersal distances were unrelated to laying date, clutch size or number of fledglings produced in the subsequent breeding attempt (*P*>0.45 in all cases). Similarly, probability of parasitism was unrelated to breeding dispersal distance movements of males (χ^2_1=0.11, *P*=0.73).

Table 3. Factors affecting breeding dispersal distance in magpies.

Effect	df	*F*	*P*
Females			
Parasitism	1	0.002	0.96
Year	4	1.31	0.28
NND (t)	1	7.75	0.008
Parasitism*NND(t)	1	0.15	0.70
NND (t+1)	1	0.97	0.33
Parasitism*NND(t+1)	1	0.02	0.87
Error	36		
Males			
Parasitism	1	4.28	0.046
Year	4	2.08	0.10
NND (t)	1	2.99	0.09
Parasitism*NND(t)	1	5.33	0.027
NND (t+1)	1	4.37	0.044
Parasitism*NND(t+1)	1	0.005	0.94
Error	33	4.28	0.046

Model show results of a Linear Mixed Model to study factors affecting breeding dispersal distance in females (*N*=47) and males (*N*=43) separately. Year was fitted as random factor in the two models.

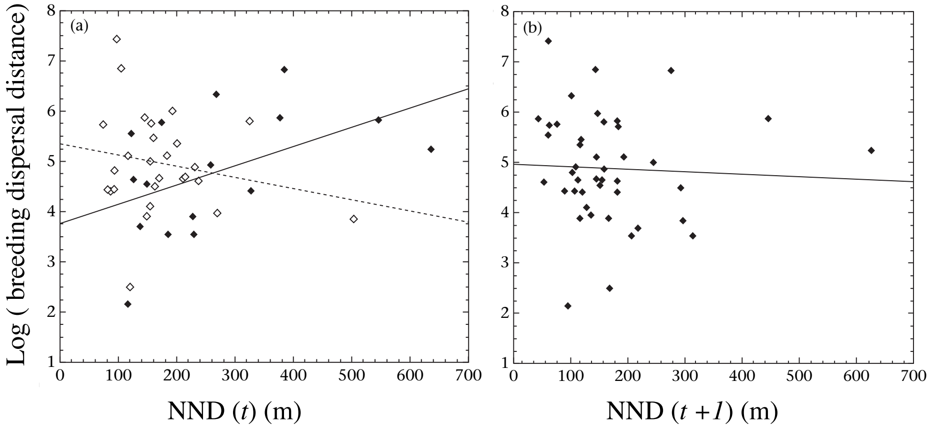

Figure 4. Relationship between male breeding dispersal distance (BDD, in log scale) and (a) average distance to the two nearer neighbor nests in year t (NND t (in meters)) and (b) average distance to the two nearer neighbor nests in year t+1 (NND t+1 (in meters). In the panel (a) cuckoo parasitized and non parasitized male magpies are depicted black (continuous line) and white (dashed line), respectively. *N*=43 males.

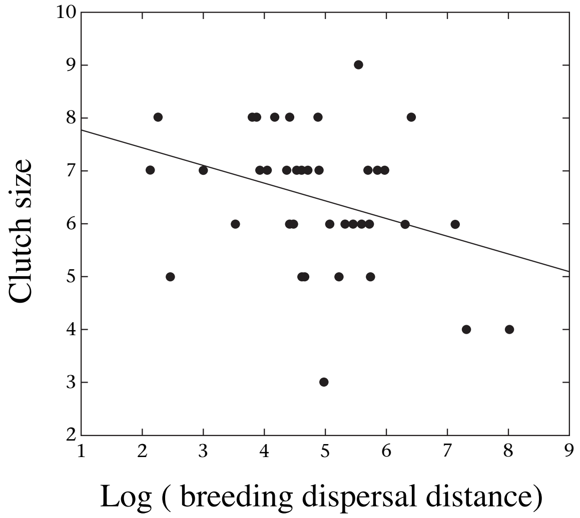

Figure 5. Correlation between clutch size of female magpies (*N*=43) in the year after they moved and log breeding dispersal distance.

Discussion

Recruitment and natal dispersal

Our results indicate that magpie's natal dispersal in a population heavily parasitized by the great spotted cuckoo is strongly influenced by fledging mass, since an individual's weight at fledging explained both its recruitment probability in the population and its natal dispersal distance once it was recruited. Magpie recruitment probability was higher and natal breeding dispersal was shorter for heavier nestlings. In UK, Eden (1987) showed that body weight had a weak influence on competitive ability in magpies when other factors or asymmetries are considered. She showed that early-hatched individuals dominated later hatching young because they gained greater experience, feeding sites and techniques enabling them to dominate younger competitors during winter (Eden 1987). In agreement with that scenario of seasonal effect on natal dispersal we have detected that recruitment probability increased for early-hatched individuals in our population. However, we still reported a positive effect of weight per se on natal dispersal that is probably due to the fact that weight at fledgling is a good predictor of survival during the few weeks after leaving the nest, and thus of recruitment probability. Magrath (1991) found that nestling weight of blackbirds *Turdus merula* itself influences juvenile survival, and proposed that in practice, the probability of survival shortly after nutritional independence might be an adequate estimate of relative probabilities of recruitment to the breeding population in many bird species. The positive effect of weight at fledging on survival during the few first months after leaving the nest has been described in passerines in general (Monrós et al. 2002) and in magpies in particular (Ponz et al. 2007). Also, weight at fledging is related in passerines to the ability to compete with other fledglings once they leave the nest (Both et al. 1999) and in general a low weight at fledging appears to be disadvantageous in most studies (Naef-Daenzer et al 2001).

Among recruits, heavier fledglings made their first breeding attempt closer to the territories where they were born. This may be related to the ability to acquire food during the postfledging period, which allows individuals to acquire enough energy to compete with conspecifics during settlement (Dufty & Belthoff 2001). On the other hand, natal dispersal distance was also related to density of conspecifics at the time of the first breeding attempt, indicating that individuals that settled closer to their natal territories bred in more saturated sites (i.e. lower distance to the two nearest neighbour nests) of the study area. This means that individuals in better physical condition (i.e. heavier chicks) are more likely found settled near their natal territories and in areas preferred as breeding grounds for other individuals (Hernández-Matias et al 2010; Kim et al 2009). Breeding near natal territories may have advantages despite the fact that leads to breeding in the proximity of other pairs. Advantages could be related to ecological factors such as the importance of familiarity with breeding grounds and/or access to high quality breeding patches (Eden 1987a, b; Forero et al 2002). The benefit of familiarity, however, was not evident in our magpie parasitized population, since among the set of recruited individuals those breeding closer to their natal nests had similar productivity and chance of being parasitized in their first breeding attempt.

There is no effect of sex on natal magpie dispersal since recruitment probability and natal dispersal distances did not differ between the two sexes.

Our data are in accordance with previous results for the species in UK population non-parasitized by cuckoos (Eden 1987a), but contrast with the general pattern of sex biased natal dispersal reported for most studied birds and mammals (Greenwood 1980; Clarke et al. 1997). Greenwood (1980) proposed that differences between sexes in the propensity to disperse may be a consequence of the species' mating systems. Whenever males defend resources to attract mates male philopatry will arise if sedentary males gain any advantage over dispersers, leading to a pattern of female-biased dispersal. Alternatively, if females may gain advantages through familiarity with their natal territories male-biased dispersal would be expected. Evidence suggests that in the magpie both sexes compete intra-sexually for breeding territory vacancies (Birkhead 1991), which makes unlikely that competitive advantage by non-dispersers will lead to sex-biased dispersal. Also, it could be argued that familiarity could be particularly advantageous for the two sexes in cuckoo parasitized magpie populations because despite parasitism non-dispersers are more able to establish a breeding territory in their natal area than elsewhere, whereas dispersers should face the costs of dispersion plus those induced by cuckoo parasitism. Finally, it cannot be discarded that the absence of sex-biased dispersal was derived from the spatial scale in which we studied magpie natal dispersal. Indeed, other studies have shown that sex-specific differences in dispersal are linked to long-distance dispersal or dispersal between habitats with very different quality whereas dispersal between areas with similar quality is associated with similar dispersal behaviour in both sexes (Verhulsts et al 1997).

We hypothesized that if dispersal was related to inbreeding risk, the sex-ratio experienced by nestlings at the nest would be a good predictor of dispersal behaviour, with individuals growing in nests with more opposite-sex siblings dispersing farthest away. This was not the case and sex-ratio had no influence on recruitment probability or natal dispersal distances in our population. This is probably due to the small number of nestlings per nest that survive and stay in the study area year to year, which will reduce the risk of inbreeding. Indeed, provided that mating was at random, sib-sib pairing probability must be highly unlikely in our population because from the whole

36 recruits only 4 individuals had a sibling of the opposite sex in the population. Thus natural selection had little opportunities to mold natal dispersal distances according to inbreeding risk.

Breeding dispersal

Magpie breeding success in cuckoo parasitized populations from south Europe is highly related to brood parasitism, since in most parasitized nests only one or a few great spotted cuckoo chicks fledge (Soler et al. 1996, 1997), which makes cuckoo parasitism a proximate factor likely to affect magpie host breeding dispersal movements.

Proximate factors explaining breeding dispersal in our cuckoo parasitized magpie population are not the same for males and females and the effect of parasitism was only evident for males. However, females and parasitized males showed a similar pattern of negative density-dependent breeding dispersion. Breeding dispersal was influenced by the proximity of neighbours (i.e. nest density) in the first year, with females and parasitized males dispersing less when they breed in close proximity to other pairs. For females, this pattern may be explained as a consequence of a high concentration of females in good areas or territories. If a particular plot within the study area was good for breeding we should expect that the plot was used by several pairs, and if individuals moved between consecutive breeding attempts (years) looking for better territories then we would expect both a smaller inter-nest distances in better plots and smaller breeding dispersal distances in individuals breeding in better plots. Supporting this argument we found that individual females that moved farther away had smaller clutch sizes than those breeding relatively closer to their previous breeding place.

Breeding close to conspecifics in a cuckoo parasitized population could be advantageous in several ways for magpie hosts. In general, these are likely to be better territories and that would be the reason why pairs breed close to each other. Both males and females could also enjoy increased opportunities for extra-pair copulations when breeding very close to other pairs (see for example Charmentier & Perret 2004). In addition, defense against brood parasites or predators is probably enhanced by the ability of groups of neighbours to detect intruders and the observed tendency of neighbors to attack intruders simultaneously (Canestrari et al. 2009). Indeed, it has been described that increased proximity to other nests and specially laying synchrony of magpie pairs both reduced the probability of being parasitized by great spotted cuckoos (Martínez et al 1996). Finally, breeding close to others offers the intriguing possibility that clever manipulation of less competent individual hosts could reduce disease prevalence (dilution effect, e.g. Hall et al. 2009).

Surprisingly, unparasitized males showed the opposite trend, and those breeding in close proximity to other pairs in year t dispersed more in year t+1 than those breeding farther away from neighbours. Differences in dispersal pattern between parasitized and unparasitized males can be explained in different non-exclusive ways. First, parasitized and unparasitized male hosts could include individuals of different age classes (Grim 2002) and/or personalities (Avilés & Parejo 2011) with different tolerance to conspecific density and dispersal propensities. Unfortunately, we don't have data to contrast this possibility at present. Secondly, it could be possible that the benefits for a host of breeding close to other conspecifics were triggered only when being parasitized, otherwise the costs of aggregation will be prevalent leading to dispersal. In this scenario, breeding close to conspecifics might benefit previously parasitized individuals by communal nest defense against cuckoos (Poiani & Elgar 1994) and/or possible dilution effects (Hall et al. 2009; Raffel et al. 2010). It still remains to be explained why female and unparasitized male magpies showed opposite sensitivity to conspecific density in our population. The fact that females tend to aggregate and males disperse from patch with high conspecific density might be related to sexual differences in the benefits of nest aggregation and clearly deserves further investigation.

Hitherto, few empirical studies have targeted how brood parasites affect host breeding dispersal, and results are contradictory. There was an effect of brown-headed cowbird parasitism both on between-year site fidelity and breeding dispersal distances in female Willow flycatcher hosts *Empidonax traillii* (Sedgwick 2004). In this case, the distance moved between consecutive years by females depended on breeding performance, being lowest for successful unparasitized females and higher for parasitized and unsuccessful females. However, breeding dispersal distances of Willow flycatcher males did not depend on breeding performance (Sedgwick 2004). In prothonotary warblers *Protonotaria citrea* adults parasitized in a given year had lower between-year return rates whereas parasitism had no effect on breeding dispersal distances of adults that returned to the study area (Hoover & Reetz 2006). Differences between studies in the effect of parasitism on dispersal may reflect either the different costs of studied brood parasites on their hosts (see introduction), or due to the fact that previous studies disregarded the possibility that the effect of parasitism on host dispersal was mediated by host density. Theoretical work had emphasized the need of taking into account density of individuals when studying the effect of proximate factors on dispersal (Greenwood & Harvey 1982). Our results support this recommendation when testing for the effect of parasitism on magpie dispersal since the effect of parasitism was mediated by host density.

In conclusion, we have found only moderate evidence supporting a role of cuckoo parasitism on magpie host dispersal and a negligible effect of variation in natal and breeding dispersal on probability of being cuckoo parasitized at the analyzed spatial scale. The level of great spotted cuckoo parasitism was relatively high during our study which reduces the chance of escaping from cuckoos through short dispersal movements. Future studies should aim to investigate dispersal events in relation to parasitism in several connected populations with different levels of cuckoo parasitism in order to ascertain whether cuckoo parasitism may affect host dispersal at larger spatial scales.

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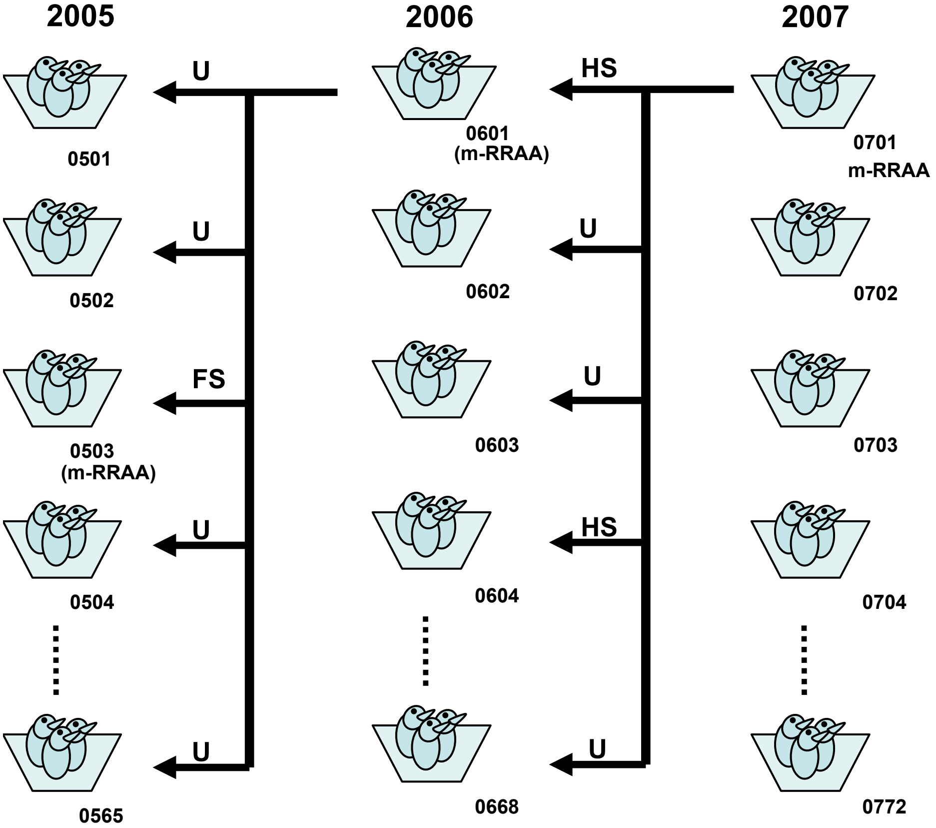
Appendixes

Table A1. Details on the variability of the loci used in the study.

Locus	N	N_A	H_O	H_E	P_E
Cpi7	78	9	0.782	0.829	0.526
MSLP4	78	8	0.731	0.738	0.663
Ase18	78	15	0.795	0.883	0.394
TG01-040	78	4	0.410	0.482	0.879
TG04-004	78	13	0.859	0.897	0.362
TG13-017	78	7	0.667	0.684	0.736
Tgu05	78	4	0.436	0.447	0.899
ZF09-005	78	5	0.397	0.481	0.883
ApCo46	78	4	0.487	0.539	0.855
PmaTGAn42	78	17	0.923	0.910	0.318
Tgu07	78	5	0.538	0.551	0.846
Tgu06	78	5	0.641	0.568	0.827
TG01-147	78	3	0.526	0.552	0.850
Ppi1	78	10	0.769	0.811	0.550
Ppi008	78	5	0.551	0.594	0.816
Sjr4	78	6	0.718	0.669	0.747
Ppi012	78	12	0.885	0.885	0.392
Ppi011	78	28	0.769	0.950	0.203
Ppi2	78	16	0.910	0.897	0.358
Ase64	78	14	0.795	0.906	0.337
Total		9.5		0.714	<0.001

N_A= number of alleles found in adult magpies, observed (H_O) and expected (H_E) heterozygosity, average non-exclusion probability (P_E) for each locus (calculated using CERVUS).

Figure A2. An example of nest/family matching following the methods explained in the text. All the chicks in nest 0601 were found to be full siblings of all the chicks in nest 0503, and so we can conclude that nests 0503 and 0601 correspond to the same magpie pair. Chicks in the nest 0701 were found to be half siblings of the chicks in two 2006 nests, 0601 and 0604. We captured the male RRAA in the nest 0701 and the paternity analyses showed that male RRAA was the father of all the chicks in that nest. Paternity analyses also showed that RRAA was the father of chicks in nest 0601 and 0503 (shown with m-RRAA between brackets). All together the data suggest that RRAA bred in nest 0503 and 0601 with the same unmarked female and in nest 0701 with a different female, and also that this female was breeding in nest 0604 in 2006. U= unrelated, HS= half siblings, FS= full siblings.



Capítulo 9

Discusión General

Discusión General

Uno de los mayores retos de la ecología evolutiva actual es entender la dinámica espacial y temporal de las interacciones parásito - hospedador e identificar los factores que afectan a la evolución de las defensas y la virulencia de los parásitos (Thompson 2006).

Esta tesis doctoral pretende contribuir a explicar la dinámica de la interacción críalo-urraca mediante estudios a largo plazo y la consideración simultánea de factores tanto bióticos como abióticos, lo que proporcionan una visión inédita sobre la dinámica de esta interacción (Capítulo 3, 4 y 5). Además se aborda la importancia de los factores que afectan a la dinámica de la interacción desde un punto de vista poblacional (Capítulo 3, Capítulo 4) e individual, mediante el estudio de la relación con el parasitismo a lo largo de la vida de los individuos hospedadores (Capítulo 4, Capítulo 5).

Los resultados de esta tesis sugieren que el patrón de parasitismo, estudiado a nivel poblacional, respondería a un proceso probabilístico basado en la disponibilidad espacio-temporal de nidos de hospedador y la abundancia de parásitos en la población (Capítulo 3). El desacople reproductivo en la fecha de puesta entre el críalo y la urraca, promovido por factores climáticos, ayudaría a entender estos resultados (Capítulo 4) ya que proporciona una explicación más a la variación anual en la probabilidad de parasitismo basada en la dinámica temporal de disponibilidad potencial de nidos de hospedador. El estudio de los mismos individuos en distintas temporadas, muestra además que existe una estructuración moderada del parasitismo a nivel individual. Algunas hembras con una combinación particular de rasgos (tamaño de nido, fecha de puesta y características del hábitat) fueron exitosas evitando el parasitismo, lo que podría explicarse en términos de conspicuidad: las hembras que crían en los momentos de mayor disponibilidad de nidos en zonas más cerradas y con nidos más pequeños no son detectadas por los críalos. Además, el mayor éxito reproductivo de estas hembras podría explicar por qué se dispersan menos en promedio a lo largo de su vida que las hembras que son parasitadas (Capítulo5).

En su conjunto, el estudio de los patrones de parasitismo desde la perspectiva poblacional e individual mostraría una importancia alta de las características del hábitat para explicar los patrones de parasitismo a nivel poblacional, lo que apoya la hipótesis de la estructura espacial del hábitat propuesta por Røskaft *et al.* (2002) (Capítulo 3 y 5). Hay sin embargo discrepancias en la importancia de la densidad de hospedadores y del tamaño de los nidos. La densidad de conespecíficos, que se ha sugerido que podría actuar "diluyendo" el riesgo de parasitismo (Martínez *et al.* 1996), no influyó en la probabilidad de parasitismo en el análisis poblacional ni en el individual, pero si en el análisis individual en interacción con la fecha de puesta, de forma que en el momento central de la temporada, cuando la disponibilidad de nidos de hospedador es mayor, el riesgo de parasitismo es menor, en particular para las hembras que crían en áreas más cerradas y tienen nidos más pequeños (Capítulo 5). El tamaño del nido es una de las variables que ha sido propuesta para explicar una selección activa de nidos por parte del parásito en esta especie (Soler *et al.* 1995), basada en que los nidos de mayor tamaño mostrarían mayor calidad de los hospedadores adultos para criar pollos de críalo. Los resultados del estudio longitudinal en hembras sugieren que la relación parasitismo-tamaño de nido estaría más bien basada en cambios en la conspicuidad de los nidos en relación con la fecha de puesta (capítulo 5). Además, el estudio a nivel poblacional muestra que este rasgo no explicaría ni la probabilidad ni la intensidad de parasitismo. Una posibilidad es que la varianza en el tamaño de los nidos en nuestra población de estudio sea tan pequeña que el tamaño no sea ya un indicativo adecuado de las diferencias entre parejas, o que la conspicuidad de los nidos sea muy similar al ser todos de tamaño muy parecido.

El estudio del efecto del clima en la interacción críalo-urraca ha mostrado que la plasticidad en la fecha de puesta en relación al clima observada en la población estudiada no fue debida a plasticidad fenotípica individual (Capítulo 5). La explicación más plausible para este desacuerdo entre la respuestas a nivel poblacional e individual a la variación ambiental podría estar relacionada con la alta conectividad genética y migratoria de la población de estudio con las circundantes que son ecológicamente diferentes y con la baja previsibilidad de las condiciones climáticas que hemos encontrado en nuestra población, lo que podría favorecer que la selección actuara más sobre la dispersión a corta distancia que en la plasticidad en la fecha de puesta en respuesta a cambios climáticos. Estos resultados sugieren un papel fundamental de factores abióticos en la dinámica temporal de la interacción entre el críalo y la urraca.

Los resultados de esta tesis apoyan la existencia de un patrón de parasitismo estructurado moderado (Capítulo 5) con hembras que consiguen evitar el parasitismo a lo largo de su vida, hembras que son parasitadas reiteradamente y otras que fueron parasitadas algunas veces. Esto supone la existencia de una *transmisión horizontal limitada* del parasitismo (sensu Kilner 2005, Hoover *et al.* 2006), implicando que la selección a favor de las defensas de los hospedadores no es uniforme dentro de la población, ya que no todos los individuos se ven afectados por el parasitismo ni con la misma intensidad a lo largo de su vida. Las hembras que no fueron nunca parasitadas se podían caracterizar en términos del tipo de hábitat que usaban y del tamaño de su nido en interacción con la fecha de puesta y tenían mucho más éxito reproductivo que aquellas parasitadas al menos una vez en su vida. El parasitismo estructurado supone en teoría que la selección favorezca a los individuos que presentan estas características, pero la interacción con la fecha de puesta favorecería una selección diversificadora en tamaño de nido y tipo de hábitat usado: dependiendo de cuando críen, es más ventajosa un área u otra o tener nidos más grandes o más pequeños. Sin embargo, no existe una diferencia entre las capacidades defensivas entre las hembras nunca parasitadas y aquellas que lo fueron a veces o siempre. Una explicación a este resultado podría provenir del muestreo insuficiente de cada hembra, lo que produciría una categorización errónea como rechazadora o aceptora (ver Capítulo 7), aunque es evidente que el comportamiento defensivo es uno más de los determinantes del parasitismo, por lo que muchas hembras aceptoras no serán parasitadas debido a sus otras características relacionadas con el parasitismo y por lo tanto la relación defensa/ parasitismo podría no ser tan directa.

La expresión de las defensas varía con la presión de parasitismo, entre poblaciones y dentro de una misma población (ver Thorogood y Davies 2013). Las defensas que han evolucionado en las urracas y en otras especies hospedadoras para contrarrestar los efectos del parasitismo son, principalmente, la defensa de nidos frente a parásitos antes de que estos pongan sus huevos y el reconocimiento y rechazo de huevos extraños (Moksnes *et al.* 1991, Soler *et al.* 1999, Roskaft *et al.* 2002, Davies *et al.* 2003, Welbergen y Davies 2008). Existe muy poca información, sin embargo, de como se expresan estos comportamientos a nivel individual y de su posible covariación entre individuos pese a que estudios teóricos recientes han sugerido la posibilidad de que los parásitos de cría pudieran seleccionar grupos de comportamientos defensivos (síndromes sensu Sih *et al.* 2004) en sus hospedadores (Avilés y Parejo 2011). Los resultados de esta tesis muestran diferencias entre las parejas en relación a la intensidad de defensa del nido y la propensión a acudir al nido en presencia de un intruso potencial, así como evidencia de una relación entre el comportamiento de rechazo de huevos y el comportamiento de defensa del nido (Capítulo 6). En concreto, el rechazo de huevos se correlacionó con una tendencia general a ser agresivo en los machos. Si fuera la hembra la que rechaza el huevo parásito, estos resultados podrían explicarse por un apareamiento sesgado, mostrando las hembras rechazadoras predilección por machos más agresivos. Sin embargo, si fueran los machos los que rechazaran, los resultados se podrán explicar por la existencia de un síndrome comportamental en la defensa frente al parásito.

El mecanismo de defensa contra el parasitismo más extendido entre los hospedadores de los parásitos de cría es reconocimiento y rechazo de huevos (Rothstein 1990, Davies 2000, Soler 2013). Uno de los principales enigmas en los estudios de las interacciones entre parásitos de cría y sus hospedadores es conocer por qué algunos hospedadores aceptan los huevos parásitos a pesar del severo coste que el parasitismo causa, ya que reduce dramáticamente su éxito reproductivo (Stokke *et al.* 2005). El estudio de la consistencia de ese comportamiento a lo largo de la vida del individuo nos ofrece indicios importantes para resolver esta pregunta. Los resultados de este estudio muestran que hay hembras en la población que siempre aceptan, pero que el 53% de las hembras que fueron muestreadas durante tres años o más cambiaron su respuesta a los huevos miméticos, y siempre lo hicieron siguiendo un patrón fijo, durante los primeros años aceptaban y cambiaron a rechazar, lo que indica que el cambio está relacionado con la edad, siendo hembras más viejas las que han desarrollado las habilidades cognitivas necesarias para el reconocimiento y rechazo. No se ha encontrado relación entre el cambio en la respuesta al huevo mimético y el riesgo de parasitismo en la población o la experiencia de parasitismo, por lo que la plasticidad fenotípica no parece jugar un papel importante. Los resultados muestran además que las hembras en su primer intento de cría siempre aceptan, por lo que la fracción de hembras muestreadas que siempre rechazó, es probablemente un grupo de hembras de cierta edad que ya habría cambiado de aceptar a rechazar, cuyo cambio no habría sido detectado por un muestreo insuficiente. Todos estos resultados finalmente sugerirían que no habría rechazadores puros en la población.

Los resultados de este trabajo muestran la importancia de muestrear a los individuos a lo largo de su vida, al menos en especies de vida moderadamente larga, como las urracas, para obtener una evaluación fiable de sus capacidades defensivas contra los parásitos, ya que las hembras pueden necesitar varios años para desarrollar las habilidades necesarias para la discriminación y rechazo de huevos parásitos. En el contexto de las dinámicas coevolutivas de las interacciones parásito-hospedador los hallazgos encontrados sugieren que la ontogenia puede jugar un papel fundamental en la expresión de la defensa, en particular para especies de larga vida. Además estos resultados explicarían la coexistencia de rechazo y aceptación de huevos parásitos en una población en base a la estructura de edades de la población, sugiriendo un escenario de equilibrio evolutivo (Rothstein 1990, Davies 2000, Lotem *et al.* 1992). En trabajos anteriores se ha considerado que el equilibrio viene dado por el parasitismo diferencial de los hospedadores de primer año y adultos (por ejemplo, Lotem *et al.* 1992), sin embargo, nuestros resultados sugieren que los parásitos usarían a los individuos de un rango de edades amplio, hasta la edad en la que se hubieran desarrollado las habilidades necesarias para el reconocimiento y rechazo de huevos. Por ello, entre otros motivos (relacionados con los factores que explican el riesgo de parasitismo, ver más arriba) no encontramos diferencias en la tasa de parasitismo entre individuos de primer año y adultos.

En este sistema no hay una clara evidencia de que los hospedadores intenten minimizar los costes asociados al parasitismo a través de la dispersión reproductiva (Capítulo 8), tal y como se ha demostrado en otros sistemas (Sedgwick 2004). El efecto del parasitismo en la distancia de dispersión reproductiva estuvo mediado por la densidad de hospedadores, y fue solo evidente en los machos. Los machos parasitados y las hembras mostraron un patrón similar de dispersión reproductiva dependiente de la densidad, se dispersaron menos cuando habían criado cerca de otras parejas. Este resultado contrasta con el hallado en el Capítulo 5, cuando se estudió la dispersión reproductiva en las hembras a largo plazo, entonces ésta sí que se relacionó con el parasitismo, con movimientos más cortos entre intentos de cría en hembras no parasitadas a lo largo de su vida.

Una conclusión importante que se deriva de esta tesis es la importancia del estudio de la consistencia a lo largo de la vida del individuo tanto de la expresión de los rasgos fenotípicos como de los comportamientos defensivos. A lo largo de esta tesis se ha mostrado que unos cambian y otros no, y esto necesita ser considerado a la hora de entender la ecología, evolución y ontogenia de la dinámica de la interacción parásito de cría- hospedador. Los rasgos fenotípicos de las hembras de urraca, tales como el tamaño del nido, volumen medio de los huevos por puesta, el tamaño de puesta, la fecha de puesta, y dispersión reproductiva, fueron repetibles (Capítulo 4, 5 y 7) mostrando que estos rasgos estarían relativamente poco influenciados por el ambiente. De hecho se ha mostrado que el clima no influye a nivel individual en la fecha de puesta a lo largo de los años en que han sido estudiadas esas hembras (Capítulo 4). Estos resultados, por tanto, restan importancia a la plasticidad fenotípica a nivel individual como factor que pueda explicar la variabilidad encontrada a nivel de población como respuesta al clima. Que algunas hembras cambien su comportamiento de rechazo a lo largo de su vida debe ser considerado de gran interés, aunque este cambio no debería considerarse un ejemplo de plasticidad fenotípica ya que no hemos detectado ninguna variable ambiental asociada al cambio (Capítulo 7).

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Conclusiones/Conclusions

Conclusiones

1. El patrón de parasitismo a nivel poblacional, responde a un proceso probabilístico basado en la disponibilidad espacio-temporal de nidos de hospedador, rasgos del hábitat y la abundancia de parásitos en la población.

2. Las condiciones climáticas en la zona de estudio afectan la fenología del hospedador (urraca) y el ajuste fenológico del parásito (críalo) con éste.

3. La plasticidad en la fecha de puesta en respuesta al clima que se observa en la población hospedadora no se debe a plasticidad fenotípica individual.

4. Existe una estructuración moderada del parasitismo a nivel individual dentro de la población. Una combinación de características del hábitat y tamaño de los nidos en interacción con la fecha de puesta permite a algunas hembras de urraca escapar consistentemente del parasitismo dentro de la población.

5. Existen diferencias entre parejas de urraca en la intensidad de defensa de sus nido y la propensión a acudir a defenderlos en presencia de un intruso potencial, así como evidencia de una relación directa entre el comportamiento de rechazo de huevos y el comportamiento de defensa del nido.

6. Algunas hembras siempre aceptan los huevos modelo a lo largo de la vida. Sin embargo, otras cambian la respuesta al modelo durante su vida, siempre siguiendo un patrón fijo de aceptar a rechazar, lo que indicaría que la transición de aceptar a rechazar está relacionada con la edad.

7. Las hembras siempre aceptan el parasitismo en su primer intento de cría, independientemente de lo que hicieron sus madres lo cual sugiere que no hay rechazadores puros en la población.

8. No se encuentran evidencias a la escala estudiada de que el parasitismo de cría sea un factor determinante en los movimientos dispersivos de la urraca.

9. De esta tesis se deriva la importancia de los estudios longitudinales en la expresión de los rasgos fenotípicos, en los comportamientos defensivos de los hospedadores, así como del patrón de uso del hospedador por los parásitos para alcanzar un entendimiento más completo de los costos y beneficios de las estrategias implicados en la interacción parásito de cría - hospedador.

Conclusions

1. The parasitism pattern reported at the population level responds to a probabilistic process based on spatial-temporal availability of host nests, habitat features and the abundance of parasites in the population.

2. Climatic conditions in the study area affect host breeding phenology and cuckoo-host phenological mismatch.

3. Population plasticity in laying date in response to climate variation cannot be explained by host phenotypic plasticity at the individual level.

4. Parasitism within the population is moderately structured at the individual level. Magpie females holding territories with a particular combination of habitat characteristics and nests size in interaction with laying date consistently escaped from parasitism within the population.

5. Magpie pairs differ in their intensity of nest defense and in their propensity to approach the nest after detecting a potential intruder. Also, it is reported evidence for a direct relationship between egg rejection and nest defense behavior in magpies nests.

6. Some females always accept model eggs throughout their life. However, some others modify their response to model eggs during their lives and in all instances following a fixed pattern of always switching from accepting to rejecting parasitic eggs, suggesting that the change from acceptance to rejection is age- related.

7. First breeder females always accept the model eggs, regardless of the response that was reported for their mothers, suggesting that there would not be pure rejecters in the population.

8. There is no evidence, at the analysed spatial scale, of a role for great spotted cuckoo parasitism on magpie dispersal movements.

9. It seems critical to perform longitudinal studies about the expression of host phenotypic traits, host defense and the patterns of host use by parasites in order to achieve a more complete understanding of the costs and benefits involved in the interactions between avian brood parasites and their hosts.

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