Defensas y Contradefensas en el Parasitismo de Cría en Aves: Reconocimiento de Huevos y Comportamiento Petitorio

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Resumen

Esta tesis trata sobre dos importantes comportamientos que ocurren durante la reproducción en algunas especies de aves: el reconocimiento y la expulsión de huevos ajenos, y el comportamiento petitorio; los cuales han sido objeto de muchos estudios en el contexto del parasitismo de cría.

Una de las principales defensas de los hospedadores frente a los parásitos de cría es el reconocimiento y expulsión de los huevos parásito. Debido a que el parásito de cría obligado necesita individuos hospedadores apropiados para su reproducción, este mecanismo defensivo del hospedador simultáneamente selecciona contradefensas en los parásitos de cría, causando una guerra de armamentos coevolutiva entre hospedadores y parásitos. Aunque los modelos coevolutivos asumen que las defensas y contradefensas están genéticamente influenciadas, un componente genético de estos comportamientos nunca ha sido demostrado en los sistemas parásitos de cría-hospedador. En esta tesis, encontramos que las urracas (*Pica pica,* Linnaeus 1758), que son el principal hospedador del Críalo (*Clamator glandarius*, Linnaeus 1758) en Europa, tienen alelos de un microsatélite (Ase64) que segrega entre aceptoras y expulsoras de huevos parásitos experimentales. Además, diferencias en la tasa de expulsión entre poblaciones del hospedador explotadas por el parásito covariaron significativamente con la distancia genética calculada para este locus.

A pesar de la ventaja selectiva que la habilidad de reconocer y expulsar el huevo parásito da al hospedador de los parásitos de cría, muchas individuos de poblaciones explotadas no tiene esta defensa. Dos hipótesis han sido propuestas para explicar este hecho. La hipótesis del retraso evolutivo, que afirma que la presencia de fenotipos no defensivos es debido a un retraso temporal en la evolución/distribución de la expulsión. Contrariamente, la hipótesis del equilibrio evolutivo asume que la expulsión de huevos es costosa, y que la aceptación de huevos podría ser la mejor opción en algunas situaciones. Aquí, por primera vez, utilizamos a Ase64 como un marcador genético del comportamiento de expulsión en urracas, para discernir entre las dos hipótesis explicando la variación en tasa de expulsión entre subpoblaciones de la población de urracas de Guadix. Encontramos que tal variación fue la consecuencia de diferencias en abundancia de individuos que son genéticamente capaces de expulsar. Los críalos, al

seleccionar a las parejas de alta calidad parental, podrían ser responsables de este escenario. Además, nuestros resultados predicen un incremento en el nivel de defensas conforme la duración de las interacciones coevolutivas entre urracas y críalos aumenta, tal como la hipótesis del retraso evolutiva afirma.

Respecto al comportamiento de petición de comida, se acepta que los pollos parásitos poseen adaptaciones para explotar el cuidado parental. Por ejemplo, un comportamiento petitorio exagerado en los parásitos de cría es menos costoso que para la descendencia de las especies no parásitas, debido a que los parásitos no comparten nido con hermanos y son criados por adultos no relacionados. Sin embargo, las evidencias sobre los costes asociados a un exagerado comportamiento petitorio son escasas.

Aquí, utilizamos una droga diluida en agua, la ciproheptadina hidroclorato, para incrementar experimentalmente el nivel de hambre de algunos pollos de urraca en el nido, mientras el resto fueron tratados con agua como control. La ciproheptadina hizo que los pollos perdieran más peso, pero ganaran más comida, mientras llevaban unos collares en sus cuellos que les impedían tragar la comida. Esto sugiere tanto costes extra como beneficios al mostrar un nivel deshonesto del nivel hambre a través del comportamiento de petición. Por lo tanto, nuestra aproximación experimental nos permitió tener en cuenta los costes y beneficios asociados a un nivel deshonesto de hambre, y explorar su balance neto sobre diferentes componentes de la eficacia biológica de los pollos. En consecuencia, encontramos que los pollos de urraca que recibieron ciproheptadina fueron de mayor tamaño (peso, tarso, y longitudes de ala y cola) y mostraron una mejor respuesta inmune a la edad de volar que los controles. Por lo tanto, aunque una exageración de la petición es costosa, nuestros resultados indican que el balance neto entre costes y beneficios es positivo. Estos resultados sugieren que otros costes en la naturaleza deben estar impidiendo una exageración del comportamiento de petición, tal como una perdida en eficacia biológica inclusiva, o costes asociados a la crianza de una progenie egoísta cuando adulto.

Una vez hemos detectado los beneficios y costes asociados a un aumento experimental del nivel de hambre en los pollos del hospedador, comprobamos la hipótesis de si la deshonestidad al mostrar el nivel de hambre es menos cotosa para los parásitos de cría que para la descendencia de las especies no parásitas. Hicimos esto mediante la comparación de los efectos del tratamiento con ciproheptadina entre urracas y críalos. Nuestros resultados sugieren que tanto urracas y críalos recibieron comida en relación a sus niveles de hambre pero, al contrario que los pollos de urraca, un incremento en las señales de petición del críalo no tuvo asociado costes metabólicos adicionales. Por lo tanto, un pollo de críalo incrementando la intensidad de la petición en relación al nivel de hambre podría obtener más comida sin pagar un coste metabólico adicional. Sin embargo, esto implica que algunos otros factores pueden jugar un importante papel determinando el nivel óptimo del comportamiento de petición en los críalos.

Al contrario que el críalo, el pollo de cuco común (*Cuculus canorus,* Linnaeus 1758) monopoliza el cuidad parental de su padres adoptivos expulsando el contenido del nido. Aunque el cuco común engaña a sus padres adoptivos asegurándose suficiente comida, no se sabe si el comportamiento de petición representa una ventaja sobre aquel del hospedador en un hipotético escenario competitivo. Aquí, nosotros comprobamos esta hipótesis en nidos de alzacola (*Cercotrichas galactotes*, Temminck 1820), comparando entre nidadas naturales y experimentales, done un cuco y una nidada de hospedador eran simultáneamente presentados a los padres. Los pollos de cuco en condiciones naturales recibieron una cantidad total de comida similar a aquella recibida por una nidada de hospedador completa. Sin embargo, cuando el pollo de cuco común y los pollos del hospedador eran presentados a los padres, el pollo de cuco recibió una cantidad de comida considerablemente menor que aquella recibida en nidos no manipulados. Estos resultados sugieren que el comportamiento de petición del pollo de cuco común no es útil para dejar fuera de combate a los pollos de hospedador, al menos cuando parasita a esta especie. Y por lo tanto, esto implica beneficios para el costoso comportamiento de expulsión de los pollos de cuco común.

Abstract

This thesis deal with two important behaviours happening during reproduction of some bird species: recognition and rejection of foreign eggs, and the begging behaviour of nestlings; which have been subject of many studies in the context of brood parasitism.

One of the main host defences against brood parasites is the recognition and rejection of parasitic eggs. Because obligated brood parasites need appropriated individuals hosts for reproduction, such host defence-mechanisms simultaneously select for counter-defences in brood parasites, causing a coevolutionary arms race between hosts and parasites. Although coevolutionary models assume that defences and counter-defences are genetically influenced, a genetic component of these behaviours has never been demonstrated in brood parasite–host systems. In this thesis, we find that magpies (*Pica pica,* Linnaeus 1758), that are the main host of the great spotted cuckoo (*Clamator glandarius*, Linnaeus 1758) in Europe, have alleles of one microsatellite locus (Ase64) that segregate between accepters and rejecters of experimental parasitic eggs. Furthermore, differences in rejection rate among host populations exploited by the brood parasite covaried significantly with the genetic distance for this locus. Both results are strong evidences of egg-ejection behaviour having a genetic component in magpies.

Despite the selective advantage that the ability of recognition and ejection of parasitic egg confer to the hosts of brood parasites, many individuals from exploited populations have not got such defence. Two hypotheses have been proposed to explain this fact. The evolutionary lag hypothesis, which poses that the presence of non-defensives phenotypes, is due to a time lag in the evolution/spread of rejection behaviour. In contrast, the evolutionary equilibrium hypothesis assumes that, because egg rejection is costly, egg acceptance could be the best option in some situations. Here, for the first time, we use Ase64 as a genetic marker of rejection behaviour in magpies, in order to discern between both hypotheses explaining the variation in rejection rate between subpopulations of the magpie population of Guadix. We found that such variation was consequence of a differential abundance of individuals that are genetically able to reject. Great spotted cuckoos, by selecting magpie pairs of high parental, may be responsible of this scenario. Moreover, our results predict an increase in defensive level

as the duration of coevolutionary interaction between magpies and cuckoos increases, such as the evolutionary lag hypothesis asserts.

In relation to begging behaviour, it is accepted that parasitic nestlings possess adaptations to exploit the host parental care. For example, an exaggerated begging behaviour in brood parasite nestlings is less costly than that for offspring of nesting species, because parasites are sharing nest with non-siblings and reared by non-related adults. However, evidences of costs associated to an exaggerated begging behaviour are scarce.

Here, we used a drug diluted in water, cyproheptadine hydrochloride, in order to increase the level of hunger of some magpie nestlings in a nest, while the rest were treated with water as a control. Cyproheptadine caused that nestlings lost more weight, but gained more food, while they wore a neck collars that impeded the food swallowing. It suggests both extra-costs and benefits of showing a dishonest level of hunger through begging behaviour. Therefore, our experimental approach allowed us to point out costs and benefits associated to a dishonest level of hunger, and to explore its net balance on different components nestling fitness. Accordingly, we found that magpie nestlings that were fed with cyproheptadine were of larger size (weight, tarsus, and wing and tail lengths) and demonstrated better immune response at fledging age than control nestlings. Thus, although an exaggeration of begging is costly, our results indicate that the net balance between costs and benefits is positive. These results suggest that other costs in nature should be impeding a further exaggeration of begging behaviour, such as a loss in inclusive fitness, or costs associated to raise selfish offspring when adult.

Once we detected benefits and costs associated to an experimental increase of level of hunger in host nestlings, we tested the hypothesis that begging behaviour dishonestly showing level of hunger is less costly for brood parasitic nestlings than for offspring of nesting species. We did it by comparing the effects of cyproheptadine treatment on nestlings of magpies and great spotted cuckoos. Our results suggest that both great spotted cuckoos and magpies received food in relation to their hunger levels, but in contrast to magpie nestlings, an increase of hunger level of cuckoo had not additional associated metabolic costs. Therefore, a great spotted cuckoo nestling, by increasing the intensity of begging display in relation to level of hunger could obtain more food without paying additional metabolic costs. However, it also implies that some

other factors may play an important role determining optimal level of begging behaviour in great spotted cuckoos.

Contrary to great spotted cuckoo, common cuckoo (*Cuculus canorus,* Linnaeus 1758) nestling monopolizes the parental care of its foster parents by evicting the nest content. Although common cuckoo nestlings trick their foster parents ensuring enough food, it is unknown whether cuckoo begging behaviour represents an advantage over that of the host nestlings in a hypothetic competitive scenario. Here, we tested this hypothesis in nests of rufous bush robins (*Cercotrichas galactotes*, Temminck 1820), by comparing between naturals and experimental broods where a cuckoo together with a host brood were simultaneously presented to parents. Cuckoo chicks at natural parasitized nests received a total amount of food similar to that received by a whole host brood in natural conditions. However, when common cuckoo and host chicks were simultaneously presented to adult hosts, cuckoos receive an amount of food considerably lower than that at unmanipulated nests. These results suggest that begging behaviour of common cuckoo nestlings is not useful to out-compete host chicks, at least when parasitizing this host species. Therefore, it implies associated benefits to the costly eviction behaviour of common cuckoo chicks.

Introducción General

Parasitismo de Cría

Definición y Tipos

El parasitismo es un tipo de interacción biológica entre dos organismos, en la que uno de ellos (el parásito) obtiene recursos del otro (el hospedador). Aunque para la mayoría de los parásitos, el hospedador es en sí la fuente de alimento y/o el lugar donde reproducirse (ej. microorganismos), hay otro tipo de individuos que lo que parasitan son los cuidados parentales del hospedador, dando lugar a la estrategia reproductora denominada parasitismo de cría. El parasitismo de cría, aunque se ha estudiado fundamentalmente en aves (ver por ejemplo. Payne 1977, Davies 2000), también aparece en otros grupos de vertebrados (ej. peces, (Sato 1986, Baba *et al.* 1990)) y en varios ordenes de insectos (Himenóptera (ej. Büschinger 1986, Brockmann 1993, Zamora-Muñoz *et al.* 2003, Fanelli *et al.* 2005), Coleóptera (Rasa y Heg 2004) y Heteróptera (ej. Monaco *et al.* 1998)).

En la aves, el parasitismo de cría puede ser facultativo, si el individuo parásito es capaz tanto de criar a parte de su descendencia como además de parasitar a otros individuos, como ocurre en algunas especies de cuclillos americanos del genero *Coccyzus*, (Cuculidae) (Payne 1997). Por otra parte, el parasitismo sería obligado, si toda la descendencia del parásito es criada por los individuos hospedadores, como ocurre en el cuco común *(Cuculus canorus*) (Wyllie 1981). Si el hospedador es de la misma especie que el parásito, se denomina parasitismo de cría intraespecífico, el cual esta bastante extendido, sobre todo en especies con pollos nidífugos o especies coloniales (hasta la fecha se han encontrado hembras parásitas en 234 especies de aves, Yom-Tov 2001); en cambio, si el parásito utiliza como hospedador a individuos de otras especies, entonces se habla de parasitismo de cría interespecífico (unas cien especies en aves, Davies 2000). Más aún, dependiendo del numero de especies de hospedador, el parásito puede ser especialista, utilizando una o unas pocas especies de hospedador (ej.: las especies de pinzones parásitos africanos del genero *Vidua* (ver abajo), que solamente parasitan a una única especie); o generalista, si éste parasita a un gran número de especies (por ejemplo, del tordo-cuco canadiense (*Molothrus ater*) se han encontrado huevos en nidos de más de 200 especies de aves, (ej. Friedman 1971)).

Orígenes y Distribución del Parasitismo de Cría en Aves

Se han propuesto tres hipótesis para explicar el origen del parasitismo intraespecífico, las cuales han recibido apoyo en diferentes especies (revisado en Petrie y Møller 1991, Rothstein y Robinson 1998, Davies 2000). La primera hipótesis postula que los parásitos serían individuos que, por ejemplo no tengan territorio, o hayan sufrido la depredación de su puesta, o que en general son de peor calidad fenotípica; que recurren a poner sus huevos en nidos de otros individuos, para así aumentar su eficacia biológica (ej. Sorenson 1998, Sandell y Diemer 1999). La segunda hipótesis, sugiere que el parasitismo intraespecífico podría ser una estrategia evolutivamente estable en la población, con beneficios similares a los de criar su propia descendencia (ej. Eadie y Fryxell 1992). Y la tercera hipótesis, considera que el parasitismo intraespecífico sería una estrategia adicional a la del cuidado de su progenie, y que algunos individuos la podrían utilizar para disminuir la competencia en su nido, y/o disminuir el número de pollos a alimentar, aumentando así su éxito reproductivo (Møller 1987, Jackson 1993). En cualquier caso, las ventajas asociadas al parasitismo van a depender del comportamiento de los otros individuos de la población, no sólo porque pueden reconocer huevos extraños y expulsarlos de sus nidos, sino también porque el individuo parásito puede ser a su vez hospedador de otros (ej. Takasu 2004).

En relación con el parasitismo de cría interespecífico, y según recientes análisis moleculares, se cree que se ha originado en siete ocasiones diferentes (ver Sorenson y Payne 2002): 1 vez en la familia Indicatoridae (indicadores de la miel, 17 especies), 1 vez en la familia Anatidae (1 especie, pato rinconero (*Heteronetta atricapilla*)), tres veces en la familia Cuculidae (cucos, 57 especies parásitas); dos en paseriformes: 1 en la familia Icteridae (tordos americanos o garrapateros, 5 especies parásitas) y 1 en la familia Estrildidae (pinzones africanos parásitos, 20 especies).

En cuanto al origen del parasitismo de cría interespecífico, fundamentalmente existen dos hipótesis: *i)* que provenga del parasitismo intraespecífico, donde una elevada competencia entre individuos parásitos, o la falta de conespecíficos hospedadores favoreciera el salto a parasitar a otras especies (ej. Payne 1977, Yamauchi 1995, Robert y Sorci 2001); o *ii)* que directamente se originara para explotar el cuidado parental de otras especies de menor tamaño o con mayores tiempos de incubación, donde los pollos parásitos podrían experimentar una ventaja asociada a su mayor tamaño en la competencia por ser alimentado (Slagsvold 1998).

De las cien especies de aves parásitas obligadas, en Europa sólo se encuentran dos, y ambas pertenecen a la subfamilia Cuculinae (Fam. Cuculidae) (Payne 1997): el cuco común (*Cuculus canorus,* Linnaeus 1758) y el críalo europeo (*Clamator glandarius*, Linnaeus 1758). Aunque cercanos filogenéticamente, sus hábitos parásitos difieren en algunos aspectos y se han originaron de forma independiente (Aragón *et al.* 1999, Sorenson y Payne 2002, aunque ver Hughes 2000). El cuco común es un parásito generalista, cuyo pollo, al poco tiempo de eclosionar, expulsa los huevos y pollos del hospedador quedándose solo en el nido (Wyllie 1981). El críalo, en cambio, parasita principalmente a la urraca (*Pica pica,* Linnaeus 1758) en Europa, y su pollo tiene que competir con los del hospedador por la comida (Soler y Soler 2000).

Coste del Parasitismo de Cría en Aves

Hay muy posos casos en los que un parásito de cría beneficie al hospedador. Por ejemplo, el parasitismo intraespecífico podría beneficiar al hospedador si el pollo parásito está emparentado con los padres adoptivos (ver por ejemplo Lyon y Eadie 2000); y el más llamativo de todos es el caso del tordo americano gigante (*Scaphidura oryzivora)*, cuyo pollo beneficia a los pollos del hospedador al alimentarse de las larvas de mosca que estos portan (Smith 1968). Sin embargo, como normal general, en las interacciones parásito de cría-hospedador, el hospedador ve disminuido su éxito reproductor en mayor o menor medida como consecuencia del parasitismo (Rothstein y Robinson 1998).

Los costes que sufren los hospedadores en el parasitismo intraespecífico son similares, aunque menos severos, a aquellos sufridos en el interespecífico (Petrie y Møller 1991, Power 1998). En primer lugar, el hospedador puede ver disminuido el número de sus huevos como consecuencia del comportamiento de puesta del parásito. Algunos parásitos suele poner su huevo desde una cierta altura y de forma apresurada, dañando con frecuencia algún huevo del hospedador (Payne 1977). Además, las hembras de muchas especies parásitas, suelen quitar uno de los huevos del hospedador antes de poner el suyo, que luego normalmente comerá (revisado en Sealy 1992, Soler *et al.* 1997);

o incluso, puede adrede causar roturas en los huevos del hospedador y así disminuir la competencia que sufrirá el pollo parásito al eclosionar (ej. Soler y Martínez 2000).

Una vez eclosiona el pollo parásito, este puede competir con los pollos del hospedador por la comida. Esta competencia ocurre en la mayoría de las especies de parásitos de cría, exceptuando a los indicadores de la miel y algunas especies de cucos (ej. Rothstein y Robinson 1998, aunque ver Dearborn 1996). El coste del parasitismo varía enormemente entre las especies hospedadoras de estos parásitos, dependiendo fundamentalmente de la diferencia en tamaño y en tiempo de incubación entre el pollo parásito y el del hospedador (ej. Soler y Soler 1991, Soler *et al.* 1996, Payne 1998, Hauber 2003a, Kilner 2003). Así por ejemplo, en los hospedadores de los tordos americanos, el coste del parasitismo varía ampliamente, desde aquellos hospedadores de mayor tamaño donde el pollo del parásito recibe menos comida que el del hospedador (ej. Lichtenstein 2001a), hasta a aquellas especies de menor tamaño donde la competencia entre pollos termina provocando la muerte de los pollos del hospedador (ver revisión en Kilner 2003).

En cambio, en los indicadores de la miel (Indicatoridae) y en algunas especies de cucos (Cuculidae) (ver Payne 1997, Davies 2000, Krüger y Davies 2002 para más detalles), el pollo parásito poco después de eclosionar mata a toda la descendencia del hospedador, quedándose solo en el nido. En los indicadores de la miel (p.ej. Vernon 1987) y en el cuclillo crespín (*Tapera naevia*) (Cuculidae) (Morton y Farabaugh 1979), este comportamiento es llevado a cabo gracias a su poderoso pico en forma de garfio, que utiliza como arma para infringir graves heridas a los pollos del hospedador hasta matarlos. En el resto de los cucos, poco después de eclosionar, el pollo expulsa a todos los huevos y pollos del hospedador fuera del nido (Payne 1997). Consecuentemente, el coste del parasitismo de cría en los hospedadores de estas especies es muy elevado.

Además de la reducción en la reproducción actual causada por el parasitismo de cría, las probabilidades de supervivencia y de reproducción futura de la pareja hospedadora pueden verse afectadas negativamente, sobre todo en especies hospedadores pequeñas, como consecuencia del gran esfuerzo que puede suponer la crianza de un pollo parásito, tanto como por su intensidad como por su duración (ej. Dearborn *et al.* 1998, Kilpatrick 2002, Grim y Honza 2001). Como consecuencia de los costes mencionados, cualquier mecanismo o carácter que redujera estos costos, se seleccionaría rápidamente en la población de hospedador dando lugar a defensas contra el parasitismo de cría. Por otra parte, puesto que los parásitos de cría obligados dependen de los hospedadores para reproducirse, las defensas en el hospedador seleccionarán parásitos con estrategias que las contrarresten, las cuales, a su vez seleccionarán nuevas defensas en el hospedador, desencadenándose una carrera de armamentos coevolutiva entre parásitos de cría y hospedadores (Dawkins y Krebs 1979).

Coevolución y Parasitismo de cría

La coevolución es el resultado evolutivo de interacciones recíprocas entre especies, y por definición, ocurre cuando un rasgo de una especie evoluciona en respuesta a un rasgo de otra especie, el cual a su vez, influye en la evolución del rasgo de la primera (Janze 1980, Thompson 1994). El entendimiento de los procesos coevolutivos es actualmente de gran interés en biología evolutiva (Thompson 1999b), ya que estos procesos podrían ser los responsables de gran parte de la biodiversidad (Thompson 1999a), y por tanto, su estudio sería importante en temas tan diversos como *i)* la evolución de la resistencia de los patógenos hacia los antibióticos, *ii)* la conservación de especies amenazadas, *iii)* el control biológico de enfermedades y plagas, etc. (Thompson 1999b). Sin embargo, aunque se conocen muchos ejemplos de evolución reciproca, sobre todo entre plantas y sus polinizadores; estos procesos coevolutivos son difíciles de estudiar, debido sobretodo, a que frecuentemente muchas especies están interactuando, haciéndose muy difícil aislar las presiones selectivas, e identificar los rasgos (adaptaciones y contradaptaciones) que han dado lugar (Thompson 1994, 1999b). Por ello, los sistemas biológicos donde interactúan un número pequeño de especies son considerados como los mejores para llevar a cabo estudios sobre coevolución (Futuyma 1998).

El parasitismo interespecífico de cría en aves es uno de los sistemas biológicos donde mejor se ha estudiado las interacciones coevolutivas (Davies y Brooke 1988, Moksnes *et al.* 1990, Sealy 1996, Ortega 1998, Davies 2000, Soler y Soler 2000). Siguiendo a Rothstein (1990), la idoneidad del parasitismo interespecífico de cría para estudiar coevolución radica en cuatro aspectos: *i*) el número de especies interactuando es normalmente pequeño; *ii*) las presiones selectivas originadas entre parásitos y hospedadores pueden llegar a ser muy severas, *iii*) la mayoría de las interacciones parásito hospedador acontecen en el nido del hospedador, facilitando su estudio; y *iv*), los mecanismos defensivos y contra-defensivos desarrollados por estas aves son fácilmente detectados por los humanos, ya que normalmente involucran señales visuales o sonoras, al contrario de lo que sucede en insectos donde las señales que predominan son las olfativas y táctiles (ej. Davies *et al.* 1989). Sin embargo, a pesar de la relativa abundancia de especies donde el parasitismo de cría ha evolucionado, la mayoría de ellas están poco estudiadas, e incluso de algunas especies de cuco e indicadores de la miel se carece por completo de datos relativos a su reproducción (Payne 1997, Davies 2000). No obstante, hay otras especies que han sido objeto de un gran número de estudios sobre los procesos coevolutivos que acontecen entre estos y sus hospedadores, destacando el cuco común (*Cuculus canorus*) y al críalo (*Clamator glandarius*) en Europa, y al tordo-cuco canadiense (*Molothrus ater)* en América (revisado en Davies 2000).

De entre lo mecanismos que se han seleccionado en los hospedadores como defensa frente el parasitismo de cría, destaca la vigilancia y la defensa del nido (ej. Moksnes *et al.* 1990, Briskie *et al.* 1992, Soler *et al.* 1999b, Røskaft *et al.* 2002a, Davies *et al.* 2003), y el reconocimiento y expulsión del huevo parásito. Una vez el hospedador ha detectado el huevo parasito, puede responder de cuatro maneras diferentes: *i)* abandonando su puesta (ej. Davies y Brooke 1989a, Gill 1998, Parker 1999), *ii)* ocultando el huevo con material del nido (ej. Sealy 1995), *iii)* expulsando el huevo parásito fuera del nido (Rothstein 1975, Davies y Brooke 1989a, Moksnes *et al.* 1990, Soler *et al.* 2001), o incluso *iv*) aceptando e incubando el huevo parásito, si ésta es mejor opción que la expulsión (Zahavi 1979, Davies *et al.* 1996, Soler *et al.* 1999c). Una vez el pollo parásito ha eclosionado, el hospedador puede reconocerlo como extraño expulsándolo del nido o abandonado a las crías. Sin embargo, el reconocimiento de pollos, a diferencia del reconocimiento de huevos, ha evolucionado en muy pocas especies, sobre todo en los hospedadores de los pinzones africanos parásitos (Payne 1998, aunque ver también Soler *et al.* 1995c, Lichtenstein 2001a, Grim *et al.* 2003, Langmore *et al.* 2003); quizás, debido al gran coste que podría suponer para el hospedador un error durante la impronta de la apariencia de sus propios pollos (ver Lotem 1993).

Una vez los fenotipos defensivos se expanden en la población, se seleccionan mecanismos contra-defensivos en los parásitos que contrarresten las defensas del hospedador, como comportamientos de distracción durante la puesta del hospedador (ej. "estrategia de distracción" en el críalo, Arias de Reina 1998), el mimetismo de los huevos parásitos a los del hospedador (ej. Davies y Brooke 1989a, Moksnes y Røskaft 1995, Avilés y Møller 2004), o comportamientos que penalicen la expulsión del huevo parásito, forzando así su aceptación (comportamiento mafioso, Zahavi 1979, Soler *et al.* 1995d, Soler *et al.* 1999c). Respecto a los pollos parásitos, las adaptaciones que estos poseen les permiten conseguir una cantidad suficiente de comida del hospedador, mediante la exageración de las señales de petición (ej. Davies *et al.* 1998, Dearborn 1998, Lichtenstein y Sealy 1998, Kilner y Davies 1999, Hauber 2003b, Soler *et al.* 1995b, Tanaka y Ueda 2005) o eliminando a los pollos del hospedador (ver arriba).

La aparición de mecanismos contra-defensivos en los parásitos, seleccionará la mejora o la aparición de nuevas defensas en el hospedador, estando hospedador y parásito inmersos en una carrera de armamentos coevolutiva (Dawkins y Krebs 1979). Por ejemplo, en el caso del mimetismo de los huevos del parásito, ésta contra-defensa selecciona en el hospedador una disminución en la variación de la apariencia de los huevos, para así facilitar la detección del huevo parásito (Stokke *et al.* 1999, Stokke *et al.* 2002, pero ver también Karcza *et al.* 2003, Avilés *et al.* 2004).

Según Davies (2000) y en relación al reconocimiento y expulsión de huevos, la carrera de armamentos entre el parásito y su hospedador tendría 4 estadios diferentes (Figura 1).

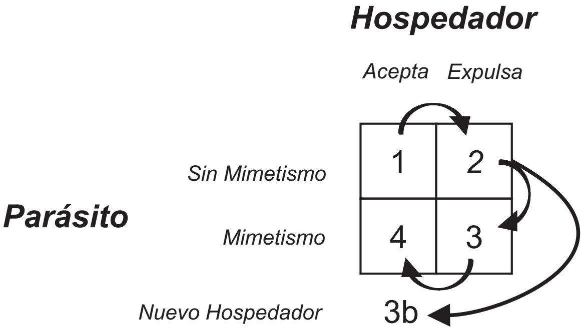

***Figura 1.-** Evolución de la expulsión y mimetismo de huevos en la carrera de armamentos coevolutiva entre hospedadores y parásitos de cría. Modificado de Davies (2000)*

El primer estadio (1, Figura 1) se correspondería con la colonización por parte del parásito de una nueva especie de hospedador, la cual carece de defensas frente al parasitismo de cría, aceptando todos los huevos del parásito. A favor de este punto de partida estarían por ejemplo el hecho de que las especies no apropiadas para ser parasitadas por el cuco común mayoritariamente aceptan los huevos del parásito (como especies granívoras o que criando en oquedades, Davies y Brooke 1989a, Moksnes *et al.* 1990, Langmore *et al.* 2005); la aceptación que muestran las especies que llevan poco tiempo siendo parasitadas por el tordo-cuco canadiense (Rothstein 1990, Hosoi y Rothstein 2000, Peer y Sealy 2004) como consecuencia de su rápida expansión a través de Norteamérica (Mayfield 1965); o la aceptación de especies hospedadoras en poblaciones en alopatría con el parásito (Davies y Brooke 1989a, Briskie *et al.* 1992, Soler *et al.* 1999a, Soler *et al.* 2001, Avilés y Møller 2003). Debido a los severos costes del parasitismo (ver arriba), en el hospedador llega a ser ventajoso el reconocimiento y la expulsión de los huevos parásitos, expandiéndose esta defensa a través de la población del hospedador (2, Figura 1). La velocidad de tal expansión dependerá de factores tales como la tasa de parasitismo (Davies y Brooke 1989b, Soler y Møller 1990, Nakamura *et al.* 1998, Soler *et al.* 2001) o el flujo génico entre poblaciones (Martínez *et al.* 1999, Soler *et al.* 1999a, Røskaft *et al.* 2002b), entre otros (ver por ejemplo algunos modelos teóricos que estudian tal expansión, Kelly 1987, Takasu *et al.* 1993, Servedio y Lander 2003, Barabas *et al.* 2004). Una vez el hospedador presenta defensas frente al parasitismo, aquellos parásitos poniendo huevos con apariencia más similar a los del hospedador podrán escapar a tales defensas, evolucionando el mimetismo en los huevos del parásito (estadio 3, Figura 1); como ocurre en algunas especies de cuco (ver apéndice en Krüger y Davies 2002, para una revisión). Sin embargo, el parasito puede también eludir las defensas cambiando de especie de hospedador, una vez la expulsión ha evolucionado (estadio 3b, Figura 1), que explicaría por ejemplo, el porqué especies que no son parasitadas en la actualidad son capaces de expulsar huevos (Rothstein 2001). Sin embargo, estos antiguos hospedadores pueden con el tiempo perder la capacidad de expulsión (ej. Cruz y Wiley 1989) como consecuencia de la existencia de costes asociados a la expulsión o a errores de reconocimiento de sus propios huevos (Davies *et al.* 1996).

Una vez el mimetismo evoluciona en el parásito, la expansión de éste mecanismo contra-defensivo será más rápida que la expansión del reconocimiento de huevos en el hospedador, ya que la presión selectiva en el parásito es mayor (siempre se tiene que enfrentar a un hospedador) que en el hospedador (puede o no ser parasitado) (efecto del enemigo raro, Dawkins y Krebs 1979). Pudiéndose llegar a un grado tal de mimetismo con los huevos del hospedador, que los hospedadores sean incapaces de detectar el huevo parásito, perdiéndose por tanto la ventaja de la expulsión y pasando el hospedador a aceptar los huevos parásitos (ver por ejemplo Takasu *et al.* 1993) (estadio 4, Figura 1). Esto se debe principalmente a que el grado de mimetismo de los huevos parásitos influye positivamente en los costos de reconocimiento y expulsión, mientras que los beneficios asociados a la expulsión de estos huevos van a depender de diversos factores ecológicos que incluyen la probabilidad de parasitismo.

Otro aspecto importante en la evolución de defensas y contra-defensas es la posibilidad de que la expresión de la defensa sea plástica, dependiendo de las condiciones ambientales (Lotem *et al.* 1992, 1995, Álvarez 1996, Davies *et al.* 1996, Brooke *et al.* 1998, Moksnes *et al.* 2000, Bártol *et al.* 2002). Esta plasticidad haría que el carácter defensivo sólo se exprese si las condiciones ambientales (ej. presencia de parásitos) indican que los posibles beneficios asociados a la defensa van a superar los posibles costes (Davies *et al.* 1996). En este sentido, en situaciones de bajo riesgo de parasitismo, la plasticidad fenotípica de las defensas anularía los costes asociados al reconocimiento y/o expulsión de huevos, ya que no se expresaría el carácter. Consecuentemente, el carácter expulsor se podría mantener en las poblaciones de hospedador no explotadas por el parásito como un carácter neutro por largos periodos de tiempo. Por tanto, el estudio de la base genética del carácter defensivo, así como la influencia de distintos factores ambientales en su expresión es de vital importancia para entender los procesos coevolutivos.

Petición de Comida y Parasitismo de Cría

El conflicto familiar y el Comportamiento Petitorio

Las diferencias genéticas entre hermanos y entre padres y hermanos, junto con el desarrollo del la teoría de la selección de parentesco desarrollada por Hamilton (1964), permitió a Trivers (1974) desarrollar su famoso modelo sobre el conflicto de intereses existente dentro de las relaciones familiares. Debido a las diferencias genéticas entre padres e hijos, los padres tenderían a repartir sus recursos por igual entre sus hijos, ya que están igualmente relacionados con todos y cada uno de ellos; sin embargo, un hijo podría obtener una mayor cantidad de recursos a costa de *i)* reducir la cantidad recibida por sus hermanos, o *ii)* a costa de hacer trabajar en demasía a sus padres. Es decir los intereses de padres, hijos y hermanos difieren dando lugar a los conflictos paterno-filiares (Figura 2). Según este argumento, la manera tan llamativa y aparentemente costosa, que tiene la descendencia de algunas especies de pedir alimento, habría evolucionado para tratar de conseguir una cantidad de recursos mayor de la que sus padres estarían dispuestos a dar (Trivers 1974), o para competir por ellos con sus hermanos (Harper 1986).

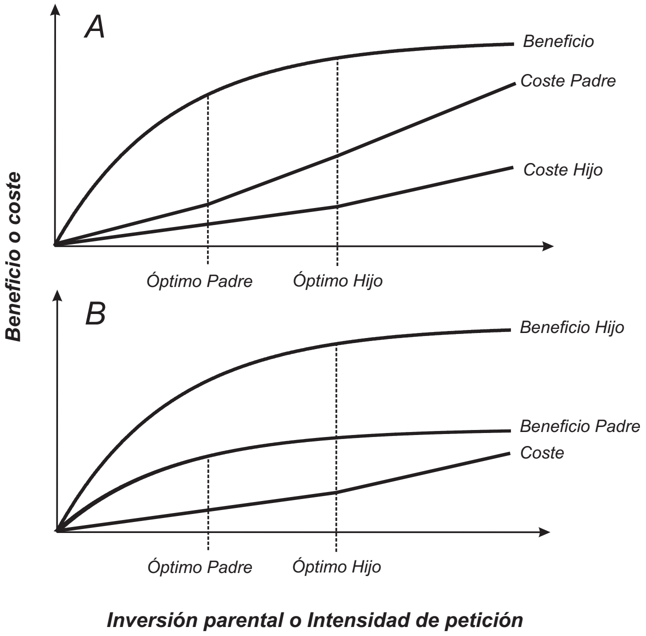

Figura 1.- *(A) Conflicto paterno-filial según Trivers (1974): los beneficios de la inversión parental son los mismos para el padre que para el hijo; sin embargo, los costes para el hijo son la mitad que para los padres debido a la relación de parentesco de 0.5 entre ambos. (B) Modificación de Lazarus y Inglis (1986) del modelo de Trivers, expresando el coste de la inversión parental en términos de reducción del número de hermanos, y por tanto siendo el coste igual para el padre que para el hijo (relación de 0.5 entre hermanos y entre padre e hijos), sin embargo, el beneficio del hijo es el doble del padre ya que es el hijo quién recibe la inversión parental.*

La pregunta que surge como consecuencia de la existencia del conflicto de intereses entre padres e hijos, es si hay un ganador o si por el contrario existe un equilibrio evolutivamente estable en un punto intermedio entre los óptimos de ambas partes. En ese sentido, a partir del trabajo de Trivers, se han venido desarrollado modelos teóricos que intentan determinar si los padres e hijos se encuentran en un equilibrio evolutivamente estable, y si este equilibrio es dependiente de factores tales como el sistema de emparejamiento de los padres (ej. Macnair y Parker 1978, Parker y Macnair 1978, Parker 1985), de costes asociados a las señales de petición y al aporte de comida (ej. Harper 1986, Eshel y Feldman 1991, Godfray 1991, 1995, Yamamura y Higashi 1992, Johnstone y Grafen 1993, Johnstone 1998, 1999), o del nivel de competencia entre hermanos (ej. Macnair y Parker 1979, Rodríguez-Gironés *et al.* 1996, Rodríguez-Gironés 1999, Johnstone 1999, 2004).

Los comportamientos de petición de recursos por parte de la descendencia han sido considerados como señales que informan sobre el nivel de necesidad, para así, poder los padres regular el aporte y la distribución de su inversión. Sin embargo, debido la existencia de un conflicto de intereses entre los miembros de la familia, los procesos de selección favorecerían a los hijos que mintieran, exagerando las señales y recibiendo diferencialmente más recursos, por lo que el comportamiento petitorio no serviría como indicador honesto de la necesidad de los hijos (revisado en Johnstone y Godfray 2002). Sin embargo, si el comportamiento petitorio implicara unos costos, las señales de petición podrían honestamente indicar el nivel de necesidad de los hijos, que los padres podrían utilizar en decisiones sobre el aporte de sus recursos (Godfray 1991, 1995, Johnstone y Grafen 1993). Esta argumentación se podría resumir en que los costes de la petición serían compensados por los beneficios de la inversión, y que cuanto mayor es la necesidad por los recursos, mayores son los beneficios que aporta (ver por ejemplo Godfray 1991).

Los modelos de señalización de la necesidad a través del comportamiento petitorio establecen tres predicciones claras (revisado en Kilner y Johnstone 1997), de las cuales dos han recibido bastante apoyo empírico: *i)* que las señales de petición deben variar con el nivel necesidad de los recursos (en aves: Redondo y Castro 1992a, Saino *et al.* 2000, Quillfeldt 2002, Sacchi *et al.* 2002, en mamíferos: Jensen *et al.* 1998, Quillfeldt *et al.* 2004, en insectos: Rauter y Moore 1999); y *ii)* que los padres ajustan su inversión dependiendo de la intensidad de las señales emitidas por sus hijos (en aves: ej. Ottosson *et al.* 1997, Burford *et al.* 1998, Granadeiro *et al.* 2000, en mamíferos: Manser y Avey 2000, en insectos: Rauter y Moore 1999, Creemers *et al.* 2003). La tercera predicción sería que las señales de petición para ser honestas deberían tener unos costes asociados a su producción que penalicen su exageración, aparte de los asociados a la reducción de eficacia biológica familiar. Sin embargo, los estudios que han intentado medir la intensidad de tales costes a veces no han dado los resultados esperados (ver por ejemplo: Kilner y Johnstone 1997, Royle *et al.* 2002).

Uno de los costes propuestos para el mantenimiento de la honestidad de la petición estaría en relación con el aumento en el riesgo de depredación asociado a la exageración de la señal. Sin embargo, aunque se ha comprobado que el riesgo de depredación aumenta conforme a la intensidad de las señales (Redondo y Castro 1992b, Haskell 1994, 1999, Leech y Leonard 1997, Briskie *et al.* 1999, Dearborn 1999), este coste no podría explicar la honestidad de la petición en sistemas donde más de un hijo es criado a la vez (camadas en mamíferos o polladas en aves nidícolas), ya que la penalización para el individuo egoísta no sería mayor que para el conjunto de la camada/nidada (Rodríguez-Gironés *et al.* 2001).

El cuanto al gasto energético durante la producción señales de petición, las mediciones directas de consumo de oxigeno no han dado los resultados esperados, obteniéndose valores cercanos a los correspondientes al consumo de oxigeno en reposo (Leech y Leonard 1996, McCarty 1996, 1997, Bachman y Chappell 1998, Soler *et al.* 1999d). Sin embargo, estos valores tan bajos podrían ser consecuencia del uso del metabolismo anaeróbico durante la petición (Weathers *et al.* 1997, aunque ver McCarty 1997). Otra posibilidad, es que los costos asociados a un aumento en la intensidad de petición podrían no ser detectables a corto plazo, pero que provocaran unos efectos importantes a largo plazo (Verhulst y Wiersma 1997). En este sentido, Rodríguez-Gironés *et al.* (2001) y Kilner (2001) estudiando pollos de urracas (*Pica pica*) y canarios (*Serinus canaria*) respectivamente, detectaron una reducción en la tasa de crecimiento asociada a un aumento en el esfuerzo de petición. En cambio, Leonard *et al.* (2003) no encontró tal coste en crecimiento en las crías de la golondrina de los árboles (*Tachycineta bicolour*). Además, debido a la imposibilidad de manipular el nivel de petición en condiciones naturales, estos experimentos han sido realizados en laboratorio, por lo que no se ha podido estudiar si los beneficios asociados a una exageración del comportamiento petitorio compensan los costos asociados a la misma. Por tanto, el desarrollo y la validación de metodologías que permitan el estudio de estas señales, y la determinación de costos y beneficios asociados en condiciones naturales es de gran importancia para comprobar las predicciones de modelos de comunicación padres-hijos.

Petición de Comida en Parásitos de Cría

Las adaptaciones que poseen los pollos de los parásitos de cría encaminadas a conseguir una cantidad suficiente de comida de sus hospedadores han sido objeto de un gran número de estudios (por ejemplo, en cucos: Davies 2000, en tordos parásitos: Dearborn y Lichtenstein 2002, en pinzones parásitos: Payne y Payne 2002). El interés que ha motivado estos trabajos se centra fundamentalmente en dos aspectos: *i)* en la determinación de las adaptaciones del pollo parásito asociadas a la manipulación del hospedador, en el contexto de una carrera de armamentos coevolutiva entre parásitos y hospedadores (ej. Soler *et al.* 1995b, Kilner *et al.* 1999, Kilner *et al.* 2004, Tanaka y Ueda 2005), y *ii)* en la utilización de los sistemas parásito de cría-hospedador como "experimentos naturales" donde abordar cuestiones acerca del mantenimiento de la honestidad de la petición y el conflicto paterno-filial (ver más arriba) (revisado en Budden y Wright 2001).

Los pollos parásitos de cría al no estar emparentados con los individuos hospedadores que los alimentan, poseen una ventaja respecto a los pollos del hospedador, ya que sus señales de petición están libres del coste asociado a la reducción de la eficacia biológica inclusiva (ej. Lotem 1998, Kilner y Davies 1999, Lichtenstein 2001b, Rodríguez-Gironés *et al.* 2002, Lichtenstein y Dearborn 2004). De hecho, un pollo parásito no vería afectada en absoluto su eficacia biológica, si a consecuencia del esfuerzo que supone su crianza para los hospedadores, éstos ven severamente reducida su probabilidad de supervivencia (Brooke y Davies 1989). Por tanto, la producción de las señales para los parásitos sería más barata que para los pollos del hospedador (ver Davies y Brooke 1988, Lotem 1998, Soler *et al.* 1999d, Lichtenstein 2001b, Lichtenstein y Dearborn 2004). En este sentido, los pollos de estas especies parásitas se caracterizan por poseer un comportamiento petitorio más intenso que el de los pollos del hospedador (Davies *et al.* 1998, Dearborn 1998, Lichtenstein y Sealy 1998, Kilner y Davies 1999, Soler *et al.* 1999d, Rodríguez-Gironés *et al.* 2002, Hauber 2003b), lo que les permitiría explotar el cuidado parental de sus hospedadores y así obtener una cantidad de comida adecuada para su desarrollo (Kilner *et al.* 1999, Grim y Honza 2001, Butchart *et al.* 2003, Tanaka y Ueda 2005), o una ventaja en la competencia con los pollos del hospedador (ej. Soler *et al.* 1995b, Lichtenstein y Sealy 1998).

En el caso del pollo de cuco común, que expulsa el contenido del nido justo después de eclosionar (Wyllie 1981), se ha comprobado que ni el vívido color rojo de la cavidad bucal (Noble *et al.* 1999), ni su gran mayor tamaño en comparación con los pollos del hospedador (Davies *et al.* 1998), son los responsables de conseguir la suficiente comida para su desarrollo. El carácter principal responsable del éxito de estos pollos parásitos es la frecuencia de petición (número de llamadas/s) que el pollo es capaz de emitir (Kilner *et al.* 1999, Kilner y Davies 1999). En cambio, para los pollos de tordos parásitos americanos, que tienen que competir con los del hospedador (Payne 1997, pero ver Dearborn 1996) es la intensidad de petición de comida y el tamaño corporal respecto a los pollos del hospedador, los principales factores que explican las ventajas de los pollos parásitos (Dearborn 1998, Lichtenstein y Sealy 1998, Lichtenstein 2001b, Hauber 2003a). Además, el éxito de los pollos parásitos va a depender del esfuerzo parental, que a su vez, va a depender del número de pollos en el nido. Debido a que los pollos parásitos tendrían ventaja sobre los de hospedador en conseguir el alimento que los padres aportan al nido, la presencia de algunos pollos de hospedador en el nido podría ser beneficioso para los parásitos (Kilner 2003, Kilner *et al.* 2004).

En el críalo europeo, al igual que en los tordos, el pollo no expulsa a los del hospedador (Soler y Soler 1991, Soler y Soler 2000). El críalo, además de poseer un comportamiento petitorio más intenso en comparación con el del su principal hospedador, la urraca (Redondo 1993, Soler *et al.* 1999d), eclosiona antes (Cramp 1985, 1994), posee una tasa de crecimiento mayor (Soler y Soler 1991) y presenta unas papilas bucales que actúan como un superestimulo (Soler *et al.* 1995b). Todas estas características, hacen que los críalos en condiciones naturales, sean preferentemente alimentados por las urracas (Soler *et al.* 1995b), mientras que los pollos de urraca mueren de hambre en los nidos parasitados (Soler *et al.* 1996).

Es posible que a pesar del menor coste de las señales de petición de comida de los pollos parásitos, todavía estos pollos varíen la intensidad de petición en función de sus niveles de necesidad (ver Kilner y Davies 1999, Hauber y Ramsey 2003, Lichtenstein y Dearborn 2004), o puede que sus señales de petición de comida sean deshonestas e independientes de la necesidad (ver ej. Redondo y Zuñiga 2002, Lichtenstein y Dearborn 2004). En este sentido, estudios comparando los factores que modulan la petición de pollos de parásitos de cría y los de sus hospedadores aclararían esta cuestión. Además, aunque los pollos de los parásitos poseen una petición exagerada, no se conocen con exactitud qué factores hacen que los pollos de algunas especies opten por realizar costosos comportamientos encaminados a matar la descendencia del hospedador, en vez de competir con los pollos del hospedador.

Justificación y Objetivos

Reconocimiento y Expulsión de Huevos

El reconocimiento y la expulsión de huevos parásitos es quizás la principal defensa de los hospedadores (ver por ejemplo Davies y Brooke 1989a, Rothstein y Robinson 1998, Davies 2000), por lo que se espera que la mayoría de los individuos de una población explotada por parásitos de cría sean capaces de reconocer huevos extraños en sus nidos. Sin embargo, esto no ocurre así en muchas especies o poblaciones estudiadas (ver Rothstein y Robinson 1998). Por ejemplo, muchos de los hospedadores, tanto de tordos americanos como del cuco europeo, no son capaces de reconocer los huevos parásitos aún siendo muy diferentes a los suyos (Rothstein 1975, 1982b, revisado en Davies 2000). Además, dentro de especies o poblaciones de hospedadores que son explotadas por parásitos de cría, no todos los individuos son capaces de expulsar los huevos del parásito, habiendo un porcentaje más o menos elevado de individuos no defensivos (revisado en Rothstein y Robinson 1998). Esta carencia de defensas presentada por algunos hospedadores del parasitismo de cría ha sido tradicionalmente explicada por dos hipótesis: *i)* la *hipótesis del retraso evolutivo*, y *ii)* la *hipótesis del equilibrio evolutivo*. La primera considera que la expulsión de huevos es siempre ventajosa para el hospedador, y explica la carencia de éste comportamiento en base a un retraso temporal en su evolución (Rothstein 1975, Dawkins y Krebs 1979, Davies y Brooke 1989b, Moksnes *et al.* 1990). En cambio, la hipótesis del equilibrio evolutivo, postula que debido a la existencia de una serie de costes de expulsión y/o de reconocimiento, la expulsión del huevo parásito no es siempre la mejor opción (Rohwer y Spaw 1988, Brooker y Brooker 1990, Lotem *et al.* 1992, Marchetti 1992, Lotem y Nakamura 1998). A favor de esta última hipótesis estarían también los estudios que han demostrado que el comportamiento de expulsión de huevos es un carácter plástico, y que la expresión de tal defensa depende de factores relativos a los costos de la expulsión (Lotem *et al.* 1992, 1995, Álvarez 1996, Davies *et al.* 1996, Brooke *et al.* 1998, Moksnes *et al.* 2000, Bártol *et al.* 2002). Sin embargo, la plasticidad fenotípica o las defensas inducidas por condicionantes ambientales relacionados con las presiones selectivas son en sí mismas defensas, que además pueden tener una base genética (Tollrian y Harvell 1999), por lo que podrían ser considerados como un grado defensivo más en la carrera de armamentos.

Debido a que hay evidencias apoyando estas dos hipótesis, se ha sugerido que ambas podrían ser correctas, aunque actuando en diferente escala espacial y/o temporal (Davies *et al.* 1996, Soler *et al.* 1998, Davies 1999, Servedio y Lander 2003). Así, en estudios que abarquen una escala temporal o espacial grande (por ejemplo comparando entre especies de hospedador o entre poblaciones más o menos aisladas) será más probable poner de manifiesto que las diferencias en el nivel de defensas sean consecuencia de diferencias en duración de las interacciones coevolutivas, tal como la hipótesis del retraso evolutivo predice. En cambio, en aquellos estudios llevados a cabo a una escala temporal y/o espacial más pequeña (ej. dentro de una población), la probabilidad de reconocimiento y expulsión de huevos parásitos será mejor explicada por características fenotípicas del hospedador, directa o indirectamente relacionadas con los costes de la defensa, como predice la hipótesis del equilibrio evolutivo (revisado en Rothstein y Robinson 1998). Además de la escala utilizada, un aspecto importante a tener en cuenta en estos tipos de estudios, es la dinámica meta-poblacional del hospedador, ya que la presencia de un nivel de mal-adaptación (individuos no defensivos) en poblaciones parasitadas podría ser la consecuencia de la migración entre poblaciones sufriendo diferentes presiones de parasitismo (Thompson *et al.* 2002, Barabas *et al.* 2004). Consecuentemente, estudios encaminados a comparar el nivel de defensas entre diferentes poblaciones de hospedadores, debería controlar el posible flujo génico entre poblaciones (Nuismer *et al.* 1999, Soler y Soler 2000, Soler *et al.* 2001). Incluso en estudios intra-poblacionales, es posible que los individuos defensivos (o no defensivos) sean fundamentalmente migrantes procedente de otras poblaciones (Martínez *et al.* 1999, Soler *et al.* 1999a), lo que afectaría profundamente a nuestras conclusiones sobre el proceso coevolutivo entre parásito y hospedador (ver Lotem y Rothstein 1995).

En el sistema críalo–urraca, estudios previos sugieren que las diferencias en frecuencia de expulsión de huevos entre diferentes poblaciones covarían con diferencias en la duración de las interacciones coevolutivas, estándo de acuerdo con la hipótesis de retraso evolutivo (Soler y Møller 1990, Soler *et al.* 1999a, Soler *et al.* 2001). En este sentido, uno de los objetivos (**objetivo 1)** de esta tesis es estudiar los factores responsables del nivel defensas mostradas por distintas subpoblaciones geográficamente aisladas dentro de la población de Guadix, para comprobar si las diferencias en defensas se asocian con el nivel de presión selectiva o tasa de parasitismo como ocurre a una escala geográfica mayor (Soler *et al.* 2001). Además se controlaríaa por el posible flujo génico entre subpoblaciones mediante la utilización de marcadores genéticos neutrales (microsatélites) **(Manuscrito II).**

Aunque la base genética en las defensas y contradefensas asociadas al parasitismo de cría es normalmente asumida por los modelos teóricos (ej. Takasu *et al.* 1993, Takasu 1998, Robert *et al.* 1999, Servedio y Lander 2003), hasta la fecha solamente existen evidencias indirectas en parásitos de cría (Marchetti *et al.* 1998, Gibbs *et al.* 2000, Payne *et al.* 2002) y en hospedadores (Rothstein 1975, Rothstein 1982a, Briskie *et al.* 1992, Soler *et al.* 1999a). Un estudio sobre el control genético de estos caracteres sería importante para validar esta importante asunción. Además, si un marcador de la capacidad genética fuese hallado, éste facilitaría enormemente el entendimiento de los procesos coevolutivos entre parásitos de cría y hospedador; ya que por ejemplo se podría discernir entre aceptación como consecuencia de una carencia de la capacidad genética para expulsar, o entre una aceptación condicional motivada por los costes de la expulsión (ver Lotem y Nakamura 1998, Sorenson y Payne 2002). En este sentido, en la urraca se ha comprobado que la expulsión de huevos es un rasgo plástico (Soler *et al.* 1999c), dependiendo su expresión de costes asociados a la expulsión (ver arriba), por lo que estos costos podrían explicar la presencia de individuos no defensivos y predecir un equilibrio evolutivo entre críalo y urraca. Así, la existencia de un marcador genético de la habilidad de reconocer huevos extraños nos permitiría distinguir entre esas dos posibilidades (**Manuscrito II**). Debido a la importancia de encontrar un marcador de reconocimiento, nos planteamos explorar una posible asociación genética entre el reconocimiento de huevos en urracas y los microsatélites utilizados para caracterizar la dinámica poblacional de la población de Guadix **(objetivo 2)**. Esto es debido a que una asociación estadística entre un carácter y un marcador genético es considerada normalmente como evidencia de una cercanía física entre el marcador y algún gen influenciando dicho carácter (Silverman y Palmer 2000, Cardon y Bell 2001, Pritchard y Donnelly 2001). De esta manera, además pondríamos de manifiesto la asumida base genética del reconocimiento y expulsión de huevos en urracas **(Manuscrito I)**.

Comportamiento Petitorio

Como ya se expuso anteriormente, Rodríguez-Gironés *et al.* (2001) y Kilner (2001) recientemente han detectado un efecto negativo asociado a un aumento en la intensidad de petición durante el desarrollo de pollos de urraca (*Pica pica*) y de canario (*Serinus canaria*) respectivamente. Estos resultados validan los modelos de señalización en las relaciones paterno-filiares; sin embargo, ambos trabajos fueron realizados en laboratorio. En ambas aproximaciones, los pollos experimentales eran forzados a pedir más para ser alimentados que los pollos controles, y ambos tipos de pollos recibían la misma cantidad de comida. Por lo tanto, aunque ambos estudios son claros manifestando el coste asociado a las señales de petición, con esa aproximación no es posible estudiar si los costos asociados a una mayor intensidad de petición podrían contrarrestar los posibles beneficios de un mutante egoísta. Además, este tipo de aproximaciones experimentales no tienen en cuenta otros posibles costes del comportamiento de petición, tal como la competencia entre hermanos por ser alimentados (Johnstone 1999, Rodríguez-Gironés 1999, Royle *et al.* 2002, Neuenschwander *et al.* 2003), ni el relacionado con la vigilancia de la llegada de los padres (Roulin 2001).

La única posibilidad de realizar esos estudios teniendo en cuenta todos los costes asociados a una petición exagerada y los posibles beneficios en recursos recibidos de los padres es realizar experimentos en el campo en los que se manipule el nivel de hambre o de petición de los pollos. Por ello, otro objetivo **(objetivo 3)** de esta tesis es desarrollar y validar un nuevo tipo de aproximación experimental para modificar la intensidad de hambre, y por lo tanto la petición en pollos nidícolas. Esta aproximación consiste en la utilización de un estimulador del apetito (ciproheptadina clorhidrato) el cual es un potente antihistamínico e inhibidor de la serotina, teniendo por tanto un efecto estimulador del apetito (Chakrabarty *et al.* 1967). La ciproheptadina ha sido usada como estimulante del apetito en pacientes de varias enfermedades necesitados de un incremento en peso corporal (ver Homnick *et al.* 2004, 2005), y su uso como estimulante del apetito en humanos (ej. Periatin©), e incluso en otros animales incluyendo aves (ej. Vita-Vrot-c©) está muy extendido. Gracias a esta aproximación, los pollos pueden ser alimentados por sus padres, tomando en cuenta tanto los beneficios de una mayor intensidad de petición, como otras fuentes de coste de petición (ver arriba). La validación de este método, así como el estudio a corto plazo de los costes (perdida de peso) y beneficios (alimento conseguido) de aumentar el nivel de hambre de los pollos se tratan en el **Manuscrito III**. Además, si el efecto de la ciproheptadina es el esperado, podremos determinar si un aumento en la petición tiene efectos netos negativos medidos durante el desarrollo, o si por el contrario, los beneficios asociados al aumento de petición (mayor ingesta de comida) compensan los costes asociados **(objetivo 4, Manuscrito IV)**.

Comportamiento Petitorio en Parásitos de Cría

Aunque la falta de relación genética de los pollos parásitos de cría con los adultos y pollos hospedadores predice un nivel de petición diferencialmente mayor en parásitos de cría (ver Davies y Brooke 1988, Lotem 1998, Soler *et al.* 1999d, Lichtenstein y Dearborn 2004), es posible tanto que el parásito varíe la intensidad de petición en función de sus niveles de necesidad (ver Kilner y Davies 1999, Hauber y Ramsey 2003, Lichtenstein y Dearborn 2004), o como que pida de manera deshonesta e independientemente de la necesidad (ver ej. Redondo y Zuñiga 2002, Lichtenstein y Dearborn 2004). Para intentar diferenciar entre estas dos posibilidades, estudiaremos si el efecto (relación costos/beneficios) de la ciproheptadina en pollos de críalo es similar al efecto provocado en los pollos de su hospedador **(objetivo 5, Manuscrito V).**

Por otra parte, si los pollos de los parásitos de cría se caracterizan por poseer un comportamiento petitorio más intenso que el de los pollos del hospedador (Davies *et al.* 1998, Dearborn 1998, Lichtenstein y Sealy 1998, Kilner y Davies 1999, Soler *et al.* 1999d, Rodríguez-Gironés *et al.* 2002, Hauber 2003b) y los padres deciden a quién alimentar en función de las señales de petición (revisado en Kilner y Johnstone 1997), el pollo de cuco común podría ser alimentado preferentemente incluso compartiendo nido con los pollos del hospedador; lo que provocaría la muerte por inanición a los pollos del hospedador, como ocurre en otras especies de parásitos. Sin embargo, si esto fuera así, los pollos parásitos de cría no tendrían que realizar el costoso comportamiento de expulsión de posibles competidores del nido. Nosotros estudiamos las causas que explicarían este costoso comportamiento de expulsión comparando la alimentación en nidadas controles parasitadas y no parasitadas, respecto a nidadas donde experimentalmente se hizo competir para ser alimentado un pollo de cuco común con una nidada de alzacola (*Cercotrichas galactotes*, Temminck 1820), un hospedador frecuente del cuco común en sur de España (Álvarez 1994, Palomino *et al.* 1999) **(objetivo 6, Manuscrito VI).**

Material y Métodos

En este apartado destaco los aspectos más importantes y generales utilizados para la realización de esta tesis que permiten entender en un contexto adecuado los principales resultados encontrados, y expuestos en el siguiente apartado. Para aspectos más concretos, el material y los métodos utilizados se especifican con más detalle en cada uno de los manuscritos. Igualmente, las zonas de estudio y las características de las especies estudiadas importantes para cada uno de nuestros objetivos, se tratan también en los manuscritos.

Reconocimiento de huevos, bases genéticas y ambientales

Las tasas de expulsión de las distintas localidades o subpoblaciones se estimaron experimentalmente utilizando modelos de escayola, realizados a partir de moldes de látex obtenidos de huevos reales de críalo. Estos modelos, una vez secos, se pintaron simulando los patrones de coloración de los huevos parásitos (para más información ver Soler y Soler 2000). Los experimentos de reconocimiento se realizaron durante el periodo de puesta, y el carácter expulsor o aceptor se estimó después de 4-6 días. Las tasas de parasitismo y fecha de puesta se obtuvieron como resultado de visitas periódicas a los nidos en la zona de estudio.

Los análisis genéticos se realizaron con muestras de sangre de pollos de urraca (uno por nido). Las muestras las recogíamos cuando los pollos tenían unos 16-18 días de edad de la vena braquial. El protocolo seguido, así como las características de los 11 microsatélites utilizados en los análisis se especifican claramente en los **manuscritos I y II**.

Comportamiento petitorio: manipulación experimental y estimación de costos y beneficios.

El nivel de hambre se manipuló utilizando la ciproheptadina, fármaco que como ya hemos mencionado se utiliza en medicina para incrementar el nivel de hambre en humanos (y también en animales) que necesitan aumento en su masa corporal. Las características de este fármaco y su forma de acción se explican en el **manuscrito III**.

El experimento consistió en dividir la pollada en pollos experimentales y controles a los dos días después de la eclosión. A los pollos experimentales le suministrábamos 0.1mg de ciproheptadina disuelto en 0.25ml de agua, mientras que los controles recibían 0.25ml de agua. Cada dos días visitábamos los nidos para administrar nuevas dosis hasta la edad de 16-18 días. Cuando los pollos tuvieron entre 10 y 12 días de edad, les colocamos unos collares que les impedían tragar la comida que les daban sus padres. Después de unas dos horas y medias, recogíamos las muestras de comida acumuladas en las bocas de los pollos y las llevábamos al laboratorio donde se estimaba su peso seco. Además, los pollos fueron pesados antes y después de la aplicación de los collares. Posteriormente, cuando los pollos tuvieron 16-18 días tomamos medidas de variables asociadas con la eficacia biológica, como son distintas medidas corporales y el grado de respuesta inmune mediada por linfocitos T.

Este protocolo se repitió en polladas experimentales compuestas por 2 pollos de urraca y otros dos de críalo, confeccionadas con pollos recién eclosionados, en los que un pollo de cada especie recibía dosis de ciproheptadina mientras que el otro las recibía de agua. En estos nidos estimamos la posibilidad de que el aumento experimental del nivel de hambre tuviera efectos distintos en las dos especies

Por último, el comportamiento de las parejas de alzacola alimentando a sus propias nidadas (parasitadas y no parasitadas) y a nidadas experimentales de un pollo de cuco común y 2-3 pollos de hospedador, se estudió mediante grabaciones en video de unas 2-4 horas de duración. Las nidadas experimentales se confeccionaron sólo para las grabaciones y los pollos del parásito y los de hospedador se disponían en soportes (nidos) distintos, pero juntos entre si (ver **manuscrito VI** para una información mas detallada del protocolo experimental).

Resultados y Discusión

Base genética del reconocimiento de huevos (Manuscrito I)

Comparamos el genotipo obtenido para once microsatélites entre los pollos procedentes de parejas (un solo pollo por nido) que expulsaron y aquellos de parejas que aceptaron un modelo experimental con apariencia similar a la de un huevo de críalo. Obtuvimos que para uno de estos microsatélites, Ase64 (Richardson *et al.* 2000), las frecuencias alélicas y genotípicas fueron diferentes entre expulsoras y aceptadoras. Estas diferencias fueron fundamentalmente causadas por dos alelos, uno más frecuente en parejas aceptoras (alelo 429bp) y otro más frecuente en parejas expulsoras (alelo 457bp) (Figura 3).

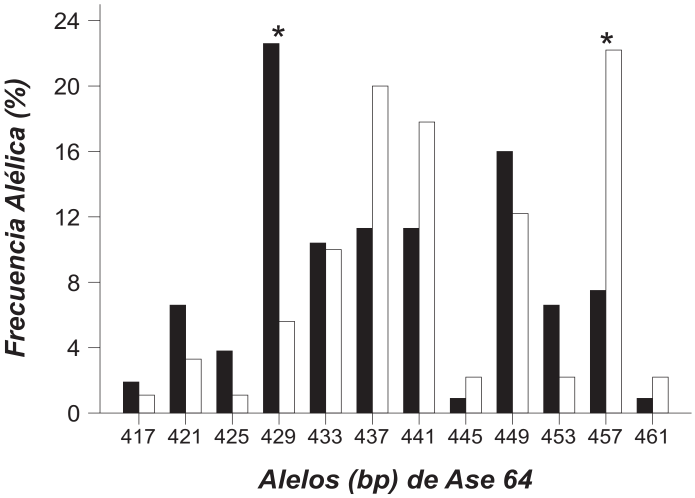

Figura 3.- *Frecuencias alélicas (%) para Ase64 en pollos de parejas aceptoras (■) y en pollos de parejas expulsoras (□). Se utilizaron muestras de un solo pollo por nido. Los símbolos indican los alelos que difirieron significativamente entre aceptadores y expulsores.*

Estos resultados, podrían haber sido originados por la presencia de una estructura genética, originada por la migración y/o por diferencias genéticas entre las subpoblaciones de urracas de Guadix con distinto nivel de rechazo de huevos. Sin embargo, no encontramos estructura génica, y por tanto, la explicación más probable de nuestros resultados es la cercanía física entre Ase64 y algún gen influenciando el carácter expulsor en urracas.

Adicionalmente, extendimos el análisis realizado en Guadix a otras poblaciones Europeas, y obtuvimos que las diferencias en tasa de expulsión y las distancias genéticas calculadas para Ase64 covariaron significativamente para las poblaciones de urracas en simpatría con el críalo; incluso después de controlar por las distancias genéticas calculadas a partir de tres loci neutrales y por las separaciones geográficas entre poblaciones (Figura 4)

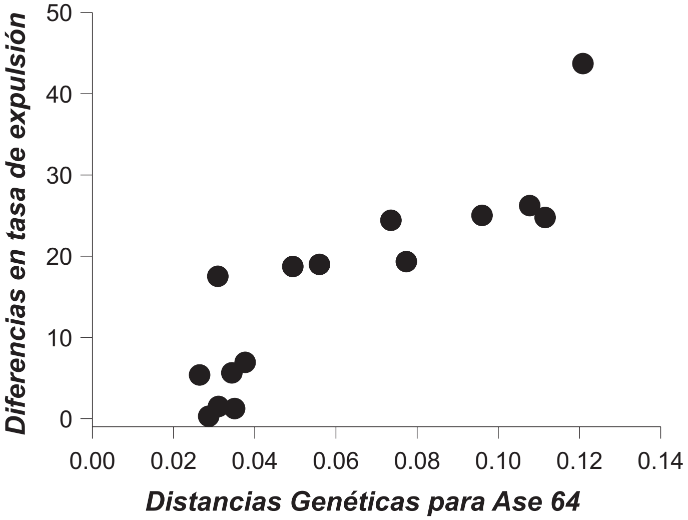

Figura 4.- *Relación entre las diferencias en tasa de expulsión (%) y distancias genéticas para Ase64 en las poblaciones en simpatría con el críalo (N = 15 diferencias entre 6 poblaciones) (Test de Mantel: r = 0.88, P < 0.001,* R^2 *= 0.78).*

Estos resultados sugieren una base genética para el reconocimiento y expulsión de huevos en urracas, validando por tanto una importante asunción teórica. Nuestros datos deberían considerarse como conservativos ya que: *i)* usamos datos genéticos procedentes de pollos (uno por nido) y no de adultos, y *ii)* debido a la existencia de una cierta plasticidad fenotípica en el comportamiento de expulsión de huevos en urracas (Soler *et al.* 1999c). Este trabajo crea la base necesaria para llevar a cabo estudios futuros sobre el control genético de este comportamiento defensivo, tanto en urraca como en otras especies de hospedadores de parásitos de cría. Aunque la distribución de los alelos de Ase64 no predice completamente el comportamiento de expulsión de las urracas, este locus podría empezarse a utilizar como un marcador de la capacidad genética del reconocimiento y expulsión de huevos en urracas, o como punto de partida para desarrollar otros marcadores mejores.

Factores ambientales y genéticos del comportamiento de expulsión de huevos en urracas (Manuscrito II)

Estudiamos las causas de variación en el nivel de defensas mostrado entre subpoblaciones de urracas dentro de la población de Guadix. Como resultado obtuvimos que las diferencias en tasa de expulsión entre subpoblaciones de urracas se relacionaron con aquellas variables que podrían influenciar en los costes del comportamiento de expulsión, como son: el riesgo de parasitismo y la fecha de puesta (ver Davies y Brooke 1988, Moksnes y Røskaft 1989, Lotem *et al.* 1992, Lotem *et al.* 1995, Álvarez 1996, Brooke *et al.* 1998, Rodríguez-Gironés y Lotem 1999) (Figura 5).

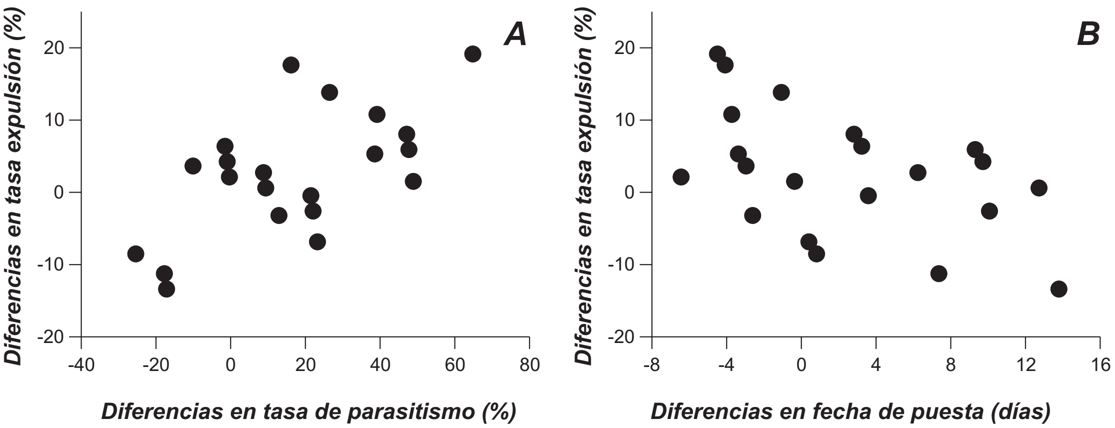

Figura 5.- *Correlaciones entre las diferencias en tasa de expulsión con (**A**) las diferencias en tasa de parasitismo (Test de Mantel: $r = 0.62$, $R^2 = 0.39$, $P = 0.006$) y con (**B**) las diferencias en fecha de puesta (Test de Mantel: $r = -0.49$, $R^2 = 0.24$, $P = 0.025$) entre las subpoblaciones de Guadix.*

Por otra parte, ni las distancias genéticas estimadas para 10 microsatélites neutrales, ni las distancias geográficas entre subpoblaciones de urracas explicaron las diferencias en tasa de expulsión mostradas entre las subpoblaciones de Guadix. En cambio, utilizando a Ase64 como marcador de la capacidad genética de expulsión en urracas **(MS I)**, obtuvimos que las diferencias en frecuencias del alelo asociado al carácter expulsor (457bp), explicaron satisfactoriamente las diferencias en tasa de expulsión entre subpoblaciones (Figura 6). Asimismo, las diferencias en frecuencias del alelo 457bp se relacionaron con las diferencias en tasa de parasitismo y con las diferencias en fecha de puesta entre subpoblaciones de una manera similar a como estas variables se relacionaron con las diferencias en porcentaje de expulsión (Figura 7).

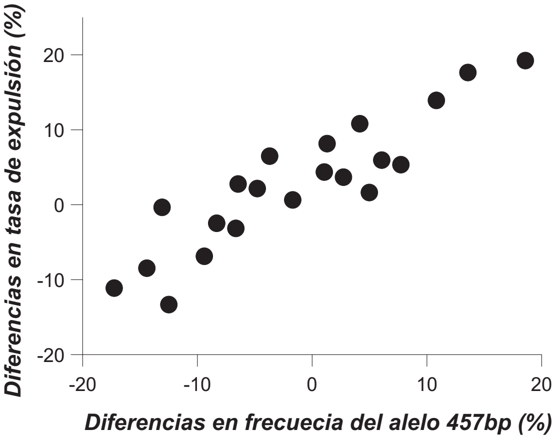

Figura 6.- Relación entre las diferencias en tasa expulsión y las diferencias en la frecuencia del alelo 457 bp de Ase64 (Test de Mantel: r = 0.91, R^2 = 0.82, P < 0.001) entre las subpoblaciones de Guadix.

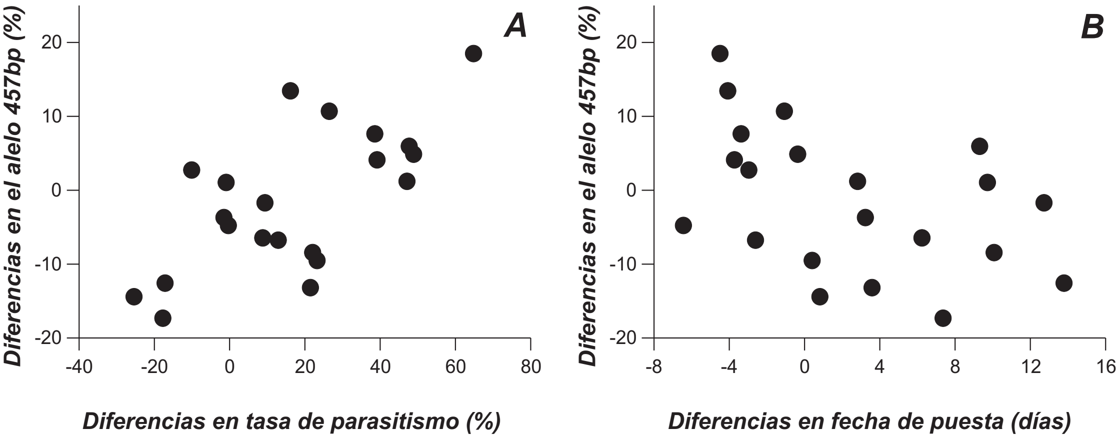

***Figura 7.-** Correlaciones entre las diferencias en frecuencia del alelo 457bp de Ase64 con (**A**) las diferencias en tasa de parasitismo (Test de Mantel: r = 0.68, R^2 = 0.46, P = 0.002) y con (**B**) las diferencias en fecha de puesta (Test de Mantel: r = -0.45, R^2 = 0.20, P= 0.035) entre las subpoblaciones de Guadix.*

Estos resultados indican que las diferencias en porcentaje de expulsión entre subpoblaciones de urracas es una consecuencia de diferencias en abundancia de urracas con la capacidad genética de expulsar, y que de alguna forma, la tasa de expulsión de una subpoblación se relaciona con las variables ambientales tenidas en cuenta.

Nosotros creemos que este escenario puede ser explicado por el efecto de los críalos seleccionando a urracas de gran calidad parental (Soler *et al.* 1995a). Estas urracas serían más frecuentes en territorios de mejor calidad (con una menor fecha de puesta). En estos territorios, debido a la presión de parasitismo, las urracas expulsoras tendrían un mayor éxito reproductor que las aceptoras (relación entre fecha de puesta y tasa de expulsión), lo que a su vez explicaría la relación positiva entre la tasa de expulsión y presión de parasitismo. Por último, este escenario en las subpoblaciones de Guadix sugiere un aumento en el nivel de defensas (tasa de expulsión) de la población total, conforme la duración de las interacciones coevolutivas incrementa, tal como predice la hipótesis del retraso evolutivo.

Costes y beneficios a corto plazo asociados a un incremento experimental del nivel de hambre mediante ciproheptadina (Manuscrito III)

Como resultado del experimento, obtuvimos que los pollos tratados con ciproheptadina, y por tanto con un mayor nivel de hambre, recibieron una mayor cantidad de comida durante los collares que los pollos controles, aunque también perdieron una mayor cantidad de peso (Figura 8).

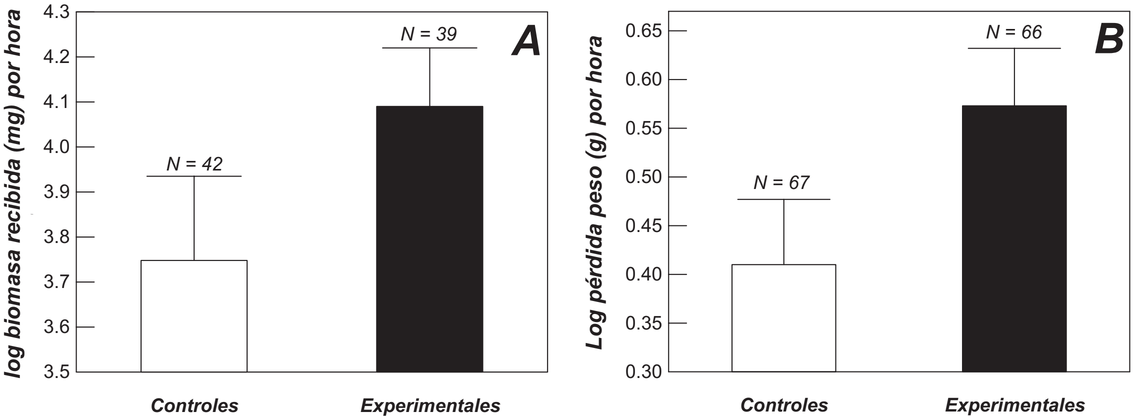

Figura 8.- *Medias ± SE de la biomasa recibida (mg)* ***(A)*** *y del peso corporal perdido (g)* ***(B)*** *por los pollos de urraca alimentados con ciproheptadina (experimentales) y aquellos alimentados con agua (controles) durante la aplicación de collares.*

Estos resultados, sugieren que la ciproheptadina mediante el incremento del nivel de necesidad, aumentó también las señales de los pollos utilizadas por los padres para decidir a qué pollo alimentar. Además, tal como predicen los distintos modelos de honestidad de las señales, el aumento de la cantidad de alimento recibido tuvo asociado un incremento en costes metabólicos, medidos aquí como perdida de peso durante los collares.

Efecto en el desarrollo de un incremento del nivel de hambre mediante ciproheptadina (Manuscrito IV)

En relación a las medidas corporales y de respuesta inmune, obtuvimos que el incremento de la necesidad en los pollos experimentales mediado por la ciproheptadina tuvo un efecto neto positivo al final de desarrollo, ya que éstos presentaban una calidad fenotípica mejor que los pollos controles (Figura 9).

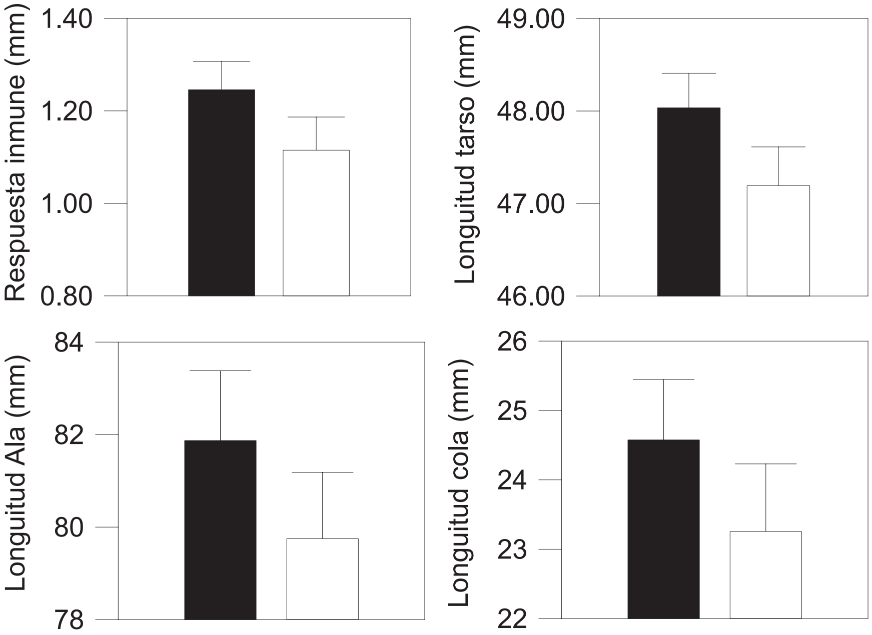

Figura 9.- *Medias ± SE de algunas variables asociadas al desarrollo de los pollos tratados con ciproheptadina (■) y de los pollos tratados con agua (□). Los análisis son modelos generales lineales (GLM) con el tratamiento como un factor dentro de grupo y el orden de la pareja de pollos (1ª o 2ª) como otro factor dentro de grupo.*

Por tanto, nuestros resultados indican que aunque la petición es costosa **(MS III)**, el balance entre costes y beneficios es positivo, indicando que otro tipo de costes, además de los metabólicos sufridos por el individuo deshonesto, estarían compensando los beneficios de una mayor intensidad de petición. Estos costes podrían ser tanto la reducción de hermanos (presentes o futuros, ver introducción), como los costes asociados a la crianza de una progenie egoísta cuando adulto (Alexander 1974).

Un aumento del nivel de hambre afecta de forma diferente a los pollos de críalo y a los pollos de urraca (Manuscrito V)

Utilizando nidadas experimentales compuestas por dos críalos y dos urracas, comparamos el efecto de un aumento de la necesidad mediado por la ciproheptadina entre pollos de las dos especies. El efecto de la ciproheptadina sobre la cantidad de alimento conseguida durante los collares no difirió entre especies; en ambos casos los pollos experimentales tendieron a conseguir una mayor cantidad de comida que los controles (Figura 10A). Sin embargo, en relación con los costes metabólicos medidos (pérdida de peso), obtuvimos que el efecto de la ciproheptadina fue diferente dependiendo de la especie: en urracas, los experimentales perdieron mayor cantidad de peso, pero esto no fue el caso en los críalos (Figura 10B).

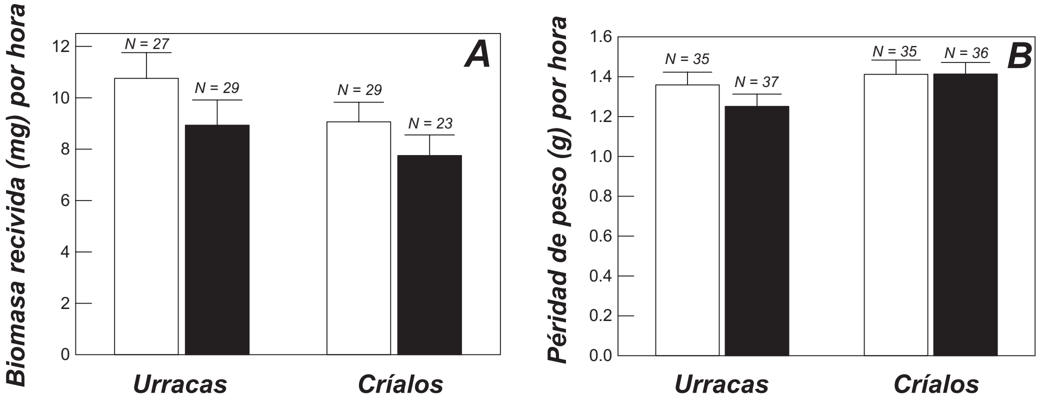

***Figura 10.-** Biomasa recibida (mg) por hora **(A)** y pérdida de masa corporal (g) por hora **(B)** en los pollos experimentals (□) y por en los pollos controles (■) mientras llevaban los collares.*

Respecto al efecto de la ciproheptadina sobre el crecimiento de los pollos, en urracas los resultados fueron similares, aunque menos evidentes que los hallados estudiando nidadas naturales de urraca no parasitadas **(MS IV)**; pero en el caso de críalo, este efecto no fue encontrado, incluso fue ligeramente diferente al efecto obtenido en las urracas.

Estos resultados sugieren que las señales de petición en el críalo, al igual que en la urraca, variarían con el nivel hambre como ocurre en otros parásitos de cría (ver Kilner y Davies 1999, Hauber y Ramsey 2003, Lichtenstein y Dearborn 2004). Además, el no haber encontrado un aumento de costes asociados a un aumento en los beneficios de incrementar el nivel de hambre, sugiere que los críalos podrían aumentar la intensidad de las señales obteniendo una mayor cantidad de alimento sin ningún coste adicional. Esta aparente contradicción, podría ser explicada por la presencia de otros costes asociados a la petición, como el riesgo de depredación o la reducción de hermanos (ya que el críalo puede a veces compartir nido con conespecíficos). Otra posibilidad es que estos resultados sean la consecuencia de que los pollos de críalo no pudieran asimilar una cantidad de comida mayor, no obteniendo beneficios de un incremento de la intensidad de petición.

Adquisición de comida por el cuco común en nidos de alzacola: beneficios de la expulsión de pollos (Manuscrito VI)

En nidos naturales de alzacola, el pollo de cuco común fue alimentado con presas de mayor tamaño, pero no recibió una tasa de cebas similar a la recibida por una nidada completa del hospedador, como ocurre en otras especies (Brooke y Davies 1989, Grim y Honza 1997). El mayor tamaño de las presas, por tanto, podrían compensar esa menor tasa de cebas. En este sentido, cuando comparamos la cantidad de alimento (tasa de cebas x por el tamaño medio de las presas), las diferencias entre nidos parasitados (con un sólo pollo de cuco) y no parasitados (nidada completa de alzacola) desaparecía.

Sin embargo, los resultados de las grabaciones de nidos experimentales donde hicimos competir un pollo de cuco común con los pollos del hospedador, demostraron que los pollos de cuco no fueron preferentemente alimentados, sino que obtuvieron una cantidad de comida similar a la obtenida por un único pollo de hospedador. Por lo tanto, los pollos de cuco en las nidadas experimentales recibieron una cantidad de comida menor que aquella conseguida por los pollos de cuco en los nidos sin manipular (Figura 11).

Estos resultados ponen de manifiesto las ventajas del costoso comportamiento de expulsión de los pollos de cuco, al menos cuando parasita al alzacola. La incapacidad manifestada por los pollos cuco común para competir exitosamente por la comida con los pollos del hospedador, podría ser la consecuencia de la perdida por parte del cuco de aquellas señales del comportamiento de petición relacionadas con la competencia entre pollos (ver por ejemplo Kacelnik *et al.* 1995, Davies *et al.* 1998, Lotem 1998); aunque resultado podría también ser debido a que los adultos de las especies que parasita el cuco común, tiendan a repartir la comida por igual entre todos sus pollos, teniendo el pollo de cuco que expulsar el contenido del nido para obtener una cantidad de comida suficiente (Soler 2001, Soler 2002).

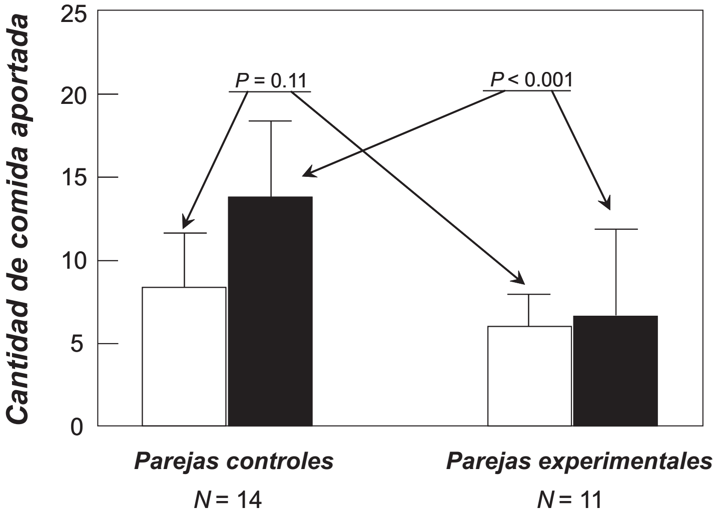

Figura 11.- *Medias ± SD de la cantidad de alimento (tasa de cebas x tamaño medio de presa) recibida por pollo de alzacola (□) y por pollo de cuco (■) en las parejas de alzacola controles y experimentales.*

Conclusiones

1. La asociación detectada entre el genotipo del microsatellite Ase64 y la expulsión de huevos realizada por las urracas frente al parasitismo del críalo supone la primera evidencia fuerte de la base genética del reconocimiento y expulsión de huevos ajenos, la cual era hasta ahora meramente asumida.

2. La variación en frecuencia de fenotipos defensivos manifestada entre las subpoblaciones de urracas de Guadix es la consecuencia de diferencias en la abundancia de individuos con la capacidad genética de reconocer y expulsar huevos parásitos. Esta distribución puede ser explicada debido a que el críalo prefiere parasitar a urracas con alta calidad parental.

3. Proponemos y validamos el uso de la ciproheptadina como una nueva herramienta experimental para manipular el nivel de hambre en el campo. Esta metodología permite el estudio de la comunicación paterno-filial en un escenario más realista, donde además de los costes, los beneficios de un nivel deshonesto de hambre puedan ser estudiados.

4. La ciproheptadina hizo que los pollos experimentales consiguieran una mayor cantidad de comida de sus padres, pero sufriendo un mayor coste metabólico. Esto sugiere que la ciproheptadina, mediante el incremento del nivel de necesidad de los pollos, también incrementó la intensidad de la petición. Estos resultados apoyan la hipótesis que afirma que el comportamiento petitorio es una señal honesta de la necesidad.

5. Durante el crecimiento de los pollos de urracas, los beneficios asociados a un nivel deshonesto de necesidad superaron a los costes. Este resultado indica que la honestidad de las señales de petición no está mantenida solamente por costes metabólicos, sino que otros costes, como la pérdida en eficacia biológica inclusiva, estarían también actuando.

6. El efecto de la ciproheptadina sobre la cantidad de comida recibida fue similar para los críalos y urracas; esto sugiere que las señales de petición en el críalo varía en función del nivel de necesidad. Sin embargo, mientras que el coste metabólico asociado al tratamiento con ciproheptadina fue detectado en urracas, esto no

ocurrió en críalos, sugiriendo la existencia de diferencias específicas en los costes asociados a un exagerado comportamiento petitorio.

7. Los pollos de cuco común que compartieron experimentalmente el cuidado parental con los pollos de alzacola, recibieron una menor cantidad de comida que los pollos de cucos que estuvieron solos. Este resultado pone de manifiesto la ventaja del comportamiento de expulsión de pollos, al menos cuando parasitan al alzacola.

Conclusions

1. The detected association between the genotype of Ase64 microsatellite and the egg-ejection performed by magpies against great spotted cuckoo parasitism is the first strong evidence on the genetic basis of recognition and ejection of foreign eggs, which was merely assumed until now.
2. Variation in frequencies of defensive phenotypes shown between magpie subpopulations of Guadix is consequence of differences in abundance of individuals with the genetic ability to recognize and eject parasitic eggs. This distribution may be explained due to great spotted cuckoo prefers parasitizing to magpies with a high parental quality.
3. We propose and validate the use of cyproheptadine as a new experimental tool to manipulate level of hunger in the field. This methodology allows the study of parent-offspring communication in a more realistic scenario, where not only costs, but also benefits associated to dishonest signal of level of hunger can be studied.
4. Cyproheptadine caused that experimental chicks obtained a greater amount of food from their parents, but paying a greater metabolic cost. It suggests that cyproheptadine, by increasing the level of chick need, also increased begging intensity. These results agree with the hypothesis asserting that begging behaviour is an honest signal of need.
5. During the growth of magpie nestlings, benefits associated to a dishonest level of need exceeded to metabolic costs. This result indicates that the honesty of begging signals is not only maintained by metabolic costs, but others costs, such as the loss in inclusive fitness, would be also acting.
6. The effect of cyproheptadine on the amount of food received was similar for great spotted cuckoos and magpies; it suggests that begging signals in great spotted cuckoo chick vary depending on level of need. However, while metabolic costs associated to cyproheptadine treatment was detected in magpies, it was not the case in cuckoos, suggesting the existence of specific differences in costs associated to an exaggerated begging behaviour.

7. Common cuckoo chicks sharing experimentally the parental care with rufous bush robin chicks, received a smaller amount of food than those cuckoos being alone. This result manifests the advantage chick-eviction behaviour of common cuckoo chick, at least when parasitizing rufous bush robin.

Perspectivas

Uno de los principales resultados que recoge esta tesis es el descubrimiento de la asociación genética entre un microsatélite (Ase64) y el reconocimiento y expulsión de huevos en la urraca. Este hallazgo pone de manifiesto la base genética de este comportamiento, y ofrece una nueva forma para abordar el estudio de las interacciones coevolutivas entre urraca y críalo. Mediante la utilización de este microsatélite como un marcador de la capacidad genética de reconocer y expulsar huevos, sería posible por primera vez estudiar esta defensa del hospedador desde un nivel genético y abordar objetivos intratables previamente. En este sentido, se podría extender el estudio llevado a cabo en Guadix a otras poblaciones de urraca, tanto en alopatría con el críalo, como a otras sufriendo diferentes presiones de parasitismo, e intentar determinar los mecanismos responsables del mantenimiento de la variación genética en las defensas del hospedador. Pudiendo por ejemplo, determinar si el hipotético gen influenciando la expulsión de huevos es solamente expresado por homocigotos, o si los individuos aceptores proceden de poblaciones no explotadas por el críalo. También, gracias este marcador genético, se podría cuantificar la eficacia biológica de los genotipos expulsores y no expulsores bajo diferentes presiones de parasitismo, y el estudio de las condiciones ambientales que afectan a la expresión del gen relacionado a la expulsión de huevos. Además, este estudio se podría extender a otras especies hospedadoras de parásitos de cría, determinando si la capacidad para reconocer y expulsar huevos en diferentes especies de hospedador radica en el mismo o distinto gen.

Recientemente, Hansson *et al.* (2005) ha determinado la posición del microsatélite Ase64 en los genomas del carricero tordal (*Acrocephalus arundinaceus)* y del pollo (*Gallus gallus*). Este descubrimiento, junto a nuestros resultados, da un marco físico donde poder buscar genes candidatos influenciando el reconocimiento y la expulsión de huevos.

Respecto a la ciproheptadina, hemos mostrado que su uso es viable para estudiar los conflictos entre padre e hijos en el campo, dando un escenario más realista que el utilizado en estudios previos. Mediante su uso hemos puesto de manifiesto los costes metabólicos asociados a una petición deshonesta, y quizás, la extensión de esta metodología a otras especies ayude a acabar con la controversia existente en relación a los costes de las señales de petición (ver introducción). Además, nuestros resultados sugieren que otros costes, además de los metabólicos, están afectando al nivel de las señales emitidas por los hijos. Con el uso de la ciproheptadina, se podría abordar estudios a largo plazo del efecto de una petición deshonesta, y determinar por ejemplo si la eficacia biológica inclusiva de individuos tratados con ciproheptadina es menor que para los individuos controles.

De la comparación del efecto de la ciproheptadina en críalos y urracas, hemos obtenido que las señales de petición del críalo no están constreñidas por costes metabólicos. Un estudio futuro, utilizando la ciproheptadina, podría intentar determinar la importancia del riesgo de depredación asociado a un aumento de la petición de los críalos. Además se podría extender nuestro estudio a otras especies parásitas de cría, y así poder determinar la generalidad de este hallazgo.

En cuanto al cuco común y al alzacola, nuestros resultados han manifestado la incapacidad que tienen los pollos de cuco común para competir exitosamente con los del hospedador. Sin embargo, nuestros resultados pueden ser la consecuencia de *i)* los pollos de cuco han perdido la capacidad para competir con los pollos de hospedador, o *ii)* debido a los patrones de reparto de comida seguidos por los hospedadores del cuco común. Por ejemplo, se podría discernir entre ambas posibilidades, llevando a cabo experimentos donde se estudie el éxito de pollos de cuco común en nidos de urraca, y el éxito de pollos de críalo en nidos de alzacola.

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Literatura Citada

Alexander, R. D. 1974. The evolution of social behaviour. *Annual Review of Ecology and Systematics*, **5,** 325-383.

Álvarez, F. 1994. A gens of cuckoo *Cuculus canorus* parasitizing rufous bush chat *Cercotrichas galactotes*. *Journal of Avian Biology*, **25,** 239-243.

Álvarez, F. 1996. Model cuckoo *Cuculus canorus* eggs accepted by Rufous bush chats *Cercotrichas galactotes* during the parasite's absence from the breeding area. *Ibis*, **138,** 340-342.

Aragón, S., Møller, A. P., Soler, J. J. y Soler, M. 1999. Molecular phylogeny of cuckoos supports a polyphyletic origin of brood parasitism. *Journal of Evolutionary Biology*, **12,** 495-506.

Arias de Reina, L. 1998. Coevolution of the great spotted cuckoo and its hosts. In: *Parastic birds and their hosts. Studies in coevolution* (Ed. by Rothstein, S. I. y Robinson, S. K.), pp. 129-142. New York, Oxford: Oxford University Press.

Avilés, J. M. y Møller, A. P. 2003. Meadow pipit (*Anthus pratensis*) egg appearance in cuckoo (*Cuculus canorus*) sympatric and allopatric populations. *Biological Journal of the Linnean Society*, **79,** 543-549.

Avilés, J. M. y Møller, A. P. 2004. How is host egg mimicry maintained in the cuckoo (Cuculus canorus)? *Biological Journal of the Linnean Society*, **82,** 57-68.

Avilés, J. M., Soler, J. J., Soler, M. y Møller, A. P. 2004. Rejection of parasitic eggs in relation to egg appearance in magpies. *Animal Behaviour*, **67,** 951-958.

Baba, R., Nagata, Y. y Yamagishi, S. 1990. Brood parasitism and egg robbing among three fresh-water fish. *Animal Behaviour*, **40,** 776-778.

Bachman, G. C. y Chappell, M. A. 1998. The energetic cost of begging behavior in nestling house wrens. *Animal Behaviour*, **55,** 1607-1618.

Barabas, L., Gilicze, B., Takasu, F. y Moskat, C. 2004. Survival and anti-parasite defense in a host metapopulation under heavy brood parasitism: a source-sink dynamic model. *Journal of Ethology*, **22,** 143-151.

Bártol, I., Karcza, Z., Moskát, C., Røskaft, E. y Kisbenedek, T. 2002. Responses of great reed warblers *Acrocephalus arundinaceus* to experimental brood parasitism: the effects of a cuckoo *Cuculus canorus* dummy and egg mimicry. *Journal of Avian Biology*, **33,** 420-425.

Briskie, J. V., Martin, P. R. y Martin, T. E. 1999. Nest predation and the evolution of nestling begging calls. *Proceedings of the Royal Society of London, Series B*, **266,** 2153-2159.

Briskie, J. V., Sealy, S. G. y Hobson, K. A. 1992. Behavioural defenses against avian brood parasitism in simpatric and allopatric host populations. *Evolution*, **46,** 334-340.

Brockmann, H. J. 1993. Parasitizing conspecifics: comparisons between Hymenoptera and birds. *Trends in Ecology and Evolution*, **8,** 2-4.

Brooke, M. de L. y Davies, N. B. 1989. Provisioning of nestling cuckoos *Cuculus canorus* by reed wabler *Acrocephalus scirpaceus* hosts. *Ibis*, **131,** 250-256.

Brooke, M. de L., Davies, N. B. y Noble, D. G. 1998. Rapid decline of host defenses in response to reduced cuckoo parasitism - behavioral flexibility of reed warblers in a changing world. *Proceedings of the Royal Society of London, Series B*, **265,** 1277-1282.

Brooker, L. C. y Brooker, M. G. 1990. Why are cuckoos host specific? *Oikos*, **57,** 301-309.

Budden, A. E. y Wright, J. 2001. Begging in nestling birds. *Current Ornithology*, **16,** 83-118.

Burford, J. E., Friedrich, T. J. y Yasukawa, K. 1998. Response to playback of nestling begging in the red-winged blackbird, *Agelaius phoeniceus*. *Animal Behaviour*, **56,** 555-561.

Büschinger, A. 1986. Evolution of social parasitism in ants. *Trends in Ecology and Evolution*, **1,** 155-160.

Butchart, S. M. H., Kilner, R. M., Fuisz, T. y Davies, N. B. 2003. Differences in the nestling begging calls of hosts and host-races of the common cuckoo, *Cuculus canorus*. *Animal Behaviour*, **65,** 345-354.

Cardon, L. R. y Bell, J. I. 2001. Association study designs for complex diseases. *Nature Reviews Genetics*, **2,** 91-99.

Chakrabarty, A. S., Pillai, R. V., Anand, B. K. y Singh, B. 1967. Effect of cyproheptadine on the electrical activity of the hypothalamic feeding centres. *Brain Research*, **6,** 561-569.

Cramp, S. 1985. *Handbook of the birds of Europe, the Middle East and north Africa. The birds of the western Palaearctic. Volume 4. Terns to woodpeckers.* Oxford: Oxford University Press.

Cramp, S. 1994. *Handbook of the birds of Europe, the Middle East and North Africa. The birds of the western Palaearctic. Volume 8. Crows to finches.* Oxford: Oxford University Press.

Creemers, B., Billen, J. y Gobin, B. 2003. Larval begging behaviour in the ant Myrmica rubra. *Ethology Ecology y Evolution*, **15,** 261-272.

Cruz, A. y Wiley, J. W. 1989. The decline of an adaptation in the absence of a presumed selection pressure. *Evolution*, **43,** 55-62.

Davies, N. B. 1999. Cuckoos and cowbirds versus hosts: Co-evolutionary lag and equilibrium. *Ostrich*, **70,** 71-79.

Davies, N. B. 2000. *Cuckoos, cowbirds, and other cheats*. London: T y A D Poyser.

Davies, N. B., Bourke, A. F. G. y Brooke, M. D. 1989. Cuckoos and parasitic ants: interespecific brood parasitism as an evolutionary arms-race. *Trends in Ecology and Evolution*, **4,** 274-278.

Davies, N. B. y Brooke, M. de L. 1988. Cuckoos versus reed warblers: adaptations and counteradaptations. *Animal Behaviour*, **36,** 262-284.

Davies, N. B. y Brooke, M. de L. 1989a. An experimental study of co-evolution between the cuckoo, *Cuculus canorus*, and its hosts. I. Host egg discrimination. *Journal of Animal Ecology*, **58,** 207-224.

Davies, N. B. y Brooke, M. de L. 1989b. An experimental study of co-evolution between the cuckoo, *Cuculus canorus*, and its hosts. II. Host egg markings, chick discrimination and general discussion. *Journal of Animal Ecology*, **58,** 225-236.

Davies, N. B., Brooke, M. de L. y Kacelnik, A. 1996. Recognition errors and probability of parasitism determine whether reed warblers should accept or reject mimetic cuckoo eggs. *Proceedings of the Royal Society of London, Series B*, **263,** 925-931.

Davies, N. B., Butchart, S. M. H., Burke, T. A., Chaline, B. y Stewart, I. R. K. 2003. Reed warblers guard against cuckoos and cuckoldry. *Animal Behaviour*, **65,** 285-295.

Davies, N. B., Kilner, R. M. y Noble, D. G. 1998. Nestling cuckoos, *Cuculus canorus*, exploit hosts with begging calls that mimic a brood. *Proceedings of the Royal Society of London, Series B*, **265,** 673-678.

Dawkins, R. y Krebs, J. R. 1979. Arms races between and within species. *Proceedings of the Royal Society of London, Series B*, **205,** 489-511.

Dearborn, D. C. 1996. Video documentation of a brown-headed cowbird nestling ejecting an indigo bunting nestling from the nest. *The Condor*, **98,** 645-649.

Dearborn, D. C. 1998. Begging behavior and food acquisition by brown-headed cowbird nestlings. *Behavioral Ecology and Sociobiology*, **43,** 259-270.

Dearborn, D. C. 1999. Brown-headed cowbird nestling vocalizations and risk of nest predation. *Auk*, **116,** 448-457.

Dearborn, D. C., Anders, A. D., Thompson, F. R. y Faaborg, J. 1998. Effects of Cowbird parasitism on parental provisioning and nestling food acquisition and growth. *Condor*, **100,** 326-334.

Dearborn, D. C. y Lichtenstein, G. 2002. Begging behaviour and host exploitation in parasitic cowbirds. In: *The evolution of begging: competition, cooperation and communication* (Ed. by Wright, J. y Leonard, M. L.), pp. 361-387. Dordrecht: Kluwer Academic.

Eadie, J. M. y Fryxell, J. M. 1992. Density dependence, frequency dependence, and alternative nesting strategies in goldeneyes. *American Naturalist*, **140,** 621-641.

Eshel, I. y Feldman, M. W. 1991. The handicap principle in parent-offspring conflict: comparison of optimality and population genetic analyses. *American Naturalist*, **137,** 167-185.

Fanelli, D., Henshaw, M., Cervo, R., Turillazzi, S., Queller, D. C. y Strassmann, J. E. 2005. The social parasite wasp *Polistes atrimandibularis* does not form host races. *Journal of Evolutionary Biology*, **18,** 1362-1367.

Friedman, H. 1971. Further Information on Host Relations of Parasitic Cowbirds. *Auk*, **88,** 239-y.

Futuyma, D. J. 1998. *Evolutionary Biology*. Sunderland: Sinauer Associates.

Gibbs, H. L., Sorenson, M. D., Marchetti, K., Brooke, M. de L., Davies, N. B. y Nakamura, H. 2000. Genetic evidence for female host-specific races of the common cuckoo. *Nature*, **407,** 183-186.

Gill, B. J. 1998. Behaviour and ecology of the shinning cuckoo *Chrysococcy lucidus*. In: *Parastic birds and their hosts. Studies in coevolution* (Ed. by Rothstein, S. I. y Robinson, S. K.), pp. 143-151. New York, Oxford: Oxford University Press.

Godfray, H. C. J. 1991. Signalling of need by offspring to their parents. *Nature*, **352,** 328-330.

Godfray, H. C. J. 1995. Evolutionary theory of parent-offspring conlict. *Nature*, **376,** 133-138.

Granadeiro, J. P., Bolton, M., Silva, M. C., Nunes, M. y Furness, R. W. 2000. Responses of breeding Cory's shearwater Calonectris diomedea to experimental manipulation of chick condition. *Behavioral Ecology*, **11,** 274-281.

Grim, T. y Honza, M. 1997. Differences in parental care of reed warbler (*Acrocephalus scirpaceus*) to its own nestlings and parasitic cuckoo (*Cuculus canorus*) chicks. *Folia Zoologica*, **46,** 135-142.

Grim, T. y Honza, M. 2001. Does supernormal stimulus influence parental behaviour of the cuckoo's host? *Behavioral Ecology and Sociobiology*, **49,** 322-329.

Grim, T., Kleven, O. y Mikulica, O. 2003. Nestling discrimination without recognition: a possible defence mechanism for hosts towards cuckoo parasitism? *Proceedings of the Royal Society of London, Series B-Biological Sciences*, **270,** S73-S75.

Hamilton, W. D. 1964. The genetical evolution of social behaviour. *Journal of Theoretical Biology*, **7,** 1-52.

Hansson, B., Akesson, M., Slate, J. y Pemberton, J. M. 2005. Linkage mapping reveals sex-dimorphic map distances in a passerine bird. *Proceedings of the Royal Society of London, Series B*, **272,** 2289-2298.

Harper, A. B. 1986. The evolution of begging: sibling competition and parent-offspring conflict. *American Naturalist*, **128,** 99-114.

Haskell, D. G. 1994. Experimental evidence that nestling begging behaviour incurs a cost due to nest predation. *Proceedings of the Royal Society of London, Series B*, **257,** 161-164.

Haskell, D. G. 1999. The effect of predation on begging-call evolution in nestling wood warblers. *Animal Behaviour*, **57,** 893-901.

Hauber, M. E. 2003a. Hatching asynchrony, nestling competition, and the cost of interspecific brood parasitism. *Behavioral Ecology*, **14,** 227-235.

Hauber, M. E. 2003b. Lower begging responsiveness of host versus parasitic Brown-Headed cowbird (*Molothrus ater*) nestlings is related to species identity but not to early social experience. *Journal of Comparative Psychology*, **117,** 24-30.

Hauber, M. E. y Ramsey, C. K. 2003. Honesty in host-parasite communication signals: the case for begging by fledgling brown-headed cowbirds *Molothrus ater*. *Journal of Avian Biology*, **34,** 339-344.

Homnick, D. N., Homnick, B. D., Reeves, A. J., Marks, J. H., Pimentel, R. S. y Bonnema, S. K. 2004. Cyproheptadine is an effective appetite stimulant in cystic fibrosis. *Pediatric Pulmonology*, **38,** 129-134.

Homnick, D. N., Marks, J. H., Hare, K. L. y Bonnema, S. K. 2005. Long-term trial of cyproheptadine as an appetite stimulant in cystic fibrosis. *Pediatric Pulmonology*, **40,** 251-256.

Hosoi, S. A. y Rothstein, S. I. 2000. Nest desertion and cowbird parasitism: evidence for evolved responses and evolutionary lag. *Animal Behaviour*, **59,** 823-840.

Hughes, J. M. 2000. Monophyly and phylogeny of cuckoos (Aves, Cuculidae) inferred from osteological characters. *Zoological Journal of the Linnean Society*, **130,** 263-307.

Jackson, W. M. 1993. Causes of couspecific nest parasitism in the northern masked weaver. *Behavioral Ecology and Sociobiology*, **32,** 119-126.

Janze, D. H. 1980. When is it coevolution? *Evolution*, **34,** 611-612.

Jensen, P., Gustafsson, M. y Augustsson, H. 1998. Teat massage after milk ingestion in domestic piglets - An example of honest begging. *Animal Behaviour*, **55,** 779-786.

Johnstone, R. A. 1998. Efficacy and honesty in communication between relatives. *American Naturalist*, **152,** 45-58.

Johnstone, R. A. 1999. Signalling of need, sibling competition, and the cost of honesty. *Proceedings of the Natural Academy of Sciences, USA*, **96,** 12644-12649.

Johnstone, R. A. 2004. Begging and sibling competition: how should offspring respond to their rivals? *American Naturalist*, **163,** E388-E406.

Johnstone, R. A. y Godfray, H. C. J. 2002. Models of begging as a signal of need. In: *The evolution of begging: competition, cooperation and communication* (Ed. by Wright, J. y Leonard, M. L.), pp. 1-20. Dordrecht: Kluwer Academic.

Johnstone, R. A. y Grafen, A. 1993. Dishonesty and the handicap principle. *Animal Behaviour*, **46,** 759-764.

Kacelnik, A., Cotton, P. A., Stirling, L. y Wright, J. 1995. Food allocation among nestling starlings: sibling competition and the scope of parental choice. *Proceedings of the Royal Society of London, Series B*, **259,** 259-263.

Karcza, Z., Moskat, C., Cherry, M. I. y Kisbenedek, T. 2003. Experimental manipulation of intraclutch variation in the great reed warbler shows no effect on rejection of parasitic eggs. *Ethology*, **109,** 15-22.

Kelly, C. 1987. A Model to explore the rate of spread of mimicry and rejection in hypothetical populations of cuckoos and their hosts. *Journal of Theoretical Biology*, **125,** 283-299.

Kilner, R. M. 2001. A growth cost of begging in captive canary chicks. *Proceedings of the Natural Academy of Sciences, USA*, **98,** 11394-11398.

Kilner, R. M. 2003. How selfish is a cowbird nestling? *Animal Behaviour*, **66,** 569-576.

Kilner, R. M. y Davies, N. B. 1999. How selfish is a cuckoo chick? *Animal Behaviour*, **58,** 797-808.

Kilner, R. M. y Johnstone, R. A. 1997. Begging the question: are offspring solicitation behaviours signals of need? *Trends in Ecology and Evolution*, **12,** 11-15.

Kilner, R. M., Madden, J. R. y Hauber, M. E. 2004. Brood parasitic cowbird nestlings use host young to procure resources. *Science*, **305,** 877-879.

Kilner, R. M., Noble, D. G. y Davies, N. B. 1999. Signals of need in parent-offspring communication and their exploitation by the common cuckoo. *Nature*, **397,** 667-672.

Kilpatrick, A. M. 2002. Variation in growth of brown-headed cowbird (Molothrus ater) nestlings and energetic impacts on their host parents. *Canadian Journal of Zoology-Revue Canadienne de Zoologie*, **80,** 145-153.

Krüger, O. y Davies, N. B. 2002. The evolution of cuckoo parasitism: a comparative analysis. *Proceedings of the Royal Society of London, Series B*, **269,** 375-381.

Langmore, N. E., Hunt, S. y Kilner, R. M. 2003. Escalation of a coevolutionary arms race through host rejection of brood parasitic young. *Nature*, **422,** 157-160.

Langmore, N. E., Kilner, R. M., Butchart, S. H. M., Maurer, G., Davies, N. B., Cockburn, A., Macgregor, N. A., Peters, A., Magrath, M. J. L. y Dowling, D. K. 2005. The evolution of egg rejection by cuckoo hosts in Australia and Europe. *Behavioral Ecology*, **16,** 686-692.

Lazarus, J. y Inglis, I. R. 1986. Shared and unshared parental investment, parent offspring conflict and brood size. *Animal Behaviour*, **34,** 1791-1804.

Leech, S. M. y Leonard, M. L. 1996. Is there an energetic cost to begging in nestling tree swallows (*Tachycineta bicolor*)? *Proceedings of the Royal Society of London, Series B*, **263,** 983-987.

Leech, S. M. y Leonard, M. L. 1997. Begging and the risk of predation in nestling birds. *Behavioral Ecology*, **8,** 644-646.

Leonard, M. L., Horn, A. G. y Porter, J. 2003. Does begging affect growth in nestling tree swallows, *Tachycineta bicolor*? *Behavioral Ecology and Sociobiology*, **54,** 573-577.

Lichtenstein, G. 2001a. Low success of shiny cowbird chicks parasitizing rufous-bellied thrushes: chick-chick competition or parental discrimination? *Animal Behaviour*, **61,** 401-413.

Lichtenstein, G. 2001b. Selfish begging by screaming cowbirds, a mimetic brood parasite of the bay-winged cowbird. *Animal Behaviour*, **61,** 1151-1158.

Lichtenstein, G. y Dearborn, D. C. 2004. Begging and short-term need in cowbird nestlings: how different are brood parasites? *Behavioral Ecology and Sociobiology*, **56,** 352-359.

Lichtenstein, G. y Sealy, S. G. 1998. Nestling competition, rather than supernormal stimulus, explains the success of parasitic brown-headed cowbird chicks in yellow warbler nests. *Proceedings of the Royal Society of London, Series B*, **265,** 249-254.

Lotem, A. 1993. Learning to recognize nestling is maladaptive for cuckoo Cuculus canorus host. *Nature*, **362,** 743-745.

Lotem, A. 1998. Manipulative begging calls by parasitic cuckoo chicks: why should true offspring not do the same? *Trends in Ecology and Evolution*, **13,** 342-343.

Lotem, A., Nakamura, H. y Zahavi, A. 1992. Rejection of cuckoo eggs in relation to host age: a possible evolutionary equilibrium. *Behavioral Ecology*, **3,** 128-132.

Lotem, A. y Rothstein, S. I. 1995. Cuckoo-host coevolution: from snapshots of an arm race to the documentation of microevolution. *Trends in Ecology and Evolution*, **10,** 436-437.

Lotem, A. y Nakamura, H. 1998. Evolutionary equilibria in avian brood parasitism. An alternative to the "arms race-evolutionary lag" concept. In: *Parastic birds and their hosts. Studies in coevolution* (Ed. by Rothstein, S. I. y Robinson, S. K.), pp. 223-235. New York, Oxford: Oxford University Press.

Lotem, A., Nakamura, H. y Zahavi, A. 1995. Constraints on egg discrimination and cuckoo-host co-evolution. *Animal Behaviour*, **49,** 1185-1209.

Lyon, B. E. y Eadie, J. M. 2000. Family matters: Kin selection and the evolution of conspecific brood parasitism. *Proceedings of the National Academy of Sciences of the United States of America*, **97,** 12942-12944.

Macnair, M. R. y Parker, G. A. 1978. Models of parent-offspring conflict. II. Promiscuity. *Animal Behaviour*, **26,** 111-122.

Macnair, M. R. y Parker, G. A. 1979. Models of parent-offspring conflict. III. Intra-brood conflict. *Animal Behaviour*, **27,** 1202-1209.

Manser, M. B. y Avey, G. 2000. The effect of pup vocalisations on food allocation in a cooperative mammal, the meerkat (*Suricata suricatta*). *Behavioral Ecology and Sociobiology*, **48,** 429-437.

Marchetti, K. 1992. Cost to host defences and the persistence of parasitic cuckoos. *Proceedings of the Royal Society of London, Series B*, **248,** 41-45.

Marchetti, K., Nakamura, H. y Gibbs, H. L. 1998. Host-race formation in the common cuckoo. *Science*, **282,** 471-472.

Martínez, J. G., Soler, J. J., Soler, M., Møller, A. P. y Burke, T. A. 1999. Comparative population structure and gene flow of a brood parasite, the great spotted cuckoo (*Clamator glandarius*), and its primary host, the magpie (*Pica pica*). *Evolution*, **53,** 269-278.

Mayfield, H. 1965. The brown-headed cowbird, with old and new hosts. *Living Bird*, **4,** 13-28.

McCarty, J. P. 1996. The energetic cost of begging in nestling passerines. *The Auk*, **113,** 178-188.

McCarty, J. P. 1997. The role of energetic costs in the evolution of begging behavior of nestling passerines. *The Auk*, **114,** 135-137.

Moksnes, A. y Røskaft, E. 1989. Adaptations of meadow pipits to parasitism by the common cuckoo. *Behavioral Ecology and Sociobiology*, **24,** 25-30.

Moksnes, A. y Røskaft, E. 1995. Egg-morphs and host preferences in the common cuckoo *(Cuculus canorus)*: an analysis of cuckoo and host eggs from European museum collections. *Journal of Zoology*, **236,** 625-648.

Moksnes, A., Røskaft, E., Braa, A. T., Korsnes, L., Lampe, H. M. y Pedersen, H. C. 1990. Behavioural responses of potential hosts towards artificial cuckoo eggs and dummies. *Behaviour*, **116,** 64-89.

Moksnes, A., Røskaft, E., Hagen, L. G., Honza, M., Mork, C. y Olsen, P. H. 2000. Common cuckoo *Cuculus canorus* and host behaviour at reed warbler *Acrocephalus scirpaceus* nests. *Ibis*, **142,** 247-258.

Møller, A. P. 1987. Intraspecific nest parasitism and antiparasite behavior in swallows, *Hirundo rustica*. *Animal Behaviour*, **35,** 247-254.

Monaco, E. L., Tallamy, D. W. y Johnson, R. K. 1998. Chemical mediation of egg dumping in the lace bug *Gargaphia solani* Heidemann (Heteroptera : Tingidae). *Animal Behaviour*, **56,** 1491-1495.

Morton, E. S. y Farabaugh, S. M. 1979. Infanticide and other adaptations of the nestling striped cuckoo *Tapera naevia*. *Ibis*, **121,** 212.

Nakamura, H., Kubota, S. y Suzuki, R. 1998. Coevolution between the common cuckoo and its major hosts in Japan. In: *Parasitic Birds and their hosts, studies in coevolution* (Ed. by Rothstein, S. I. y Robinson, S. K.), pp. 94-112. Oxford: Oxford University Press.

Neuenschwander, S., Brinkhof, M. W. G., Kolliker, M. y Richner, H. 2003. Brood size, sibling competition, and the cost of begging in great tits (*Parus major*). *Behavioral Ecology*, **14,** 457-462.

Noble, D. G., Davies, N. B., Hartley, I. R. y McRae, S. B. 1999. The red gape of the nestling cuckoo (*Cuculus canorus*) is not a supernormal stimulus fo three common hosts. *Behaviour*, **136,** 759-777.

Nuismer, S. L., Thompson, J. N. y Gomulkiewicz, R. 1999. Gene flow and geographically structured coevolution. *Proceedings of the Royal Society of London, Series B*, **266,** 605-609.

Ortega, C. P. 1998. *Cowbirds and other brood parasites*. Tucson: University of Arizona Press.

Ottosson, U., Bäckman, J. y Smith, H. G. 1997. Begging affects parental effort in the pied flycatcher, *Ficedula hypoleuca*. *Behavioral Ecology and Sociobiology*, **41,** 381-384.

Palomino, J. J., Martín-Vivaldi, M. y Soler, M. 1999. Determinants of reproductive success in the rufous bush robin (*Cercotrichas galactotes*). *Journal für Ornithologie*, **140,** 467-480.

Parker, G. A. 1985. Models of parent-offspring conflict. V. Effects of the behavior of the two parents. *Animal Behaviour*, **33,** 519-533.

Parker, G. A. y Macnair, M. R. 1978. Models of parent-offspring conflict. I. Monogamy. *Animal Behaviour*, **26,** 97-110.

Parker, T. H. 1999. Responses of bell's vireos to brood parasitism by the brown-headed cowbird in Kansas. *Wilson Bulletin*, **111,** 499-504.

Payne, R. B. 1977. The ecology of brood parasitism in birds. *Annual Review of Ecology and Systematics*, **8,** 1-28.

Payne, R. B. 1997. Family Cuculidae (cuckoos). In: *Handbook of the birds of the world. vol. 4* (Ed. by Hoyo, J., Elliott, A. y Sargatal, J.), pp. 508-607. Barcelona: BirdLife / Lynx edicions.

Payne, R. B. 1998. Brood parasitism in birds: strangers in the nest. *Bioscience*, **48,** 377-386.

Payne, R. B., Hustler, K., Stjernstedt, R., Sefc, K. M. y Sorenson, M. D. 2002. Behavioural and genetic evidence of a recent population switch to a novel host species in brood-parasitic indigobirds Vidua chalybeata. *Ibis*, **144,** 373-383.

Payne, R. B. y Payne, L. L. 2002. Begging for parental care from another species: specialization and generalization in brood parastic finches. In: *The evolution of begging:*

competition, cooperation and communication (Ed. by Wright, J. y Leonard, M. L.), pp. 429-449. Dordrecht: Kluwer Academic.

Peer, B. D. y Sealy, S. G. 2004. Correlates of egg rejection in hosts of the brown-headed cowbird. *The Condor*, **106,** 580-599.

Petrie, M. y Møller, A. P. 1991. Laying eggs in others' nests: intraspecific brood parasitism in birds. *Trends in Ecology and Evolution*, **6,** 315-320.

Power, H. W. 1998. Quality control and the important questions in avian conspecific brood parasitism. In: *Parastic birds and their hosts. Studies in coevolution* (Ed. by Rothstein, S. I. y Robinson, S. K.), pp. 376-395. New York, Oxford: Oxford University Press.

Pritchard, J. K. y Donnelly, P. 2001. Case-control studies of association in structured or admixed populations. *Theoretical Population Biology*, **60,** 227-237.

Quillfeldt, P. 2002. Begging in the absence of sibling competition in Wilson's storm-petrels, Oceanites oceanicus. *Animal Behaviour*, **64,** 579-587.

Quillfeldt, P., Masello, J. F. y Hamer, K. C. 2004. Sex differences in provisioning rules and honest signalling of need in Manx shearwaters, Puffinus puffinus. *Animal Behaviour*, **68,** 613-620.

Rasa, O. A. E. y Heg, D. 2004. Individual variation and prior experience affect the discrimination of a brood-parasite by its subsocial beetle host. *Behavioral Ecology and Sociobiology*, **57,** 155-163.

Rauter, C. M. y Moore, A. J. 1999. Do honest signalling models of offspring solicitation apply to insects? *Proceedings of the Royal Society of London, Series B*, **266,** 1691-1696.

Redondo, T. 1993. Exploitation of host mechanisms for parental care by avian brood parasites. *Etología*, **3,** 235-297.

Redondo, T. y Castro, F. 1992a. Signalling of nutritional need by magpie nestlings. *Ethology*, **92,** 193-204.

Redondo, T. y Castro, F. 1992b. The increases in risk of predation with begging activity in broods of magpies *Pica pica. Ibis*, **114,** 180-187.

Redondo, T. y Zuñiga, J. M. 2002. Dishonest begging and host manipulation by *Clamator* cuckoos. In: *The evolution of begging: competition, cooperation and communication* (Ed. by Wright, J. y Leonard, M. L.), pp. 1-20. Dordrecht: Kluwer Academic.

Richardson, D. S., Jury, F. L., Dawson, D., Salgueiro, P., Komdeur, J. y Burke, T. 2000. Fifty Seychelles warbler (*Acrocephalus sechellensis*) microsatellite loci polymorphic in Sylviidae species and their cross-species amplification in other passerine birds. *Molecular Ecology*, **9,** 2155-2234.

Robert, M. y Sorci, G. 2001. The evolution of obligate interspecific brood parasitism in birds. *Behavioral Ecology*, **12,** 128-133.

Robert, M., Sorci, G., Møller, A. P., Hochberg, M. E., Pomiankowski, A. y Pagel, M. 1999. Retaliatory cuckoos and the evolution of resistance to brood parasites. *Animal Behaviour*, **58,** 817-824.

Rodríguez-Gironés, M. A. 1999. Sibling competition stabilizes signalling resolution models of parent-offspring conflict. *Proceedings of the Royal Society of London, Series B*, **266,** 2399-2402.

Rodríguez-Gironés, M. A., Cotton, P. A. y Kacelnik, A. 1996. The evolution of begging: signaling and sibling competition. *Proceedings of the Natural Academy of Sciences, USA*, **93,** 14637-14641.

Rodríguez-Gironés, M. A. y Lotem, A. 1999. How to detect a cuckoo egg: a signal-detection theory model for recognition and learning. *American Naturalist*, **153,** 633-648.

Rodríguez-Gironés, M. A., Zuñiga, J. M. y Redondo, T. 2001. Effects of begging on growth rates of nestling chicks. *Behavioral Ecology*, **12,** 269-274.

Rodríguez-Gironés, M. A., Zuñiga, J. M. y Redondo, T. 2002. Feeding experience and relative size modify the begging strategies of nestlings. *Behavioral Ecology*, **13,** 762-785.

Rohwer, S. y Spaw, C. D. 1988. Evolutionary lag versus bill-size constraints: a comparative study of the aceptance of cowbird eggs by old hosts. *Evolutionary Ecology*, **2,** 27-36.

Røskaft, E., Moksnes, A., Stokke, B. G., Bicik, V. y Moskat, C. 2002a. Aggression to dummy cuckoos by potential European cuckoo hosts. *Behaviour*, **139,** 613-628.

Røskaft, E., Moksnes, A., Stokke, B. G., Moskat, C. y Honza, M. 2002b. The spatial habitat structure of host populations explains the pattern of rejection behavior in hosts and parasitic adaptations in cuckoos. *Behavioral Ecology*, **13,** 163-168.

Rothstein, S. I. 1975. Evolutionary rates and host defenses against avian brood parasitism. *American Naturalist*, **109,** 161-176.

Rothstein, S. I. 1982a. Mechanisms of avian egg recognition: which egg parameters elicit responses by rejecter species. *Behavioral Ecology and Sociobiology*, **11,** 229-239.

Rothstein, S. I. 1982b. Successes and failures in avian egg and nestling recognition with comments on the utility of optimality reasoning. *American Zoologist*, **22,** 547-560.

Rothstein, S. I. 1990. A model system for coevolution: avian brood parasitism. *Annual Review of Ecology and Systematics*, **21,** 481-508.

Rothstein, S. I. 2001. Relic behaviours, coevolution and the retention versus loss of host defences after episodes of avian brood parasitism. *Animal Behaviour*, **61,** 95-107.

Rothstein, S. I. y Robinson, S. K. 1998. The evolution and ecology of avian brood parasitism. In: *Parasitic Birds and their hosts, studies in coevolution* (Ed. by Rothstein, S. I. y Robinson, S. K.), pp. 3-56. Oxford: Oxford University Press.

Roulin, A. 2001. On the cost of begging vocalization: implications of vigilance. *Behavioral Ecology*, **12,** 506-511.

Royle, N. J., Hartley, I. R. y Parker, G. A. 2002. Begging for control: when are offspring solicitation behaviours honest? *Trends in Ecology and Evolution*, **17,** 434-440.

Sacchi, R., Saino, N. y Galeotti, P. 2002. Features of begging calls reveal general condition and need of food of barn swallow (*Hirundo rustica*) nestlings. *Behavioral Ecology*, **13,** 268-273.

Saino, N., Ninni, P., Incagli, M., Calza, S., Sacchi, R. y Møller, A. P. 2000. Begging and parental care in relation to offspring need and condition in barn swallow (*Hirundo rustica*). *American Naturalist*, **156,** 637-649.

Sandell, M. I. y Diemer, M. 1999. Intraspecific brood parasitism: a strategy for floating females in the European starling. *Animal Behaviour*, **57,** 197-202.

Sato, T. 1986. A brood parasitic catfish of mouthbrooding cichlid fishes in lake Tanganyika. *Nature*, **323,** 58-59.

Sealy, S. G. 1992. Removal of yellow warbler eggs in association with cowbird parasitism. *The Condor*, **94,** 40-54.

Sealy, S. G. 1995. Burial of cowbird eggs by parasitized yellow warblers: an empirical and experimental study. *Animal Behaviour*, **49,** 877-889.

Sealy, S. G. 1996. Evolution of host defenses against brood parasitism: implications of puncture-ejection by a small passerine. *Auk*, **113,** 346-355.

Servedio, M. R. y Lander, R. 2003. Coevolution of an avian host and its parasitic cuckoo. *Evolution*, **57,** 1164-1175.

Silverman, E. K. y Palmer, L. J. 2000. Case-control association studies for the genetics of complex respiratory diseases. *American Journal of Respiratory Cell and Molecular biology*, **22,** 645-648.

Slagsvold, T. 1998. On the origin and rarity of interspecific nest parasitism in birds. *American Naturalist*, **152,** 264-272.

Smith, N. G. 1968. The advantage of being parasitized. *Nature*, **219,** 690-694.

Soler, J. J., Martínez, J. G., Soler, M. y Møller, A. P. 1999a. Genetic and geographic variation in rejection behaviour of cuckoo eggs by European magpie populations: an experimental test of rejecter-gene flow. *Evolution*, **53,** 947-956.

Soler, J. J., Martínez, J. G., Soler, M. y Møller, A. P. 2001. Coevolutionary interactions in a host-parasite system. *Ecology Letters*, **4,** 470-476.

Soler, J. J. y Soler, M. 2000. Brood-parasite interactions between great spotted cuckoos and magpies: a model system for studying coevolutionary relationships. *Oecologia*, **125,** 309-320.

Soler, J. J., Soler, M., Møller, A. P. y Martínez, J. G. 1995a. Does the great spotted cuckoo choose magpie hosts according to their parenting ability? *Behavioral Ecology and Sociobiology*, **36,** 201-206.

Soler, J. J., Soler, M., Pérez-Contreras, T., Aragón, S. y Møller, A. P. 1999b. Antagonistic antiparasite defenses: nest defense and egg rejection in the magpie host of the grest spotted cuckoo. *Behavioral Ecology*, **10,** 707-713.

Soler, J. J., Sorci, G., Soler, M. y Møller, A. P. 1999c. Change in host rejection behavior mediated by the predatory behavior of its brood parasite. *Behavioral Ecology*, **10,** 275-280.

Soler, M. 2001. Begging behaviour of nestlings and food delivery by parents: the importance of the breeding strategy. *Acta ethologica*, **4,** 59-63.

Soler, M. 2002. Breeding strategy and begging intensity: influences on food delivery by parents and host selection by parasitic cuckoos. In: *The evolution of begging. Competition, cooperation and communication* (Ed. by Wright, J. y Leonard, M. L.), pp. 413-427. Dordrecht (The Netherlands): Kluwer Academic.

Soler, M. y Martínez, J. G. 2000. Is egg-damaging behavior by great spotted cuckoos an accident or an adaptation? *Behavioral Ecology*, **11,** 495-501.

Soler, M., Martínez, J. G. y Soler, J. J. 1996. Effects of brood parasitism by the great spotted cuckoo on the breeding success of the magpie host: an experimental study. *Ardeola*, **43,** 87-96.

Soler, M., Martínez, J. G., Soler, J. J. y Møller, A. P. 1995b. Preferential allocation of food by magpie *Pica pica* to great spotted cuckoo *Clamator glandarius* chicks. *Behavioral Ecology and Sociobiology*, **37,** 7-13.

Soler, M. y Møller, A. P. 1990. Duration of sympatry and coevolution between the great spotted cuckoo and its magpie host. *Nature*, **343,** 748-750.

Soler, M. y Soler, J. J. 1991. Growth and development of great spotted cuckoos and their magpie host. *The Condor*, **93,** 49-54.

Soler, M., Soler, J. J. y Martínez, J. G. 1997. Great spotted cuckoos improve their reproductive success by damaging magpie host eggs. *Animal Behaviour*, **54,** 1227-1233.

Soler, M., Soler, J. J., Martínez, J. G. y Møller, A. P. 1995c. Chick recognition and acceptance. a weakness in magpies exploited by the parasitic great spotted cuckoo. *Behavioral Ecology and Sociobiology*, **37,** 243-248.

Soler, M., Soler, J. J., Martínez, J. G. y Møller, A. P. 1995d. Magpie host manipulation by great spotted cuckoos: evidences for an avian mafia? *Evolution*, **49,** 770-775.

Soler, M., Soler, J. J., Martínez, J. G. y Moreno, J. 1999d. Begging behaviour and its energetic cost in great spotted cuckoo and magpie host chicks. *Canadian Journal of Zoology*, **77,** 1794-1800.

Soler, M., Soler, J. J., Martínez, J. G., Pérez-Contreras, T. y Møller, A. P. 1998. Micro-evolutionary change and population dynamics of a brood parasite and its primary host: the intermitent arms race hypothesis. *Oecologia*, **117,** 381-390.

Sorenson, M. D. 1998. Patterns of parasitic egg laying and typical nesting in redhead and canvasback ducks. In: *Parastic birds and their hosts. Studies in coevolution* (Ed. by Rothstein, S. I. y Robinson, S. K.), pp. 357-375. New York, Oxford: Oxford University Press.

Sorenson, M. D. y Payne, R. B. 2002. Molecular genetic perspectives on avian brood parasitism. *Integrative and Comparative Biology*, **42,** 388-400.

Stokke, B. G., Moksnes, A. y Røskaft, E. 2002. Obligate brood parasites as selective agents for evolution of egg appearance in passerine birds. *Evolution*, **56,** 199-205.

Stokke, B. G., Moksnes, A., Røskaft, E., Rudolfsen, G. y Honza, M. 1999. Rejection of artificial cuckoo (*Cuculus canorus*) eggs in relation to variation in egg appearance among reed warblers (*Acrocephalus scirpaceus*). *Proceedings of the Royal Society of London, Series B*, **266,** 1483-1488.

Takasu, F. 1998. Modelling the arms race in avian brood parasitism. *Evolutionary Ecology*, **12,** 969-987.

Takasu, F. 2004. How many eggs should be laid in one's own nest and others' in intra-specific brood parasitism? *Population Ecology*, **46,** 221-229.

Takasu, F., Kawasaki, K., Nakamura, H., Cohen, J. E. y Shigesada, N. 1993. Modeling the population dynamics of a cuckoo-host association and the evolution of host defenses. *American Naturalist*, **142,** 819-839.

Tanaka, K. D. y Ueda, K. 2005. Horsfield's hawk-cuckoo nestlings simulate multiple gapes for begging. *Science*, **308,** 653.

Thompson, J. N. 1994. *The coevolutionary process*. Chicago: The University of Chicago Press.

Thompson, J. N. 1999a. Specific hypotheses on the geographic mosaic of coevolution. *American Naturalist*, **153,** S1-S14.

Thompson, J. N. 1999b. The evolution of species interactions. *Science*, **284,** 2116-2118.

Thompson, J. N., Nuismer, S. L. y Gomulkiewicz, R. 2002. Coevolution and maladaptation. *Integrative and Comparative Biology*, **42,** 381-387.

Tollrian, R. y Harvell, C. D. 1999. *The ecology and evolution of inducible defenses*. Princeton, New Yersey: Princeton University Press.

Trivers, R. L. 1974. Parent-offspring conflict. *American Zoologist*, **14,** 249-264.

Verhulst, S. y Wiersma, P. 1997. Is begging cheap? *Auk*, **114,** 134.

Vernon, C. J. 1987. Bill hooks of *Protodiscus* nestlings. *Ostrich*, **58,** 187.

Weathers, W. W., Hodum, P. J. y Anderson, D. J. 1997. Is the energy cost of begging by nestling passerines surprisingly low? *The Auk*, **114,** 133.

Wyllie, I. 1981. *The cuckoo*. London: Batsford.

Yamamura, N. y Higashi, M. 1992. An evolutionary theory of conflict resolution between relatives: altruism, manipulation, compromise. *Evolution*, **46,** 1236-1239.

Yamauchi, A. 1995. Theory of evolution of nest parasitism in birds. *American Naturalist*, **145,** 434-456.

Yom-Tov, Y. 2001. An updated list and some comments on the occurrence of intraspecific nest parasitism in birds. *Ibis*, **143,** 133-143.

Zahavi, A. 1979. Parasitism and nest predation in parasitic cuckoos. *American Naturalist*, **113,** 157-159.

Zamora-Muñoz, C., Ruano, F., Errard, C., Lenoir, A., Hefetz, A. y Tinaut, A. 2003. Coevolution in the slave-parasite system *Proformica longiseta* - *Rossomyrmex minuchae* (Hymenoptera : Formicidae). *Sociobiology*, **42,** 299-317.

I

A quantitative trait locus for recognition of foreign eggs in the host of a brood parasite

A quantitative trait locus for recognition of foreign eggs in the host of a brood parasite

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Abstract

Avian brood parasites reduce the reproductive output of their hosts and thereby select for defence mechanisms such as ejection of parasitic eggs. Such defence mechanisms simultaneously select for counter-defences in brood parasites, causing a coevolutionary arms race. Although coevolutionary models assume that defences and counter-defences are genetically influenced, this has never been demonstrated for brood parasites. Here, we give strong evidence for genetic differences between ejector and non-ejectors, which could allow the study of such host defence at the genetic level, as well as studies of maintenance of genetic variation in defences. Briefly, we found that magpies, that are the main host of the great spotted cuckoo in Europe, have alleles of one microsatellite locus (Ase64) that segregate between accepters and rejecters of experimental parasitic eggs. Furthermore, differences in ejection rate among host populations exploited by the brood parasite covaried significantly with the genetic distance for this locus.

Keywords.- *Avian inter-specific brood parasitism, Clamator glandarius, Coevolution, Egg rejection, Genetic marker, Host defences, Microsatellites, Pica pica.*

Resumen

Las aves parásitas de cría reducen el éxito reproductivo de sus hospedadores y consecuentemente seleccionan mecanismos defensivos tal como la expulsión de los huevos parásitos. Este mecanismo defensivo simultáneamente selecciona contradefensas en los parásitos de cría, causando una carrera de armamentos coevolutiva. Aunque los modelos coevolutivos asumen que las defensas y contradefensas están genéticamente influenciadas, esto nunca ha sido demostrado para los parásitos de cría. Aquí, damos una robusta evidencia de diferencias genéticas entre expulsores y no expulsores, lo cual podría permitir el estudio de la defensa del hospedador desde un nivel genético, además de estudios sobre el mantenimiento de la variación genética en las defensas. Brevemente, hemos encontrado que las urracas, que son el principal hospedador del críalo en Europa, poseen unos alelos de un microsatellite (Ase64) que diferencian entre aceptores de expulsores de huevos parásitos experimentales. Además, las diferencias en tasa de expulsión entre poblaciones de hospedador explotadas por el parásito de cría covariaron significativamente con las distancias genéticas calculadas para este locus.

Palabras Clave.- *Parasitismo de Cría interespecífico en Aves, Clamator glandarius, Coevolución, Expulsión de huevos, Marcador genético, Defensas del hospedador, Microsatélites, Pica pica.*

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Introduction

Coevolution is a widespread process in nature that, for instance, is believed to be partly responsible for biodiversity (Thompson 1999). Therefore, understanding the coevolutionary process is of great importance in evolutionary biology. Avian brood parasitism is one of the most well studied model systems of coevolutionary interactions, and it has been proposed as a model system for studying coevolutionary processes (Rothstein 1990). This is because avian brood parasites exert strong selection pressures on their hosts and defensive and counter-defensive mechanisms (e.g. foreign egg recognition and rejection by host and egg mimicry by parasites) are easily detected by humans (Rothstein 1990). Once host defensive phenotypes spread in the population, counter-defensive mechanisms in brood parasites are of a selective advantage, giving rise to a coevolutionary arms race between host and parasite (e.g., Davies 2000).

Cultural co-evolution cannot occur, then a prime condition is a genetic basis for defences and counter-defences, otherwise evolution will not occur (Fisher 1930). However, although some evidence is consistent with a genetic basis of host (Rothstein 1975, 1982, Briskie *et al.* 1992, Soler *et al.* 1999a) and parasite behaviour (Marchetti *et al.* 1998, Gibbs *et al.* 2000, Payne *et al.* 2002), genetic control and its extent have not been directly studied. Thus, since all models assume a genetic basis of traits involved in the process (see e.g., Takasu 1998, Robert *et al.* 1999, Servedio & Lander 2003), a study of the genetic determinants of anti-parasite behaviour by hosts is essential for testing a critical assumption of theory. Thus, if a marker of, for instance, the genetic capacity of rejection of parasitic eggs is found, it would greatly contribute to a better understanding of the coevolutionary process in general and that between brood parasites and their hosts in particular (Sorenson & Payne 2002).

Modern genetic techniques allow the study of associations between neutral genetic marker (such as microsatellites or single nucleotide polymorphisms (SNPs)) and phenotypes. The basic idea of association studies is that a marker close to a certain gene influencing on the target trait may also have allele frequency differences between individuals with and without the trait. This is because recombination is less frequent between loci that are close to each other. Therefore, a statistical association between genotypes at a marker locus and the phenotype is usually considered evidence of close physical linkage between the marker and the trait loci (Silverman &

Palmer 2000, Cardon & Bell 2001, Pritchard & Donnelly 2001). A priori, this methodology may seem too simplistic for the study of complex traits such as behavioural ones, because this approach assumes that genetic differences between individuals with and without the trait would be consequence of differences in expression of one or a few genes. However, recent evidence supports such an approach. Although a vast number of genes could be influencing the expression of a complex trait, only a few loci with large effects (major genes) would be responsible for most of the genetic variation between individuals related to that trait (see Fitzpatrick *et al.* 2005). Therefore, and by using examples implying behavioural traits, we could distinguish the role of *Gp-9* gene influencing social organization in the fire ant (*Solenopsis invicta*) (Ross & Keller 1998); or the genetic association between *Pgm* locus and male mating strategy in a marine isopod (*Paracerceis sculpa*) (Shuster & Sassaman 1997); or those studies showing the influence of the *for* genes on foraging behaviour in several species (reviewed in Fitzpatrick & Sokolowski 2004).

For purposes others than finding genetic marker of rejection behaviour (i.e., estimation of gene flow among sub-populations), we used a group of polymorphic genetic markers (microsatellites) that were tested for genetic association with rejection behaviour by using available specific statistical programs allowing comparison of allelic and genotypic frequencies of nestlings from nests that accepted experimental model eggs and those that rejected them. Therefore, we did not design a specific protocol to detect a genetic association and thus a possible genetic marker of ejection behaviour. However, since we find a significant genetic association with rejection behaviour of magpies (*Pica pica* Linnaeus, 1758) (see Results) breeding in southern Spain (Guadix) where they are extensively exploited by the brood parasitic great spotted cuckoo (*Clamator glandarius* Linnaeus, 1758) (for a detailed review of this coevolutionary system, see Soler & Soler 2000), we further test whether differences in rejection rates between different European magpie populations predict differences in allelic frequencies of the hypothetical marker, as should be case if the association reflects a true genetic marker (Sorenson & Payne 2002).

Methods

Study area

Fieldwork was conducted during the breeding seasons 2000–2001 in Hoya de Guadix (37°18'N, 3°11'W). The study area is located in south-eastern Spain, at approximately

1000 m a. s. l., and comprises several study plots that vary in size and ecological characteristics (for more detailed information on the Guadix study area, see Soler *et al.* 1998a,b).

In addition, we also used data and extracted DNA from others European magpie populations sampled for previous studies (Martínez *et al.* 1999, Soler *et al.* 1999a). However, mainly due to DNA degradation, some of the samples failed to produce PCR products and, thus, only a few individuals were successfully genotyped for some populations. To avoid spurious results, we arbitrarily used those populations with at least 10 genotyped individuals (i.e. nests). In total, we used DNA of nestlings from six sympatric populations and two allopatric populations (see Tables 1 and 2). These additional samples were genotyped only for one locus (Ase64, see below).

Field procedures

At the beginning of the breeding season, we systematically explored the study area looking for magpie nests before clutch completion. Once a nest was located, it was visited twice per week, and, when at least one magpie egg was detected in the nest, one experimental model egg was added. Model eggs were made with plaster of Paris and painted resembling those of great spotted cuckoos following the protocol described in (Soler & Soler 2000). We revisited the nests after six-seven days and, if the experimental model egg remained in the nest, the pair was classified as an accepter. Otherwise, if the model egg was absent from the nest, the pair was classified as an ejector. To avoid pseudo-replication, nests used in the analyses from 2001 were from magpie territories not controlled in 2000 (magpies are generally faithful to their chosen territory (Birkhead 1991)). We only used first breeding attempts because in replacement clutches rejection decisions of egg-recognizer magpies may change to accept experimental eggs due to retaliatory behaviour of the great spotted cuckoo (Soler *et al.* 1999b). Finally, when nestlings were about 18 days old, we took a blood sample from the brachial vein of magpie nestlings and stored it in 1ml of 100% ethanol. Afterwards, in order to use only independent samples, we randomly selected one unique nestling sample per magpie nest to perform the genetic analyses.

Laboratory work

Genomic DNA was isolated from blood using the ammonium-acetate precipitation method (adapted from Bruford *et al.* 1998). We used eleven polymorphic microsatellite loci. Four had previously been isolated from magpies: Ppi1, Ppi2, Ppi3 (Martínez *et al.* 1999) and Ppi4 (Martinez J.G., Dawson D.A. & Burke T., unpublished, EMBL accession number: PPI272377); three from Seychelles warbles (*Acrocephalus sechellensis*): Ase12, Ase18 and Ase64 (Richardson *et al.* 2000); one from indigo bird (*Vidua chalybeata)*: Indi28 (Sefc *et al.* 2001); one from western crowned-warbler (*Phylloscopus occipitalis*): Pocc1 (Bensch *et al.* 1997); and two from house sparrow (*Passer domesticus*): Pdo5 and Pdo6 (Griffith *et al.* 1999). Details of the primers used in this study can be found on the Sheffield Molecular Genetics Facility Passerine primer cross-utility database, accessed via http://www.shef.ac.uk/misc/groups/molecol/birdmarkers.html.

Both DNA isolation and genotyping for these 11 microsatellites for samples from Guadix were performed in England (see Acknowledgements) during 2002. Polymerase chain reactions (PCRs) were performed with the forward primer of each marker labelled with a fluorescence dye. The reaction profile for each locus was 94 ºC for 120 s, followed by 35 cycles of 94 ºC for 30 s, 51-60 ºC (depending of each locus) for 30 s, and 72 ºC for 30 s; and then 72 ºC for 5 min. Volume of PCR reaction were 10 µl, which containing around 10 ng of DNA, 1.0 µM of each primer, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$ and 0.05 units of *Taq* DNA polymerase, in the manufacturer's buffer. The PCR products were electrophoresed through an ABI Prism 377 DNA sequencer (Applied Biosystems). The outputs were analysed using ABI Genescan software (version 3.1.2) and Genotyper DNA fragment analysis software (version 2.5).

Genotyping of Ase64 microsatellite locus for magpie chicks from different European magpie populations was performed in France (see Acknowledgements) during 2003. The profile of the PCR reaction was as follow: 94 ºC for 120 s, followed by 35 cycles of 94 ºC for 15 s, 54 ºC for 15 s, and 72 ºC for 15 s; and followed by 72 ºC for 5 min. Volume of PCR reaction were 10 µl, which containing around 15-50 ng DNA, 0.2 µM of each primer, 0.3 mM of each dNTP, 1.5 mM $MgCl_2$ and 0.25 units of *Taq* DNA polymerase, in the manufacturer's buffer. The forward primer of Ase64 locus was also fluorescently labelled and the PCR products were electrophoresed through an ABI Prims 310 Genetic Analyser (Applied Biosystems). Output was analysed using ABI Genescan software (version 3.1.2). Ten samples from Guadix with genotypes for Ase64 locus known were genotyped together with these samples from different European magpie populations, thus controlling for possible differences between the two protocols of genotyping.

Statistical analyses

Allelic frequencies (Fisher exact test) and genotypic (log-likelihood (G) based exact test) differentiation analyses between individual accepters and ejectors from the Guadix population were conduced by using GENEPOP 3.3 software (Raymond & Rousset 1995). Since deviations from Hardy-Weinberg equilibrium may affect association studies (Pritchard & Rosenberg 1999, Silverman & Palmer 2000), we performed Hardy-Weinberg exact tests for each locus for accepter and ejector magpies by using GENEPOP 3.3 (Table 3). Unbiased p-values and standard errors for all above analyses were estimated by using Markov chain with 10,000 dememorizations, 1000 batches, and 10,000 iterations per batch. To check whether a certain allele of the Ase64 locus was significantly associated with acceptance or ejection phenotypes, we performed a Pearson χ^2 test with one degree of freedom considering the number of copies of such an allele in both accepters and ejector pairs against the sum of copies of the remaining alleles.

Since population structure as well as recent population admixture could be a problem for studies of association due to the possibility of character stratification (i.e. different subgroups differing in frequency of traits under investigation; see for instance, Pritchard & Donnelly 2001, Devlin *et al.* 2001, Cardon & Palmer 2003, Zondervan & Cardon 2004), we tested for stratification of ejection behaviour using STRAT 1.1 software (Pritchard *et al.* 2000, see also, Pritchard & Rosenberg 1999) and taking into account all loci except that associated with rejection behaviour. Briefly, this population stratification test performs a χ^2 test for each of the non-associated microsatellite loci where alleles with fewer than 10 copies were grouped. Differences between allelic frequencies of accepter and ejector individuals were then obtained by summing χ^2 values obtained for each microsatellite locus used. Degrees of freedom were estimated as the addition of those from the individual tests. A non-significant result would indicate non-stratification of ejection behaviour in our magpie population.

Table 1.– Matrix of genetic distances for Ase64 microsatellite locus (above the diagonal) and matrix of differences in ejection rates (below the diagonal) between populations with ten or more samples genotyped.

Populations	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	N_{Ase64}	Alleles	Ejection rate (%)
Les Camargues (1)	---	0.077	0.121	0.096	0.108	0.112	0.085	0.137	10	8	18.75
Torres del Segre (2)	19.35	---	0.074	0.034	0.038	0.026	0.065	0.083	14	11	38.10
Logroño (3)	43.75	24.41	---	0.049	0.031	0.056	0.056	0.084	13	8	62.50
Badajoz (4)	25.00	5.66	18.75	---	0.035	0.029	0.052	0.046	22	12	43.75
Guadix (5)	26.25	6.91	17.50	1.25	---	0.031	0.033	0.102	120	12	45.00
Doñana (6)	24.73	5.38	19.02	0.27	1.52	---	0.042	0.075	24	10	43.48
*Trondheim (7)	2.08	21.43	45.83	27.08	28.33	26.81	---	0.109	20	11	16.67
*Jyväskylä (8)	11.61	30.95	55.36	36.61	37.86	36.34	9.52	---	13	7	7.14

Ejection rate, number of analysed individuals and detected alleles per population are shown. Data about ejection behaviour are from Soler *et al.* (1999a) (*) refers to populations in allopatry with the great spotted cuckoo.

Table 2. – Matrix of genetic distances estimated from three neutral loci (above the diagonal) and matrix of geographic distances (below the diagonal) between magpie populations with ten or more samples genotyped.

Populations	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Les Camargues (1)	---	0.101	0.082	0.071	0.073	0.063	0.145	0.156
Torres del Segre (2)	375	---	0.094	0.079	0.099	0.107	0.124	0.144
Logroño (3)	550	225	---	0.046	0.072	0.057	0.154	0.112
Badajoz (4)	1100	700	565	---	0.039	0.038	0.142	0.168
Guadix (5)	950	575	560	385	---	0.050	0.157	0.178
Doñana (6)	1200	800	700	225	300	---	0.193	0.195
*Trondheim (7)	2175	2450	2400	2900	3000	3150	---	0.150
*Jyväskylä (8)	2425	2775	2825	3350	3350	3550	800	---

All values are from Soler *et al.* (1999a). (*) refers to populations in allopatry with the great spotted cuckoo.

Finally, to analyze the relationship between matrices of differences in ejection rates of European magpie populations differing in selection pressure from parasitism and matrix of genetic distances between those populations, estimated from the detected genetic marker, we first used GENEPOP 3.3 to generate the allelic frequencies for the Ase64 locus per population. These allelic frequencies were used by GENDIST, included in PHYLIP 3.57c software package (Felsenstein 1993), providing a matrix of genetic distances (Cavalli-Sforza's chord distance) for Ase64 (Table 1). We also used previously published matrices of geographic distances and genetic distances estimated from three neutral loci for the same magpie populations (Table 2), trying to control for possible geographic or genetic effects. Finally, to explore the relationships between matrices, we used Mantel's and partial Mantel's tests implemented in FSTAT 2.9.3 software (Goudet 2002), with p-values estimated after 2000 randomizations. The matrix of differences in ejection rate was used as dependent variable and all the others as independent variables (for similar analyses see e.g. Soler *et al.* 2001).

To control for type-I error in multiple tests, we used a modified Bonferroni adjustment. This is because dividing alpha-value by the number of microsatellites used would be too conservative, because loci are not entirely independent in the genome (see e.g., Silverman & Palmer 2000, Cardon & Bell 2001, Freimer & Sabatti 2004). We then assigned α-value to half of that estimated from Bonferroni correction because probability of detecting type-I error for associated markers was half of that estimated for markers in equilibrium (Cardon & Bell 2001). Therefore, alpha-value for our analyses with 11 potential markers was assigned to 0.009. It should be mentioned here that because our genetic samples are from nestlings and not from adults, this level of critical alpha-value is conservative (see below).

Results

Allelic frequencies of Ase64 microsatellite (Richardson *et al.* 2000) significantly differed for magpie nestlings coming from accepter (N = 55) and ejector (N = 45) nests (Table 3). Further, genotypic frequencies of accepters and ejectors also differed for Ase64 microsatellite locus (Table 3). Those differences were mainly due to two alleles, 429bp and 457bp, the former being more frequent in accepter (χ^2_1 = 11.27, P < 0.001, Fig. 1) and the latter in rejecter magpie nests (χ^2_1 = 8.56, P = 0.003, Fig. 1).

Table 3.– Genetic comparisons and Hardy-Weinberg exact tests (HW tests) for each microsatellite locus between accepter and ejector magpies from Guadix.

Locus	Number of alleles	Allelic frequencies	Genotypic frequencies	HW tests	
				Accepters	Ejectors
Ppi1	10	0.03 (<0.001)	0.12 (<0.001)	*<0.001 (<0.001)	0.10 (0.001)
Ppi2	19	0.49 (0.002)	0.43 (0.001)	0.91 (0.002)	0.90 (0.002)
Ppi3	8	0.42 (0.001)	0.39 (<0.001)	0.42 (0.002)	0.50 (0.001)
Ppi4	5	0.31 (<0.001)	0.46 (0.001)	*<0.001 (<0.001)	*<0.001 (<0.001)
Ase12	2	1.00 (<0.001)	1.00 (<0.001)	0.26	0.66
Ase18	12	0.25 (0.001)	0.27 (0.001)	0.21 (0.001)	0.18 (0.002)
Ase64	12	*0.002 (<0.001)	*0.008 (<0.001)	0.61 (0.003)	0.09 (0.002)
Pocc1	7	0.05 (<0.001)	0.17 (<0.001)	*0.001 (<0.001)	*<0.001 (<0.001)
Pdo5	7	0.17 (<0.001)	0.18 (<0.001)	0.91 (0.001)	0.77 (0.001)
Pdo6	2	0.41 (<0.001)	0.50 (<0.001)	*0.001	0.07
Indi28	4	0.74 (<0.001)	0.77 (<0.001)	0.06	0.01

Results are p-values (S.E.). Asterisks indicate significant results after correction for multiple testing

Those results were not due to stratification of ejection behaviour (see Methods), neither genetically because STRAT software did not detect any stratification (χ^2_{38} = 47.50, P = 0.14), nor geographically because frequency of ejectors and accepters in each

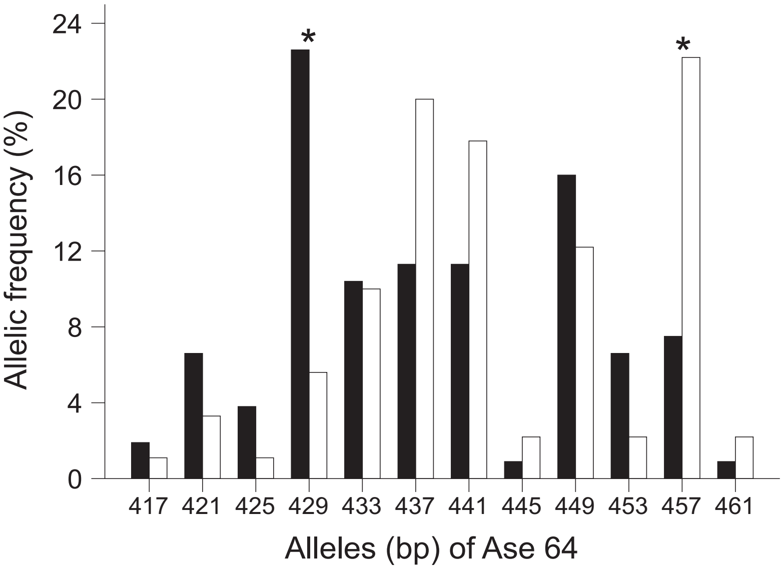

Figure 1.- *Allelic frequencies (%) for Ase64 microsatellite locus of nestlings coming from accepter and ejector nests. Symbols refer to alleles whose frequencies significantly differed between accepters and ejectors.*

sub-zone did not differ from that in the entire Guadix population ($\chi^2_6 = 4.69$, $P = 0.58$).

Moreover, in accordance with the prediction of Ase64 locus being a genetic marker of egg ejection behaviour, we found that genetic distance between populations estimated for this locus explained differences in ejection frequencies among host populations sympatric with cuckoos (N = 6, Fig. 2). That was the case even after controlling for genetic distances (from Soler *et al.* (1999a), Table 2) (Partial Mantel's tests, partial correlation coefficient (Ase64 locus) = 0.85, $P < 0.001$, $R^2 = 0.79$) or geographic distances (Table 2) (Partial Mantel's tests, partial correlation coefficient (Ase64 locus) = 0.81, $P = 0.001$, $R^2 = 0.79$). However, when also including allopatric populations (N = 2, Table 1), genetic distances estimated from Ase64 no longer explained differences in ejection rate between populations, neither alone (Mantel's test, $r = 0.35$, $P = 0.08$, $R^2 = 0.12$), even after controlling for genetic distance for the three neutral loci in a partial correlation analysis (Partial Mantel's tests, partial correlation coefficient (Ase64 locus) = 0.27, $P = 0.19$, $R^2 = 0.26$), nor when controlling for geographic distance between populations (Partial Mantel's tests, partial correlation coefficient (Ase64 locus) = 0.26, $P = 0.18$, $R^2 = 0.4$).

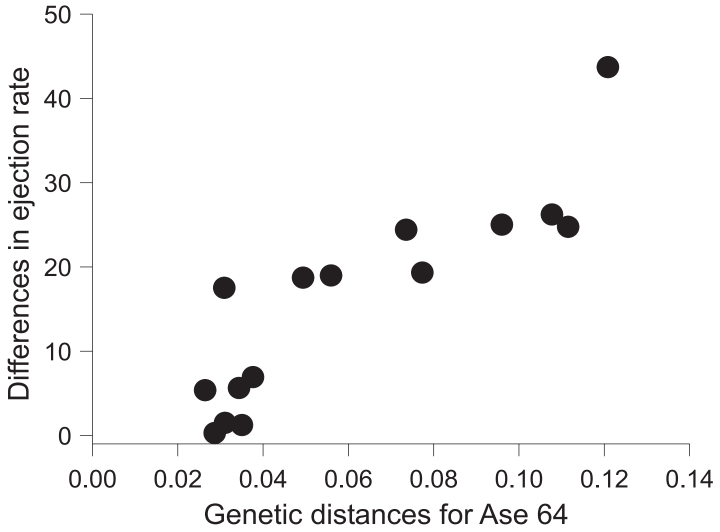

Figure 2.- *Relationship between differences in ejection rate (%) and genetic distances for the Ase64 locus for sympatric populations (N = 15 differences between 6 populations) (Mantel's test: $r = 0.88$, $P < 0.001$, $R^2 = 0.78$).*

These results were not biased due to a larger sample size and/or a larger number of alleles detected for the Guadix population compared to that obtained for other magpie populations (see Table 1) because 10 repetitions of the analyses, using randomly selected subsamples from Guadix (with approximately the average sample size used for the other populations being 17 (range of subsamples 12-22, mean 16.9)), gave similar results (Table 4). Therefore, these results strongly suggest an association between Ase64 and rejection behaviour in magpie populations in sympatry with the brood parasite.

Table 4.– Test of the relationship between genetic marker and frequency of ejection using ten different random samplings from Guadix either, when only sympatric populations (A) or all populations (B) are included in the analyses.

Test	R	P	P_{max}	P_{min}	R^2
A	0.77 (0.03)	*0.004 (0.002)	0.018	0.0005	0.59 (0.04)
After controlling for genetic distances	0.74 (0.03)	*0.005 (0.002)	0.025	0.0005	0.62 (0.04)
After controlling for geographic distances	0.68 (0.03)	*0.01 (0.004)	0.042	0.0005	0.60 (0.04)
B	0.29 (0.01)	0.140 (0.018)	0.257	0.056	0.08 (0.01)
After controlling for genetic distances	0.22 (0.01)	0.257 (0.017)	0.325	0.165	0.23 (0.01)
After controlling for geographic distances	0.22 (0.01)	0.268 (0.020)	0.381	0.171	0.38 (0.01)

Partial Mantel's test was used when statistically controlling for genetic distances from neutral loci, or when controlling for geographic distances. All values are averages (S.E.) except those indicating the maximum (P_{max}) and minimum p-values (P_{min}). Asterisks indicate the significant results.

Discussion

We found strong statistical association between one (Ase64) of the 11 microsatellite loci analysed and ejection behaviour of mimetic model eggs in magpies. This association was mainly caused by only two alleles, one more frequent in rejecter magpies (457 bp allele, Fig. 1) and another more frequent in accepter magpies (429 bp allele, Fig. 1). The detected association could however appear because of a possible genetic stratification due to gene flow of rejecter magpies from other populations (Soler *et al.* 1999a). If that were the case, rejecter magpies would be genetically different from accepters due to accepters and rejecters being from different ancestral populations, rather than to any physical association between loci. However, we can discard this possibility because we did not find any evidence of genetic stratification in our study population (see Results).

Another alternative explanation is based on geographical stratification of rejection behaviour associated with a relationship between genetic and geographical distances. Our study area is composed of several geographically isolated sub-zones, with different ecological conditions (Soler *et al.* 1998a), which could influence phenotypic expression of hypothetical egg-recognition genes (e.g., Davies *et al.* 1996, Brooke *et al.* 1998). However, rejection rates of particular sub-zones did not differ from that estimated for the entire Guadix population (see Results). Moreover, we found differences in both ejection behaviour and genetic distances for Ase64 locus between sub-zones of Guadix were explained neither by geographic nor by neutral genetic distances. Moreover, we did not found any genetic structure in magpie metapopulation of Guadix, indicating that magpie subpopulations are quite interconnected between them by gene flow (unpublished data).Therefore, a true genetic association between Ase64 locus and gene(s) involved in ejection behaviour of parasitic eggs is the most likely explanation, suggesting physical linkage in the genome.

Moreover, when analysing data from different European magpie populations in sympatry with the great spotted cuckoo, we found a significant positive relationship between population differences in rejection rates and genetic distances estimated for Ase64 microsatellite locus, even after controlling for geographic or genetic distances (from neutral microsatellite loci) (see Results and Fig. 2). However, that was not the case when including data from the two allopatric populations in the analyses. These different results, far from being contrary to the hypothesis of Ase64 microsatellite locus being a genetic marker of ejection behaviour, are what should be expected. Selection pressure due to brood parasitism only occurs in host populations that co-occur with parasites and, therefore, the spread of a mutation allowing recognition together with the ancestral genetic environment closest to the mutant gene (i.e. 457bp allele of Ase64 locus) would only occur in magpie populations subject to brood parasitism, giving rise to the detected association between the 457bp allele and ejection behaviour (E' individuals, Fig. 3). Instead, in magpie populations allopatric with the cuckoo, due to gene flow from sympatric populations (Soler *et al.* 1999a), there would also be individuals with both the genetic variant influencing on ejection of parasitic eggs and the 457bp allele of Ase64 (E' individuals, Fig. 3). However, because no selection pressure favours these ejector mutants in allopatric populations (as far as we know, there is no evidence of intraspecific brood parasitism in Eurasian magpies), relative frequencies of non-ejector vs. ejector individuals, all holding the 457bp allele of Ase64 locus (A'/E', Fig. 3), should be higher in allopatric than in sympatric population. This larger relative frequency of non-ejector individuals holding the 457bp allele of Ase64 locus (A' individuals, Fig. 3) in allopatric populations would mask the detected relationship between population differences in ejection rates and genetic distances estimated from the genetic marker for sympatric magpie populations (Fig. 3).

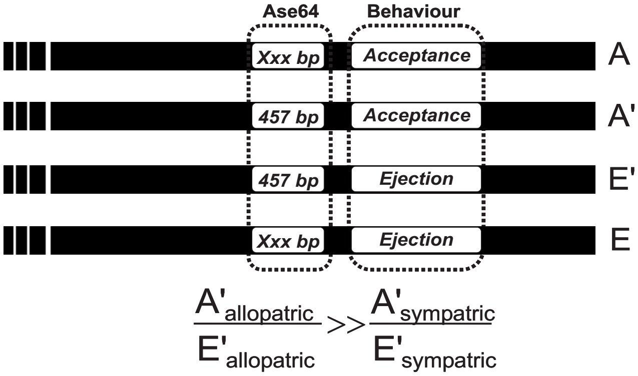

Figure 3.- *Genotypes for Ase64 microsatellite locus and the hypothetical gene involved in ejection behaviour. Xxx bp indicates any allele of Ase64 locus except the 457bp allele. Our results suggest that E' individuals descended from a mutation in an A' individual. The difference in proportions of E' and A' in sympatric and allopatric populations would be responsible for lack of association when allopatric populations are included in the analysis.*

All our results could be considered conservative because we used genetic data from chicks (one per nest) but not from parents, due to logistic problems of capturing adults, losing information due to segregation of the hypothetical genetic marker of ejection behaviour in the offspring. This is because there is no reason to think that mating preferences would be systematically biased and, thus, individual ejectors could be equally mated either with an accepter or with an ejector, and vice versa. Similarly, phenotypic plasticity due to the associated costs to cuckoo egg recognition and rejection detected for this character (Lotem *et al.* 1995, Álvarez 1996, Brooke *et al.* 1998, Soler *et al.* 1999a) would partially mask the relationship between genetic marker and phenotypes, making our findings more robust. However, we cannot rule out the possibility that the genetic association detected here includes a genetic basis for a phenotypically plastic decision rule, rather than a genetic basis of such ability to recognize and to reject foreign eggs.

Nothing is known about the genetic mechanism underlying ejection behaviour. It is possible that the genes involved in ejection behaviour in magpies are (or not) the same than those in other species suffering brood parasitism. Therefore, similar studies in other host species could be successful giving crucial information about the genetic control of this behavioural trait. At the other hand and due to this possibility, some caution should be taken when considering the neutrality of Ase64 in previous and futures population genetic studies in these host species.

The results presented here should be considered as strong evidence for a genetic influence on egg recognition and ejection, and thus validating a critical assumption of studies on coevolution between hosts and brood parasites. This evidence, however, does not mean that Ase64 was the genetic marker of recognition and ejection of foreign eggs in magpies, which would require further efforts and studies. For instance, the distribution of Ase64 alleles did not completely predict ejection behaviour of magpies and, although it could be a consequence of using genotypes of nestlings rather than those of adults, we cannot rule out the possibility of some other alleles of Ase64 (apart from 429bp and 457bp) and/or even other markers close to other genes acting on expression of this defensive trait apart from that hypothesized to be close to Ase64 locus, were important predictions of the complex behaviour of foreign egg recognition and rejection. In any case, more studies of this locus acting as a genetic marker of ejection behaviour of foreign eggs would be needed to develop a crucial genetic tool in order to test important evolutionary predictions: (*i*) tracking fitness consequences of ejector and non-ejector host genotypes under different environmental conditions; (*ii*) studying mechanisms explaining the maintenance of genetic variation in the host; and (*iii*) studying environmental conditions affecting phenotypic expression of gene(s) related to ejection of parasitic eggs, which is a plastic trait (Soler *et al.* 1999b) that affects the coevolutionary process between brood parasites and their hosts (Davies 2000).

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References

Álvarez, F. 1996. Model cuckoo *Cuculus canorus* eggs accepted by Rufous bush chats *Cercotrichas galactotes* during the parasite's absence from the breeding area. *Ibis*, **138,** 340-342.

Bensch, S., Price, T. & Kohn, J. 1997. Isolation and characterization of microsatellite loci in a *Phylloscopus* warbler. *Molecular Ecology*, **6,** 91-92.

Birkhead, T. R. 1991. *The Magpies. The ecology and behaviour of black-billed and yellow-billed magpies*. London: T & A D Poyser.

Briskie, J. V., Sealy, S. G. & Hobson, K. A. 1992. Behavioural defenses against avian brood parasitism in simpatric and allopatric host populations. *Evolution*, **46,** 334-340.

Brooke, M. de L., Davies, N. B. & Noble, D. G. 1998. Rapid decline of host defenses in response to reduced cuckoo parasitism - behavioral flexibility of reed warblers in a changing world. *Proceedings of the Royal Society of London, Series B*, **265,** 1277-1282.

Bruford, M. W., Hanotte, O., Brookfield, J. F. Y. & Burke, T. 1998. Multilocus and single-locus DNA fingerprinting. In: *Molecular genetic analysis of populations: a practical approach* (Ed. by Hoelzel, A. R.), pp. 287-336. Oxford: IRL Press.

Cardon, L. R. & Bell, J. I. 2001. Association study designs for complex diseases. *Nature Reviews Genetics*, **2,** 91-99.

Cardon, L. R. & Palmer, L. J. 2003. Population stratification and spurious allelic association. *The Lancet*, **361,** 598-604.

Davies, N. B. 2000. *Cuckoos cowbirds, and others cheats*. London: T & A D Poyser.

Davies, N. B., Brooke, M. de L. & Kacelnik, A. 1996. Recognition errors and probability of parasitism determine whether reed warblers should accept or reject mimetic cuckoo eggs. *Proceedings of the Royal Society of London, Series B*, **263,** 925-931.

Devlin, B., Roeder, k. & Bacanu, S.-A. 2001. Unbiased methods for population-based association studies. *Genetic Epidemiology*, **21,** 273-284.

Felsenstein, J. 1993. PHYLIP (Phylogeny Inference Package). *ver. 3.57c* (Seatle)

Fisher, R. A. 1930. *The genetical theory of natural selection*. Oxford: Oxford University Press.

Fitzpatrick, M. J., Ben-Shahar, Y., Smid, H. M., Vet, L. E. M., Robinson, G. E. & Sokolowski, M. B. 2005. Candidate genes for behavioural ecology. *Trends in Ecology and Evolution*, **20,** 96-104.

Fitzpatrick, M.J. & Sokolowski, M.B. 2004. In search of food: exploring the evolutionary link between cGMP-dependent protein kinase (PKG) and behaviour. *Integrative and Comparative Biology,* **44**: 28-36.

Freimer, N. & Sabatti, C. 2004. The use of pedigree, sib-pair and association studies of common diseases for genetic mapping and epidemiology. *Nature genetics*, **36,** 1045-1051.

Gibbs, H. L., Sorenson, M. D., Marchetti, K., Brooke, M. de L., Davies, N. B. & Nakamura, H. 2000. Genetic evidence for female host-specific races of the common cuckoo. *Nature*, **407,** 183-186.

Goudet, J. 2002. FSTAT, a program to estimate and test gene diversities and fixation indices. *ver. 2.9.3.* (Switzerland)

Griffith, S. C., Stewart, I. R. K., Dawson, D., Owens, I. P. F. & Burke, T. 1999. Constrasting levels of extra-pair paternity in mainland and island populations of a socially monogamous bird, the house sparrow (*Passer domesticus)*: is there an "island effect"? *Biological Journal of the Linnean Society*, **68,** 303-316.

Lotem, A., Nakamura, H. & Zahavi, A. 1995. Constraints on egg discrimination and cuckoo-host co-evolution. *Animal Behaviour*, **49,** 1185-1209.

Marchetti, K., Nakamura, H. & Gibbs, H. L. 1998. Host-race formation in the common cuckoo. *Science*, **282,** 471-472.

Martínez, J. G., Soler, J. J., Soler, M., Møller, A. P. & Burke, T. A. 1999. Comparative population structure and gene flow of a brood parasite, the Great Spotted Cuckoo (*Clamator Glandarius*), and its primary host, the magpie (*Pica pica*). *Evolution*, **53,** 269-278.

Payne, R. B., Hustler, K., Stjernstedt, R., Sefc, K. M. & Sorenson, M. D. 2002. Behavioural and genetic evidence of a recent population switch to a novel host species in brood-parasitic indigobirds Vidua chalybeata. *Ibis*, **144,** 373-383.

Pritchard, J. K. & Donnelly, P. 2001. Case-control studies of association in structured or admixed populations. *Theoretical Population Biology*, **60,** 227-237.

Pritchard, J. K. & Rosenberg, N. A. 1999. Use of unlinked genetic markers to detect population stratification in association studies. *American Journal of Human Genetics*, **65,** 220-228.

Pritchard, J. K., Stephens, M., Rosenberg, N. A. & Donnelly, P. 2000. Association mapping in structured populations. *American Journal of Human Genetics*, **67,** 170-181.

Raymond, M. & Rousset, F. 1995. Population genetics software for exact tests and ecumenicism. *J. Heredity*, **86,** 248-249.

Richardson, D. S., Jury, F. L., Dawson, D., Salgueiro, P., Komdeur, J. & Burke, T. 2000. Fifty Seychelles warbler (*Acrocephalus sechellensis*) microsatellite loci polymorphic in Sylviidae species and their cross-species amplification in other passerine birds. *Molecular Ecology*, **9,** 2155-2234.

Robert, M., Sorci, G., Møller, A. P., Hochberg, M. E., Pomiankowski, A. & Pagel, M. 1999. Retaliatory cuckoos and the evolution of resistance to brood parasites. *Animal Behaviour*, **58,** 817-824.

Ross, K.G. & Keller, L. 1998. Genetic control of social organization in an ant. *Proceedings of the Natural Academy of Sciences, USA,* **95**, 14232-14237.

Rothstein, S. I. 1975. Evolutionary rates and host defenses against avian brood parasitism. *American Naturalist*, **109,** 161-176.

Rothstein, S. I. 1982. Successes and Failures in Avian Egg and Nestling Recognition with Comments on the Utility of Optimality Reasoning. *Am. Zool.*, **22,** 547-560.

Rothstein, S. I. 1990. A model system for coevolution: avian brood parasitism. *Annual Review of Ecology and Systematics*, **21,** 481-508.

Sefc, K. M., Payne, R. B. & Sorenson, M. D. 2001. Characterisation of microsatellite loci in village indigobirds *Vidua chalybeata* and cross-species amplification in estrildid and ploceid finches. *Molecular Ecology Notes*, **1,** 252-254.

Servedio, M. R. & Lander, R. 2003. Coevolution of an avian host and its parasitic cuckoo. *Evolution*, **57,** 1164-1175.

Shuster, S.M. & Sassaman, C. 1997. Genetic interaction between male mating strategy and sex ratio in a marine isopod. *Nature,* **388**, 373-377.

Silverman, E. K. & Palmer, L. J. 2000. Case-Control Association Studies for the Genetics of Complex Respiratory Diseases. *American Journal of Respiratory Cell and Molecular Biology,* **22,** 645-648.

Soler, J. J., Martínez, J. G., Soler, M. & Møller, A. P. 1999a. Genetic and geographic variation in rejection behaviour of cuckoo eggs by European magpie populations: an experimental test of rejecter-gene flow. *Evolution*, **53,** 947-956.

Soler, J. J., Martínez, J. G., Soler, M. & Møller, A. P. 2001. Coevolutionary interactions in a host-parasite system. *Ecology Letters*, **4,** 470-476.

Soler, J. J. & Soler, M. 2000. Brood-parasite interactions between great spotted cuckoos and magpies: a model system for studying coevolutionary relationships. *Oecologia*, **125,** 309-320.

Soler, J. J., Sorci, G., Soler, M. & Møller, A. P. 1999b. Change in host rejection behavior mediated by the predatory behavior of its brood parasite. *Behavioral Ecology*, **10,** 275-280.

Soler, M., Soler, J. J. & Martínez, J. G. 1998a. Duration of sympatry and coevolution between the great spotted cuckoo (*Clamator glandarius*) and its primary host, the magpie (*Pica pica*). In: *Parasitic Birds and their hosts, studies in coevolution* (Ed. by Rothstein, S. I. & Robinson, S. K.), pp. 113-128. Oxford: Oxford University Press.

Soler, M., Soler, J. J., Martínez, J. G., Pérez-Contreras, T. & Møller, A. P. 1998b. Micro-evolutionary change and population dynamics of a brood parasite and its primary host: the intermitent arms race hypothesis. *Oecologia*, **117,** 381-390.

Sorenson, M. D. & Payne, R. B. 2002. Molecular genetic perspectives on avian brood parasitism. *Integrative and Comparative Biology*, **42,** 388-400.

Takasu, F. 1998. Modelling the arms race in avian brood parasitism. *Evolutionary Ecology*, **12,** 969-987.

Thompson, J. N. 1999. Specific hypotheses on the geographic mosaic of coevolution. *American Naturalist*, **153,** S1-S14.

Zondervan, K. T. & Cardon, L. R. 2004. The complex interplay among factors that influence allelic association. *Nature Reviews Genetics*, **5,** 89-101.

II

Environmental and genetic components of rejection behaviour of foreign eggs in magpies: the importance of using genetic markers in coevolutionary studies

Environmental and genetic components of rejection behaviour of foreign eggs in magpies: the importance of using genetic markers in coevolutionary studies.

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Abstract

Empirical evidences have been reported for those two hypotheses explaining the persistence of non-rejecter individuals in host species of brood parasites. The evolutionary lag hypothesis poses that the presence of non-defensives is due to a time lag in the evolution of rejection. In contrast, the evolutionary equilibrium hypothesis assumes that egg rejection is costly, with egg acceptance being the best option in some situations. Here, for the first time, we use a genetic marker of rejection behaviour in magpies in order to discern between both hypotheses. We found that although rejection rate covaried significantly with those variables assumed to influence rejection costs (risk of parasitism and laying date), this result was consequence of a differential abundance of individuals that are genetically able to reject. This scenario predicts an increase in defensive level as the duration of coevolutionary interaction between magpies and cuckoos increases, such as the evolutionary lag hypothesis asserts.

Keywords.- *Avian inter-specific brood parasitism, Clamator glandarius, Coevolution, Egg rejection, Evolutionary lag hypothesis, Evolutionary equilibrium hypothesis, Genetic marker, Host defences, Pica pica.*

Resumen

Se han encontrado evidencias empíricas apoyando las dos hipótesis que explican la persistencia de individuos no expulsores en especies hospedadores de parásitos de cría. La hipótesis del retraso evolutivo afirma que la presencia de no defensivos es debido a un retraso temporal en la evolución de la expulsión. Contrariamente, la hipótesis del equilibrio evolutivo asume que la expulsión es costosa, siendo la aceptación de huevos la mejor opción en algunas situaciones. Aquí, por primera vez, utilizamos un marcador genético del comportamiento de expulsión en urracas para discernir entre ambas hipótesis. Encontramos que aunque la tasa de expulsión covarió significativamente con aquellas variables asumidas a estar influenciando los costes de la expulsión (riesgo de parasitismo y fecha de puesta), este resultado fue la consecuencia de diferencias en abundancia de individuos que son capaces genéticamente de expulsar. Este escenario predice un incremento en el nivel de defensas conforme la duración de la interacción coevolutiva entre urracas y críalos incrementa, tal como la hipótesis del retraso evolutivo afirma.

Palabras Clave.- *Parasitismo de cría interespecífico en aves, Clamator glandarius, Coevolución, Expulsión de huevos, Hipótesis del retraso evolutivo, Hipótesis del equilibrio evolutivo, Marcador genético, Defensas del hospedador, Pica pica.*

Submitted

Introduction

The ability to recognize and reject eggs with odd appearance is considered as one of the main defences against avian brood parasitism (Payne 1977, Rothstein 1990). It has been demonstrated that it is a direct consequence of the selection pressure exerted by brood parasites (see e.g. Davies & Brooke 1989a, Rothstein & Robinson 1998, Davies 2000) which, in turn, selects for counter-defences in brood parasites, such as parasitic eggs mimicking the appearance of host eggs (Payne 1977, Davies & Brooke 1988, Rothstein 1990, Moksnes & Røskaft 1995). Although a proper rejection of any parasitic egg by hosts would result in great benefits (Rothstein 1990), there are many host species that lack this defence (mainly cowbird host species, e.g. Rothstein 1975b, 1982). Moreover, even for species able to recognize and reject parasitic eggs, many individuals accept them and, apparently, defensive phenotypes do not replace non-defensive individuals (see e.g. Rothstein & Robinson 1998).

Two hypotheses have been proposed to explain the absence of recognition and rejection in some host species, and the persistence of non-rejecters phenotypes in populations and species where rejection has evolved: the evolutionary lag hypothesis (ELH), and the evolutionary equilibrium hypothesis (EEH). The former explains the presence of accepter individuals or species simply arguing that hosts have had not enough time to evolve the recognition capacity and/or to spread it (Rothstein 1975a, Dawkins & Krebs 1979, Davies & Brooke 1989b, Moksnes *et al.* 1990). Instead, the EEH suggests that acceptance is maintained in host populations because there are costs associated with rejection behaviour (such as recognition errors, or breakage of some host eggs when trying to evict the parasitic egg) that counteract benefits (Rohwer & Spaw 1988, Brooker & Brooker 1990, Lotem *et al.* 1992, Marchetti 1992, Lotem & Nakamura 1998). Because there are evidences supporting these two hypotheses, it has been suggested that both could be right, although they could be acting at different levels, temporally and/or spatially (Davies *et al.* 1996, Soler *et al.* 1998b, Davies 1999, Servedio & Lander 2003). For instance, an ongoing coevolutionary process is more likely detected when studying variation in phenotypic frequencies at large temporal or spatial scales, mainly because the probability of detecting an effect size large enough to reach statistical significance increases as the temporal (i.e. evolutionary time) or spatial (i.e. larger differences in parasitism selection pressure and or evolutionary time) scales increase. On the other hand, at small temporal or spatial scales, evidences of an evolutionary equilibrium between hosts and parasites, due to costs associated with defensive traits, are more likely detected. This is because it is known that rejection ability is a plastic phenotypic trait whose expression depends on several environmental factors influencing on egg rejection costs, such as risk of parasitism (Davies & Brooke 1988, Moksnes *et al.* 2000, Álvarez 1996, Bártol *et al.* 2002) or host phenotypic quality (i.e. age, Lotem *et al.* 1992, Lotem *et al.* 1995, Rodríguez-Gironés & Lotem 1999).

Variation in rejection capacity is observed among populations of the same host species varying in level of parasitism selection pressure, and is interpreted as the result of differences in the duration of time of coevolutionary interactions and, thus, in accordance with the evolutionary lag hypothesis (Soler & Møller 1990, Briskie *et al.* 1992, Soler *et al.* 2001). However, non-defensive individuals may be present in different populations independently of parasitism selection pressure, and its frequency may depend, not only on the time of sympatry with parasites, but also on environmental conditions such as risk of parasitism and host phenotypic quality (see above), which would be in accordance with the evolutionary equilibrium hypothesis. Moreover, because those non-defensive individuals may also come from non-parasitized populations (e.g. Nuismer *et al.* 1999, Røskaft *et al.* 2002), an estimation of gene flow among these populations is necessary to distinguish between the two hypotheses explaining the presence of non-defensive phenotypes (see Soler *et al.* 2001).

However, even after controlling for gene flow among populations, a positive relationship between risk of parasitism and frequencies of defensive phenotypes can be explained by the evolutionary response to parasitism selection pressure (in accordance with the ELH), but also because the phenotypic expression of defences may be dependent on level of parasitism selection pressure (i.e. inducible defences) (in accordance with the EEH). In this case, only the use of a genetic marker of the defensive trait allowing to discern between the phenotypic expressions of rejection gene(s) (which may depend on environmental conditions) and the genetic ability to reject foreign eggs would permit testing different predictions from the above hypotheses (see e.g. Lotem & Nakamura 1998, Sorenson & Payne 2002).

We have recently found strong evidence supporting the microsatellite locus Ase64 (Richardson *et al.* 2000) as a genetic marker of egg ejection ability in magpies (*Pica pica* Linnaeus, 1758) (Martín-Gálvez *et al.* 2005, **MS I**). In particular, we have found that Ase64 has alleles that segregate between accepters and rejecters of experimental parasitic eggs; and this result was mainly due to two alleles, 429bp and 457bp, the former being more frequent in accepter and the latter in rejecter magpies. Furthermore, differences in rejection rate among host populations exploited by the brood parasite covaried significantly with the genetic distance for this locus. Therefore, for the first time, we use here this genetic marker of rejection ability of magpies, the main host of great spotted cuckoos (*Clamator glandarius* Linnaeus, 1758) in Europe, to explore the importance of environmental (i.e. parasitism risk) and phenotypic conditions (estimated as laying date, see e.g. Birkhead 1991, Soler *et al.* 1995) in explaining rejection rates of different magpie sub-populations within the well studied Guadix magpie population, (see Soler & Soler 2000, for a detailed review about general characteristics and coevolutionary interactions between both bird species). Additionally, we used 10 neutral microsatellite loci to study whether geographically isolated plots reflect genetically structured sub-populations and, thus, with a limited gene flow among them allowing us to discuss results on a well-established coevolutionary scenario.

In particular, we first study whether geographically isolated sub-population (see Fig 1) were also, at some degree, genetically isolated (i.e. genetically differentiated) because it would affect the coevolutionary process (see Thompson 1994, Nuismer *et al.* 1999). Secondly, we study the influence of genetic (i.e. genetic marker: 457bp of Ase64) and environmental (risk of parasitism and laying date) factors on rejection rates of magpies at different sub-populations, while controlling one by the other in a multiple autocorrelation analysis. The 457bp allele of Ase64 is associated to egg ejection ability (see above), thus, the differences in allelic frequencies of 457bp should explain differences in rejection ability between sub-populations. Moreover, to explore the influence of environmental factors (i.e. parasitism rate and laying date) on rejection behaviour of magpies, we include information on the allelic frequencies of the 457bp for each of the studied sub-populations. This is important because, for instance, in the case of parasitism rates being related to rejection rates, the question to distinguish between the EEH and the ELH would be whether parasitism rate is a signal used by magpies to make decisions on egg rejection (i.e. a plastic response), or whether it indicates level of parasitism selection pressure. The former interpretation would support an evolutionary equilibrium, while the later would indicate an ongoing coevolutionary arms race between magpies and great spotted cuckoos because natural selection (i.e. parasitism selection pressure) is the process explaining the differences in defensive-phenotypic frequencies between magpie sub-populations. Therefore, only the inclusion of the genetic marker of rejection behaviour would help us to differentiate between these two hypotheses.

Methods

Study Area

Magpie population of Guadix (37º18' N, 3º11' W, southern Spain) is sited in a high-altitude plateau (approx. 1000 m a.s.l.) conformed by little lands dedicated to cultivation of cereals (specially barley) and alternated with plots more or less dispersed of almond groves (mainly in the proximity of towns) or oak trees, which are preferentially used by magpies to build their nests (Soler 1990). Therefore, it is possible to differentiate clearly several geographically isolated sub-populations of breeding magpies (here we use data from seven of them, Fig. 1) with different ecological conditions and different levels of selection pressure exerted by the brood parasitic great spotted cuckoo (Soler *et al.* 1998a) that could be influencing rejection costs of foreign eggs (see introduction).

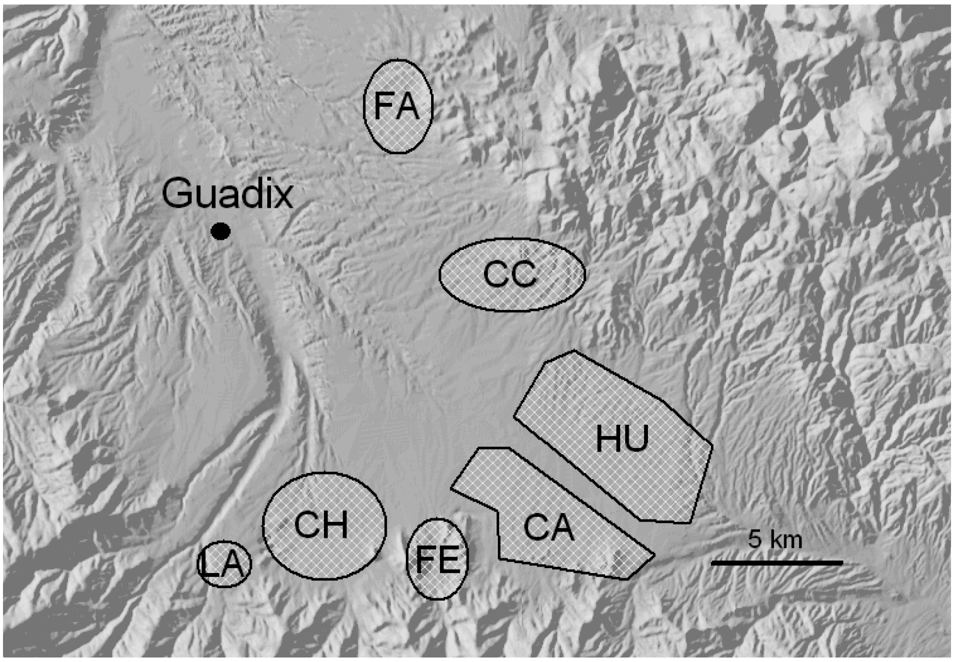

Figure 1.- *Map showing the relative situation and area estimated for the magpie sub-populations studied in the Hoya de Guadix.*

Field Procedures

Fieldwork was conducted during the breeding seasons of 2000 and 2001. At the beginning of the spring, we searched systematically the possible nest localizations, and once they were found, nests were revisited regularly to determine their laying date. When nest contained at least one magpie egg, one mimetic model egg made with plaster of Paris and painted resembling those of great spotted cuckoos was added (without removing any magpie eggs). During the laying period, we visited the nests at least each three times a week to determine variables such as clutch size and presence of great spotted cuckoo eggs. We classified the pair as accepter if experimental-model egg remained into the nest after six to seven days; otherwise, if the model disappeared from the nest or the magpie pair abandoned its clutch, it was considered as rejecter.

Due to logistic problems with capturing adults, we had to use genetic data from chicks, getting a blood sample from the brachial vein when they were 18 days old. Blood samples were stored in 1ml of 100% ethanol. Afterwards, in order to use only independent data, we randomly selected a single sample per magpie nest to perform the genetic analyses. Moreover, nests selected for the genetic analyses were also chosen randomly within the complete study area, and to avoid pseudo-replication, blood samples used in the genetic analyses from 2001 were from magpie territories not controlled in 2000. In short, we used samples coming from 101 nests in 2000 and from 21 nests in 2001. We assume that genetic composition of each magpie sub-population did not change between the two study years. Although magpie pairs are able to lay replacement clutches if their first breeding attempt fails (Birkhead 1991), for this work, we only used data from first breeding attempts because in replacement clutches egg-recognizer magpies may change their behaviour to acceptance due to retaliatory behaviour of great spotted cuckoos (Soler *et al.* 1999b).

Laboratory work

Genomic DNA was isolated from blood using the ammonium-acetate precipitation method (adapted from Bruford *et al.* (1998)). We used eleven polymorphic microsatellite loci. Four of them were isolated from magpies: Ppi1, Ppi2, Ppi3 (Martínez *et al.* 1999) and Ppi4 (Martinez J.G., Dawson D.A. & Burke T., unpublished, EMBL accession number: PPI272377); three from Seychelles warbles

(*Acrocephalus sechellensis*): Ase12, Ase18 and Ase64 (Richardson *et al.* 2000); one from indigo bird (*Vidua chalybeata)*: Indi28 (Sefc *et al.* 2001); one from western crowned-warbler *(Phylloscopus occipitalis)*: Pocc1 (Bensch *et al.* 1997); and two from house sparrow (*Passer domesticus*): Pdo5 and Pdo6 (Griffith *et al.* 1999). Details of the primers used in this study can be found on the Sheffield Molecular Genetics Facility Passerine primer cross-utility database, accessed via http://www.shef.ac.uk/misc/groups/molecol/birdmarkers.html. Since in a previous study (Martín-Gálvez *et al.* 2005, **MS I**) we detected a statistical association between the Ase64 genotypes and recognition and ejection behaviour of foreign eggs (see introduction), we use Ase64 separately from the other ten neutral microsatellite loci to perform statistical analyses.

Genotypes for these 11 microsatellites were generated during 2002. Polymerase chain reactions (PCRs) were performed with the forward primer of each marker labelled with a fluorescent dye. The reaction profile for each locus was 94 ºC for 120 s, followed by 35 cycles of 94 ºC for 30 s, the annealing T^a (depending of each locus) for 30 s, and 72 ºC for 30 s; and then 72 ºC for 5 min. Volumes of PCR reactions were 10 µl, containing approximately 10 ng of DNA, 1.0 µM of each primer, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$ and 0.05 units of *Taq* DNA polymerase, in the manufacturer's buffer. The PCR products were electrophoresed through an ABI Prism 377 DNA sequencer (Applied Biosystems). The output was analysed using ABI Genescan software (version 3.1.2) and Genotyper DNA fragment analysis software (version 2.5).

Statistical analyses

Genetic differentiation between magpie sub-populations

STRUCTURE 2.1 (Pritchard *et al.* 2000) was used to infer the population genetic structure of Guadix, by assigning each individual genotyped to one or some clusters in relation with its genotype for all microsatellite loci but Ase64. In this way, we are able to determine whether different plots of breeding magpies within the Guadix population are genetically different. In short, five different runs were performed per each number of sub-populations assumed (k=1 to k=7), with a length of burn-in period oscillating between 10,000 and 50,000, and with number of MCMC repetitions after burn-in period between 500,000 and 900,000 (see table 2 and appendix). In all simulations, we assumed that allelic frequencies are correlated between sub-populations, and that a certain individual genotype can be drawn from two or more genotypes coming from different sub-populations (i.e. model with admixture).

Allelic frequencies for each microsatellite locus and for each sub-population were calculated using GENEPOP 3.3 (Raymond & Rousset 1995). Afterwards, allelic frequencies of the ten loci (all but Ase64) were used to estimate the matrix of genetic distances (Cavalli-Sforza's cord distance) between sub-populations, a computation carried out by the program GENDIST included in the package PHYLIP 3.57c (Felsenstein 1993). We calculated in a similar way the matrix of genetic distances for Ase64. In addition, since the genetic association previously found between Ase64 and rejection behaviour was explained mainly by two alleles of that locus (being the 429bp allele more frequent in accepter individuals, and the 457bp allele more frequent in rejecter individuals, Martín-Gálvez *et al.* 2005, **MS I**), we also built the matrices of differences in the frequencies of copies between sub-populations for both alleles.

Finally, we also obtained the paired *F*st-values between sub-populations using GENEPOP 3.3 to determine whether a significant correlation between (*F*st/1-*F*st) values and geographical distances between plots exist and, thus, whether there is a significant genetic structure in Guadix due to geographical isolation (Rousset 1997).

Comparisons between magpie sub-populations

Given that we do not have direct measures of abundance of adult great spotted cuckoos in each plot during this study, estimates of parasitism selection pressure due to brood parasitism in each sub-population (i.e. probability to be parasitized) were assumed to be equivalent to parasitism rate (i.e. percentage of nests parasitized), since in a previous study in the same population both variables were highly correlated (Soler *et al.* 1998b).

There was variation between years of study in the values of phenotypic and environmental variables. Briefly, rejection rate was 51.28 % (N = 156 nests) vs. 35.93 % (N = 177 nests) during 2000 and 2001 years respectively ($\chi^2_1 = 8.97$, $P < 0.05$); parasitism rate oscillated between 26.61 % (N = 263) in 2000 and 41.24 % (N = 194) in 2001 ($\chi^2_1 = 10.82$, $P < 0.05$); and the laying date (April 1= day 1) in 2000 was in average ten days later (29.40 ± 0.70, N = 220) than for 2001 (19.11 ± 0.79, N = 168) ($F_{1,386} = 94.01$, $P < 0.001$). For this reason, we decided to standardize the values of each sub-population in each study year, by subtracting the mean values of the whole population obtained in this year. Afterwards, we averaged between both years for standardized values of each sub-population (see Table 1), and we used the resultant values to make up the matrices of differences between sub-populations. We kept the signs in the matrices of differences obtained per each phenotypic and environmental variable both when comparing between them and when comparing these with the matrices obtained from the frequencies of both 429bp and 457bp alleles of Ase64. Therefore, we were able to discern the directionality of change between these variables. However, because the values of genetic and geographical distances are always positive, we used the absolute values of the differences of phenotypic and environmental variables to compare them with genetic and geographical distances between sub-populations.

To test for the relationship between matrices of differences, we performed Mantel's tests and Partial Mantel's test using FSTAT 2.9.3 (Goudet 2002), with estimation of p-values after 2000 randomizations. Further, we used STATISTICA 6.0 (StatSoft, Inc. 2001) to carry out the remainder statistical analyses. All statistical tests were two-tailed.

Results

Genetic isolation of magpie sub-populations

There was no correlation between paired Fst/(1-Fst) values and geographical distances between plots in Guadix (Mantel's test: $r = 0.08$, $R^2 = 0.01$, $P = 0.74$), which suggests a lack of genetic differentiation due to distance. Accordingly, results from the simulations performed by STRUCTURE 2.1 using 10 polymorphic microsatellite loci indicated that Guadix population is not made up by genetically distinct subgroups. Instead, the presence of a unique genetic group in Guadix is the most probable result from each one of the performed simulations (see methods and table 2). An additional support indicating a total lack of genetic differences between magpie sub-populations of Guadix comes from the fact that no individuals were strongly assigned to a certain sub-population. Rather, all individual genotypes resulted to be a mixture roughly symmetric of genotypes coming from each one of all sub-populations (i.e. model allowing admixture, see Methods, see appendix). Therefore, these results would indicate that breeding magpie plots of Guadix are not corresponding with sub-populations genetically differentiated.

In any case, although we did not detect genetic structure we will continue calling sub-population to geographically separated magpie breeding areas within the whole Guadix population. Accordingly, risk of parasitism significantly differed between magpie sub-populations in both years (2000: $\chi^2_5 = 66.92$, $P < 0.001$; 2001: $\chi^2_4 = 15.16$, $P < 0.05$); and it also happens with respect to the laying dates, but only for the 2000 year (2000: $F_{5,214} = 7.09$, $P < 0.001$ vs. 2001: $F_{4,163} = 2.25$, $P = 0.065$). However, rejection rates of magpie sub-populations were not significantly different to the rejection rate calculated for the whole population of Guadix for each year (2000: $\chi^2_5 = 2.44$, $P = 0.79$; 2001: $\chi^2_4 = 4.41$, $P = 0.35$).

Table 1.- Mean between years of the standardized values of average laying date, parasitism rate and rejection rate per sub-population.

Zone	**Average laying date**	**Parasitism rate**	**Rejection rate**
FE	-3.54 (36)	-7.05 (46)	-3.45 (23)
FA	-10.01 (18)	-7.57 (21)	-1.28 (10)
LA	3.78 (34)	-24.84 (34)	-14.59 (28)
CA	2.68 (116)	1.57 (138)	-0.65 (117)
CH	-0.33 (126)	-8.74 (145)	3.06 (93)
HU	-0.75 (50)	39.92 (55)	4.67 (46)
CC	0.02 (8)	14.32 (18)	-3.78 (16)

Numbers between parentheses refer to sample sizes of magpie nests

Table 2.- Results from simulations made by STRUCTURE 2.1 software

Ki	**lnPr(X$\mid$K$_i$)$_1$**	**lnPr(X$\mid$K$_i$)$_2$**	**lnPr(X$\mid$K$_i$)$_3$**	**lnPr(X$\mid$K$_i$)$_4$**	**lnPr(X$\mid$K$_i$)$_5$**	**Pr(K=i)$_{\text{whole}}$**
1	*-3443.8*	*-3442.2*	*-3445.9*	*-3441.5*	*-3442*	*~1.0*
2	-3552.2	-3780	-3817	-3746.6	-3696.7	~0.0
3	-3787.7	-3597.9	-3828.1	-4014.7	-3637.3	~0.0
4	-3728.8	-3810.2	-3696.6	-3760.1	-3792.6	~0.0
5	-3855.5	-3835.5	-3788.8	-3888.4	-3844.5	~0.0
6	-4036.2	-4006	-3855.1	-4139.9	-3953.2	~0.0
7	-4114.3	-4089.7	-4098.9	-4147.7	-4261.5	~0.0
Burn-in length	10,000	20,000	30,000	40,000	50,000	
Reps.	500,000	600,000	700,000	800,000	900,000	

K$_i$ = number of sub-populations assumed. Pr(X|K$_i$) refers to the probability of data coming from a population with a number of sub-populations *i* per each one of the five simulations achieved. Pr(K=i) is the probability of K being *i*. Burn-in length and reps. are the parameters used to do each MCMC simulations

Genetic components of egg rejection behaviour

Absolute differences in rejection rate between sub-populations were explained neither by genetic distances calculated for ten microsatellite loci (Mantel's Test: $r = -0.02$, $R^2 < 0.01$, $P = 0.93$) nor by the geographical distances between sub-populations (Mantel's Test: $r = 0.03$, $R^2 < 0.01$, $P = 0.89$). Further, genetic distances calculated using Ase64 did not successfully explain the differences in rejection rate shown between sub-populations (Mantel's Test: r = 0.18, R^2 = 0.03, P = 0.45). A similar result was found after comparing the matrix of differences in the percentage of the 429bp allele of Ase64 with the matrix of differences in rejection rate (Mantel's Test: r = 0.08, R^2 = 0.07, P = 0.73). However, when using differences in the frequencies of the 457bp allele (i.e. that associated with the rejecters individuals), it significantly explained the differences in rejection rates found between sub-populations (Mantel's Test: r = 0.91, R^2 = 0.82, P < 0.001, Fig. 2). Thus, these results suggest that differences in rejection rate between sub-populations are a consequence of differences in the percentage of copies of the 457bp allele and, thus, in the genetic ability to reject foreign eggs. Hence, hereafter we use the frequencies of the 457 bp allele to control for the genetic component of rejection behaviour when comparing environmental and phenotypic variables between sub-populations.

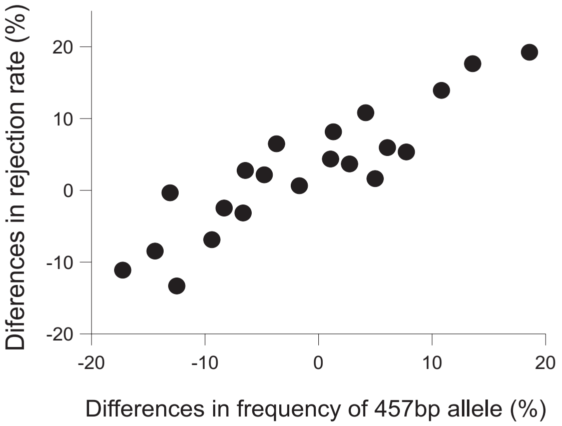

Figure 2.- *Relationship between the differences in rejection rate and the differences in frequency of the 457 bp allele of Ase64 (associated to rejection behaviour) found when comparing the magpie sub-populations of Guadix.*

Environmental components of egg rejection behaviour

The differences in probability of parasitism by great spotted cuckoos, measured as percentage of nests parasitized, covaried positively with the differences in rejection rate of mimetic model eggs between sub-populations of Guadix (mean of values standardized per sub-population and year of study; Mantel's Test: r = 0.62, R^2 = 0.39, P = 0.006, Fig. 3a). However, after controlling for differences between sub-populations in the frequencies of the 457bp allele, differences in parasitism rate did no longer covary with the differences in rejection rate (Partial Mantel's tests, partial correlation coefficient (parasitism rate) = 0.02, R^2 = 0.83, P = 0.94). This is because differences in the frequencies of the 457bp allele vs. the differences in parasitism rate covaried at a similar degree (Mantel's test: r = 0.68, R^2 = 0.46, P = 0.002, Fig. 3b) than did differences in parasitism rate and differences in rejection rate (see Figs. 3a and 3b).

On the other hand, differences in mean phenotypic quality (i.e. laying date) of individuals within each sub-population were negatively correlated with differences in rejection rate between sub-populations (mean of standardized values for each sub-population and study year; Mantel's Test: r = -0.49, R^2 = 0.24, P = 0.025; Fig. 4a). However, this was not longer the case after controlling for differences in the frequency of the 457bp allele (Partial Mantel's tests, partial correlation coefficient (average of laying date) = -0.09, R^2 = 0.83, P = 0.71). This is because differences in averages of laying date between sub-populations were also correlated with the differences in the frequencies of the 457bp allele (Mantel's Test: r = -0.45, R^2 = 0.20, P= 0.035, Fig. 4b), which predicted differences in rejection rates (see above).

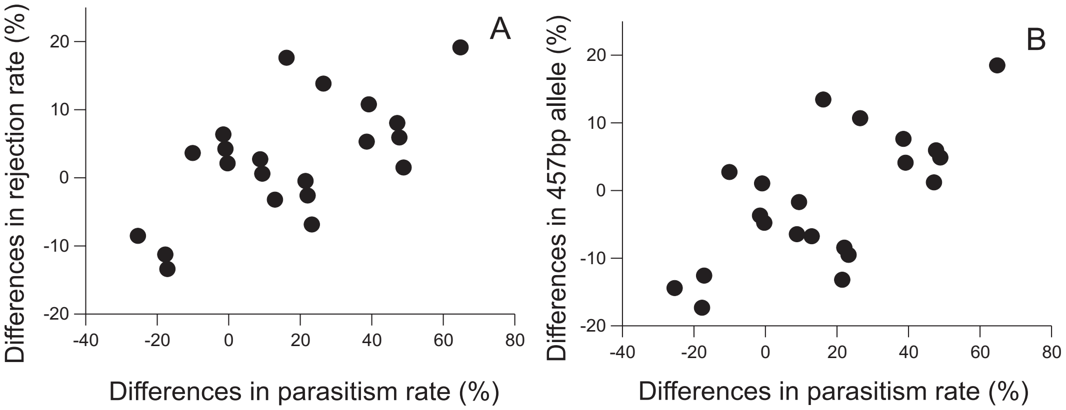

Figure 3.- *Correlations between differences in parasitism rate with **(A)** differences in rejection rate and **(B)** differences in percentage of the 457 bp allele copies obtained when comparing magpie sub-populations of Guadix.*

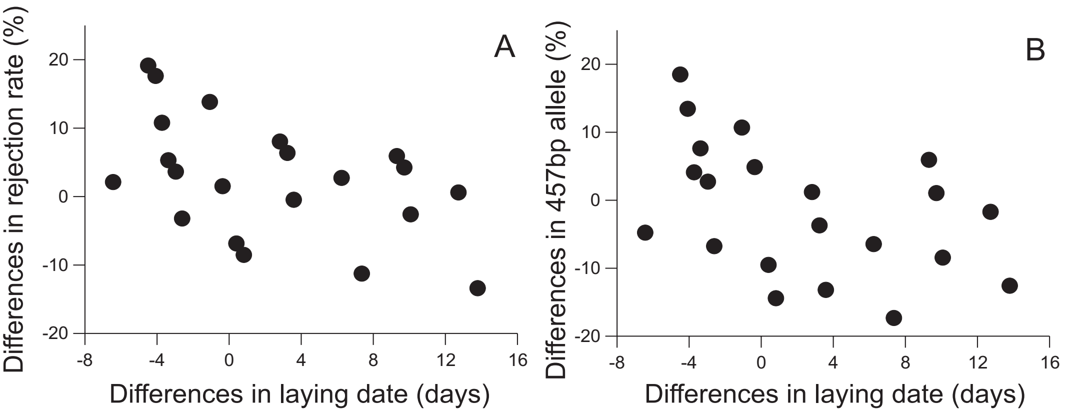

Figure 4.- *Relationships between differences in phenotypic quality of magpies breeding in different sub-populations and **(A)** differences in rejection rate and **(B)** differences in percentage of the 457 bp allele copies between magpie sub-populations of Guadix*

Discussion

Although different magpie sub-populations of Guadix are geographically isolated, experience different rate of parasitism and environmental conditions (see Soler *et al.* 1998b, Soler & Soler 2000, and also Fig. 1 and Table 1), we did not find any evidence of genetic structure associated to these sub-populations. This lack of genetic structure could be a consequence of the proximity of these sub-populations to each other, which would translate into a high amount of gene flow among them that, at least partially, would prevent them from isolated coevolutionary processes (see Thompson 1994, Nuismer *et al.* 1999) and would allow differences in the frequency of defensive phenotypes between sub-populations.

In accordance with the lack of genetic structure, the variation in defensive abilities detected between the different magpies sub-populations (see Table 1) were explained neither by genetic distances estimated for the ten neutral microsatellites loci nor by those genetic distances calculated for the non-neutral Ase64 locus (see results). However, accordingly with a genetic basis of the ejection behaviour of magpies (Martín-Gálvez *et al.* 2005, **MS I**), differences in frequencies of the allele associated with the ejection behaviour (i.e. 457bp of Ase64) significantly explained differences in rejection rates between different sub-populations (Fig. 2). Therefore, although magpie sub-populations of Guadix are not genetically structured, differences in frequencies of the allele associated to ejection behaviour predicted differences in egg rejection ability of magpies from different sub-populations.

This association, although suggesting that the 457bp allele of Ase64 is a genetic marker of ejection ability, might also be interpreted as this allele being related to the phenotypic expression of defences induced by the risk of parasitism, which is quite common in invertebrates and plants (see e.g. Agrawal *et al.* 1999). Since rejection behaviour implies associated costs, such as recognition errors, the abandoning of own offspring, or destruction of some own eggs when trying to eject parasitic eggs (see Davies 2000), a conditional defence induced by the presence of parasites would be beneficial for hosts (Davies *et al.* 1996). However, this fact cannot explain the relationship between differences in parasitism rate and allele frequencies of the 457 bp allele at different sub-populations detected here (see Results). This is because the probability of phenotypic expression of inducible defences (i.e. probability of parasitism) cannot have a genetic basis on hosts. Therefore, the detected association between differences in rejection ability and those in frequencies of the 457bp genetic marker between different sub-populations suggests a genetic basis of rejection ability. Thus, it allows us to test predictions from the EEH and ELH while controlling, at least partially, for the genetic basis of rejection behaviour when studying the influence of other – environmental – factors (see Introduction).

We have detected a positive relationship between risk of parasitism (i.e. parasitism rates) and rejection rates (Fig 3). This result may be in accordance with both the EEH and the ELH. In the first case, because it is possible that, as for some hosts of the European cuckoo (*Cuculus canorus)* (see Davies & Brooke 1988, Moksnes & Røskaft 1989, Álvarez 1996, Brooke *et al.* 1998, Moksnes *et al.* 2000, Bártol *et al.* 2002, but see also Lindholm 2000), egg rejection in magpies was a plastic phenotypic trait mainly expressed when brood parasites are present and/or abundant in the area (i.e. high risk of parasitism). In accordance with this possibility, probability of parasitism of magpies is related to number of great spotted cuckoos present in the area (Zuñiga & Redondo 1992, Soler *et al.* 1994) and, thus, the detected association may indicate that in areas where cuckoos are more abundant (i.e. high risk of parasitism) most magpies decided to reject foreign eggs from their nests. However, although we have experimentally demonstrated that rejection behaviour is a plastic trait in magpies (Soler *et al.* 1999b), great spotted cuckoo presence does not affect magpie decision of ejecting foreign eggs (Soler *et al.* 2000). On the other hand, the ELH may also predict the positive relationship between rejection and parasitism rates detected here if the risk of parasitism is a variable mainly related to the level of parasitism selection pressure at different sub-populations. Briefly, because a stronger parasitism selection pressure is predicted for non-defensive than for defensive (i.e. rejecter) genotypes, in sub-populations suffering high risk of parasitism, defensive genotypes would be more common. That could be because both/either a differential reproductive success and/or a differential migration rate. Therefore, that relationship will be the evolutionary consequence of sub-populations differing in parasitism selection pressure. In accordance with this last possibility, when including differences in frequencies of the rejecter allele (457bp) as an additional independent variable in our analysis, differences in risk of parasitism did no longer covary with the differences in rejection rate. This is because differences in risk of parasitism between sub-populations predicted differences in frequencies of the rejecter allele, which explained differences in rejection rates between sub-populations (see results).

We also found that between-sub-population differences in mean laying dates predicted differences in rates of egg rejection. Again, this relationship would be in accordance with level of rejection rates being in an evolutionary equilibrium because laying date in magpies is reflecting phenotypic or territory quality (Birkhead 1991, Soler *et al.* 1995, Sorci *et al.* 1997) and individual phenotypic quality may affect costs of recognition and rejection of parasitic eggs (Lotem 1993, Lotem *et al.* 1995, Rodríguez-Gironés & Lotem 1999, but also Davies & Brooke 1988, Sealy 1995, Amundsen *et al.* 2002, Stokke *et al.* 2004). However, it could also be in agreement with the ELH if the relationship found between the differences in laying dates and the differences in rejection rate between sub-populations is a by-product of a differential level of parasite selection pressure suffered at different sub-populations. It is known that great spotted cuckoos preferentially parasitizes magpies with greater parental ability (Soler *et al.* 1995), which is detected by nest size (Soler *et al.* 1999a, De Neve *et al.* 2004b) and is related to laying date (Högstedt 1981, Birkhead 1991, Goodburn 1991, De Neve *et al.* 2004a). Thus, great spotted cuckoos parasitizing preferentially sub-populations with magpies with a high phenotypic quality may be the cause of the detected negative association between laying date and egg rejection rate. Therefore, although differences in laying date were not related to differences in parasitism rates between sub-populations (Mantel's Test: $r = -0.28$, $R^2 = 0.08$, $P = 0.22$), because it can be predicted an association between magpie phenotypic quality (i.e. laying date) and parasitism selection pressure (Soler *et al.* 1999a), the relationship between laying date and rejection rate should be corrected for the frequencies of the 457bp rejecter allele.

Thus, when including differences in frequencies of 457bp allele as an additional independent variable explaining differences in rates of egg rejection between magpie sub-populations, the laying date no longer explained a significant proportion of variance. That was the case because matrices of differences in allelic frequencies and those of laying date are negatively related. Laying date is a condition dependent trait that in magpies, as well as in most bird species (see e.g. Price *et al.* 1988, Lotem *et al.* 1992, Svensson 1997), varies depending on age and general phenotypic quality of individuals (Birkhead 1991, Soler *et al.* 1995, De Neve & Soler 2002, De Neve *et al.* 2004a). Since a genetic association between laying date and the ability to recognize and reject parasitic eggs is very unlikely, the relationships between laying date and the genetic marker of rejection behaviour, and between laying date and rejection rate, could be explained if, for instance, individuals bearing rejecter alleles were of better general phenotypic quality than individuals lacking these rejecter alleles. In any case, because differences in rejection rates are explained exclusively by differences in frequencies of 457bp allele between different sub-populations, which is depending on parasitism selection pressure (see above) and predict mean laying date, the above results are not in agreement with the EEH.

We believe that the relationships between allelic frequencies and risk of parasitism and laying date can be explained by great spotted cuckoos selecting magpies of superior phenotypic quality. This hypothetical cuckoo preference would result in some magpie sub-populations being under stronger selection pressure than others, where fitness benefits for defensive individuals would be relatively larger than for non-defensive individuals. In other words, coevolutionary hot spots (sensu Thompson 1994, Gomulkiewicz *et al.* 2000) are also possible in a geographically but not genetically structured population (Nuismer *et al.* 1999). Obviously, in this case, it could be beneficial for magpies to abandon heavily parasitized plots looking for other areas with less parasitism selection pressure, but the benefits associated to this option would be relatively higher for non-defensive individuals. Therefore, the relationship between the frequency of the defensive allele (457 bp) and laying date can be explained by rejecter magpies being able to breed in areas of high habitat quality because cost of parasitism is relatively lower for them than for non-defensive magpies, for which the best option would be to look for breeding areas with relatively lower parasitism selection pressure. From this scenario, it can be predicted that frequencies of defensive genotypes should be higher in sub-populations under strong selection pressure, which is in accordance with our results. On the other hand, under the parasite point of view, it could be beneficial to abandon areas with a high proportion of defensive phenotypes when, for instance, loss due to egg rejection by magpies surpass those benefits arising from the use of hosts of relatively high parental quality. If this is the case, since gene flow among these sub-populations is quite high, giving rise to a non-genetically structured population, and because of the reasons exposed above, the frequency of defensive genotypes would increase rapidly in new hypothetical re-colonized areas by great spotted cuckoos. Therefore, differential migration of magpies with and without the defensive genotypes in relation to cuckoo preference for parasitizing different sub-populations may explain the results presented here. This scenario clearly predicts an increase in the frequency of defensive genotypes at the whole population level with evolutionary time (i.e. in agreement with the evolutionary lag hypothesis). Moreover, the existence of coevolutionary hot spots within the whole magpie population of Guadix is consistent with the intermittent arms race hypothesis between parasites and their hosts proposed some years ago (Soler *et al.* 1998b). This is because sub-populations suffering from a high parasitism should change gradually from one year to another giving rise to an increase in defensive phenotypes as the evolutionary time progress.

In summary, we have detected evidence of environmental conditions explaining the level of host defence of different sub-populations within the whole magpie population of Guadix. However, the relationship between environmental conditions (i.e. laying date and risk of parasitism) and frequency of the defensive 457bp allele reveals that the fact that environmental conditions explaining host rejection behaviour is the consequence of different magpie sub-populations suffering different level of parasitism selection pressures (such as ELH assert), rather than a direct influence of environmental conditions on the phenotypic expression of magpie defensive traits (in according to EEH).

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References

Agrawal, A. A., Tuzun, S. & Bent, E. 1999. *Induced plant defenses against pathogens and herbivores. Biochemistry, ecology, and agriculture.* St. Paul, Minn.: APS Press.

Álvarez, F. 1996. Model cuckoo *Cuculus canorus* eggs accepted by rufous bush robin chats *Cercotrichus galactotes* during the parasite's absence from the breeding area. *Ibis*, **138,** 340-342.

Amundsen, T., Brobakken, P. T., Moksnes, A. & Røskaft, E. 2002. Rejection of common cuckoo Cuculus canorus eggs in relation to female age in the bluethroat (*Luscinia svecica*). *Journal of Avian Biology*, **33,** 366-370.

Bártol, I., Karcza, Z., Moskát, C., Røskaft, E. & Kisbenedek, T. 2002. Responses of great reed warblers Acrocephalus arundinaceus to experimental brood parasitism: the effects of a cuckoo *Cuculus canorus* dummy and egg mimicry. *Journal of Avian Biology*, **33,** 420-425.

Bensch, S., Price, T. & Kohn, J. 1997. Isolation and characterization of microsatellite loci in a *Phylloscopus* warbler. *Molecular Ecology*, **6,** 91-92.

Birkhead, T. R. 1991. *The Magpies. The ecology and behaviour of black-billed and yellow-billed magpies*. London: T & A D Poyser.

Briskie, J. V., Sealy, S. G. & Hobson, K. A. 1992. Behavioural defenses against avian brood parasitism in simpatric and allopatric host populations. *Evolution*, **46,** 334-340.

Brooke, M. de L., Davies, N. B. & Noble, D. G. 1998. Rapid decline of host defenses in response to reduced cuckoo parasitism - behavioral flexibility of reed warblers in a changing world. *Proceedings of the Royal Society of London, Series B*, **265,** 1277-1282.

Brooker, L. C. & Brooker, M. G. 1990. Why are cuckoos host specific? *Oikos*, **57,** 301-309.

Bruford, M. W., Hanotte, O., Brookfield, J. F. Y. & Burke, T. 1998. Multilocus and single-locus DNA fingerprinting. In: *Molecular genetic analysis of populations: a practical approach* (Ed. by Hoelzel, A. R.), pp. 287-336. Oxford: IRL Press.

Davies, N. B. 1999. Cuckoos and cowbirds versus hosts: Co-evolutionary lag and equilibrium. *Ostrich*, **70,** 71-79.

Davies, N. B. 2000. *Cuckoos cowbirds, and others cheats*. London: T & A D Poyser.

Davies, N. B. & Brooke, M. de L. 1988. Cuckoos versus reed warblers: adaptations and counteradaptations. *Animal Behaviour*, **36,** 262-284.

Davies, N. B. & Brooke, M. de L. 1989a. An experimental study of co-evolution between the cuckoo, *Cuculus canorus*, and its hosts. I. Host egg discrimination. *Journal of Animal Ecology*, **58,** 207-224.

Davies, N. B. & Brooke, M. de L. 1989b. An experimental study of co-evolution between the cuckoo, *Cuculus canorus*, and its hosts. II. Host egg markings, chick discrimination and general discussion. *Journal of Animal Ecology*, **58,** 225-236.

Davies, N. B., Brooke, M. de L. & Kacelnik, A. 1996. Recognition errors and probability of parasitism determine whether reed warblers should accept or reject mimetic cuckoo eggs. *Proceedings of the Royal Society of London, Series B*, **263,** 925-931.

Dawkins, R. & Krebs, J. R. 1979. Arms races between and within species. *Proceedings of the Royal Society of London, Series B*, **205,** 489-511.

De Neve, L. & Soler, J. J. 2002. Nest building activity and laying date influence female reproductive investment in magpies. An experimental study. *Animal Behaviour,* **63,** 975-980.

De Neve, L., Soler, J. J., Soler, M. & Pérez-Contreras, T. 2004a. Differential maternal investment counteracts for late breeding in magpies *Pica pica*: an experimental study. *Journal of Avian Biology*, **35,** 237-245.

De Neve, L., Soler, J. J., Soler, M. & Pérez-Contreras, T. 2004b. Nest size predicts the effect of food supplementation to magpie nestlings on their immunocompetence: an experimental test of nest size indicating parental ability. *Behavioral Ecology*, **15,** 1031-1036.

Felsenstein, J. 1993. PHYLIP (Phylogeny Inference Package). *ver. 3.57c* (Seattle)

Gomulkiewicz, R., Thompson, J. N., Holt, R. D., Nuismer, S. L. & Hochberg, M. E. 2000. Hot spots, cold spots, and the geographic mosaic theory of coevolution. *American Naturalist*, **156,** 156-174.

Goodburn, S. F. 1991. Territory quality or bird quality? factors determining breeding success in the magpie (*Pica pica*). *Ibis*, **133,** 85-90.

Goudet, J. 2002. FSTAT, a program to estimate and test gene diversities and fixation indices. *ver. 2.9.3.* (Switzerland)

Griffith, S. C., Stewart, I. R. K., Dawson, D., Owens, I. P. F. & Burke, T. 1999. Contrasting levels of extra-pair paternity in mainland and island populations of a socially monogamous bird, the house sparrow (*Passer domesticus)*: is there an "island effect"? *Biological Journal of the Linnean Society*, **68,** 303-316.

Högstedt, G. 1981. Effect of additional food on reproductive success in the magpie (*Pica pica*). *Journal of Animal Ecology*, **50,** 219-229.

Lindholm, A. K. 2000. Tests of phenotypic plasticity in reed warbler defences against cuckoo parasitism. *Behaviour*, **137,** 43-60.

Lotem, A. 1993. Learning to recognize nestling is maladaptive for cuckoo Cuculus canorus host. *Nature*, **362,** 743-745.

Lotem, A., Nakamura, H. & Zahavi, A. 1992. Rejection of cuckoo eggs in relation to host age: a possible evolutionary equilibrium. *Behavioral Ecology*, **3,** 128-132.

Lotem, A. & Nakamura, H. 1998. Evolutionary equilibria in avian brood parasitism. An alternative to the "arms race-evolutionary lag" concept. In: *Parasitic birds and their hosts. Studies in coevolution* (Ed. by Rothstein, S. I. & Robinson, S. K.), pp. 223-235. New York, Oxford: Oxford University Press.

Lotem, A., Nakamura, H. & Zahavi, A. 1995. Constraints on egg discrimination and cuckoo-host co-evolution. *Animal Behaviour*, **49,** 1185-1209.

Marchetti, K. 1992. Cost to host defences and the persistence of parasitic cuckoos. *Proceedings of the Royal Society of London, Series B*, **248,** 41-45.

Martín-Gálvez, D., Soler, J. J., Martínez, J. G., Krupa, A. P., Richard, M., Soler, M., Møller, A. P. & Burke, T. 2005. A quantitative trait locus for recognition of foreign eggs in the host of a brood parasite. *Journal of .Evolutionary Biology (in press).*

Martínez, J. G., Soler, J. J., Soler, M., Møller, A. P. & Burke, T. A. 1999. Comparative population structure and gene flow of a brood parasite, the Great Spotted Cuckoo (*Clamator Glandarius*), and its primary host, the magpie (*Pica pica*). *Evolution*, **53,** 269-278.

Moksnes, A. & Røskaft, E. 1989. Adaptations of meadow pipits to parasitism by the common cuckoo. *Behavioral Ecology and Sociobiology*, **24,** 25-30.

Moksnes, A. & Røskaft, E. 1995. Egg-morphs and host preferences in the common cuckoo *(Cuculus canorus)*: an analysis of cuckoo and host eggs from European museum collections. *Journal of Zoology*, **236,** 625-648.

Moksnes, A., Røskaft, E., Braa, A. T., Korsnes, L., Lampe, H. M. & Pedersen, H. C. 1990. Behavioural responses of potential hosts towards artificial cuckoo eggs and dummies. *Behaviour*, **116,** 64-89.

Moksnes, A., Røskaft, E., Hagen, L. G., Honza, M., Mork, C. & Olsen, P. H. 2000. Common cuckoo Cuculus canorus and host behaviour at Reed Warbler Acrocephalus scirpaceus nests. *Ibis*, **142,** 247-258.

Nuismer, S. L., Thompson, J. N. & Gomulkiewicz, R. 1999. Gene flow and geographically structured coevolution. *Proceedings of the Royal Society of London, Series B*, **266,** 605-609.

Payne, R. B. 1977. The ecology of brood parasitism in birds. *Annual Review of Ecology and Systematics*, **8,** 1-28.

Price, T., Kirkpatrick, M. & Arnold, S. J. 1988. Directional selection and the evolution of breeding date in birds. *Science*, **240,** 798-799.

Pritchard, J. K., Stephens, M. & Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics*, **155,** 945-959.

Raymond, M. & Rousset, F. 1995. Population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86,** 248-249.

Richardson, D. S., Jury, F. L., Dawson, D., Salgueiro, P., Komdeur, J. & Burke, T. 2000. Fifty Seychelles warbler (*Acrocephalus sechellensis*) microsatellite loci polymorphic in Sylviidae species and their cross-species amplification in other passerine birds. *Molecular Ecology*, **9,** 2155-2234.

Rodríguez-Gironés, M. A. & Lotem, A. 1999. How to detect a cuckoo egg: a signal-detection theory model for recognition and learning. *American Naturalist*, **153,** 633-648.

Rohwer, S. & Spaw, C. D. 1988. Evolutionary lag versus bill-size constraints: a comparative study of the acceptance of cowbird eggs by old hosts. *Evolutionary Ecology*, **2,** 27-36.

Røskaft, E., Moksnes, A., Stokke, B. G., Moskat, C. & Honza, M. 2002. The spatial habitat structure of host populations explains the pattern of rejection behavior in hosts and parasitic adaptations in cuckoos. *Behavioral Ecology*, **13,** 163-168.

Rothstein, S. I. 1975a. Evolutionary rates and host defenses against avian brood parasitism. *American Naturalist*, **109,** 161-176.

Rothstein, S. I. 1975b. Mechanisms of avian egg-recognition: do birds know their own eggs. *Animal Behaviour*, **23,** 268-&.

Rothstein, S. I. 1982. Successes and Failures in avian egg and nestling recognition with comments on the utility of optimality reasoning. *American Zoologist*, **22,** 547-560.

Rothstein, S. I. 1990. A model system for coevolution: avian brood parasitism. *Annual Review of Ecology and Systematics*, **21,** 481-508.

Rothstein, S. I. & Robinson, S. K. 1998. The evolution and ecology of avian brood parasitism. In: *Parasitic Birds and their hosts, studies in coevolution* (Ed. by Rothstein, S. I. & Robinson, S. K.), pp. 3-56. Oxford: Oxford University Press.

Rousset, F. 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics*, **145,** 1219-1228.

Sealy, S. G. 1995. Burial of Cowbird Eggs by Parasitized Yellow Warblers - An Empirical and Experimental-Study. *Animal Behaviour*, **49,** 877-889.

Sefc, K. M., Payne, R. B. & Sorenson, M. D. 2001. Characterisation of microsatellite loci in village indigobirds *Vidua chalybeata* and cross-species amplification in estrildid and ploceid finches. *Molecular Ecology Notes*, **1,** 252-254.

Servedio, M. R. & Lander, R. 2003. Coevolution of an avian host and its parasitic cuckoo. *Evolution*, **57,** 1164-1175.

Soler, J. J., Martínez, J. G., Soler, M. & Moller, A. P. 1999a. Host sexual selection and cuckoo parasitism: an analysis of nest size in sympatric and allopatric magpie *Pica pica* populations parasitized by the great spotted cuckoo Clamator glandarius. *Proceedings of the Royal Society of London, Series B*, **266,** 1765-1771.

Soler, J. J., Martínez, J. G., Soler, M. & Møller, A. P. 2001. Coevolutionary interactions in a host-parasite system. *Ecology Letters*, **4,** 470-476.

Soler, J. J. & Soler, M. 2000. Brood-parasite interactions between great spotted cuckoos and magpies: a model system for studying coevolutionary relationships. *Oecologia*, **125,** 309-320.

Soler, J. J., Soler, M., Møller, A. P. & Martínez, J. G. 1995. Does the great spotted cuckoo choose magpie hosts according to their parenting ability? *Behavioral Ecology and Sociobiology*, **36,** 201-206.

Soler, J. J., Sorci, G., Soler, M. & Møller, A. P. 1999b. Change in host rejection behavior mediated by the predatory behavior of its brood parasite. *Behavioral Ecology*, **10,** 275-280.

Soler, M. 1990. Relationships between the great spotted cuckoo *Clamator glandarius* and its corvid hosts in a recently colonized area. *Ornis Scandinavica*, **21,** 212-223.

Soler, M. & Møller, A. P. 1990. Duration of sympatry and coevolution between the great spotted cuckoo and its magpie host. *Nature*, **343,** 748-750.

Soler, M., Soler, J. J. & Martínez, J. G. 1998a. Duration of sympatry and coevolution between the great spotted cuckoo (*Clamator glandarius*) and its primary host, the magpie (*Pica pica*). In: *Parasitic Birds and their hosts, studies in coevolution* (Ed. by Rothstein, S. I. & Robinson, S. K.), pp. 113-128. Oxford: Oxford University Press.

Soler, M., Soler, J. J., Martínez, J. G. & Møller, A. P. 1994. Micro-evolutionary change in host response to a brood parasite. *Behavioral Ecology and Sociobiology*, **35,** 295-301.

Soler, M., Soler, J. J., Martínez, J. G., Pérez-Contreras, T. & Møller, A. P. 1998b. Micro-evolutionary change and population dynamics of a brood parasite and its primary host: the intermittent arms race hypothesis. *Oecologia*, **117,** 381-390.

Soler, M., Soler, J. J. & Møller, A. P. 2000. Effect of great spotted cuckoo presence on magpie rejection behaviour. *Behaviour*, **137,** 213-220.

Sorci, G., Soler, J. J. & Møller, A. P. 1997. Reduced immunocompetence of nestlings in replacement clutches of the european magpie (*Pica pica*). *Proceedings of the Royal Society of London, Series B*, **264,** 1593-1598.

Sorenson, M. D. & Payne, R. B. 2002. Molecular genetic perspectives on avian brood parasitism. *Integrative and Comparative Biology*, **42,** 388-400.

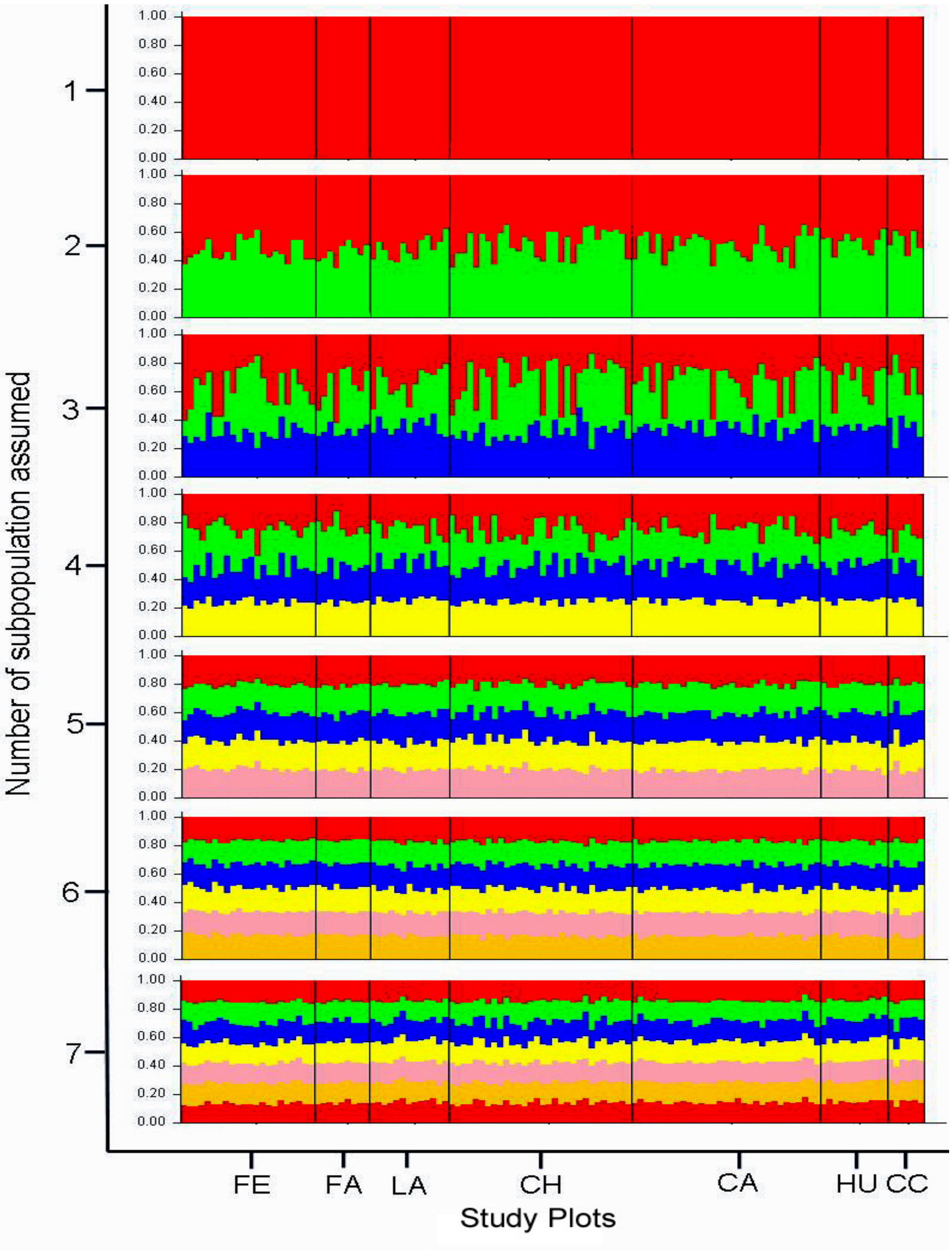
Stokke, B. G., Rudolfsen, G., Moksnes, A. & Røskaft, E. 2004. Rejection of conspecific eggs in chaffinches: The effect of age and clutch characteristics. *Ethology*, **110,** 459-470.

Svensson, E. 1997. Natural selection on avian breeding time: causality, fecundity-dependent, and fecundity-independent selection. *Evolution*, **51,** 1276-1283.

Thompson, J. N. 1994. *The coevolutionary process*. Chicago: The University of Chicago Press.

Zuñiga, J. M. & Redondo, T. 1992. No evidence for variable duration of sympatry between the great spotted cuckoo and its magpie host. *Nature*, **359,** 410-411.

Appendix



Graphic showing results from one of the five simulations made by STRUCTURE software. Each slice coloured of each vertical bar correspond with the percentage of genome of each individual assigned to each subpopulation assumed.

Costs and benefits associated to an experimental increase of level of hunger in natural conditions: a new experimental approach by using an appetite enhancer

III

Costs and benefits associated to an experimental increase of level of hunger in natural conditions: a new experimental approach by using an appetite enhancer

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Abstract

Models of parent-offspring conflict and communication assume that begging behaviour is costly and that it influences parental feeding behaviour. While there exist extensive literature on the begging affecting parental decisions, evidences of costs associated to an increase (dishonest) of begging behaviour of some nestlings within a brood are scarce. Here, for the first time, we used a drug, cyproheptadine hydrochloride, diluted in water to increase experimentally the level of hunger of some magpie (*Pica pica*) nestlings in a nest, while the rest were fed with water as a control. This drug directly acts on the hunger centre at the hypothalamus and is broadly used in humans and in pets as appetite enhancer. Cyproheptadine can be used to increase nestlings' hunger-level in the field because our experimental nestlings received more food from parents that control nestlings during a two and a half hours of experiment with neck collars, Moreover, experimental nestlings lost more weight but gain more food than control ones, which respectively strongly suggests extra-costs and benefits of showing a dishonest level of hunger through begging behaviour. Therefore, our experimental approach allow us to point out cost and benefit associated to an dishonest level of hunger, but also open the possibility to directly manipulate in the field the level of hunger and exploring its effect on different components of nestling and adult fitness or related variables.

Key words.- *Begging, benefits of begging, Costs of begging, Cyproheptadine, feeding behaviour, honest signalling.*

Resumen

Los modelos del conflicto paterno-filial y comunicación asumen que el comportamiento de petición es costoso y que éste influencia la alimentación por los padres. Mientras que existe una extensiva literatura sobre la petición afectando a las decisiones de los padres, evidencias sobre los costes de un incremento (deshonesto) del comportamiento de petición de algunos pollos dentro de una nidada son escasas. Aquí, por primera vez, utilizamos un droga, la ciproheptadina clorhidrato, diluida en agua para experimentalmente incrementar el nivel de hambre de algunos pollos de urraca (*Pica pica*) en un nido, mientras el resto era alimentado con agua como un control. Esta droga actúa directamente sobre el centro del hambre en el hipotálamo y es ampliamente utilizado como en humanos y en animales domésticos como un estimulante del apetito. La ciproheptadina puede ser utilizada para incrementar el nivel de hambre de los pollos en el campo debido a que nuestros pollos experimentals recibieron más comida de los padres que los pollos controles durante dos horas y media con collares. Además, los pollos experimentales perdieron más peso, pero ganaron más comida que los controles, lo cual respectivamente sugiere costes extras y beneficios al mostrar un nivel deshonesto de hambre a través del comportamiento de petición. Por lo tanto, nuestra aproximación experimental nos permite tener en cuenta los costes y beneficios asociados a un nivel deshonesto de hambre, y también abre la posibilidad de manipular directamente en el campo el nivel de hambre en el campo y explorar su efecto sobre diferentes componentes de la eficacia biológica de adultos y pollos o variables relacionadas

Palabras Clave.- *Comportamiento Petitorio, Beneficios de la petición, Costes de la peticion, Ciproheptadina, Comportamiento de alimentación, Señalización honesta.*

Submitted

Introduction

Since Trivers (1974) proposed the existence of parent-offspring conflict, several models have tried to explain the resolution of that conflict in general and the evolution of begging behaviour in particular (e.g., Mock & Parker 1997). All of these models assume that begging is costly and, thus, begging behaviour should honestly signal offspring needs. Therefore, parents should use offspring level of begging when adjusting parental effort and allocating food within a brood (Godfray 1991, 1995a, 1995b). In accordance with this hypothesis, it has been showed that parents increase their feeding effort as a response to playback (e.g. Ottosson *et al.* 1997, Burford *et al.* 1998, but see Clark & Lee 1998) or brood size experiments (e.g., Smith *et al.* 1988), and preferentially feed the offspring that begs more vigorously (see Kilner & Johnstone 1997). On the other hand, there exist evidences on the predicted relationship between level of needs and begging characteristics (Sacchi *et al.* 2002), but also on costs associated to begging (see Roulin 2001) that could explain the honesty of the signal.

Perhaps, the clearest evidences on costs associated to begging behaviour are related to predation because of the increase in nest detectably (e.g., Redondo & Castro 1992, Haskell 1994, Leech & Leonard 1997, Briskie *et al.* 1999, Dearborn 1999). However, because those costs are suffered by the whole brood, and therefore begging and non-begging chicks within the same brood experience identical predatory costs, these kinds of costs cannot explain the evolution and stabilization of begging behaviour (Godfray 1995b). Thus, different costs for nestlings that beg and do not beg are necessary for explaining the evolution of honest-begging behaviour (see Rodríguez-Gironés (2001) for further discussion).

On the other hand, although an energetic cost of begging is likely, mainly because it consists on conspicuous calls and extravagant movements such as stretching of the neck, wing shivering and gaping, which apparently could imply energetic costs associated to muscular activity of nestlings, all studies have failed to find out that begging is an energetically costly activity. For instance, direct measures of oxygen consumption during food solicitation of nestling birds have shown values close to resting metabolic rate (Leech & Leonard 1996, McCarty 1996, Soler *et al.* 1999, Bachman & Chappell 1998), which is certainly much lower than those corresponding to behaviours considered as energetically expensive, such as short flights (Carlson & Moreno 1992) or singing (Eberhardt 1994; but see Horn *et al.* 1995). However, it is possible that energy for begging activity comes from anaerobic metabolism, thereby explaining the low rate of oxygen consumption (Weathers *et al.* 1997, but see McCarty 1997). Another possibility to explain the low rate of oxygen consumption during begging is that, although it can be seem inexpensive in the short-terms, it would have detectable costs during longer periods, for instance, affecting to nestling growth or general phenotypic quality at fledging (Verhulst & Wiersma 1997). Accordingly with this possibility, Rodríguez-Gironés *et al.* (2001) and Kilner (2001) found that an experimental increase of begging behaviour resulted in a negative effect on nestling growth rate when studying magpie (*Pica pica*) and canary (*Serinus canaria*) nestlings, respectively (but see also Leonard *et al.* 2003). In both experimental approaches, some nestlings were forced to beg for food more than other control-nestlings, but control and experimental nestlings obtained the same amount of food. Thus, although both articles are clear in detecting costs of begging behaviour, because both experiments were performed in the laboratory, nothing can be said about possible benefits associated to the experimental increases of begging activity. This is important because, although the existence of costs associated to the signals indicate the existence of equilibrium, the evolution of honest signalling of need by offspring would depend not only on the costs, but also on the associated benefits.

Here, we for the first time test a new methodology allowing the manipulation of nestling's level of hunger in natural conditions, and therefore, to test predictions of the evolution of parent-offspring conflict and the honesty of begging signals. We used cyproheptadine hydrochloride, a drug that directly acts on the hunger centre at the hypothalamus (Chakrabarty *et al.* 1967) and that is broadly used in human as appetite enhancer (Homnick *et al.* 2004). Briefly, we supplied cyproheptadine to a half of the nestlings in magpie broods during the nestling period and, subsequently, we compared the effect of treatment on the amount of food received (i.e. benefits), and weight lost (i.e. costs), for each nestling within the same nest during a two and a half hours trial with neck collars that impeded food swallowing by nestlings.

If cyproheptadine is useful for manipulating the level of hunger and, thereby the intensity of begging behaviour, we should find that experimental nestlings receive more food and lose more weight than control nestlings. This is because, for nestlings of similar phenotypic quality, parents should deliver more food to the hungriest nestling, and level of hunger should be honestly signalled by begging behaviour (i.e. should be costly). Therefore, in the case of finding support for these predictions, our results would add evidence to the assumption that begging behaviour is a costly activity, but for the first time, in natural conditions.

Material and Methods

Study area and species

Fieldwork was performed during the springs of 2002 and 2003 in a magpie population breeding in the Hoya of Guadix (37º18' N, 3º11' W, southern Spain). This place is a high-altitude plateau (approx. 1000 m a.s.l.) with cereal crops (especially barley) that alternate with more or less dispersed plots of almond groves or oak trees, which are preferentially used by magpies to build their nests (Soler 1990).

Magpie is a territorial, sedentary, and relatively long-lived passerine bird with a well-studied biology (reviewed in Birkhead 1991). It occurs throughout large parts of the Holarctic region where in some places it becomes abundant. Magpie lays a single clutch between March and May with 5-7 eggs (rank 3-10). Female starts to incubate before clutch completion (usually once the fourth egg is laid), so resulting in broods that hatch asynchronously, where some of the smallest nestlings starve soon after hatching (Birkhead 1991).

Field procedures

At the beginning of the breeding season, we searched for new nest localizations and, once a nest was located, we visited it regularly to determine laying date. During the laying period, nests were checked at least twice per week to determine clutch size and occurrence of brood parasitism. Although brood parasitism is quite common in the area (Soler & Soler 2000), we did not use any parasitized nest in this study.

After hatching, when nestlings were about 2-4 days old we weighted (Pesola spring valance, accuracy 0.1g) and marked all nestlings within a brood by painting the tarsus with non-toxic waterproof pens of different colours (Uni Posca, PC-8k). Nestlings were ranked according to their weight and alternately assigned to the experimental and control treatments (see Soler *et al.* (2003) and De Neve *et al.* (2004) for a further description of the experimental design). Moreover, nestlings with experimental treatment were dosed with 0.1 mg of cyproheptadine hydrochloride

(Acofarma, Inc.) diluted in 0.25 ml of water, while control nestlings were administrated with 0.25 ml of water. Drug dilution is the same than that used for children (0.4 mg/ml), since we calculated the dose based on those recommended for children and extrapolated to one nestling of 100 grams (0.05 mg/day). Every two days, we visited experimental nests to re-colour tarsus, weight, and feed nestlings with cyproheptadine or water.

When nestlings were about 10-11 days old (after the fifth dose), they were weighted and we placed neck collars in all the chicks in the nest. The ligature wire around the neck was tight enough to hinder the swallowing of food and loose enough to avoid strangling the chicks (see Soler *et al.* (1995) for a further description of the use of neck-collars method in magpies). Nestlings wore the neck collar for a period of 2 to 2.5 hours. This period is approximately one hour shorter than that previously used in magpies (Soler *et al.* 1995). Although this shorter experimental period would reduce the hypothetical differences between nestlings of different treatments, it would also reduce the possibility of some nestlings vomiting boluses of food that would not be possible to assign to any nestling. However, because that sometimes occurs, we did not use information from nestlings in nests where more than one bolus of food were found, which were not possible to be assigned to any individual nestlings. After neck collar period, food was extracted from each nestling and kept in absolute ethanol until its analysis in the lab. Moreover, nestlings were weighted before and after neck-collar period to estimate body-mass loss during that time. As a measure of weight loss independent of the time that nestlings were fitted with neck collars, we used differences in body mass (before minus after the experiment) divided by the time of the experiment (in hours). Moreover, because a gain in nestling body mass during neck collar application would mean the ingestion of some food indicating that the neck-collar was not correctly placed, we used only those nestlings that at least lost one gram of body mass. Finally, there exist possibility of adult magpies removing from the nest boluses of food vomited by nestlings, and thus, we ignored nestlings without assigned food from the analyses.

For estimations of biomass of food received for each nestlings (mg dry weight), food samples were placed in an oven at 60ºC for a period of 24 hours (i.e. to constant mass) and, later on, weighted on a precision balance (Adam equipment 120/0.0001g).

Sample sizes and statistical analyses

Because in some nests we were unable to assign some boluses of food to any of the nestlings, sample sizes for biomass received (81 nestlings from 27 nests, but only 17 nests with information for both experimental and control nestlings) and body-mass loss (133 nestlings from 31 nests, all nests except one with information for both experimental and control nestlings) differed. Moreover, although we repeated the experiment in 2002 (number of experimental nests: for biomass received = 17; for body-mass loss = 20) and 2003 (number of experimental nests: for biomass received = 10; for body-mass loss = 11) no significant differences between years appeared with respect to biomass received (GLM with year as a fixed factor, nest identity (nested within year) as a random factor and body mass before the experiment as a covariable; year effect: F = 0.23, df = 1,35.3, P = 0.63) or body-mass loss during the experiment (GLM with year as a fixed factor, nest identity (nested within year) as a random factor and body mass before the experiment as a covariable; year effect: F = 0.10, df = 1,31.5, P = 0.77). Further, the effect of the experiment did not differ significantly between years for any of the dependent variables (GLM with year and experimental treatment as a fixed factors, nest identity (nested within year) and the interaction between experimental treatment and nest identity as a random factors, and body mass before the experiment as a covariable. Interaction between year and experimental treatment: biomass received, F = 0.01, df = 1,20.1, P = 0.98; body mass loss: F = 0.03, df = 1,31.8, P = 0.85). Therefore, for the analyses we pooled data from these two years.

After natural log(e) transformations, distribution of frequencies of both biomass received and body-mass loss (Kolmogorov-Smirnov test for continuous variables; P > 0.2) did not differ significantly from normal distribution and, therefore, we used parametric statistical tests. In short, we used General Linear Models trying to explain, either, biomass received or mass loss for each nestling (dependent variables) on the basis of treatment effect (fixed effect) and nest identity (random effect) (independent variables). Moreover, we also included body mass before the neck-collar trials and brood size (i.e. number of hatched nestlings) as covariables in our models. The former because adult magpies preferentially feed nestlings of larger size (Soler *et al.* 1995) and body mass could also affect weight loss during the time of the experiment. The later, because begging strategies is affected by feeding experience (Kedar *et al.* 2000, Rodríguez-Gironés *et al.* 2002) and level of nestling competition (i.e. number of hatchling) (e.g., Mock & Parker 1997). Further, we also test for the interaction between experimental treatment and nest identity because a non-significant interaction would indicate a similar effect of treatment in most experimental nests, but also because by introducing that interaction degrees of freedom are conservatively adjusted to approximately the number of experimental nests. The error terms were calculated by the Satterthwaite's method. The type-I sums of squares and the over-parameterized model to code the two categorical factors were used.

Since predictions were *a priory* and completely directional, one-tail α values were used to calculated statistical significance. Values are weighted means ± SE.

Results

Following our predictions, we found that nestlings experimentally provided with cyproheptadine received more food, but lost more weight, than control-water fed nestlings (Fig. 1 and Table 1). It was the case after controlling for brood size and nestling body-mass before the experiment. This last factor explained a significant proportion of variance of biomass received, but also of body-mass loss during the standardized time of the experiment (Table 1).

Moreover, the interaction factor between experimental treatment and nest identity resulted far from significant when trying to explain either biomass received or body mass lost (Table 1). Therefore, cyproheptadine had similar effects independently of nest identity.

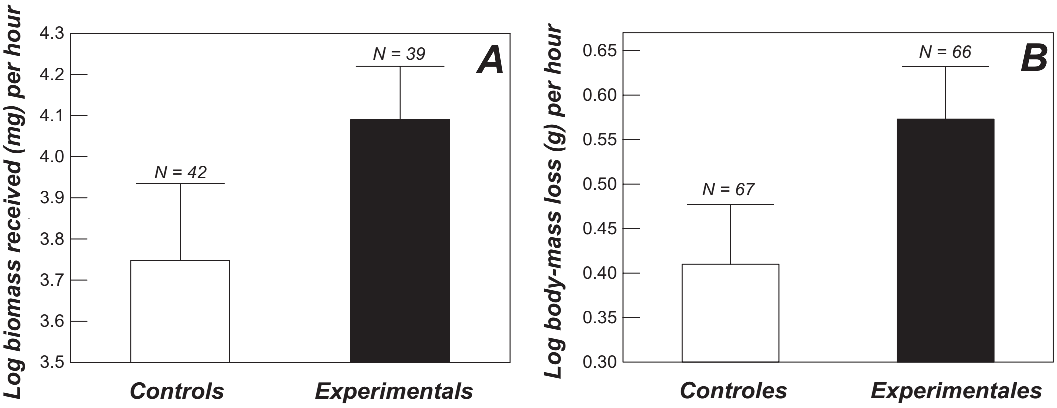

Figure 1*.- Means ± SE of biomass received (A) and body-mass loss (B) by nestling experimentally fed with the appetitive stimulant cyproheptadine (experimental), and those feed with water (control).*

Table 1: Results of GLM models exploring the effects of experimental feeding with cyproheptadine (fixed factor), on biomass received by parents, and body-mass loss of nestlings (dependent variables) during two and a half hours of experiment with neck collars. The models also included nest identity as random factor, and nestling body mass before the experiment and brood size as covariable.

	MS	**Error MS**	**df**	**F**	**P**
BIOMASS RECEIVED					
Body mass (Cov.)	16.09	0.846	1, 33.6	19.03	0.0001
Brood size (Cov.)	0.001	0.801	1, 20.3	0.01	0.99
Treatment (Fixed)(1)	2.369	0.588	1, 10.8	4.03	0.070
Nest identity (Random)(2)	0.796	0.659	25, 16.1	1.21	0.35
Interaction (1*2)	0.658	1.074	16, 36	0.61	0.85
Error	1.074		36		
BODY-MASS LOSS					
Body mass (Cov.)	1.642	0.367	1, 38.0	4.472	0.041
Brood size (Cov.)	0.009	0.408	1, 29.9	0.021	0.89
Treatment (Fixed)(1)	0.854	0.235	1, 23.7	3.625	0.069
Nest identity (Random)(2)	0.414	0.225	29, 26.5	1.843	0.058
Interaction (1*2)	0.224	0.205	29, 71	1.094	0.37
Error	0.205		71		

Discussion

In this article, we have used cyproheptadine to increase the level of hunger of some nestlings, leaving other nestlings within the same nest as a control. The use of this drug as appetitive enhancer is well founded because this drug is a powerful antihistaminic and serotonin antagonist, with the secondary effect of appetite stimulation (see Homnick *et al.* 2004, and references therein). Cyproheptadine acts directly on the hypothalamus, because cats administrated with this drug demonstrated an increased neuronal activity at this site (Chakrabarty *et al.* 1967). Although the mechanism of action is unknown, it appears not to be due to a hypoglycaemic-induced hyperphagia or to an increase in endogenous growth hormone (Bergen 1964, Stiel *et al.* 1970). Rather, its action as appetite stimulation is probably mediated by an alteration of the activity of serotonin on the appetite centre at the hypothalamus (Delitala *et al.* 1975), as it has also been suggested for pigeons (Gunturkun *et al.* 1989). Due to this effect of cyproheptadine, it has been studied and broadly used as appetitive stimulant in patients of several diseases that need of an increase in body weight (e.g. cancer, HIV, cystic fibrosis, anorexia, etc.) with the advantage that this drug has minimal side effects consisting primarily of transient drowsiness (see Homnick *et al.* 2004, and references therein). Moreover, it has also been demonstrated that this weight gain in healthy human adults is principally due to an increase in food intake, although a decrease in activity resulting from the sedative effect of the drug might play an additional minor role (Stiel *et al.* 1970). Thus, cyproheptadine has been included in commercial medicines as appetite stimulant for humans (e.g. Periatin©), but also for other animals including birds (e.g. Vita-Vrot-c©).

Given all properties mentioned above, it can be assumed that, by using cyproheptadine, we increased level of hunger of some nestling in the nest. This experimentally increased level of hunger should affect begging behaviour of experimental nestlings, which in turn should result in these nestlings being preferentially fed by parents (see Mock & Parker 1997, Budden & Wright 2001, Kilner & Johnstone 1997). However, since the relationship between level of hunger and parent feeding behaviour is theoretically mediated by begging intensity being honest and, therefore, costly (e.g. Godfray 1991, 1995a, 1995b), experimental nestlings should experience larger costs than control nestlings. In accordance with these predictions, we found that experimental nestlings received more food, but lost more weight than control nestlings (see Fig. 1) while wearing neck collars that impeded them to swallow food received by parents.

We did not film experimental nests and thus we do not know whether experimental and control nestlings differed in their begging behaviour. Experimental nestlings may have received more food by, for instance, increasing begging call when parents are deciding which chick to feed (e.g., Smith & Montgomerie 1991). It is also possible that competition between siblings for controlling the space where adults preferentially feed was also important explaining or results (e.g., McRae *et al.* 1993). In any case, independently of the aspect(s) of begging behaviour that cyproheptadine affects, it is clear from our result that our experiment affects parental distribution of food, which was biased towards experimental nestlings (i.e. experimental nestling received more food than control nestlings). Furthermore, On the basis of the extensive literature on the influence of level of hunger on begging behaviour (e.g., Smith & Montgomerie 1991, Kacelnik *et al.* 1995, Kilner 1995, Cotton *et al.* 1996, Kölliker *et al.* 1998,), but also on the use of begging by parents to decide the chick to feed (see Budden & Wright 2001, and references therein), the effect of our experiment on biomass received by each nestling should be mediated by a change in begging behaviour associated to the experimentally modified hunger level.

Therefore, these results guarantee the use of cyproheptadine as an appetitive enhancer that affects nestling's begging behaviour and parental distribution of food among siblings in natural conditions. This is very important because it opens the possibility of direct manipulation of the level of hunger in the field and of exploring its effect on different components of nestling begging behaviour and fitness, but also on feeding behaviour of parents and its consequences on present and future reproduction as have been modelled by different authors (see Mock & Parker 1997).

The second result of our experiment is that experimental nestlings lost more weight than control nestlings during the two and a half hours that wore neck collars (Fig. 1b). Drowsiness, which is the main secondary effect of cyproheptadine (see above), cannot be the responsible of the higher level of weight loss of experimental nestlings during the neck-collar experiment, because its expected influence on weight loss is just the opposite (i.e., weight gain, see Stiel *et al.* 1970). Moreover, we included body mass before de experiment as a covariable in our model. The inclusion of this variable would statistically control for any variation in body mass loss due to differences in physiological activity of nestlings that vary in body mass (i.e. water loss, weight of faeces). Therefore, the detected differential weight loss of experimental nestlings is statistically controlled for possible allometric effects and, thus, should be related to a higher activity (i.e. begging behaviour) provoked by the experimentally increased level of hunger. Since we manipulated level of hunger, this result is a strong evidence of costs associated to begging behaviour.

Until now, experimental evidences of costs associated to begging behaviour come from laboratory studies where some nestlings were forced to increase their breeding effort but received the same amount of food than control nestlings (Kilner 2001, Rodríguez-Gironés *et al.* 2001), but also from brood size manipulations in the field (Neuenschwander *et al.* 2003). This is however the first time that level of hunger is directly manipulated in the field resulting in larger weight loss (costs) and amount of food received (benefits) of experimentally hungry nestlings. The allocation of food by parents depends not only on offspring solicitation signals (e.g., conspicuous calls, stretching of the neck, wing shivering and gaping), but also on the result of intra-brood competition for focal places in the nest, which is also costly (i.e. sibling competition) and thus also provides information of a nestling's relative need (see Budden & Wright 2001, Ostreiher 2001). Thus, costs associated to signals of need, which reliably reflect hunger level of nestlings and, therefore, explain honesty of the signal (i.e. begging), should also include those costs associated to nestling competition for food delivered by parents. Costs detected here included those from sibling competition since the experiments were performed in natural conditions allowing nestlings to compete with siblings. Moreover, because with this experimental approach we were able to detect benefits associated with the experimental increase level of hunger, it is also possible to study whether benefits counteract costs associated to an exaggerated level of hunger (i.e. "dishonest" signals) by studying its influence on variables related to fitness in fledglings, but also in adults.

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References

Bachman, G. C. & Chappell, M. A. 1998. The energetic cost of begging behavior in nestling house wrens. *Animal Behaviour*, **55,** 1607-1618.

Bergen, S. S. 1964. Appetite stimulating properties of cyproheptadine. *American Journal of Diseases of Children*, **108,** 270-&.

Birkhead, T. R. 1991. *The Magpies. The ecology and behaviour of black-billed and yellow-billed magpies*. London: T & A D Poyser.

Briskie, J. V., Martin, P. R. & Martin, T. E. 1999. Nest predation and the evolution of nestling begging calls. *Proceedings of the Royal Society of London, Series B*, **266,** 2153-2159.

Budden, A. E. & Wright, J. 2001. Begging in nestling birds. *Current Ornithology*, **16,** 83-118.

Burford, J. E., Friedrich, T. J. & Yasukawa, K. 1998. Response to playback of nestling begging in the red-winged blackbird, *Agelaius phoeniceus*. *Animal Behaviour*, **56,** 555-561.

Carlson, A. & Moreno, J. 1992. Cost of short flights in the willow tit measured with doubly-labelled water. *Auk*, **109,** 389-393.

Chakrabarty, A. S., Pillai, R. V., Anand, B. K. & Singh, B. 1967. Effect of cyproheptadine on the electrical activity of the hypothalamic feeding centres. *Brain Research*, **6,** 561-569.

Clark, A. B. & Lee, W. H. 1998. Red-winged blackbird females fail to increase feeding in response to begging call playbacks. *Animal Behaviour*, **56,** 563-570.

Cotton, P. A., Kacelnik, A. & Wright, J. 1996. Chick begging as a signal: are nestling honest? *Behavioral Ecology*, **7,** 178-182.

De Neve, L., Soler, J. J., Soler, M. & Pérez-Contreras, T. 2004. Nest size predicts the effect of food supplementation to magpie nestlings on their immunocompetence: an experimental test of nest size indicating parental ability. *Behavioral Ecology*, **15,** 1031-1036.

Dearborn, D. C. 1999. Brown-headed cowbird nestling vocalizations and risk of nest predation. *Auk*, **116,** 448-457.

Delitala, G., Masala, A., Alagna, S. & Devilla, L. 1975. Effect of cyproheptadine on spontaneous diurnal-variations of plasma Acth-cortisol and Acth-Gh secretion Induced by L-Dopa. *Biomedicine Express*, **23,** 406-409.

Godfray, H. C. J. 1991. Signalling of need by offspring to their parents. *Nature*, **352,** 328-330.

Godfray, H. C. J. 1995a. Evolutionary theory of parent-offspring conflict. *Nature*, **376,** 133-138.

Godfray, H. C. J. 1995b. Signalling of need between parents and young: parent-offspring conflict and sibling rivalry. *American Naturalist*, **146,** 1-24.

Gunturkun, O., Grothues, A., Hautkappe, A., Vise, F., Wawrzyniak, N. & Zwilling, U. 1989. Serotonergic modulation of ingestive behavior in pigeons. *Pharmacology Biochemistry and Behavior*, **32,** 415-420.

Haskell, D. 1994. Experimental evidence that nestling begging behaviour incurs a cost due to nest predation. *Proceedings of the Royal Society of London, Series B,* **257,** 161-164.

Homnick, D. N., Homnick, B. D., Reeves, A. J., Marks, J. H., Pimentel, R. S. & Bonnema, S. K. 2004. Cyproheptadine is an effective appetite stimulant in cystic fibrosis. *Pediatric Pulmonology*, **38,** 129-134.

Kacelnik, A., Cotton, P. A., Stirling, L. & Wright, J. 1995. Food allocation among nestling starlings: sibling competition and the scope of parental choice. *Proceedings of the Royal Society of London, Series B* **259,** 259-263.

Kedar, H., Rodríguez-Gironés, M. A., Yedvab, S., Winkler, D. W. & Lotem, A. 2000. Experimental evidence for offspring learning in parent-offspring communication. *Proceedings of the Royal Society of London, Series B*, **267,** 1723-1727.

Kilner, R. 1995. When do canary parents respond to nestling signals of need. *Proceedings of the Royal Society of London, Series B*, **260,** 343-348.

Kilner, R. M. & Johnstone, R. A. 1997. Begging the question: are offspring solicitation behaviours signals of need? *Trends in Ecology and Evolution*, **12,** 11-15.

Kilner, R. M. 2001. A growth cost of begging in captive canary chicks. *Proceedings of the National Academy of Science, USA*, **98,** 11394-11398.

Kölliker, M., Richner, H., Werner, I. & Heeb, P. 1998. Begging signals and biparental care: nestling choice between parental feeding locations. *Animal Behaviour*, **55,** 215-222.

Leech, S. M. & Leonard, M. L. 1996. Is there an energetic cost to begging in nestling tree swallows (*Tachycineta bicolor*)? *Proceedings of the Royal Society of London, Series B*, **263,** 983-987.

Leech, S. M. & Leonard, M. L. 1997. Begging and the risk of predation in nestling birds. *Behavioral Ecology*, **8,** 644-646.

Leonard, M. L., Horn, A. G. & Parks, E. 2003. The role of posturing and calling in the begging display of nestling birds. *Behavioral Ecology and Sociobiology*, **54,** 188-193.

McCarty, J. P. 1996. The energetic cost of begging in nestling passerines. *Auk*, **113,** 178-188.

McCarty, J. P. 1997. The role of energetic costs in the evolution of begging behavior of nestling passerines. *Auk*, **114,** 135-137.

McRae, S. B., Weatherhead, P. J. & Montgomerie, R. 1993. American robin nestlings compete by jockeying for position. *Behavioral Ecology and Sociobiology*, **33,** 101-106.

Mock, D. W. & Parker, G. A. 1997. *The evolution of sibling rivalry*. Oxford: Oxford University Press.

Neuenschwander, S., Brinkhof, M. W. G., Kolliker, M. & Richner, H. 2003. Brood size, sibling competition, and the cost of begging in great tits (*Parus major*). *Behavioral Ecology*, **14,** 457-462.

Ostreiher, R. 2001. The importance of nestling location for obtaining food in open cup-nests. *Behavioral Ecology and Sociobiology*, **49,** 340-347.

Ottosson, U., Backman, J. & Smith, H. G. 1997. Begging affects parental effort in the pied flycatcher, *Ficedula hypoleuca. Behavioral Ecology and Sociobiology*, **41,** 381-384.

Redondo, T. & Castro, F. 1992. The increase in risk of predation with begging activity in broods of magpies *Pica pica. Ibis*, **134,** 180-187.

Rodríguez-Gironés, M. A., Zuñiga, J. M. & Redondo, T. 2001. Effects of begging on growth rates of nestling chicks. *Behavioral Ecology and Sociobiology*, **12,** 269-274.

Rodríguez-Gironés, M. A., Zuniga, J. M. & Redondo, T. 2002. Feeding experience and relative size modify the begging strategies of nestlings. *Behavioral Ecology*, **13,** 782-785.

Roulin, A. 2001. On the cost of begging vocalization: implications of vigilance. *Behavioral Ecology*, **12,** 506-511.

Sacchi, R., Saino, N. & Galeotti, P. 2002. Features of begging calls reveal general condition and need of food of barn swallow (*Hirundo rustica*) nestlings. *Behavioral Ecology*, **13,** 268-273.

Smith, H. G., Kallander, H., Fontell, K. & Ljungstrom, M. 1988. Feeding frequency and parental division of labour in the double-brooded great tit *Parus major*. Effects of manipulating brood size. *Behavioral Ecology and Sociobiology*, **22,** 447-453.

Smith, H. G. & Montgomerie, R. 1991. Nestling American robins compete with siblings by begging. *Behavioral Ecology and Sociobiology*, **29,** 307-312.

Soler, J. J., De Neve, L., Pérez-Contreras, T., Soler, M. & Sorci, G. 2003. Trade-off between immunocompetence and growth in magpies: an experimental study. *Proceedings of the Royal Society of London B, Biological Sciences*, **270,** 241-248.

Soler, J. J. & Soler, M. 2000. Brood-parasite interactions between great spotted cuckoos and magpies: a model system for studying coevolutionary relationships. *Oecologia*, **125,** 309-320.

Soler, M. 1990. Relationships between the great spotted cuckoo *Clamator glandarius* and its corvid hosts in a recently colony area. *Ornis Scandinavica*, **21,** 212-223.

Soler, M., Martínez, J. G., Soler, J. J. & Møller, A. P. 1995. Preferential allocation of food by magpie *Pica pica* to great spotted cuckoo *Clamator glandarius* chicks. *Behavioral Ecology and Sociobiology*, **37,** 7-13.

Soler, M., Soler, J. J., Martinez, J. G. & Moreno, J. 1999. Begging behaviour and its energetic cost in great spotted cuckoo and magpie host chicks. *Canadian Journal of Zoology*, **77,** 1794-1800.

Stiel, J. N., Liddle, G. W. & Lacy, W. W. 1970. Studies of mechanism of cyproheptadine-induced weight gain in human subjects. *Metabolism*, **19,** 192-200.

Trivers, R. L. 1974. Parent-Offspring Conflict. *American Zoologist*, **14,** 249-264.

Verhulst, S. & Wiersma, P. 1997. Is begging cheap? *Auk*, **114,** 134.

Weathers, W. W., Hodum, P. J. & Anderson, D. J. 1997. Is the energy cost of begging by nestling passerines surprisingly low? *Auk*, **114,** 133.

IV

Parents may compensate energetic costs associated to a dishonest level of need. An experimental study

Parents may compensate energetic costs associated to a dishonest level of need. An experimental study

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Abstract

Theoretical models about parent-offspring communication postule that dishonest begging should have fitness costs. Here, we test this hypothesis by administrating magpie nestlings with a drug (cyproheptadine), which increases the level of hunger. Thanks to this new approach, we manipulated level of hunger of magpie nestlings while they were raised in the field, fed by their parents and competing for food with their sibs. Consequently and in contrast to previous studies, we were able to estimate not only costs, but also possible benefits associated with an experimentally increased level of hunger. As result, we found that nestlings that received cyproheptadine were of larger size (weight, tarsus, wing and tail lengths) and demonstrated better immune response than control nestlings at fledging. Thus, although exhibit a dishonesty level of hunger is costly, our results indicate that the net balance between costs and benefits is positive. We discuss additional possible costs in nature that impede a further exaggeration of begging behaviour.

Keywords.- *Begging behaviour, Parent-offspring conflict, Honest signalling, Begging costs, Pica pica, Cyproheptadine*

Resumen

Los modelos teóricos sobre la comunicación entre padres e hijos postulan que la petición deshonesta debería tener unos costes en eficacia biológica. Aquí, comprobamos esta hipótesis mediante la administración a pollos de urraca de una droga (la ciproheptadina), la cual incrementa el nivel de hambre. Gracias a esta nueva aproximación, manipulamos el nivel de hambre de los pollos de urraca mientras que fueron criados en el campo, alimentados por sus padres mientras y competiendo por la comida con sus hermanos. Consecuentemente y al contrario que estudios previos, fuimos capaces de no solo estimar los costes, sino también los beneficios asociados con un incremento experimental del nivel de hambre. Como resultado, encontramos que los pollos que recibieron ciproheptadina fueron de mayor tamaño (peso, tarso y longitudes de ala y cola) y demostraron una mejor respuesta inmune que los controles a la edad de volar. Por lo tanto, aunque demostrar un nivel deshonesto de necesidad es costoso, nuestros resultados indican que el balance neto entre costes y beneficios es positivo es positive. Nosotros discutimos los posibles costes adicionales en la naturaleza que impiden una exageración del comportamiento de petición.

Palabras Clave.- *Comportamiento petitorio, Conflicto paterno-filial, Señalización honesta, Costes de la petición, Pica pica, Ciproheptadina*

Submitted

Introduction

In species with parental care, offspring signal their needs by a more or less complex begging behaviour allowing to parent to adjust feeding effort accordingly to offspring solicitation (see Kilner & Johnstone 1997, Mock & Parker 1997, Royle *et al.* 2002). However, mainly because of differences in genetic relatedness between parent and offspring, and between siblings, offspring want to obtain more resources than parents are selected to give, appearing the parent-offspring conflict (Trivers 1974). The evolution of conspicuous and extravagant offspring signals during solicitation of care has been explained in relation to this parent-offspring conflict (reviewed in Godfray 1995, Mock & Parker 1997). Initially, the exaggeration of begging behaviour was considered as a way to obtain additional resources from parents, which do nothing to evade this psychological manipulation (e.g. Trivers 1974, Parker & Macnair 1978, but see Alexander 1974). In this scenario, accordingly with Hamilton's rule (see e.g. Mock & Parker 1997), the selfish behaviour of a certain offspring would be only limited by costs related to the loss in inclusive fitness due to the genes shared with the parents and siblings. Subsequently, resolution models of parent-offspring conflict have postulated that parents and offspring should be in an evolutionary equilibrium that is maintained by the direct costs associated to offspring and/or parental behaviours (Harper 1986, Eshel & Feldman 1991, Godfray 1991, Yamamura & Higashi 1992, Rodríguez-Gironés 1999). For instance, some of these models suggest that, due to costs associated to begging behaviour, offspring honestly show to parents their condition and, thus, parents are able to assess offspring's need and adjust properly their effort as well as food allocation among siblings (Harper 1986, Godfray 1991, Rodríguez-Gironés 1999). According to this interpretation of begging as a honest signal of need, a number of experimental studies have shown both that food deprivation causes an increase of begging levels, and that parents increase provisioning rate in response to increased levels of begging (reviewed in Kilner & Johnstone 1997, Mock & Parker 1997).

Evidence on the expected costs associated to offspring begging behaviour is scarce and sometimes controversial (reviewed in Kilner & Johnstone 1997, Roulin 2001, although see also Royle *et al.* 2002). Perhaps the clearest evidence on such costs comes from two recently published articles (Kilner 2001, Rodríguez-Gironés *et al.* 2001) that found that an experimental increase of begging behaviour resulted in a negative effect on nestling growth rate when studying canary (*Serinus canaria*) and magpie (*Pica pica*) nestlings, respectively (but see also Leonard *et al.* 2003). In these experimental approaches, some nestlings were forced to beg for food more that other control-nestlings, but control and experimental nestlings obtained the same amount of food. Thus, although both articles are clear detecting a physiological cost of begging behaviour, nothing can be said about the net balance of costs and benefits of an increase in begging behaviour. This is important because theoretical models predict that, due to the honesty of begging behaviour, parents should feed preferentially offspring with the most exaggerated begging and, thus, it is at least possible that parents compensate metabolic costs associated with an exaggerated begging increasing their feeding effort accordingly. In other words, even if a cost associated to begging behaviour is detected, because parents may compensate such as costs, a certain level of begging-behaviour exaggeration may result in a net benefit for offspring. Therefore, an interesting question remaining to be answered, is if benefits associated to an exaggerated begging behaviour compensate or not the associated physiological costs.

Here, we try to answer this question by experimentally increasing hunger level of some nestlings within a brood of magpies (*Pica pica*), and consequently their begging behaviour (see below). This was done by the administration of cyproheptadine hydrochloride, a drug that directly acts on the hunger centre at the hypothalamus (Chakrabarty *et al.* 1967). We have previously found strong evidences suggesting that this drug affects begging behaviour of magpies because experimental nestlings received more food (benefits) but their body mass decreased more than that of control nestlings (costs) (Soler *et al.*, submitted, **MS III**). Briefly, we supplied cyproheptadine to half the nestlings in magpie broods during the nestling period and, subsequently, we compared the effect of treatment with control nestlings on variables related to nestling probability of recruitment, such as immune response (reviewed in Saino & Møller 2002), body mass, tarsus length, tail length and wing length (reviewed in Lindström 1999). The advantage of using this methodology (i.e. drug) is that nestlings are in their natural nests, fed by their parents and, thus, possible net benefits (or costs) associated with a "dishonest" begging of the experimental nestlings can be estimated. In addition, this experimental design takes also into account the effect of competition between siblings (e.g. Johnstone 1999, Rodríguez-Gironés 1999, Royle *et al.* 2002, Neuenschwander *et al.* 2003) and the possible costs related to the nestling vigilance for parent arrival (Roulin 2001) on the estimation of the net balance of an increased begging behaviour.

Methods

Area and species of study

Fieldwork was performed during the spring of 2002 in a magpie population breeding in the Hoya of Guadix (37º18' N, 3º11' W, southern Spain). This place is a high-altitude plateau (approx. 1000 m a.s.l.) shaped by small cereal crops (specially barley) and alternated with plots more or less dispersed of almond groves (mainly in the proximity of towns) or oak trees, which are preferentially used by magpies to build their nests (Soler 1990).

The magpie is a territorial, sedentary, and relatively long-lived passerine bird with a well-studied biology (reviewed in Birkhead 1991). It occurs throughout large parts of Holarctic region being very abundant in some areas. Magpies lay a single clutch between March and May with 5-7 eggs (rank 3-10). Females start to incubate before clutch completion (usually once the fourth egg is laid), which result in broods that hatch asynchronously, where some of the smallest nestlings starve soon after hatching (Birkhead 1991, Cramp 1994).

Field procedures

At the beginning of the breeding season, we searched for new nest localizations and, once a nest was located, we visited it regularly to determine laying date. During the laying period, nests were checked at least twice per week to determine, between others, clutch size and laying date. Although brood parasitism is quite common in the area (Soler & Soler 2000), we did not use any parasitized nest in this study.

After hatching, when nestlings were about 2-4 days old, we weighted (Pesola spring valance, accuracy 0.1g) and marked all nestlings within a brood by painting the tarsus with a non-toxic waterproof pen (Uni Posca, PC-8k). Nestlings were ranked according to their weight, and alternately assigned to the experimental and control treatment. Thus, we obtained pairs of experimental-control chicks of similar weight within broods. Further, we alternated between nests the order of assignation of the experimental and control treatment and, thus, in some broods the heavier nestlings of the pairs were assigned to the experimental treatment, and in other broods they were control (for a similar experimental design, see e.g. Soler *et al.* 2003, De Neve *et* *al.* 2004). Experimental nestlings were fed with 0.1 mg of cyproheptadine hydrochloride (Acofarma, Inc.) diluted in 0.25 ml of water, while control nestlings were administrated with 0.25 ml of water. Drug concentration was the same used for children (0.4 mg/ml), while we calculated the dose based on those recommended for children and extrapolated to one nestling of 100 grams (0.05 mg/day).

After the first doses, nests were revisited every two days, in order to re-colour the tarsi, weight the nestlings, and supply to them with the experimental (i.e. cyproheptadine) or control (i.e. water) doses. All nestlings received the treatment six times. Four days after the last dose (ca. 16-18 days old), nests were visited to weight nestlings (Pesola 300g ± 1g) and measure tarsi (with a digital calliper, ± 0.01 mm), wings, and tail lengths (using a ruler, ± 0.1 cm). At this age, we also evaluated the in vivo T-cell-mediated immune response of nestlings by a phytohemagglutinin (PHA-P, Reference number: L8754, Sigma Chemical Co.) injection in the wing web (Cheng & Lamont 1988). Briefly, we injected fledglings subcutaneously in the right wing web with 0.5 mg of PHA dissolved in 0.1 ml of physiological saline solution (Bausch & Lomb). As a control, the left wing web was injected with 0.1 ml of physiological saline solution. Before and 24 h after the injection, we measured the thickness of each wing web at the injection site with a digital pressure-sensitive micrometer (Mitutoyo, models 547-301, ± 0.01 mm). The T-cell-mediated immune response (here after, immune response variable) was then estimated as the change in thickness of the right wing web (PHA injection) minus the change in thickness of the left wing web (Lochmiller *et al.* 1993). We repeated measurements of each wing web three times, and used the mean values to calculate the change in thickness.

Sample sizes and statistical analyses

After excluding nests that failed to rear at least two nestling to the age of PHA-injection, sample sizes were reduced from 71 to 61 nests. All dependent variables demonstrated no significant differences in variance estimated for control and experimental groups (i.e. homogeneous variance; Levene test of homogeneity of variances; $P > 0.05$) and, thus, we used General Lineal Models to analyze the effect of treatment on dependent variables (immune response; nestling weights in each visit; wing, tail and tarsus lengths at fledging age). The model consisted on two categorical factors: the nest identity, which we assumed to have random effects, and the treatment (i.e. experimental or control) with an assumed fixed effect on the dependent variables. Moreover, we also estimated the interaction between nest identities and treatment because a non-significant interaction would imply that the effect of our experiment did not differ between nests and, thus, the generality of the effects detected. The Error terms were calculated by the Satterthwaite's method, and type III sums of squares and the over-parameterized model to code the two categorical factors were used.

Additionally, to check whether the treatment effects on nestling development were masked by the variation of dependent variables due to nestling hierarchy in body mass, we repeated analyses by using a GLM with two within factors, each one with two levels (i.e. repeated measures): the treatment as the first within factor, and the order of nestling pair (first or second) as the second within factor, and thus acting each nest as an independent case. This design allowed us to determine whether the treatment effect was different for different nestling hierarchy (interaction factor). Sigma-restricted model was used to code categorical factors and the type VI (unique) to get the sums of squares. For these analyses we only used those nests where at least the two first pairs (i.e. two experimentals and two controls) of magpie nestlings had survived to fledging age (N = 37). Moreover, we performed a multivariable approach by using the previous design but including jointly into the analysis all dependent variables associated to nestling development (i.e. Wilks lambda test).

All statistical analyses were performed using STATISTICA 6.0 (StatSoft, Inc. 2001) and all tests were two-tailed. Values are means ± SE.

Table 1.– Effects of the treatment with cyproheptadine on those variables measured in magpie nestlings close to fledging age. Analyses are GLMs with nest identity (random effects) and experimental treatment (fixed effects) as between factors.

		Effect	df effect	MS effect	df error	MS Error	F	P
Immune response	Treatment	F	1	0.60	68.78	0.12	4.89	0.030
	Nest identity	R	59	0.41	57.12	0.12	3.36	< 0.001
	Interaction	R	55	0.12	132	0.12	0.998	0.49
Tarsus length	Treatment	F	1	0.48	70.12	8.3	0.06	0.81
	Nest identity	R	60	20.16	55.68	8.18	2.46	< 0.001
	Interaction	R	55	8.17	140	9.38	0.87	0.72
Wing length	Treatment	F	1	8.95	70.22	84.36	0.11	0.75
	Nest identity	R	60	273.73	55.68	83.09	3.29	< 0.001
	Interaction	R	55	83.02	140	95.89	0.87	0.73
Tail length	Treatment	F	1	46.34	77.01	23.43	1.98	0.16
	Nest identity	R	60	93.02	55.44	22.06	4.22	< 0.001
	Interaction	R	55	22.03	133	34.94	0.63	0.97
Final Weight	Treatment	F	1	4.76	66.86	397.91	0.01	0.91
	Nest identity	R	60	889.59	55.48	402.77	2.135	0.002
	Interaction	R	55	403.01	139	355.11	1.14	0.28

Results

Magpie nestlings experimentally fed with ciproheptadine and, thus, with a level of hunger experimentally increased, demonstrated a larger T-cell-mediated immune response than control nestlings (1.24 ± 0.03 vs. 1.13 ± 0.03; Table 1).

Moreover, when including information on position on the nestling hierarchy (larger or smaller nestlings) by using GLMs with a repeated measures design (i.e. pair order as first within factor and treatment as the second within factor), the positive effect of the experiment was detected not only for T-cells mediated immune response (1.25 ± 0.06 vs. 1.12 ± 0.07), but also for weight, tarsus, wing, and tail lengths (Fig. 1, Fig 2, and table 2). Therefore, experimental magpie nestlings had in average better phenotypic quality than their sibling control nestlings within the same nest.

Table 2.- Analyses of cyproheptadine effect when including information on position on the nestling hierarchy (larger or smaller nestlings)

	MS effect	MS Error	df	F	P
Immune response					
Pair order	0.06	0.13	1,34	0.44	0.51
Treatment	0.60	0.14	1,34	4.14	0.0497
Interaction	0.01	0.11	1,34	0.08	0.78
Tarsus length					
Pair order	36.14	8.73	1,36	4.14	0.049
Treatment	26.18	3.58	1,36	7.31	0.010
Interaction	0.01	5.55	1,36	0.001	0.97
Wing length					
Pair order	566.68	85.44	1,36	6.63	0.014
Treatment	166.76	31.80	1,36	5.24	0.028
Interaction	0.76	37.97	1,36	0.02	0.89
Tail length					
Pair order	241.83	31.34	1,34	7.72	0.0084
Treatment	60.98	14.02	1,34	4.35	0.0446
Interaction	0.69	21.48	34	0.03	0.86
Final Weight					
Pair order	1418.78	347.85	1,35	4.08	0.051
Treatment	1296	180.27	1,35	7.19	0.011
Interaction	182.25	200.89	1,35	0.91	0.35
All variables	*Value test (Wilks lambda test)*				
Pair order	0.70		5,27	2.28	0.075
Treatment	0.66		5,27	2.79	0.037
Interaction	0.93		5,27	0.42	0.83

We used GLMs with pair order as first within factor and treatment as the second within factor.

Moreover, the effects of cyproheptadine on nestling weight were mainly detected after the sixth visit (i.e. 12-14 days old) as showed in Fig. 2, which suggests that benefits associated to an increased level of hunger accumulated along the nestling period and becoming statistically detected only when nestlings were close to fledging.

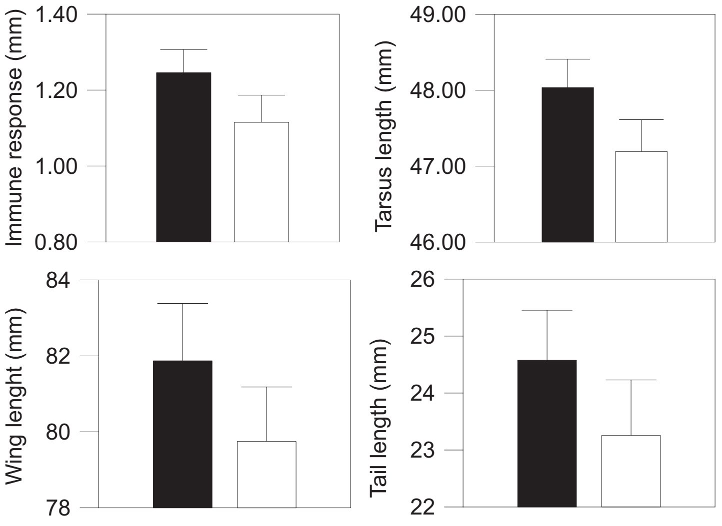

Figure 1.- *Effect of cyproheptadine on some of variables associated to the development of nestlings. Analyses are GLMs with treatment variable as one within factor and the order nestling pairs as second within factor. Black and open bars correspond to experimental and control treatment respectively; whiskers indicate the standard errors of means.*

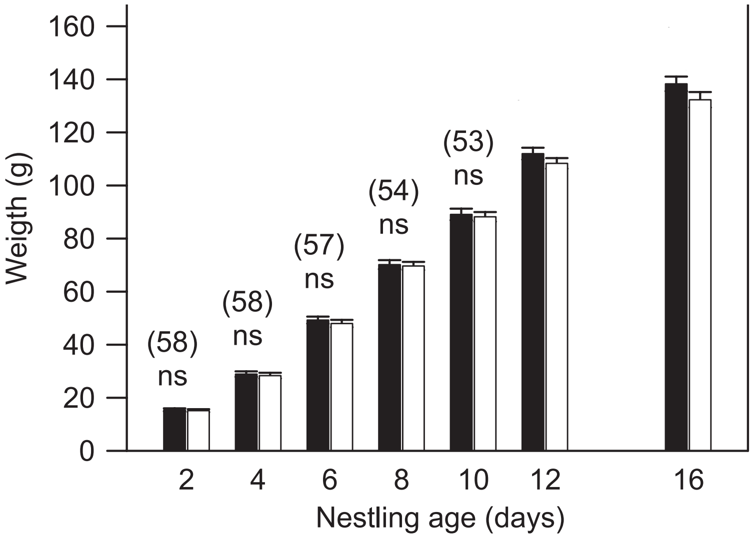

Figure 2.- *Comparisons between each treatment of the nestling weight obtained during every nest visit when including information on nestling hierarchy (i.e. GLM with a repeated measures design). Between parentheses is the number of nests used in each analysis.* ***(ns)*** *indicates if test was non-significant, instead* ***(*)*** *is used when p-value was lower than 0.05.*

Discussion

In this article, we have tested whether metabolic costs associated to signal production maintain the honesty of offspring food solicitation by using a new experimental approach, which allowed us to have a scenario quite more realistic than that in previous studies (see introduction). This experimental manipulation consisted in the increase of level of hunger in the experimental nestlings, but not by means of modifying the amount of food consumed by nestlings or the time necessary to obtain it. Instead, we increased directly the nestling need by using cyproheptadine hydrochloride. This drug is a powerful antihistaminic and serotonin antagonist, with the secondary effect of appetite stimulation (Chakrabarty *et al.* 1967). Cyproheptadine has been studied and used as appetitive stimulant in patients of several diseases that need of an increase in body weight with the advantage that this drug has minimal side effects consisting primarily of transient drowsiness (see Homnick *et al.* 2004, 2005 and references therein). Moreover, cyproheptadine has also been included in commercial medicines as appetite stimulant for humans (e.g. Periatin©), but also for other animals including birds (e.g. Vita-Vrot-c ©). Therefore, the use of this drug as enhancer of hunger level of nestling is justified. In agreement with this assumption, we have previously showed that magpie nestlings treated with cyproheptadine obtained a greater amount of food than control ones (Soler et al. submitted, **MS III**). Therefore, since parents use signal emitted by their offspring to decide food allocation (reviewed in Kilner & Johnstone 1997), the greater amount of food received by experimental nestling indicate us that cyproheptadine, by increasing the level of nestling hunger, also increase the begging intensity. Moreover, the experimental magpie nestlings suffered additional metabolic costs, which is in conformity with that predicted about the honesty begging signals (see above). Therefore, it can be assumed that here we directly increased levels of begging behaviour of some nestlings by using cyproheptadine hydrochloride.

Our main result is that, when nestlings were about to abandon the nest, experimental chicks were of better phenotypic quality than their control siblings. Briefly, after controlling the effect of the experiment for body-size hierarchy, nestlings that were fed with cyproheptadine during the nestling period had larger body mass, T-cell mediated immune response, and tarsus, tail and wing lengths. These results suggest that an increase begging behaviour had not negative, but positive effects on the variables used to estimate nestling phenotypic quality (i.e. immune response, corporal measures, and weight gain).

We can assume that, because our experiment was performed in the field, all energetic costs associated to an exaggerated begging behaviour, including sibling competition, are controlled in our results. Therefore, if the metabolic costs were the responsible for the theoretically optimum level of begging behaviour (e.g. Harper 1986, Eshel & Feldman 1991, Godfray 1991, Yamamura & Higashi 1992, Rodríguez-Gironés 1999), we should find a negative effect of our experiment in nestling phenotypic quality. This is because in that scenario, costs associated to an exaggeration of the signal would always surpass associated benefits. Thus, since our experiment suggests that benefits surpass costs associated to a "dishonest" begging behaviour, an interesting evolutionary question arises, namely, why not do magpie nestlings beg more? The answer to this question should be related to the existence of other costs apart from the energetic ones that may prevent from escalation of begging signals in magpie nestlings.

One of these costs is related to risk of predation, because predators would more easily detect nests with nestlings that beg loudly (Haskell 1994, Leech & Leonard 1997). However, because those costs are suffered by the whole brood, and different costs for nestlings that beg and do not beg are necessary for explaining the evolution of honest-begging behaviour (see Rodríguez-Gironés *et al.* 2001, for a detailed discussion), this cost would not prevent an escalation of begging signals. Another cost that influences the evolution of begging behaviour is that related to the loss of inclusive fitness (Hamilton 1964, Trivers 1974). A nestling by begging dishonestly would receive additional resources at expense of decreasing the amount of food delivered to its siblings, and/or force its parents to increase parental effort, which may reduce the probability of survival of both parents and siblings for future reproductions (e.g. Briskie *et al.* 1994, Johnstone 1998, Lotem 1998). However, these costs are quite difficult to test in natural conditions, mainly because it would need to study several generations, but also because variation in nestling phenotypic quality between nests is on average much larger than that within a nest and, thus, the effect size necessary to find statistically significant differences between nestlings that grew with and without dishonest siblings is probably much larger than the effect of the experiment.

Another additional cost for the dishonesty, which may also be contributing to our results in addition to the loss of inclusive fitness, would be related to the criticism reported by Alexander (1974) to the concept of parent-offspring conflict developed by Trivers (1974). In short, Alexander (1974) affirmed that a certain allele causing a conflicting offspring that receive more food than the parental optimum could not spread because the advantage of this allele when young would be counteracted by the disadvantage of producing conflicting offspring when adult (hereafter titled as selfish-offspring cost). Although this idea has been quite debated concluding that such costs do not prevent the evolution of parent-offspring conflict (see e.g. Dawkins 1976, Macnair & Parker 1978, Parker & Macnair 1978, Dawkins & Krebs 1979), it has also been suggested that such factor could be well founded and, thus, it should be added as a further cost in the equation explaining the maintenance of signal honesty (see e.g. Dawkins 1976). However, as the previous described costs, its demonstration would imply a long-term study. In any case, with the results reported here, we are not able to discuss the relative importance of costs related to producing a conflicting progeny and those related to the loss in inclusive fitness.

In conclusion, our results strongly suggest that begging honesty is not exclusively maintained by the energetic costs associated to the signal emission and measured during the nestling development. In fact, the experimental administration of an appetite enhancer had a positive effect on fitness-related variables of magpie nestlings. Therefore, it suggests a major role of costs related to the loss in inclusive fitness, and/or those related to the disadvantages of raising selfish offspring when adult.

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References

Alexander, R. D. 1974. The evolution of social behaviour. *Annual Review of Ecology and Systematics*, **5,** 325-383.

Birkhead, T. R. 1991. *The Magpies. The ecology and behaviour of black-billed and yellow-billed magpies*. London: T & A D Poyser.

Briskie, J. V., Naugler, C. T. & Leech, S. M. 1994. Begging intensity of nestling birds varies with sibling relatedness. *Proceedings of the Royal Society of London, Series B*, **258,** 73-78.

Chakrabarty, A. S., Pillai, R. V., Anand, B. K. & Singh, B. 1967. Effect of cyproheptadine on the electrical activity of the hypothalamic feeding centres. *Brain Research*, **6,** 561-569.

Cheng, S. & Lamont, J. 1988. Genetic analysis of immunocompetence measures in a white leghorn chicken line. *Poultry Science*, **67,** 989-995.

Cramp, S. 1994. *Handbook of the birds of Europe, the Middle East and North Africa. The birds of the western Palaearctic. Volume 8. Crows to finches*. Oxford: Oxford University Press.

Dawkins, R. 1976. *The selfish gene*. Oxford: Oxford University Press.

Dawkins, R. & Krebs, J. R. 1979. Arms races between and within species. *Proceedings of the Royal Society of London, Series B*, **205,** 489-511.

De Neve, L., Soler, J. J., Soler, M. & Pérez-Contreras, T. 2004. Nest size predicts the effect of food supplementation to magpie nestlings on their immunocompetence: an experimental test of nest size indicating parental ability. *Behavioral Ecology*, **15,** 1031-1036.

Eshel, I. & Feldman, M. W. 1991. The handicap principle in parent-offspring conflict: comparison of optimality and population genetic analyses. *American Naturalist*, **137,** 167-185.

Godfray, H. C. J. 1991. Signalling of need by offspring to their parents. *Nature*, **352,** 328-330.

Godfray, H. C. J. 1995. Evolutionary theory of parent-offspring conlict. *Nature*, **376,** 133-138.

Hamilton, W. D. 1964. The genetical evolution of social behaviour. *Journal of Theoretical Biology*, **7,** 1-52.

Harper, A. B. 1986. The evolution of begging: sibling competition and parent-offspring conflict. *American Naturalist*, **128,** 99-114.

Haskell, D. G. 1994. Experimental evidence that nestling begging behaviour incurs a cost due to nest predation. *Proceedings of the Royal Society of London, Series B*, **257,** 161-164.

Homnick, D. N., Homnick, B. D., Reeves, A. J., Marks, J. H., Pimentel, R. S. & Bonnema, S. K. 2004. Cyproheptadine is an effective appetite stimulant in cystic fibrosis. *Pediatric Pulmonology*, **38,** 129-134.

Homnick, D. N., Marks, J. H., Hare, K. L. & Bonnema, S. K. 2005. Long-term trial of cyproheptadine as an appetite stimulant in cystic fibrosis. *Pediatric Pulmonology*, **40,** 251-256.

Johnstone, R. A. 1998. Efficacy and honesty in communication between relatives. *American Naturalist*, **152,** 45-58.

Johnstone, R. A. 1999. Signaling of need, sibling competition, and the cost of honesty. *Proc. Natl. Acad. Sci. USA*, **96,** 12644-12649.

Kilner, R. M. 2001. A growth cost of begging in captive canary chicks. *Proceedings of the Natural Academy of Sciences, USA*, **98,** 11394-11398.

Kilner, R. M. & Johnstone, R. A. 1997. Begging the question: are offspring solicitation behaviours signals of need? *Trends in Ecology and Evolution*, **12,** 11-15.

Leech, S. M. & Leonard, M. L. 1997. Begging and the risk of predation in nestling birds. *Behavioral Ecology*, **8,** 644-646.

Leonard, M. L., Horn, A. G. & Porter, J. 2003. Does begging affect growth in nestling tree swallows, *Tachycineta bicolor*? *Behavioral Ecology and Sociobiology*, **54,** 573-577.

Lindström, J. 1999. Early development and fitness in birds and mammals. *Trends in Ecology and Evolution*, **14,** 343-348.

Lochmiller, R. L., Vestey, M. R. & Boren, J. C. 1993. Relationship between protein nutritional status and immunocompetence in northern bobwhite chicks. *Auk*, **110,** 503-510.

Lotem, A. 1998. Manipulative begging calls by parasitic cuckoo chicks: why should true offspring not do the same? *Trends in Ecology and Evolution*, **13,** 342-343.

Macnair, M. R. & Parker, G. A. 1978. Models of Parent-Offspring Conflict. II. Promiscuity. *Anim. Behav.*, **26,** 111-122.

Mock, D. W. & Parker, G. A. 1997. *The evolution of sibling rivalry.* New York: Oxford University Press.

Neuenschwander, S., Brinkhof, M. W. G., Kolliker, M. & Richner, H. 2003. Brood size, sibling competition, and the cost of begging in great tits (*Parus major*). *Behavioral Ecology*, **14,** 457-462.

Parker, G. A. & Macnair, M. R. 1978. Models of Parent-Offspring Conflict. I. Monogamy. *Anim. Behav.*, **26,** 97-110.

Rodríguez-Gironés, M. A. 1999. Sibling competition stabilizes signalling resolution models of parent-offspring conflict. *Proceedings of the Royal Society of London, Series B*, **266,** 2399-2402.

Rodríguez-Gironés, M. A., Zuñiga, J. M. & Redondo, T. 2001. Effects of begging on growth rates of nestling chicks. *Behavioral Ecology*, **12,** 269-274.

Roulin, A. 2001. On the cost of begging vocalization: implications of vigilance. *Behavioral Ecology*, **12,** 506-511.

Royle, N. J., Hartley, I. R. & Parker, G. A. 2002. Begging for control: when are offspring solicitation behaviours honest? *Trends in Ecology and Evolution*, **17,** 434-440.

Saino, N. & Møller, A. P. 2002. Immunity and begging. In: *The evolution of begging: competition, cooperation and communication* (Ed. by Wright, J. & Leonard, M. L.), pp. 245-267. Dordrecht: Kluwer Academic.

Soler, J. J., De Neve, L., Pérez-Contreras, T., Soler, M. & Sorci, G. 2003. Trade-off between immunocompetence and growth in magpies: an experimental study. *Proceedings of the Royal Society of London, Series B*, **270,** 241-248.

Soler, J. J. & Soler, M. 2000. Brood-parasite interactions between great spotted cuckoos and magpies: a model system for studying coevolutionary relationships. *Oecologia*, **125,** 309-320.

Soler, M. 1990. Relationships between the great spotted cuckoo *Clamator glandarius* and its corvid hosts in a recently colonized area. *Ornis Scandinavica*, **21,** 212-223.

Trivers, R. L. 1974. Parent-offspring conflict. *American Zoologist*, **14,** 249-264.

Yamamura, N. & Higashi, M. 1992. An evolutionary theory of conflict resolution between relatives: altruism, manipulation, compromise. *Evolution*, **46,** 1236-1239.

V

Experimental manipulation of hunger level results in a different outcome for great spotted cuckoo and magpie nestlings

Experimental manipulation of hunger level results in a different outcome for great spotted cuckoo and magpie nestlings

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Abstract

Begging behaviour of brood parasite nestlings is predicted to be less costly than that of offspring of nesting species. This is because parasites are sharing nest with non-siblings and are reared by non-related adults. However, although theoretical differences in costs of begging between parasitic and non-parasitic species may explain the commonly exaggerated begging behaviour in parasite nestlings, it does not preclude that begging behaviour of cuckoos was dependent of individual need (i.e. honest). Here, we test this hypothesis by studying the costs and benefits of an experimental increase of need in the brood parasite great spotted cuckoo (*Clamator glandarius*) nestlings, and in its main host in Europe and magpie (*Pica pica*) nestlings, both growing in the same nests (i.e. under identical environmental conditions). We found that the amount of food received by both great spotted cuckoo and magpies was related to their level of hunger. However, contrarily to magpie nestlings, benefits related to a increasing of level of need, were not associated with increase metabolic costs; suggesting that a dishonest cuckoo begging signals of need would have benefits but not additional costs. We discuss the possibility of other factors that our experiment did not take into account (i.e. begging costs related to risk of predation, or inclusive fitness, or possible physiological constraints such as limited food assimilation) to explain why cuckoos received food in accordance with their level of need, which therefore should be honestly signalled by begging behaviour.

Keywords.- *Begging behaviour, begging cost, brood parasitism, Clamator glandarius, cyproheptadine, Pica pica, honest signalling*

Resumen

El comportamiento petitorio de los parásitos de cría es predicho a ser menos costoso que aquel para la descendencia de las especies no parásitas. Esto es debido a que estos parásitos no comparten nido con hermanos y son criados por adultos no relacionados. Sin embargo, aunque las diferencias teóricas en costes de petición entre especies parásitas y no parásitas pueden explicar el habitual exagerado comportamiento de petición en pollos parásitos, esto no impide que el comportamiento de petición sea dependiente de la necesidad individual (es decir honesto). Aquí, comprobamos esta hipótesis mediante el estudio de los costes y beneficios de un incremento experimental de la necesidad de pollos de críalo (*Clamator glandarius*) y urraca (*Pica pica*) creciendo en el mismo nido (por lo tanto, bajo idénticas condiciones ambientales). Encontramos que la cantidad de comida recibida por los críalos y urracas estuvo relacionada con sus niveles de necesidad. Sin embargo, al contrario a los pollos de urraca, los beneficios de un incremento de nivel de necesidad no estuvieron asociados con un incremento de los costes metabólicos; sugiriendo que señales deshonestas en el cuco de petición tendrían beneficios sin costes adicionales. Discutimos la posibilidad de que otros factores que nuestro experimento no tiene en cuenta (ej. costes de la petición relacionados con el riesgo de depredación, eficacia biológica inclusiva, o posibles limitaciones fisiológicas tal como una limitada asimilación de comida) para explicar porqué los críalos recibieron comida acorde su nivel de necesidad, la cual debería ser honestamente indicada por el comportamiento de petición.

Palabras Claves.- *Comportamiento de petición, Coste de la petición, Parasitismo de cría, Clamator glandarius, Ciproheptadina, Pica pica, Señalización honesta*

Submitted

Introduction

In species with parental care, parents use signals emitted by their offspring to regulate their effort and resource allocation (e.g. Kilner & Johnstone 1997, Mock & Parker 1997). Because parents and offspring are not genetically identical, a parent-offspring conflict exists on the amount of parental inversion selected to give and receive, respectively (Trivers 1974). Resolution of this conflict, and hence the evolutionary stability of this system, is achieved by the existence of costs that penalize any dishonest offspring behaviour (see e.g. Godfray & Johnstone 2000, for a review of resolution theoretic models of this conflict). This is because possible benefits of a dishonest offspring (i.e. obtaining a greater amount of parental resources) are counteracted by associated costs. In altricial birds, offspring solicitation is manifested during parental arrivals as conspicuous calls and extravagant movements such as stretching of the neck, wing shivering and gaping (e.g. Redondo & Castro 1992a), but also by scramble competition between siblings (VanHeezik & Seddon 1996, Neuenschwander *et al.* 2003). Thus, offspring solicitation would imply direct costs (affecting on own reproductive success), such as: *i)* an additional energetic expense which would have negative effects on development (Kilner 2001, Rodríguez-Gironés *et al.* 2001); *ii)* a greater chance to be predated as a consequence of an increase in detectably during solicitation (Redondo & Castro 1992b, Haskell 1994, 1999, Leech & Leonard 1997, Briskie *et al.* 1999, Dearborn 1999), and *iii)* costs related to the disadvantage of raising selfish offspring when adult (Alexander 1974). Moreover, begging behaviour would also imply indirect costs; those related to a loss in inclusive fitness by depriving relatives from food (i.e. present or future siblings) (see e.g. Briskie *et al.* 1994).

However, offspring of some species of altricial birds are free of several of the begging costs listed above. These species are the obligated avian brood parasites, such as cuckoos and cowbirds (e.g. Lotem 1998, Kilner & Davies 1999, Rodríguez-Gironés *et al.* 2002, Lichtenstein & Dearborn 2004), which lay their eggs in nests of other species (hosts) that take care of the parasitic offspring (see Payne 1977, Rothstein 1990, Davies 2000). Parasitic nestlings are non-relatives of both adults and nestmates, and thus, their begging behaviour would be free of costs associated to inclusive fitness. Therefore, begging signals are predicted to be cheaper for parasitic nestlings than for nestlings of nesting species (see Davies & Brooke 1988, Lotem

1998, Soler *et al.* 1999, Lichtenstein & Dearborn 2004). In accordance, parasite nestlings typically possess an exaggerated begging behaviour in comparison to that of host nestlings (Davies *et al.* 1998, Dearborn 1998, Lichtenstein & Sealy 1998, Kilner & Davies 1999, Soler *et al.* 1999, Rodríguez-Gironés *et al.* 2002, Hauber 2003), that ensures an adequate provisioning rate in the case of evictor parasites (see Kilner *et al.* 1999, Grim & Honza 2001, Butchart *et al.* 2003), and confers advantages when competing for food with host nestlings in the case of non evictor parasites (e.g. Soler *et al.* 1995, Lichtenstein & Sealy 1998).

Although brood parasites would be able to exaggerate their begging behaviour due to lower costs than those suffered by their hosts, their begging could still be honestly indicating level of need (see Kilner & Davies 1999, Hauber & Ramsey 2003, Lichtenstein & Dearborn 2004). This is because begging behaviour in brood parasites could still be both energetically expensive to produce (see Soler *et al.* 1999) and attractive to predators (Dearborn 1999, but see Arcese *et al.* 1996). On the other hand, it is also possible that brood parasite nestlings begged for food dishonestly (i.e. with fewer benefits than costs associated to an exaggerated begging behaviour) since costs concerning inclusive fitness do not exist for brood parasites, and any adaptation exploiting the host signal-system (e.g. Kilner *et al.* 1999) and/or reducing energetic costs for the production of solicitation signals (see e.g. Lotem 1998) would be of selective advantage. In addition, the amplification of other components involved in begging behaviour, such as visual signals (Soler *et al.* 1995, Tanaka & Ueda 2005) or other aspects related with sibling competition, such as improving the vigilance for parental arrivals or scramble competition (see Roulin 2001, Dearborn 1998, Lichtenstein & Sealy 1998, Hauber 2003), would reduce predation risk as a consequence of begging display. Consequently, parasite nestlings could be able to beg at a superior level than that associated to their true need without a negative balance, resulting in dishonest begging and independent of nestling need (see e.g. Redondo & Zuñiga 2002, Lichtenstein & Dearborn 2004).

In this article, we study possible interespecific differences in costs and benefits associated to an exaggerated level of hunger of a brood parasite, the great spotted cuckoo *Clamator glandarius*, and those of its main host in Europe, the magpie *Pica pica*. To increase nestling need, we used a new approach consisting in the administration of a drug (cyproheptadine hydrochloride, Soler *et al.* submitted, **MS III**), which acts on the hunger centre sited on the hypothalamus (Chakrabarty *et al.*

1967). In a previous article, we have reported that cyproheptadine causes an increase in the amount of food received by magpie nestlings, likely due to an increase in begging behaviour, although at the expenses of additional begging metabolic costs (Soler *et al.* submitted, **MS III**). Therefore, differences with respect to the benefit/costs balance associated to an experimental increase of need of magpies and cuckoos sharing environmental conditions (i.e. nest) can be interpreted as differences mediated specific begging behaviour.

Our design consisted of experimental broods of two great-spotted cuckoo and two magpie chicks of similar weight, where one chick of each species was fed with cyproheptadine during the nestling period. The other two nestlings were fed with water and used as control. Afterwards, we tested the effect of cyproheptadine (i.e. increased level of need) both in the short term (ca. two and a half hours), by measuring amount of food received and body-mass loss, and in the long term (i.e. nestling period), by measuring variables related to nestling fitness, such as immune response (reviewed in Saino & Møller 2002), and corporal measures (reviewed in Lindström 1999), when nestlings were about to leave the nest. Then, if begging behaviour of great spotted cuckoo nestlings is a honest signal, modulated by begging costs and nestling need (hypothesis 1), an experimental increase of nestling need should have a similar outcome (i.e. relative differences between experimental and control nestlings) for the studied variables in nestlings of both species (prediction 1). On the other hand, if begging behaviour of great spotted cuckoo nestlings is dishonest and independent of nestling need (hypothesis 2), the outcome of our experimental increase of nestling need should differ between magpie and great spotted cuckoo nestlings (prediction 2).

Methods

Study species

The magpie is a territorial, sedentary, and relatively long-lived passerine bird with a well-studied biology (reviewed in Birkhead 1991). It occurs throughout large parts of the Holarctic region where in some places it becomes abundant. Magpies lay a single clutch between March and May with 5-7 eggs (rank 3-10). Females start to incubate before clutch completion (usually once the fourth egg is laid), so resulting in broods that hatch asynchronously, where some of the smallest nestlings starve soon after hatching (Birkhead 1991, Cramp 1994).

The great spotted cuckoo is an obligate brood parasite, which in Europe mainly parasitizes magpie nests (in our study area 36.4 % (N= 272) and 36.14 % (N=324) of magpie nests were parasitized during the breeding seasons of 2002 and 2003, respectively). Unlike common cuckoos, the great spotted cuckoo chicks do not evict eggs or host hatchlings and, thus, they must compete for food with foster siblings (Soler & Soler 1991, Soler & Soler 2000). However, some characteristics confer great spotted cuckoo nestlings with crucial advantages in this competition that usually results in the starvation of host chicks in the parasitized nests (Soler *et al.* 1996). Theses cuckoo characteristics are: a shorter incubation period (12-14 vs. 21-22 days, Cramp 1985, 1994), a growth rate much higher than that of host chicks (Soler & Soler 1991), the presence of a gape with well-developed palatal papillae that acts as a super-stimulus (Soler *et al.* 1995), and an exaggerated begging behaviour compared to that of host nestlings (Redondo 1993, Soler *et al.* 1999). For a more detailed review of the peculiarities of this system, see Soler & Soler (2000).

Field procedures

Fieldwork was carried out during the springs of 2002 and 2003 in a magpie population breeding in the Hoya of Guadix (37º18' N, 3º11' W, southern Spain). This place is a high-altitude plateau (approx. 1000 m a.s.l.) shaped by small cereal crops (specially barley) and alternated with dispersed plots of almond groves (mainly in the proximity of villages) or oak trees, which are preferentially used by magpies to build their nests (Soler 1990). At the beginning of the breeding season, we searched for new nest locations and, once a nest was located, we visited it regularly to determine laying date. During the laying period, nests were visited at least twice a week to determine clutch size and presence of brood parasitism.

Experimental broods

In order to avoid the starvation of host nestlings due to out-competence (see above), we eliminated in our experimental design the difference in hatching time between both species by creating experimental broods containing two great spotted cuckoos and two magpie chicks of a similar weight. We used hatchlings coming from different magpie pairs, both parasitized and non-parasitized by great spotted cuckoos. In short, from 97 experimental broods 21, 69, and 7 broods held nestlings from two, three, and four different magpie pairs, respectively. The experiment was performed when nestlings were about 2-4 days old. We avoided the possibility of using the same magpie pairs by not using the same magpie territories in both years to raise the experimental broods. It was so because magpies are generally faithful to their territory (Birkhead 1991).

Once the experimental brood set up, nestlings were weighted and marked by colouring one of their tarsi with non-toxic and waterproof ink (Uni Posca, PC-8k). Nestlings of both species were assigned to the experimental or control treatment; and between nests, we alternately performed all of the four possible combinations according to differences in weight between nestlings and species (i.e. the heaviest ones in both species being experimental or control, and the heaviest magpies and the lightest cuckoos being experimental or control). Treatments consisted in the oral administration to experimental nestlings of 0.1 mg of cyproheptadine hydrochloride (Acofarma, Inc.) diluted in 0.25 ml water, and the administration of 0.25 ml of water to control nestlings. Drug concentration was the same than that used for children (0.4 mg/ml), but we calculated the dose based on those recommended for children and extrapolated to one nestling of 100 grams (0.05 mg/day).

Subsequently, we revisited nests every two days, in order to re-colour the tarsi, to weight and supply nestlings with the experimental (i.e. cyproheptadine) or control (i.e. water) doses. All nestlings received the treatment six times. Two days after the last dose for the great spotted cuckoo chicks (ca. 14-16 days old) and four days after the last dose for the magpie chicks (ca. 16-18 days old), nests were revisited to weight nestlings (Pesola 300 ± 1 g) and measure tarsi (with a digital calliper, Mitutoyo, accuracy ± 0.01 mm), wings, and tail lengths (by a ruler, ± 0.1 cm) of surviving fledglings. At this age, we also evaluated the in vivo T-cell-mediated immune response of nestlings by a phytohemagglutinin (PHA-P, Reference number: L8754, Sigma Chemical Co.) injection in the wing web (Cheng & Lamont 1988). In short, we injected fledglings subcutaneously in the right wing web with 0.5 mg of PHA dissolved in 0.1 ml of physiological saline solution (Bausch & Lomb). As a control, left wing web was injected with 0.1 ml of physiological saline solution. Before and 24 h after the injection, we measured the thickness of each wing web at the injection site with a digital pressure-sensitive micrometer (Mitutoyo, models 547-301, ± 0.01 mm). The T-cell-mediated immune response (here after, immune response variable) was then estimated as the change in thickness of the right (PHA injection) minus that of the left wing web (Lochmiller *et al.* 1993). We repeated measurements of each wing web three times, and used the mean values to calculate the change in thickness.

Neck-collars trials

We used neck-collars (Kluijver 1933) to determine both benefits (i.e. food acquisition) and costs (i.e. metabolic expend) in the short term of the experimental increase of nestling need. When nestlings were 10-12 days old, we applied neck-collars to all nestlings in part of our experimental broods (N=49). Neck-collars consisted on pieces (ca. 80 mm) of fine wire (ø 1 mm.) lined with plastic, which were meticulously placed around nestling necks allowing normal respiration but avoiding the ingestion of food delivered by adult magpies. After ca. two and a half hours (141.76 ± 14.60 min (mean ± SE.)), we revisited the nests to remove collars and collect food samples from nestlings' throats. Food samples were stored in absolute ethanol until their desiccation in the laboratory (about 24 hours at 60 ºC). Afterwards, we weighted dry samplings using a precision scale (Adam equipment 120/0.0001g). In addition, we weighted nestlings before and after this treatment in order to determine body-mass loss of nestlings during the time of application of neck-collars. This value would be indicative of metabolic costs associated to nestling behaviour during the time of experiment (e.g. water loss, see Kilner 2001). Since collars should prevent nestlings from swallowing any food, (i.e. a gain in weight during neck collars application means some food ingestion) we used only data from nestlings that lost weight during the neck-collars period.

No nestlings died or resulted injured as a consequence of the use of neck-collars. As far as we could determine, we did not find any long-term negative effects on nestling development, since all measured variables of nestlings close to fledging (i.e. immune response, weight, wing length, and the tarsus and tail lengths (both square root-transformed)) did not significantly differ between nests used for the placing of neck-collars (N=49) and nests not used (N=36). These analyses were performed by GLMs with year of study, nestling species and collar treatment as between factors with fixed effects assumed; and nest identity (nested in year and in collar treatment) also as a between factor but assuming random effects.

Statistical procedures

Since we used data from two different breeding seasons, and we detected differences between them for some variables analyzed (see year effect in tables 1 and 2), we included the year of study and its interaction with experimental treatment in all the analyses performed in this article.

Most dependent variables possessed an approximately normal distribution of their frequencies (Kolmogorov-Smirnov test for continuous variables; $P > 0.2$), and variances of groups considered for the statistical analyses that do not differ significantly (Levene test of homogeneity of variances, $P > 0.05$). After square root transformations, the frequencies and variances between groups of those variables measured during neck-collars application (i.e. biomass received, body-mass loss) and the lengths of nestling tarsus and tail were normalized and homogenized, respectively.

Therefore, we decided to use parametric statistic, in particular General Lineal models (sensu Nelder & Wedderburn 1972). The effect of cyproheptadine treatment on biomass received and body-mass loss by nestlings per hour of application of neck-collars was estimated by means of a General Linear Model with year of study, treatment effect, and nestling species as fixed between factors, and nest identity (nested in year of study) as a random between factor. We also included body mass before the experiment as a covariable, because adult magpies preferentially feed nestlings of larger size (Soler *et al.* 1995) and nestling body mass could also affect weight loss during the time of neck-collars. Besides including the interaction between treatment and year of study (see above), we tested for the interaction between experimental treatment and nestling species, which indicated if treatment with cyproheptadine affected differentially (prediction 2), or similarly (prediction 1) magpie and great spotted cuckoo nestlings (see introduction). Afterwards, posthoc least significant difference tests (LSD tests) were used to analyze the effect of experimental treatment within each nestling species. Error terms were calculated by the Satterthwaite's method, and we used the type-III sums of squares and the over-parameterized model to code the two categorical factors.

Nonetheless, we were able to explore the effect of experimental treatment on those variables associated to nestling fitness by using a General Lineal Model with a repeated design (i.e. repeated measures), although it implies a reduction of our sample sizes (from 85 to 61 nests with nest of the two species an with the two treatments). Briefly, such model had two within factors with two levels each one: the nestling species (great spotted cuckoos and magpies) and the treatment (experimentals and controls); and year of study as a between factor, thus acting each nest as an independent case. This design allowed us to determine whether the treatment effect was different (prediction 2) or similar (prediction 1) between nestling species (interaction factor). Afterwards, in order to analyze the effect inside each nestling species, we also used post-hoc comparisons (LSD test). Sigma-restricted model was used to code categorical factors and the type VI (unique) to get the sums of squares. Moreover, we performed a multivariable approach by using the previous design but including jointly into the analysis all dependent variables associated to nestling development (i.e. Wilks lambda test).

All statistical analyses were performed by using STATISTICA 7.0 software (StatSoft, Inc. 2004) and all tests were two-tailed. Values are weighted means ± SD.

Results

Effect of ciproheptadine during neck-collars trials

Cyproheptadine treatment affected positively the amount of food received by nestlings during neck-collars, and this effect was not different depending on nestling species (see Table 1A and Fig. 1A). Accordingly, experimental magpies tended to received more food than control magpies (square root of amount of food received by experimental nestlings: 10.76 ± 1 mg/h (N=27), and by control ones: 8.93 ± 0.98 mg/h (N = 29); LSD test: MS = 9.87, df = 9, P = 0.06). We found a similar outcome for great spotted cuckoo nestlings, although the differences between treatments did not reach statistical significance (experimental nestlings: 9.07 ± 0.76 mg/h (N = 29), control ones: 7.75 ± 0.8 mg/h (N=23); LSD test: MS = 9.87, df = 9, P = 0.17).

In relation to body-mass loss, experimental nestlings tended to experience a greater body-mass loss than control nestlings (Table 1B). Moreover, the effect of cyproheptadine on body-mass loss differed depending on nestling species (significant interaction between treatment and nestling species in Table 1B and Fig. 1B). While experimental magpie nestlings tended to lose more weight than controls (square root of weight loss: 1.36 ± 0.06 g/h (N=35) vs. 1.25 ± 0.06 g/h (N = 37); LSD test: MS = 0.56, df. = 23, P = 0.06) that was not the case for great spotted cuckoo nestlings (experimental cuckoo nestlings: 1.41 ± 0.07 g/h (N = 35), control cuckoos. 1.41 ± 0.06 g/h (N=36); LSD test: MS = 0.56, df = 23, P = 0.98). Finally, the effect of the experiment was similar in both years (see interaction between treatment and year in

Table 1), and in most of experimental broods (see interaction between treatment and nest identity in Table 1).

Table 1.- Result from GLMs analyzing the cyproheptadine effect while nestlings wore neck-collars on the square root of Biomass received per hour (A) and the square root of Body-mass loss per hour (B).

A) Biomass received

Factors	**Effects**	**MS**	**Error MS**	**df**	**F**	**P**
Body mass	Cov.	0.07	9.87	1, 9.07	0.01	0.93
Year	Fixed	97.90	21.92	1, 45.64	4.47	0.040
Treatment	Fixed	52.99	12.25	1, 26.49	4.33	0.047
Nestling species	Fixed	4.49	13.69	1, 28.75	0.33	0.57
Nest (nested in year)	Random	23.69	21.28	40, 15.85	1.11	0.42
Year * Treatment	Fixed	3.97	12.12	1, 28.22	0.33	0.57
Nestling species * Treatment	Fixed	2.05	9.87	1, 9.00	0.21	0.66
Treatment * Nest (nested in year)	Random	12.50	9.87	23, 9.00	1.27	0.37

B) Body-mass loss

Factors	**Effects**	**MS**	**Error MS**	**df**	**F**	**P**
Body mass	Cov.	0.01	0.06	1, 23	0.16	0.69
Year	Fixed	0.92	0.22	1, 43.28	4.17	0.047
Treatment	Fixed	0.24	0.07	1, 46.24	3.66	0.06
Nestling species	Fixed	0.01	0.08	1, 55.34	0.13	0.72
Nest (nested in year)	Random	0.25	0.10	40, 22.52	2.43	0.014
Year * Treatment	Fixed	0.07	0.07	1, 46.57	1.01	0.32
Nestling species * Treatment	Fixed	0.43	0.06	1, 23	7.61	0.011
Treatment * Nest (nested in year)	Random	0.07	0.06	36, 23	1.22	0.31

Asterisks are used to indicate interaction between factors.

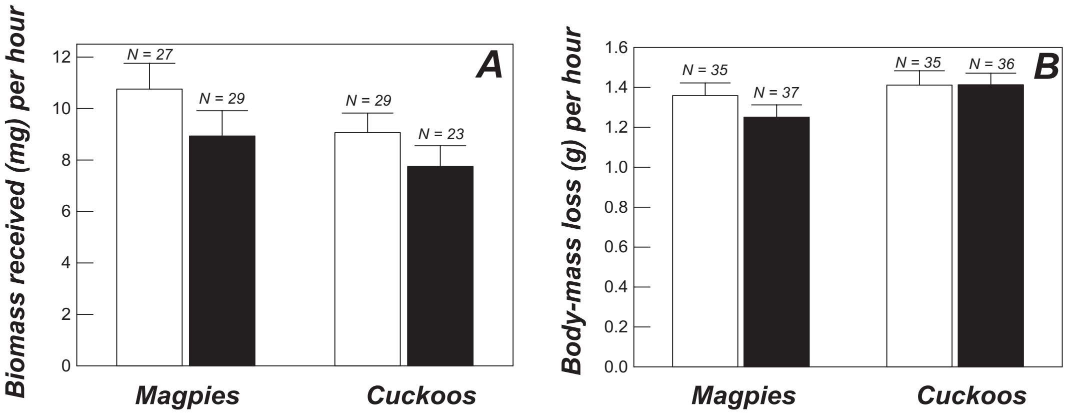

Figure 1.- *Square roots of biomass received (mg) per hour* ***(A)*** *and body-mass loss (g) per hour (B) by experimental (□) and control (■) nestlings while wore neck-collars.*

Effect of cyproheptadine on nestling development

After controlling for variation between species, we did not find any effect of the cyproheptadine treatment when considering nestlings of both species jointly (Table 2). Nonetheless, the administration of cyproheptadine affected tarsus length in a different way depending on nestling species (significant interaction between nestling species and treatment, Table 2). Experimental magpie nestlings tended to have a longer tarsus than control ones, but this was not the case for great spotted cuckoo nestlings (see Table 3).

Table 2.- Univariable and multivariable analyses of the cyproheptadine effect on variables associated to nestling development

	MS	MS Error	df	F	P
Immune response					
Year of Study	9.67	0.30	1, 54	32.76	< 0.001
Nestling species	1.12	0.23	1, 54	4.90	0.031
Treatment	0.09	0.17	1, 54	0.51	0.48
Year of study * Treatment	0.01	0.17	1, 54	0.05	0.82
Nestlings species * Treatment	0.02	0.21	1, 54	0.12	0.73
(Tarsus length)$^{1/2}$					
Year of Study	0.53	0.07	1, 59	7.58	0.008
Nestling species	132.70	0.04	1, 59	3147.26	<0.001
Treatment	0.02	0.03	1, 59	0.88	0.35
Year of study * Treatment	0.07	0.03	1, 59	2.56	0.12
Nestlings species * Treatment	0.13	0.03	1, 59	4.05	0.049
Wing length					
Year of Study	686.07	173.70	1, 59	3.95	0.05
Nestling species	468.45	104.33	1, 59	4.49	0.038
Treatment	68.10	52.18	1, 59	1.31	0.26
Year of study * Treatment	32.32	52.18	1, 59	0.62	0.43
Nestlings species * Treatment	71.99	69.91	1, 59	1.03	0.31
(Tail length)$^{1/2}$					
Year of Study	11.07	1.19	1, 58	9.28	0.003
Nestling species	120.18	0.55	1, 58	217.69	< 0.001
Treatment	0.03	0.30	1, 58	0.09	0.76
Year of study * Treatment	0.00	0.30	1, 58	0.00	0.96
Nestlings species * Treatment	0.70	0.42	1, 58	1.68	0.20
Final Weight					
Year of Study	10.51	563.86	1, 59	0.02	0.89
Nestling species	62798.82	387.48	1, 59	162.07	< 0.001
Treatment	327.74	198.78	1, 59	1.65	0.20
Year of study * Treatment	401.73	198.78	1, 59	2.02	0.16
Nestlings species * Treatment	55.63	255.55	1, 59	0.22	0.64
All variables (Wilks lambda test)	*Value test*				
Year of Study	0.50		5,46	9.17	<0.001
Nestling species	0.01		5,46	1221.64	<0.001
Treatment	0.96		5,46	0.34	0.89
Year of study * Treatment	0.94		5,46	0.54	0.74
Nestlings species * Treatment	0.94		5,46	0.61	0.69

Asterisks indicate the interaction between factors

Moreover, when we analyzed the effect of cyproheptadine only for magpie nestlings, using those experimental nest where at least both magpie nestling survived to fledging, thus increasing degrees of freedom, we found that the effect on tarsus length was hold (GLM with treatment as within factor and year as between factor: $F_{1,66}$ = 5.43, P = 0.023; experimental nestlings: 7.01 ± 0.03 mm. and control nestlings: 6.95 ± 0.03 mm). Furthermore, we found a similar outcome on magpie wing lengths (GLM with treatment as within factor and year as between factor: between factor: $F_{1,66}$ = 4.18, P = 0.045; experimental nestlings: 87.10 ± 0.91 mm, and control nestlings: 85.02 ± 1.15 mm).

In the case of great spotted cuckoo nestlings, when analyzing the effect of cyproheptadine separately (i.e. data from nests where at least both nestlings reached fledging age (N= 75)), results were not different to those obtained from the global analysis including both nestlings species presented above (GLM with treatment as within-group factor and year as between-group factor: treatment effect for all dependent variables with P values greater than 0.05).

Table 3.- Means ± SE of the variables associated to nestling development of each species nestlings and cyproheptadine treatments. Posthoc Least Significant Differences tests (LSD tests) between treatments within each nestling species are also shown.

	Experimentals	**Controls**	**MS**	**df**	**P**
Magpies					
Immune response	1.51 ± 0.06	1.53 ± 0.08	0.21	54	0.76
$(\text{Tarsus length})^{1/2}$	7.02 ± 0.03	6.96 ± 0.03	0.03	59	0.055
Wing length	87.22 ± 0.92	84.90 ± 1.18	69.91	59	0.13
$(\text{Tail length})^{1/2}$	5.17 ± 0.08	5.07 ± 0.09	0.42	58	0.40
Final Weight	152.09 ± 2	148.78 ± 2.34	255.55	59	0.28
Cuckoos					
Immune response	1.35 ± 0.07	1.41 ± 0.07	0.21	54	0.52
$(\text{Tarsus length})^{1/2}$	5.49 ± 0.03	5.52 ± 0.03	0.03	59	0.34
Wing length	83.00 ± 1.45	83.36 ± 1.55	69.91	59	0.81
$(\text{Tail length})^{1/2}$	6.46 ± 0.12	6.61 ± 0.13	0.42	58	0.24
Final Weight	118.39 ± 2.70	117.86 ± 2.60	255.55	59	0.75

Discussion

In this study, we experimentally tested the hypothesized difference in costs associated to signalling a dishonest level of hunger between nestlings of brood parasites and those of their hosts. This was done that by exploring predictions from the hypothesis about the existence of interespecific differences between magpies and great spotted cuckoo nestlings on the effects of an appetitive stimulant drug. Our experimental approach allowed to work with magpie and great spotted cuckoo nestlings raised by magpie pairs in the field; thus, the experimental design did not underestimate other important factors apart from the begging display explaining the cost of food solicitation, such as the competition between siblings (e.g. Johnstone 1999, Rodríguez-Gironés 1999, Royle *et al.* 2002, Neuenschwander *et al.* 2003) or the possible costs related to the nestling vigilance for parent arrival (Roulin 2001)

By means of a neck-collar trial, we found that, independently of the species, nestlings treated with cyproheptadine, and thus with an increased level of need, obtained a greater amount of food than controls (see Results). This is in accordance with previous results from non-parasitized magpies broods (Soler *et al.* submitted, **MS III**), and indicates that cyproheptadine, by increasing the level of hunger, increased the intensity of signals of need detected by parents (i.e. begging behaviour) resulting in experimental nestlings of both species being preferentially feed (see Fig. 1A). These results are in accordance with prediction 1 and suggest that the intensity of begging behaviour in great spotted cuckoo nestlings, as in host nestlings (reviewed in Kilner & Johnstone 1997, Mock & Parker 1997, Budden & Wright 2001), would be modulated by the level of food requirements. This is because, such as in others brood parasites (see Kilner & Davies 1999, Hauber & Ramsey 2003, Lichtenstein & Dearborn 2004), great spotted cuckoos received food in relation to their hunger level, which should be mediated by the interpretation of foster parents of a honest begging behaviour of parasitic nestlings. Additionally, we found that great spotted cuckoo nestlings did not receive a greater amount of food than magpie nestlings (see nestling species factor in Table 1) as can be predicted. However, it is also known that great spotted cuckoo chicks were not preferentially fed when they were smaller than magpie chicks (Soler *et al.* 1995), and that the asymptotic weight of magpie nestlings is larger than that of cuckoos (Soler & Soler 1991). Thus, our results can be explained because at the age of neck-collars trials (ca. 10-12 days old), parasitic nestlings had already across its maximum growth rate and became smaller (99.27 ± 1.69 g (N=96)) than magpies nestlings (105.21 ± 1.64 (N= 96)) (GLM with year of study, treatment effect, and nestling species as fixed between factors, and nest identity (nested in year of study) as a random between factor: $F_{1, 46.53}= 7.1$, $P = 0.011$).

When considering both species, experimental nestlings tended to lose more weight during neck-collar trials than control nestlings, indicating a greater metabolic costs (Table 1). However, the significant interaction between treatment and species showed that this effect only occurred in magpie nestlings (see Results and Fig. 1B). Therefore, great spotted cuckoo nestlings, dissimilarly to magpie nestlings, did not experience an increment of metabolic costs associated to the experimental increase of level of hunger (i.e. begging behaviour). However, with our data, we cannot discuss differences in metabolic costs between magpie and greats potted cuckoo nestlings, because these could be due to differences in physiology and growth strategies. However, those differences in body mass loss between controls and experimental nestlings of the two species (i.e. interaction treatment species) would indicate that costs associated to a dishonest level of hunger differ between species.

In accordance to these results, a great spotted cuckoo nestling, by dishonestly showing level of hunger (i.e. increasing the intensity of begging display) could obtain more food, but paying differentially less additional metabolic costs than magpie nestlings. Then, an important question arises, namely: why do cuckoos not increase their begging behaviour and get the associated additional food? One possible explanation would be the existence of others than the metabolic costs, such as the increased predation risk. For instance, Dearborn (1999) found in indigo bunting (*Passerina cyanea*), that those broods parasitized by brown headed cowbird (*Molothrus ater*) were more frequently predated than non parasitized broods, concluding that such difference was partially caused by the noisy begging calls of cowbird nestlings. In relation to the magpie-great spotted cuckoo system, although in nonparasitized magpie broods, the noisiest ones were also the most frequently preyed (Redondo & Castro 1992b), there is no difference in predation rates between broods parasitized by great spotted cuckoo and those nonparasitized (Redondo & Zuñiga 2002). However, great spotted cuckoo nestlings outcompete nestmates more often than cowbird nestlings (see Davies 2000) and thus, the level of noise at the nest would depend not only on the presence of great spotted cuckoos, but also on whether host nestlings are also begging. Thus, it is possible that when host and great spotted cuckoo nestlings are sharing the nest, cuckoos modulated their begging behaviour because of the associated predation cost. Other type of costs that could prevent an increase in signal of need of cuckoos is associated to the loss in inclusive fitness. Great spotted cuckoo females may lay more than one egg per host nest (e.g. Martínez *et al.* 1998) and thus, the exaggeration of begging behaviour of nestlings of this species could also be constrained if implies a deprivation of food of relatives in the same nest (Rodríguez-Gironés *et al.* 2002). However, according to this possibility we should find an outcome for cuckoo nestlings similar to that for magpie nestlings at the end of nestling period, which was not the case (see below).

Another possibility explaining why cuckoo nestlings do not naturally increase their begging behaviour could be that they are already obtaining all the food that they can properly assimilate (i.e. they are growing at maximum rate at the age of the experiment). Therefore, begging for additional food for cuckoos, although relatively less expensive than for magpies, it would not involve any fitness benefits. We believe that this is the most likely possibility, and accordingly, we found that whereas experimental magpies showed a higher phenotypic quality than control ones at the end of nestling period (i.e. tarsus and wing length, see Results), great spotted cuckoos did not show such differences between treatments. Moreover, we found that the effect of cyproheptadine on tarsus length was significantly different for cuckoos and magpies nestlings (Table 2).

The effect of cyproheptadine on the development of magpie nestlings was similar to that previously reported in non-parasitized broods (i.e. a higher phenotypic quality of experimental nestlings, Martín-Gálvez *et al.* submitted, **MS IV**), which would suggest that begging behaviour in magpies is not only maintained by metabolic costs; instead, other costs such as the loss in inclusive fitness or costs related to the disadvantage of raising selfish offspring when adult, should be also maintaining the honesty of food solicitation signals in magpies (Martín-Gálvez *et al.*, submitted, **MS IV**). However, as a difference with this previous article, here, cyproheptadine effect on magpie nestling development was not manifested in all variables measured. Perhaps, this reduced effect on magpie nestling development could be a consequence of a greater sibling competition in these experimental broods caused by the two parasite nestlings, in comparison to the competence suffered in non-parasitized broods.

In a similar study, Redondo & Zuñiga (2002) found that begging intensity of great spotted cuckoo chicks did not vary in relation to the duration (0.5, 1 and 2.5 hours) of food deprivation (see Table 1 therein), while that of magpie nestlings did. However, they did not study the statistical interaction between nestling species and time of food deprivation (i.e. a differential effect of food deprivation on begging intensity depending on nestling species). Moreover, in a subsequent experiment where nestlings were trained to beg for food at different rates (see Rodríguez-Gironés *et al.* 2002), experimental cuckoo nestlings of different treatments consistently increase begging intensity depending of time of food deprivation (0.5, 1 and 2.5 hours) (Rodríguez-Gironés, Unpublished data, Com. Pers.) and thus, honestly signalling of level of nestling need.. Moreover, Soler *et al.* (1999) found both great spotted cuckoo and magpies nestlings being hand-reared stopped to beg when they had received enough food, thereby, suggesting that begging behaviour in both species could be indicating levels of nestling need. Our results are in agreement with these last two studies, since cyproheptadine by increasing the hunger level of cuckoos and magpies, also increased in a similar way the amount of food received by experimental nestlings (non-significant interaction between nestling species and treatment). Therefore, because we have directly manipulated hunger level, our results suggest that cuckoo begging signals depend on nestling need (see above).

Summarizing, our results suggest that although begging behaviour of great spotted cuckoo varies with level of need, such as it has been observed in other brood parasite species, an increase of cuckoo level of hunger has not additional metabolic costs associated. Therefore, a great spotted cuckoo nestling, by dishonestly showing level of hunger, would obtain more food without paying additional metabolic costs. However, it could not be beneficial for great spotted cuckoo nestlings because the existence of others costs (such as predation risk or loss in inclusive fitness) or because physiological limitation in processing and assimilation of nutrients.

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References

Alexander, R. D. 1974. The evolution of social behaviour. *Annual Review of Ecology and Systematics*, **5,** 325-383.

Arcese, P., Smith, J. N. M. & Hatch, M. I. 1996. Nest predation by cowbirds and its consequences for passerine demography. *Proceedings of the Natural Academy of Sciences, USA*, **93,** 4608-4611.

Birkhead, T. R. 1991. *The Magpies. The ecology and behaviour of black-billed and yellow-billed magpies*. London: T & A D Poyser.

Briskie, J. V., Martin, P. R. & Martin, T. E. 1999. Nest predation and the evolution of nestling begging calls. *Proceedings of the Royal Society of London, Series B*, **266,** 2153-2159.

Briskie, J. V., Naugler, C. T. & Leech, S. M. 1994. Begging intensity of nestling birds varies with sibling relatedness. *Proceedings of the Royal Society of London, Series B*, **258,** 73-78.

Budden, A. E. & Wright, J. 2001. Begging in nestling birds. *Current Ornithology*, **16,** 83-118.

Butchart, S. M. H., Kilner, R. M., Fuisz, T. & Davies, N. B. 2003. Differences in the nestling begging calls of hosts and host-races of the common cuckoo, *Cuculus canorus*. *Animal Behaviour*, **65,** 345-354.

Chakrabarty, A. S., Pillai, R. V., Anand, B. K. & Singh, B. 1967. Effect of cyproheptadine on the electrical activity of the hypothalamic feeding centres. *Brain Research*, **6,** 561-569.

Cheng, S. & Lamont, J. 1988. Genetic analysis of immunocompetence measures in a white leghorn chicken line. *Poultry Science*, **67,** 989-995.

Cramp, S. 1985. *Handbook of the birds of Europe, the Middle East and north Africa. The birds of the western Palaearctic. Volume 4. Terns to woodpeckers.* Oxford: Oxford University Press.

Cramp, S. 1994. *Handbook of the birds of Europe, the Middle East and North Africa. The birds of the western Palaearctic. Volume 8. Crows to finches.* Oxford: Oxford University Press.

Davies, N. B. 2000. *Cuckoos, cowbirds, and other cheats.* London: T & A D Poyser.

Davies, N. B. & Brooke, M. de L. 1988. Cuckoos versus reed warblers: adaptations and counteradaptations. *Animal Behaviour*, **36,** 262-284.

Davies, N. B., Kilner, R. M. & Noble, D. G. 1998. Nestling cuckoos, *Cuculus canorus*, exploit hosts with begging calls that mimic a brood. *Proceedings of the Royal Society of London, Series B*, **265,** 673-678.

Dearborn, D. C. 1998. Begging behavior and food acquisition by brown-headed cowbird nestlings. *Behavioral Ecology and Sociobiology*, **43,** 259-270.

Dearborn, D. C. 1999. Brown-headed Cowbird nestling vocalizations and risk of nest predation. *Auk*, **116,** 448-457.

Godfray, H. C. J. & Johnstone, R. A. 2000. Begging and bleating: the evolution of parent-offspring signalling. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, **355,** 1581-1591.

Grim, T. & Honza, M. 2001. Does supernormal stimulus influence parental behaviour of the cuckoo's host? *Behavioral Ecology and Sociobiology*, **49,** 322-329.

Haskell, D. G. 1994. Experimental evidence that nestling begging behaviour incurs a cost due to nest predation. *Proceedings of the Royal Society of London, Series B*, **257,** 161-164.

Haskell, D. G. 1999. The effect of predation on begging-call evolution in nestling wood warblers. *Animal Behaviour*, **57,** 893-901.

Hauber, M. E. 2003. Lower begging responsiveness of host versus parasitic Brown-Headed cowbird (*Molothrus ater*) nestlings is related to species identity but not to early social experience. *Journal of Comparative Psychology*, **117,** 24-30.

Hauber, M. E. & Ramsey, C. K. 2003. Honesty in host-parasite communication signals: the case for begging by fledgling brown-headed cowbirds *Molothrus ater. Journal of Avian Biology*, **34,** 339-344.

Johnstone, R. A. 1999. Signalling of need, sibling competition, and the cost of honesty. *Proceedings of the Natural Academy of Sciences, USA*, **96,** 12644-12649.

Kilner, R. M. 2001. A growth cost of begging in captive canary chicks. *Proceedings of the Natural Academy of Sciences, USA*, **98,** 11394-11398.

Kilner, R. M. & Davies, N. B. 1999. How selfish is a cuckoo chick? *Animal Behaviour*, **58,** 797-808.

Kilner, R. M. & Johnstone, R. A. 1997. Begging the question: are offspring solicitation behaviours signals of need? *Trends in Ecology and Evolution*, **12,** 11-15.

Kilner, R. M., Noble, D. G. & Davies, N. B. 1999. Signals of need in parent-offspring communication and their exploitation by the common cuckoo. *Nature*, **397,** 667-672.

Kluijver, H. N. 1933. Bijdrage tot de biologie en de ecologie van den spreeuw (*Sturnus vulgaris* L.) gedurende zijn voortplantingstijd. *Versl Meded Plantenziekt Wageningen*, **69,** 1-145.

Leech, S. M. & Leonard, M. L. 1997. Begging and the risk of predation in nestling birds. *Behavioral Ecology*, **8,** 644-646.

Lichtenstein, G. & Dearborn, D. C. 2004. Begging and short-term need in cowbird nestlings: how different are brood parasites? *Behavioral Ecology and Sociobiology*, **56,** 352-359.

Lichtenstein, G. & Sealy, S. G. 1998. Nestling competition, rather than supernormal stimulus, explains the success of parasitic brown-headed cowbird chicks in yellow warbler nests. *Proceedings of the Royal Society of London, Series B*, **265,** 249-254.

Lindström, J. 1999. Early development and fitness in birds and mammals. *Trends in Ecology and Evolution*, **14,** 343-348.

Lochmiller, R. L., Vestey, M. R. & Boren, J. C. 1993. Relationship between protein nutritional status and immunocompetence in northern bobwhite chicks. *Auk*, **110,** 503-510.

Lotem, A. 1998. Manipulative begging calls by parasitic cuckoo chicks: why should true offspring not do the same? *Trends in Ecology and Evolution*, **13,** 342-343.

Martínez, J. G., Soler, J. J., Soler, M. & Burke, T. 1998. Spatial patterns of egg laying and multiple parasitism in a brood parasite: a non-territorial system in the great spotted cuckoo (Clamator glandarius). *Oecologia*, **117,** 286-294.

Mock, D. W. & Parker, G. A. 1997. *The evolution of sibling rivalry*. New York: Oxford University Press.

Nelder, J. A. & Wedderburn, R. W. M. 1972. Generalized linear models. *Journal of the Royal Statistical Society, Series A*, **135,** 370-384.

Neuenschwander, S., Brinkhof, M. W. G., Kolliker, M. & Richner, H. 2003. Brood size, sibling competition, and the cost of begging in great tits (*Parus major*). *Behavioral Ecology*, **14,** 457-462.

Payne, R. B. 1977. The ecology of brood parasitism in birds. *Annual Review of Ecology and Systematics*, **8,** 1-28.

Redondo, T. 1993. Exploitation of host mechanisms for parental care by avian brood parasites. *Etología*, **3,** 235-297.

Redondo, T. & Castro, F. 1992a. Signalling of nutritional need by magpie nestlings. *Ethology*, **92,** 193-204.

Redondo, T. & Castro, F. 1992b. The increase in risk of predation with begging activity in broods of magpies *Pica pica*. *Ibis*, **114,** 180-187.

Redondo, T. & Zuñiga, J. M. 2002. Dishonest begging and host manipulation by *Clamator* cuckoos. In: *The evolution of begging: competition, cooperation and communication* (Ed. by Wright, J. & Leonard, M. L.), pp. 1-20. Dordrecht: Kluwer Academic.

Rodríguez-Gironés, M. A. 1999. Sibling competition stabilizes signalling resolution models of parent-offspring conflict. *Proceedings of the Royal Society of London, Series B*, **266,** 2399-2402.

Rodríguez-Gironés, M. A., Zuñiga, J. M. & Redondo, T. 2001. Effects of begging on growth rates of nestling chicks. *Behavioral Ecology*, **12,** 269-274.

Rodríguez-Gironés, M. A., Zuñiga, J. M. & Redondo, T. 2002. Feeding experience and relative size modify the begging strategies of nestlings. *Behavioral Ecology*, **13,** 762-785.

Rothstein, S. I. 1990. A model system for coevolution: avian brood parasitism. *Annual Review of Ecology and Systematics*, **21,** 481-508.

Roulin, A. 2001. On the cost of begging vocalization: implications of vigilance. *Behavioral Ecology*, **12,** 506-511.

Royle, N. J., Hartley, I. R. & Parker, G. A. 2002. Begging for control: when are offspring solicitation behaviours honest? *Trends in Ecology and Evolution*, **17,** 434-440.

Saino, N. & Møller, A. P. 2002. Immunity and begging. In: *The evolution of begging: competition, cooperation and communication* (Ed. by Wright, J. & Leonard, M. L.), pp. 245-267. Dordrecht: Kluwer Academic.

Soler, J. J. & Soler, M. 2000. Brood-parasite interactions between great spotted cuckoos and magpies: a model system for studying coevolutionary relationships. *Oecologia*, **125,** 309-320.

Soler, M. 1990. Relationships between the great spotted cuckoo *Clamator glandarius* and its corvid hosts in a recently colonized area. *Ornis Scandinavica*, **21,** 212-223.

Soler, M., Martínez, J. G. & Soler, J. J. 1996. Effects of brood parasitism by the great spotted cuckoo on the breeding success of the magpie host: an experimental study. *Ardeola*, **43,** 87-96.

Soler, M., Martínez, J. G., Soler, J. J. & Møller, A. P. 1995. Preferential allocation of food by magpie *Pica pica* to great spotted cuckoo *Clamator glandarius* chicks. *Behavioral Ecology and Sociobiology*, **37,** 7-13.

Soler, M. & Soler, J. J. 1991. Growth and development of great spotted cuckoos and their magpie host. *The Condor*, **93,** 49-54.

Soler, M., Soler, J. J., Martínez, J. G. & Moreno, J. 1999. Begging behaviour and its energetic cost in great spotted cuckoo and magpie host chicks. *Canadian Journal of Zoology*, **77,** 1794-1800.

StatSoft, I. 2004. STATISTICA (data analysis software system). *ver. 7*

Tanaka, K. D. & Ueda, K. 2005. Horsfield's hawk-cuckoo nestlings simulate multiple gapes for begging. *Science*, **308,** 653.

Trivers, R. L. 1974. Parent-Offspring conflict. *American Zoologist*, **14,** 249-264.

VanHeezik, Y. M. & Seddon, P. J. 1996. Scramble feeding in jackass penguins: Within-brood food distribution and the maintenance of sibling asymmetries. *Animal Behaviour*, **51,** 1383-1390.

VI

Food acquisition by common cuckoo nestlings in rufous bush robin nests and the advantage of the eviction behaviour

Food acquisition by common cuckoo nestlings in rufous bush robin nests and the advantage of the eviction behaviour

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Abstract

The common cuckoo, *Cuculus canorus*, is a brood parasite that monopolizes parental care of its host species: soon after hatching, the chicks remove the host offspring. Although cuckoo chicks trick their foster parents into providing enough food, it is unknown whether cuckoo begging behaviour represents an advantage over that of the host chicks in a hypothetical competitive scenario. We studied the feeding behaviour of rufous bush robins, *Cercotrichas galactotes*, when rearing their own and parasitic chicks in natural and in experimental nests where a cuckoo and a host brood were presented simultaneously to parents. In natural parasitized and nonparasitized nests, the feeding rate for cuckoo chicks did not differ from that of a single host chick of the same age, but cuckoos were fed with a different diet and with larger prey. Thus, cuckoo chicks received a similar amount of food to that received by a whole host brood. Cuckoo chicks in experimental nests did not receive a diet, prey size or feeding rate different to that received by a single host chick and thus received considerably less food than cuckoo chicks in unmanipulated nests. These results suggest that cuckoo chicks could not outcompete host chicks, at least when parasitizing this host species, and thus that their eviction behaviour is beneficial. We discuss various explanations for the inability of cuckoos to outcompete host chicks.

Keywords.- *Brood parasitism, Cercotrichas galactotes, Cuculus canorus, Chick eviction, Sibling competition, Provisioning behaviour*

Resumen

El cuco común, *Cuculus canorus*, es un parásito de cría que monopoliza el cuidado parental de sus especies hospedadoras: poco después de eclosionar, los pollos expulsan la descendencia del hospedador. Aunque el pollo de cuco engaña a sus padres adoptivos para que les den suficiente comida, no se sabe si el comportamiento de petición del cuco representa un ventaja sobre aquel de los pollos del hospedador en un hipotético escenario competitivo. Nosotros estudiamos el comportamiento de alimentación del alzacola, *Cercotrichas galactotes*, criando a su propios pollos y criando a pollos parásitos en nidos naturales, y en nidos experimentales donde un pollo de cuco y una nidada del hospedador eran presentadas simultáneamente a los padres. En los nidos naturales no parasitados y parasitados, la tasa de cebas para el cuco no fue diferente que la de un único pollo de la misma edad, en cambio los cucos fueron alimentados con una dieta diferente y con presas de mayor tamaño. Consecuentemente, los pollos de cuco recibieron una cantidad similar de comida que la recibida por una nidada completa. Los pollos de cuco en los nidos experimentales no recibieron ni una dieta, ni tamaño de presas y ni una tasa de cebas diferentes a la recibida por un único pollo de hospedador, y por lo tanto recibieron considerablemente menos comida los pollos de cucos en los nidos no manipulados. Estos resultados sugieren que el pollo de cuco no podría competir exitosamente con los pollos del hospedador, al menos cuando parasita a esta especie de hospedador, y por lo tanto su comportamiento de expulsión es beneficioso. Discutimos varias explicaciones sobre la incapacidad del cuco para competir con los pollos de hospedador

Keywords.- *Parasitismo de cría, Cercotrichas galactotes, Cuculus canorus, Expulsión de pollos, Competencia entre hermanos, Comportamiento aprovisionador*

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Introduction

Brood parasitism is a reproductive strategy in which certain individuals, the parasites, receive parental care from unrelated individuals, the hosts (Rothstein 1990). The common cuckoo, *Cuculus canorus*, is an obligate brood parasite which parasitizes about a hundred species in Europe, mainly small and abundant passerines with open nests and short nestling periods (Soler *et al.* 1999), although the frequency of parasitism varies between species (Wyllie 1981; Moksnes & Røskaft 1995). The common cuckoo female lays one egg per host nest, but usually removes and eats one of the host's eggs. In general, the cuckoo egg hatches before those of the host and, in a few hours (8–36), cuckoo chicks start evicting host offspring from the nest (hereafter eviction behaviour) without any host parent obstruction, thereby remaining alone in the host nest. This urge to eject nest contents dissipates after 4 days (Wyllie 1981).

Eviction behaviour is likely to be costly, because of the great relative weight that the cuckoo chick has to move in a short time at a considerable height, and because of the risk of falling from the nest while trying to eject foster siblings (Wyllie 1981). Kleven *et al.* (1999) provided an indirect measure of such costs: they found that although the increase in weight of cuckoo chicks reared by reed warblers, *Acrocephalus scirpaceus*, was generally lower than that of chicks reared by great reed warblers, *Acrocephalus arundinaceus*, the opposite occurred during the first 3 days of the nestling period. Kleven *et al.* suggested that the larger eggs and deeper nests of great reed warblers increased the energy cost associated with the ejection behaviour.

It is generally assumed that the high begging rate and large gape of the cuckoo chick, compared with those of a host chick (Kilner & Davies 1999; Kilner *et al.* 1999), trick the foster parents into increasing their feeding effort up to the level needed for its normal development (Davies 2000). Several mechanisms have been proposed to explain how such exaggerated cues would affect parental effort. (1) A cuckoo chick may imitate the begging signals of a complete host brood (Davies *et al.* 1998). (2) A cuckoo chick may compensate for its deficient visual cue (only one gape) by exaggerating its call rate, through tuning into the sensory predispositions of the adult host (Kilner & Davies 1999; Kilner *et al.* 1999). (3) A cuckoo chick could also be a supernormal stimulus, which would prevent its recognition as a parasitic chick by adult hosts despite its odd appearance (Dawkins & Krebs 1979; Redondo 1993). Finally, (4) the exaggerated begging behaviour of a cuckoo chick could merely indicate its greater need compared to the need of a single host chick (Soler & Soler 1999; Grim & Honza 2001) and thus exploit the honest signalling system between chicks and parents.

If the exaggerated feeding behaviour of foster parents towards a single cuckoo chick is related to the higher begging intensity of cuckoos (reviewed in Kilner & Johnstone 1997), parasitic chicks should be able to outcompete host chicks if they shared the nest with foster siblings. This is the case for chicks of some nonevictor parasites (e.g. brown-headed cowbird, Molothrus ater: Dearborn 1998; Lichtenstein & Sealy 1998; some Cuculidae species: Davies 2000). Furthermore, cuckoo chicks could even benefit from assistance by host chicks in soliciting a higher provisioning rate (Kilner 2003). Taking into account both the high energetic costs associated with the eviction behaviour (see above) and the ability of cuckoo chicks to trick their foster parents, an important evolutionary question arises: what advantage does eviction behaviour confer on cuckoo chicks? The answer to this question could be related to an inability of cuckoo chicks to stimulate foster parents to feed them preferentially in a hypothetical competitive scenario with host chicks.

To test this hypothesis, we examined the feeding behaviour of the rufous bush robin, *Cercotrichas galactotes*, a small passerine (ca. 23 g) frequently used as a host by the common cuckoo in southern Iberia (Álvarez 1994a; Palomino *et al.* 1999), in natural and experimental nests where cuckoo and host chicks were presented together to adults. Since the increase in weight of a brood of rufous bush robins does not differ from that of a single cuckoo chick (Álvarez 1994b), relative food provisioning to cuckoos and complete host broods should be similar in parasitized and nonparasitized natural nests (Wyllie 1981; Kilner & Davies 1999). However, if cuckoo chicks were not able to outcompete host siblings in a hypothetical competitive scenario, cuckoo chicks in experimental nests should receive less food than in naturally parasitized nests.

Methods

Study Area and General Field Procedures

We carried out the fieldwork during 1994–1995 in a wine-growing region in Los Palacios, southern Spain (37º9'N, 2º14'W; 12 m above sea level). The study area comprised vineyards with scattered fruit trees, and with small patches used for vegetable production. Rufous bush robins show a marked preference for breeding in these vineyards. This region has a Mediterranean climate, with rainy autumns and springs, and hot, dry summers.

We found nests by following the breeding activities of pairs and looking in suitable nest sites, mainly in vineyards. We checked all nests twice a week during incubation and every 2 days during the nestling period to collect data on laying date, clutch size, presence or absence of brood parasitism, number of hatched eggs and number of fledglings. Rufous bush robin and cuckoo chicks were weighed at 9 and 15 days, respectively. Subsequently, they were ringed (under licences from the Consejería de Medio Ambiente de la Junta de Andalucía) with a numbered aluminium ring and a colour ring (different every year).

Video Recordings

The nests were videorecorded to estimate feeding variables. In total, we recorded 25 pairs: four unmanipulated parasitized pairs (cuckoo chick alone); 10 unmanipulated nonparasitized pairs (two with four rufous bush robin chicks and eight with three); and 11 experimental pairs (one with three rufous bush robin and one cuckoo chicks, and 10 with two rufous bush robin and one cuckoo chicks). Because excluding the experimental nest with three host chicks did not change the results, we included this nest in the analyses. Eight of the 11 experimental pairs were previously not parasitized, whereas three of them were. Host chicks were 5–9 days old (X ± SD: 7.10 ± 1.3 days) and cuckoo chicks 5–10 days old (7.20 ± 1.57 days). Differences in age between experimental rufous bush robin and experimental cuckoo chicks varied between 0 and 4 days: for five experimental pairs there was no difference between host and cuckoo chicks; for two pairs host chicks were older than cuckoo chicks; and in four pairs cuckoo chicks were older than host chicks. All nonexperimental nests were recorded only once. However, for recording the 11 experimental pairs, we used only seven cuckoo chicks in 11 host nests, mainly because we found too few cuckoo chicks of the right age. Although reusing chicks could be seen as a pseudoreplication problem, we did not find significant differences associated with cuckoo chick identity for any feeding variable (see below), even after controlling for age (one-way ANCOVA with age as covariate: provisioning rate: $F_{6,3} = 1.77$, $P = 0.342$; prey size: $F_{6,3} = 1.42$, $P = 0.416$; amount of food: $\mathrm{F}_{6,3}= 2.20$, $P = 0.276$). Whether chicks had experience of sharing parental care with foster siblings did not affect our results since residuals of provisioning rate, or amount of food received, after we controlled for age did not vary between experimental cuckoos with and without experience (t test: provisioning rate: t_9 = -0.34, P = 0.737; amount of food: t_9 = -0.46, P = 0.653). Thus, because feeding variables were mainly related to foster parents, and not to individual cuckoo characteristics, the use of some cuckoo chicks in more than one experimental nest did not affect our results.

We videorecorded unmanipulated nests for 2–2.5 h and experimental nests for about 4 h, divided into two sessions of 2 h (see below). All recordings were started between 0700 and 0930 hours (local time), except for one unmanipulated parasitized nest for which recording started at 1600 hours (excluding this late-filmed nest from the analyses did not change our results, although the significant differences in provisioning rate found between cuckoo chick and unmanipulated host brood (see below) become marginally significant, from 0.035 to 0.08). The video camera was installed 0.5–1 m from the nests.

To carry out the experiment, we placed cuckoo and host chicks into two separate nests (i.e. one additional nest close to the natural nest), thereby preventing the cuckoo from evicting the rufous bush robin chicks (see Davies & Brooke 1988, for a similar experimental design). A single nest also does not provide sufficient space for one cuckoo chick plus two or three host chicks. Additional nests were empty nests collected in the same area that had suffered from natural predation. We placed the additional nest close to and at the same height as the natural nest. Since naturally parasitized pairs always rear a single cuckoo chick, our experimental approach required us to use chicks from two pairs for each recording, one parasitized and one nonparasitized (Fig. 1). To avoid nest desertion during the experiment, when we used a nonparasitized pair for video recording (Fig. 1a), we cross-fostered one host chick from this pair to the experimental cuckoo chick nest and thus the rufous bush robin chick was fed by the pair not used in the video recording. When we recorded a naturally parasitized pair (Fig. 1b), we used all but one host chicks from the nonparasitized pair. By using the host brood minus one chick, we imitated a rufous bush robin brood parasitized by a nonevictor cuckoo chick where the cuckoo female had removed a host egg. Thus, the experimental scenario was one cuckoo chick in one randomly chosen nest and the rufous bush robin brood minus one host chick in the other nest. At mid-recording time (2 h), the contents of each nest were exchanged to control for possible effects of position.

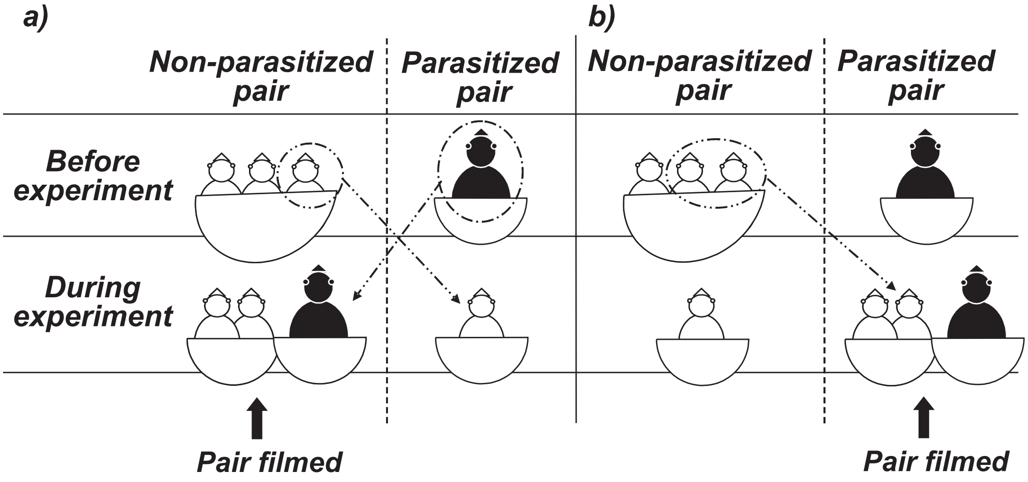

Figure 1. *Diagram showing how experimental chicks were moved between nests. When we recorded the nonparasitized pair (a), one host chick (white chick symbol) replaced the cuckoo chick (black chick symbol) in the parasitized pair. In contrast, when we filmed the parasitized pair (b), all but one of the host chicks were moved to the parasitized experimental pair's nest.*

The adults seemed not to be disturbed by the presence of the recording material or by the additional nest, and resumed normal activity within a few minutes. When the recordings were finished, chicks were returned to their original nests.

Ethical Note

The population studied is concentrated in an agricultural area. The vineyards were frequently visited by the farmers (more than two visits per week, in some cases daily) in the breeding season so our study pairs were used to human disturbance. Moving chicks between nests took about 0.5–1 h and the high ambient temperatures (mean daytime air temperature of around 27ºC) meant that chicks did not require additional warming during transport or while they were in the additional nests.

To assess possible negative impacts of our manipulations on chicks and pairs, we compared mean body mass of host chicks and brood size at 9 days old, just before fledging. We used only host chicks because most cuckoo chicks were recorded and thus manipulated. The average weight did not differ between host chicks from filmed (19.30 ± 1.89 g, $N = 17$ pairs) and nonfilmed (18.99 ± 3.71 g, $N = 42$ pairs) broods (one-way ANCOVA with number of successful fledglings as covariate: $F_{1,59} = 0.17$, $P = 0.685$). Number of successful fledglings did not differ between filmed and nonfilmed broods (filmed broods: 3.12 ± 0.49; nonfilmed broods: 2.79 ± 0.80; one-way ANOVA: $F_{1,60} = 2.50$, $P = 0.119$). In addition, we did not find any differences in average body mass of host chicks between experimental broods (whose nest contents were rearranged and transferred; 18.97 ± 2.05 g, N =

7), control broods (whose nest contents were only recorded; 19.53 ± 1.84 g, N = 10) and nonrecorded broods (18.99 ± 3.7 g, N = 42; one-way ANCOVA with number of successful fledglings as covariate: $F_{2,58} = 0.15$, P = 0.861; all post hoc comparisons NS). Finally, differences in fledging success between these three groups of nests were not statistically significant (experimental broods: 3.00 ± 0.58; control broods: 3.20 ± 0.42; nonfilmed broods: 3.12 ± 0.49; one-way ANOVA: $F_{2,59} = 1.39$, P = 0.258; all post hoc comparisons NS). Therefore, no negative effects on nestlings were detected in this study as a consequence of our manipulations.

Fieldwork was done with special permission of the Andalusia government (Consejería de Medio Ambiente de la Junta de Andalucía).

Analyses

We obtained the following data from each recording: total duration; time between feeding trips; number of chicks that begged for per adult feeding visit; type of prey carried (larva, grasshopper, etc., when distinguishable; Table 1); size of prey relative to adult bill length (1: smaller; 2: similar; 3: larger); and the chick chosen to be fed. The same person made all prey determinations. Because it was difficult to identify small prey (determined versus undetermined prey size: 1.99 ± 0.77 (N = 485) versus 1.35 ± 0.52 (N = 222); t test: $t_{705} = -11.27$, P < 0.001) a large number of food items were included in an undetermined category. However, since prey size is taxon dependent we included this prey category in the analysis of differences in diets of cuckoo and host chicks.

Provisioning rate was estimated as the number of prey brought to the nests per hour from the first to the last feeding trip recorded. Since we visited experimental nests to change chick positions, we estimated values of provisioning rate (as well as other variables) separately for each of the two recording sessions. We used number of prey brought to the nests instead of the number of feeding trips because on some occasions the adults carried more than one prey (usually two or three, occasionally even four) to the nest in a single trip (multiple feedings). Multiple feedings occurred mainly in natural nonparasitized nests (nonparasitized: 9.47%; parasitized: 0%) and in experimental nests (3.08%). When multiple feeding occurred, prey were smaller than when adults carried a single prey (multiple feedings: 1.47G 0.65 (N = 91); single feedings: 1.82 ± 0.76 (N = 574); t test: $t_{663} = -4.48$, P < 0.001) and, thus, we used number of prey together with prey size in the analyses related to parental effort. We calculated the total amount of food carried to the nest per hour by multiplying the provisioning rate by average prey size. We estimated diet by considering the total number of prey received per nest and per chick species. Then, we estimated relative percentages of each prey type per nest in unmanipulated nests or per nest and species of chick in experimental nests. Afterwards, we determined the average of the relative percentages of each prey type received by cuckoo and rufous bush robin chicks in both the unmanipulated nests and the experimental nests (Table 1).

Statistical Procedures

Distributions of most feeding variables did not differ significantly from normal distributions (Kolmogorov Smirnov test: NS). Therefore, for these variables we used parametric tests following Sokal & Rohlf (1995). We used a nonparametric analysis when distributions of variables differed significantly from normal distributions (Kolmogorov Smirnov test: $P < 0.05$). We used a chi-square test to compare the diets of cuckoo and rufous bush robin chicks provided by both unmanipulated and experimental pairs. Expected frequencies in this last analysis were the average of the observed frequencies for cuckoo and rufous bush robin chicks and, thus, either of the two diets (cuckoo or rufous bush robin) can be used in the analysis as observed frequencies (Table 1).

We used one-way ANCOVA with nestling age as covariate to test for differences in feeding variables (provisioning rate, prey size and the total amount of food) between unmanipulated parasitized and unmanipulated nonparasitized pairs, thereby controlling for differences in chick age, which could affect feeding variables. We also used this analysis to compare the amount of food delivered by the different types of rufous bush robin pairs (i.e. experimental, unmanipulated nonparasitized and unmanipulated parasitized pairs). Instead, to evaluate the amount of food delivered per chick by unmanipulated and experimental pairs, we used two-way ANCOVA with chick age as covariate, species as one between-groups factor and experimental or non experimental status as the second between-groups factor. Post hoc least significant difference (LSD) tests were used to contrast groups within an analysis.

To test for differences in feeding variables between cuckoo and rufous bush robin chicks in the experimental nests, we used repeated measures ANOVAs with two within-group factors: original or additional nests and chick species. Nest parasitism status (i.e. parasitized or nonparasitized before the experiment) was included as a between-groups factor and the difference in age between cuckoo and host chicks (age of cuckoo chicks minus age of host chicks) as covariate. Thus, this analytical design has the advantages of controlling for possible differences in feeding behaviour between experimental pairs (i.e. paired tests) and of allowing us to explore the interactions between different factors included in the model.

This statistical design was also used for testing whether the begging signal of one species was chosen preferentially in experimental nests (repeated measures ANOVAs with nest parasitism status as a between-groups factor and age differences as covariate). In this case, the repeated measures were the percentage of feeds received by one chick species when the other one was or was not begging as one within-group factor, and the original or additional nest as the second one.

For all statistical analyses we used STATISTICA version 6.0 (Statsoft Inc., www.statsoft.com) and all tests were two tailed. Values are means ± SD.

Results

Feeding in Natural Conditions

Provisioning rate to cuckoos in naturally parasitized nests was on average less than a half of that to host broods in nonparasitized nests (nonparasitized: 16.97 ± 8.21 prey/h; parasitized: 6.45 ± 2.10 prey/h; one-way ANCOVA with nestling age as covariate: $F_{1,11} = 5.76$, $P = 0.035$). However, parasitized pairs provided cuckoo chicks with larger prey than nonparasitized pairs gave to their own young (cuckoo chicks: 2.15 ± 0.18; host broods: 1.65 ± 0.28; one-way ANCOVA with nestling age as covariate: $F_{1,11} = 9.70$, $P = 0.010$). Unmanipulated pairs provided significantly different diets to cuckoos and rufous bush robins (chi-square test: χ^2_9 = 17:23, P = 0.045; Table 1).

Although nonparasitized broods received on average more parental effort (total amount of food carried/h) than parasitized ones, these differences were not statistically significant (nonparasitized nests: 27.59 ± 14.41; parasitized nests: 13.82 ± 4.57; one-way ANCOVA with nestling age as covariate: $F_{1,11} = 3.05$, $P = 0.108$).

Table 1. – Average percentages of each prey type received by rufous bush robin and cuckoo chicks in unmanipulated and experimental nests

Prey type	% In unmanipulated nests		% In experimental nests	
	Cuckoo chicks	Host broods	Cuckoo chicks	Host broods
Larvae	51.87	36.00	51.32	41.83
Arachnida	2.08	0.74	0.40*	1.00
Diptera	0*	0.17	0	0
Neuroptera	0*	0.28	2.52	4.18
Odonata	0*	0.40	0	0
Dermaptera	0*	0.48	0.00*	0.57
Orthoptera: Caeliphera	1.47	7.59	4.10	4.01
Orthoptera: Ensiphera	0	2.10	7.06	6.15
Lepidoptera	0*	1.83	2.27	0.89
Homoptera	1.47*	0.17	0	0
Heteroptera	0	2.95	0	0
Pupae	0*	0.72	0.00*	1.14
Himenoptera	0	2.35	2.60	0.94
Coleoptera	6.33	1.41	0.00	2.31
Chilopoda	0*	0.17	0	0
Diplopoda	0*	1.67	0	0
Grape	16.89	3.74	0.40	2.75
Undetermined	19.89	37.24	29.34	34.22

*Prey type included within a category ‘others’ for statistical analyses when the average percentage for both cuckoo chicks and host broods was below 2%.

Feeding in Experimental Conditions

We found no significant differences between the first and second recording sessions (see Methods) for any variable related to parental effort of experimental pairs (repeated measures ANOVA with nest parasitism status as between-groups factor and age differences as covariate: all NS). However, adults preferred to feed chicks of both species in their original nests (Table 2), although this preference differed between species (interactions between chick species and original or additional nest factors, see Table 2). The percentage of feedings to rufous bush robin chicks when some of them were begging was higher when they were in the original nest than when they were in the additional nest (Table 2), but this was not the case for cuckoo chicks (Table 2). However, since we used values for both sessions, and compared cuckoo and host chick variables from the same experimental nest, the effect of the kind of nest (i.e. original or additional nest) is partially controlled for in the rest of the analyses.

Table 2. – Results of comparisons of variables related to parental effort of experimental pairs on their original nests or additional nests, and the relative success of cuckoo chicks and host broods in both type of nest.

Variables	Original nest vs. additional nest				Cuckoo chicks				Host broods			
	Type nest effect		Chick species interaction		Original nest	Additional Nest	LSD test*		Original nest	Additional nest	LSD test*	
	$F_{1,8}$	*P*	$F_{1,8}$	*P*			*MS*	*P*			*MS*	*P*
Preys carried per hour	13.48	0.006	7.87	0.023	5.86 ± 5.69	1.36 ± 1.30	2.98	< 0.001	9.99 ± 3.11	3.21 ± 2.78	2.98	< 0.001
Prey sizes	< 0.01	0.998	0.05	0.824	1.71 ± 0.69	1.67 ± 0.99	0.60	0.898	1.86 ± 0.23	1.89 ± 0.73	0.60	0.927
Amount of food carried per hour	12.57	0.008	12.81	0.007	10.59 ± 9.88	2.7 ± 2.47	7.90	< 0.001	18.88 ± 6.23	6.95 ± 5.13	7.90	< 0.001
Percentage of feeds to which chicks begged	3.74	0.089	0.04	0.853	76.84 ± 31.31	56.31 ± 32.29	501.96	0.064	90.03 ± 8.88	66.46 ± 20.96	501.96	0.039

Values are means ± SD. *Least significant difference test, df= 8.

Host experimental broods received almost twice as many prey/h as the experimental cuckoo chicks (Table 3). There were no significant differences in prey sizes (Table 3) or diet received by experimental cuckoo chicks and host experimental broods (chi-square test: χ^2_9 = 4:50, P = 0.876; Table 1). Cuckoo chicks received on average half the total amount of food received by host experimental broods (Table 3). In addition, on average, a single experimental host chick received an average amount of food very similar to that received by the experimental cuckoo chick (Table 3). These results were not influenced by whether the experimental pairs were or were not parasitized before the experiment, nor by the differences in age between cuckoo and host chicks fed by the same experimental pair (interactions in Table 3).

Table 3. – Comparisons of the variables related to parental effort between cuckoo chicks and host broods fed by the experimental pairs.

Variables	Host broods	Cuckoo chicks	Chick species Effect		Parasitism status interactions		Age difference interactions	
			$F_{1,8}$	P	$F_{1,8}$	P	$F_{1,8}$	P
Preys carried/h	6.60 ± 1.77	3,61 ± 3.21	12.79	0.007	1.37	0.276	1.86	0.209
Average of prey carried per h and per chick	3.19 ± 0.93	3,61 ± 3.21	0.17	0.695	0.74	0.414	2.34	0.165
Prey sizes	1.88 ± 0.21	2.00 ± 0.375	0.11	0.753	4.93	0.057	4.3	0.072
Amount of food carried/h	12.42 ± 3.65	6.68 ± 5.21	16.46	0.004	1.70	0.229	2.62	0.144
Average of amount of food carried per h and per chick	6.01 ± 1.90	6.68 ± 5.21	0.33	0.581	1.11	0.323	2.91	0.126

Values are means ± SD.

Which Chick is Selected to be Fed?

Both species responded to adult feeding in the experimental nests by begging for food at a similar rate (percentage of total feedings to which cuckoo chicks responded by begging: 74.29%; host chicks: 71.43%). However, the cuckoo chick was preferentially fed by adults only when no host chick was begging for food (percentage of feedings to cuckoo chick when no host chick was begging: 63.81 ± 38.15%; when at least one host chick was begging: 22.55 ± 29.43%; repeated measures ANOVA: $F_{1,8}$ = 8.77, P = 0.018; all interactions NS). On the other hand, the percentage of prey obtained by host broods did not depend on whether the cuckoo chick was begging or not (percentage of feedings obtained by host chicks when cuckoo chick was not begging: 45.24 ± 38.36%; when cuckoo chick was begging: 50.22 ± 38.67%; repeated measures ANOVA: $F_{1,8}$ = 0.03, P = 0.860; all interactions NS).

Natural versus Experimental Conditions

There were differences in the amount of food carried by the pairs in each treatment (Fig. 2); unmanipulated nonparasitized pairs delivered more food than both unmanipulated parasitized pairs and experimental pairs (least significant difference, LSD, post hoc comparisons in Fig. 2). The average amount of food received by a single rufous bush robin chick did not differ in unmanipulated (8.36 ± 3.29, N = 10) or experimental nests (6.01 ± 1.90, N = 11; LSD post hoc comparison: P = 0.110). However, cuckoo chicks received more food in naturally parasitized nests (13.82 ± 4.57, N = 4) than in experimental nests (6.68 ± 5.21, N = 11; LSD post hoc comparison: P = 0.001). Thus, the interaction between species and nest treatment (i.e. experimental or unmanipulated) was statistically significant (Fig. 3).

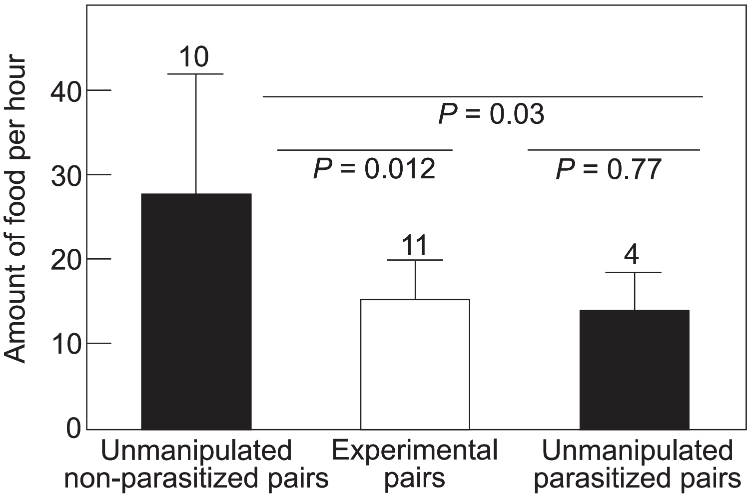

Figure 2.- *Amount of food carried by rufous bush robin adults in each treatment + SD. One-way ANCOVA with chick age as covariate:* $F_{2,21}$ = *3.9, P = 0.036. Least significant difference post hoc comparisons between groups are shown. Sample sizes are shown above the bars.*

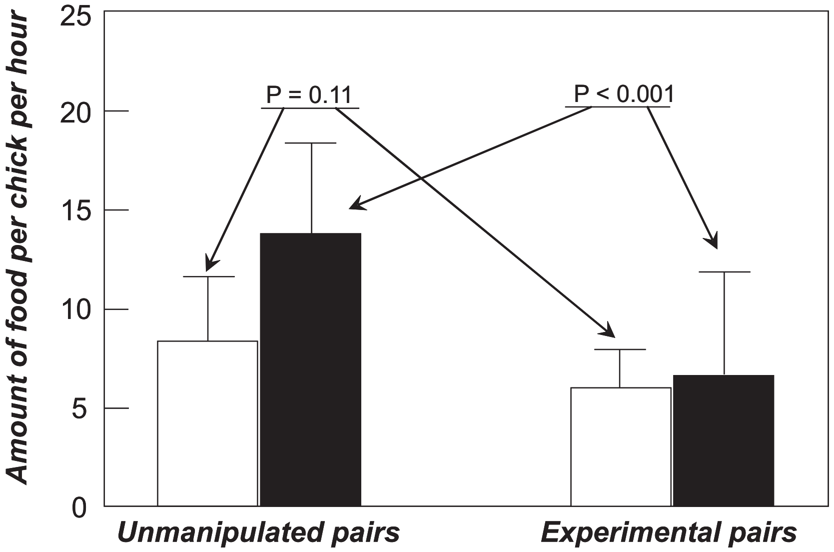

Figure 3.- *Mean + SD amount of food received/chick per h in the unmanipulated (N = 14) and experimental (N = 11) nests. Two way ANCOVA with control or experimental pairs and species as between-groups factors and chick age as interaction factor: covariate:* $F_{1,31} = 4.74$, $P = 0.037$*. Least-significant difference post hoc comparisons between unmanipulated and experimental nests for each chick species are also shown. (□) Host chick; (■) cuckoo chick.*

Discussion

Most studies on common cuckoo chick feeding behaviour by adult hosts have been carried out on a single host species, the reed warbler (for other hosts see e.g. Wyllie 1981; Khayutin *et al.* 1982; Álvarez 1994b; Kleven *et al.* 1999; Butchart *et al.* 2003). Reed warbler adults feed cuckoo chicks and a whole brood of their own chicks with a similar diet and at a similar rate (Brooke & Davies 1989; Grim & Honza 1997). Hence, apparently, cuckoo chicks are able to trick host parents when they are alone in the nest to carry enough food to feed an unusually large chick with an extended nestling period.

Álvarez (1994b) found that rates of weight increase of common cuckoo chicks and broods of rufous bush robin were not significantly different, but, in his figure, growth rates of cuckoo chicks were between those of a whole brood and of a single rufous bush robin chick. We found that parental effort (amount of food delivered) was more than double in nonparasitized than in parasitized nests, but statistical differences did not reach significance. However, the statistical power of this analysis is low (0.34) and nonsignificant results could be caused by the small sample size (sample size necessary to reach a significant result with the detected effect size Z

17 per species). In natural parasitized nests, the cuckoo chick received larger prey on average, but fewer than half of the feeds received by whole host broods in nonparasitized nests. So it is possible that the larger prey compensated for the lower feeding rate. The fewer feeds, together with the differences in diet (i.e. prey size) of cuckoo and host chicks (see Results), could be explained by adults being more selective when capturing and/or carrying prey to parasitic chicks. In accordance with that interpretation, we found that for unmanipulated pairs, nonparasitized parents brought a greater variety of prey to the nest than did parasitized ones (Table 1). However, this result could be a by-product of parasitized nests receiving fewer feeds than nonparasitized nests. Although these results contradict those found by Brooke & Davies (1989) and Grim & Honza (1997) in reed warblers (see above), they could be reconciled if rufous bush robins and reed warblers forage differently, the latter being more opportunist than the former (Cramp 1988, 1992). Therefore, these two species may respond in different ways to the same problem (to feed a single, large and hungry chick): increasing prey size (rufous bush robin) or increasing feeding rate (reed warbler).

Why are Experimental Cuckoos not Successful?

When both cuckoo and host chicks were present, the two species received a similar diet and prey size, but the provisioning rate to parasitic chicks was lower than to all host chicks considered together (see Results). As we had predicted, cuckoo chicks in experimental nests received less food than those in naturally parasitized nests. This is unlikely to have been because the parents could not meet the extra demands of mixed broods. Cuckoo, but not host, chicks were negatively affected by being in a mixed species brood (Fig. 3, interaction factor). Furthermore, in contrast to reed warbler hosts (see e.g. Davies *et al.* 1998; Kilner *et al.* 1999), those pairs feeding mixed broods did not work harder than the nonmanipulated pairs; surprisingly, the experimental pairs carried a similar amount of food to unmanipulated parasitized pairs, and less than that carried by unmanipulated nonparasitized pairs (Fig. 2).

In experimental nests, we found that when some of the rufous bush robin chicks were begging, they were selected to be fed by parents independently of whether the cuckoo chick was begging or not. In addition, experimental cuckoo chicks were fed more frequently than their foster siblings only when the rufous bush robin chicks did not beg. Finally, we found no differences on average in feeding rates to single host chicks and cuckoo chicks. Therefore, cuckoo begging behaviour is not enough to elicit rufous bush robin foster parents to feed the cuckoo chick preferentially in an experimental competitive scenario with foster siblings. That conclusion is not affected by a possible bias in feeding preference of host parents, as occurs in other systems (Soler *et al.* 1995), because naturally parasitized pairs did not feed cuckoo chicks preferentially during the experiments.

Our experimental results agree with those obtained by Davies & Brooke (1988), who found that reed warbler pairs did not feed cuckoo chicks preferentially when they were also feeding one or two reed warbler chicks (but see Grim & Honza 2001 for problems related to the experimental design). Furthermore, in nests of redstarts, *Phoenicurus phoenicurus*, a cavity-nesting bird species, 46% of cuckoo chicks failed to evict nestmates, and most of these cuckoos (56%) died (presumably by starvation), whereas at least one redstart chick from every mixed brood left the nest (Rutila *et al.* 2002). All these results can be explained by a lower ability of parasitic chicks to compete with host chicks, but also by parental discrimination of parasitic chicks, which can occur even in nonevictor brood-parasitic species (Lichtenstein 2001). However, even in the case of parasitic chick recognition, all these results suggest an inability of cuckoo chicks to outcompete foster siblings.

The inability of cuckoos to elicit foster parents to feed them preferentially may have preceded or followed the evolution of eviction behaviour. In the former case the eviction behaviour would be the consequence of that inability, and in the latter the inability to compete with experimental siblings would be the consequence of the eviction behaviour. Although it is difficult to distinguish between these two possibilities with the available information, a discussion of possible scenarios is of interest because it implies different selection pressures favouring the evolution of eviction behaviour.

The first possibility is related to potential hosts distributing food among siblings independently of chick traits (i.e. begging intensity, size, etc.). This seems to be the case for those species possessing a synchronously hatching brood and where typically all chicks fledge (i.e. clutch size adjuster species, Soler 2001). In nests of such species, a parasitic chick sharing the nest with foster siblings should receive an amount of food similar to that received by a single host chick (Soler 2002). Therefore, because a nonevictor cuckoo chick would not be able to ensure enough food for its development when parasitizing these species, it is possible that the evolution of eviction behaviour in cuckoos can be explained mainly by the specific rules followed by adult hosts among their offspring. In agreement with this possibility, the common cuckoo parasitizes adjuster host species that are smaller than itself and a cuckoo chick needs more food than a single host chick; thus, the only possibility for the cuckoo chick to monopolize parental care and receive enough food is the evolution of eviction behaviour (Soler 2002). However, in disagreement with this possibility, the nonevictor brood-parasitic cowbirds also commonly exploit smaller host species (see e.g. Davies 2000) and the parasitic chick also needs more food than a single host young. There is no evidence that the hosts of cowbirds and common cuckoos differ in the degree of hatching asynchrony.

The second possibility, that the inability of cuckoos to elicit preferential feeding followed the evolution of eviction behaviour, is related to the possibility that the competitive ability of cuckoos was lost after the evolution of eviction behaviour because of the costs associated with some of the begging signals. Some components of begging signals (e.g. call rate) are involved in stimulating food delivery rate to the nest, whereas others (e.g. postures) are concerned with sibling competition and food allocation (see e.g. Kacelnik *et al.* 1995; Johnstone 2004). Evictor cuckoo chicks could have lost begging signals related to sibling competition. In accordance with that scenario, cuckoo chicks are not able to stretch the legs and neck, as chicks of various host species do in order to place the head as close as possible to the feeding parents. However, evictor cuckoo chicks, by exaggerating their call rate, and because they receive all the food brought to the nest, are able to trick the foster parents and obtain enough food for optimal growth (Kilner *et al.* 1999; Soler & Soler 1999; Grim & Honza 2001; Butchart *et al.* 2003). Because common cuckoo chicks do not need to compete with foster siblings, they could have lost the other begging signals (i.e. postures), since these could increase the risk of falling from a small nest (Davies *et al.* 1998) and/or losing them could reduce the cost of producing an exaggerated call rate (Lotem 1998). In agreement with this idea, although our experiment prevented cuckoo and host chicks from competing for positions related to parent arrival, or sibling aggression, they never moved in relation to parent arrivals in natural or experimental nests; they simply opened their gapes and gave begging calls. Thus, although we cannot exclude the possibility that cuckoo chicks compete for a position with experimental foster siblings, this is unlikely.

One way to estimate the probability of occurrence of the two possibilities discussed above is to study eviction behaviour in a phylogenetic context looking at contrasts (i.e. events of appearance and disappearance) of eviction behaviour within a brood-parasitic clade. However, whether using phylogenies inferred from either osteological (Hughes 2000) or molecular data (Aragón *et al.* 1999; Sorenson & Payne 2002), the number of contrasts detected is very low. Although eviction behaviour is unknown for some species of cuckoos (for instance, lesser cuckoo, Cuculus poliocephalus; see also Payne 1997; or appendix in Krüger & Davies 2002), contrasts appear only within the common koel, *Eudynamys scolopacea*, clade, for which some Indian subspecies do not evict host siblings. None the less, it is clear that, before brood parasitism appeared within a clade, the ancestral cuckoos shared the nest with siblings and therefore competed with them to be fed by parents, although we do not know whether that ancestral competitive ability was effective after brood parasitism evolved. It is possible that, depending on the rules followed by host parents for distributing food among chicks, eviction behaviour appeared only in clades parasitizing hosts that distribute food evenly among offspring, whereas for other brood parasitic clades parasitizing hosts with high sibling competition, brood-parasitic chicks had an advantage because of some specific traits (larger size, short incubation period, etc.; Soler 2002). Although natural selection would favour the loss of competitive ability in the first case because of the associated costs, in the second case natural selection would favour the exaggeration of some begging component related to sibling competition. Then, only by detecting begging signals related to sibling competition in non brood-parasitic cuckoos and studying those target signals in brood-parasitic cuckoos with and without ejection behaviour, would it be possible to know whether or not the inability of some cuckoos to elicit preferential feeding preceded the evolution of ejection behaviour.

In conclusion, our results suggest that the costly eviction behaviour of cuckoos is of selective advantage because it prevents competition with foster siblings for parental care where its exaggerated begging behaviour is not enough to obtain the food needed for development. These results could be explained by (1) cuckoo chicks lacking begging signals related to sibling competition, (2) host adults tending to distribute food evenly among the brood, and/or (3) host adults recognizing a cuckoo chick when their own chicks are present.

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References

Álvarez, F. 1994a. A gens of cuckoo *Cuculus canorus* parasitizing rufous bush chat *Cercotrichas galactotes*. *Journal of Avian Biology*, **25,** 239-243.

Álvarez, F. 1994b. Rates of weight increase of cuckoo (*Cuculus canorus)* and host (*Cercotrichas galactotes)* chicks. *Ardeola*, **41,** 63-65.

Aragón, S., Møller, A. P., Soler, J. J. & Soler, M. 1999. Molecular phylogeny of cuckoos supports a polyphyletic origin of brood parasitism. *Journal of Evolutionary Biology*, **12,** 495-506.

Brooke, M. de L. & Davies, N. B. 1989. Provisioning of nestling cuckoos *Cuculus canorus* by reed wabler *Acrocephalus scirpaceus* hosts. *Ibis*, **131,** 250-256.

Butchart, S. M. H., Kilner, R. M., Fuisz, T. & Davies, N. B. 2003. Differences in the nestling begging calls of hosts and host-races of the common cuckoo, *Cuculus canorus*. *Animal Behaviour*, **65,** 345-354.

Cramp, S. 1985. *Handbook of the birds of Europe, the Middle East and north Africa. The birds of the western Palaearctic. Volume 4. Terns to woodpeckers*. Oxford: Oxford University Press.

Davies, N. B. 2000. *Cuckoos, cowbirds, and other cheats*. London: T & A D Poyser.

Davies, N. B. & Brooke, M. de L. 1988. Cuckoos versus reed warblers: adaptations and counteradaptations. *Animal Behaviour*, **36,** 262-284.

Davies, N. B., Kilner, R. M. & Noble, D. G. 1998. Nestling cuckoos, *Cuculus canorus*, exploit hosts with begging calls that mimic a brood. *Proceedings of the Royal Society of London, Series B*, **265,** 673-678.

Dawkins, R. & Krebs, J. R. 1979. Arms races between and within species. *Proceedings of the Royal Society of London, Series B*, **205,** 489-511.

Dearborn, D. C. 1998. Begging behavior and food acquisition by brown-headed cowbird nestlings. *Behavioral Ecology and Sociobiology*, **43,** 259-270.

Grim, T. & Honza, M. 1997. Differences in parental care of reed warbler (*Acrocephalus scirpaceus*) to its own nestlings and parasitic cuckoo (*Cuculus canorus*) chicks. *Folia Zoologica*, **46,** 135-142.

Grim, T. & Honza, M. 2001. Does supernormal stimulus influence parental behaviour of the cuckoo's host? *Behavioral Ecology and Sociobiology*, **49,** 322-329.

Hughes, J. M. 2000. Monophyly and phylogeny of cuckoos (Aves, Cuculidae) inferred from osteological characters. *Zoological Journal of the Linnean Society*, **130,** 263-307.

Johnstone, R. A. 2004. Begging and sibling competition: how should offspring respond to their rivals? *American Naturalist*, **163,** E388-E406.

Kacelnik, A., Cotton, P. A., Stirling, L. & Wright, J. 1995. Food allocation among nestling starlings: sibling competition and the scope of parental choice. *Proceedings of the Royal Society of London, Series B*, **259,** 259-263.

Kilner, R. M. 2003. How selfish is a cowbird nestling? *Animal Behaviour*, **66,** 569-576.

Kilner, R. M. & Davies, N. B. 1999. How selfish is a cuckoo chick? *Animal Behaviour*, **58,** 797-808.

Kilner, R. M. & Johnstone, R. A. 1997. Begging the question: are offspring solicitation behaviours signals of need? *Trends in Ecology and Evolution*, **12,** 11-15.

Kilner, R. M., Noble, D. G. & Davies, N. B. 1999. Signals of need in parent-offspring communication and their exploitation by the common cuckoo. *Nature*, **397,** 667-672.

Kleven, O., Moksnes, A., Røskaft, E. & Honza, M. 1999. Host species affects the growth rate of cuckoo (*Cuculus canorus*) chicks. *Behavioral Ecology and Sociobiology*, **47,** 41-46.

Krüger, O. & Davies, N. B. 2002. The evolution of cuckoo parasitism: a comparative analysis. *Proceedings of the Royal Society of London, Series B*, **269,** 375-381.

Lichtenstein, G. & Sealy, S. G. 1998. Nestling competition, rather than supernormal stimulus, explains the success of parasitic brown-headed cowbird chicks in yellow warbler nests. *Proceedings of the Royal Society of London, Series B*, **265,** 249-254.

Lotem, A. 1998. Manipulative begging calls by parasitic cuckoo chicks: why should true offspring not do the same? *Trends in Ecology and Evolution*, **13,** 342-343.

Moksnes, A. & Røskaft, E. 1995. Egg-morphs and host preferences in the common cuckoo *(Cuculus canorus)*: an analysis of cuckoo and host eggs from European museum collections. *Journal of Zoology*, **236,** 625-648.

Palomino, J. J., Martín-Vivaldi, M. & Soler, M. 1999. Determinants of reproductive success in the rufous bush robin (*Cercotrichas galactotes*). *Journal für Ornithologie*, **140,** 467-480.

Payne, R. B. 1997. Family Cuculidae (cuckoos). In: *Handbook of the birds of the world. vol. 4* (Ed. by Hoyo, J., Elliott, A. & Sargatal, J.), pp. 508-607. Barcelona: BirdLife / Lynx edicions.

Redondo, T. 1993. Exploitation of host mechanisms for parental care by avian brood parasites. *Etología*, **3,** 235-297.

Rothstein, S. I. 1990. A model system for coevolution: avian brood parasitism. *Annual Review of Ecology and Systematics*, **21,** 481-508.

Rutila, J., Latja, R. & Kostela, K. 2002. The common cuckoo *Cuculus canorus* and its cavity nesting host, the redstarts *Phoenicurus phoenicurus*: a peculiar cuckoo-host system? *Journal of Avian Biology*, **33,** 414-419.

Sokal, R. R. & Rohlf, F. J. 1995. *Biometry. The principles and practice of statistics in biological research.* San Francisco, USA: W. H. Freeman.

Soler, J. J., Møller, A. P. & Soler, M. 1999. A comparative study of host selection in the European cuckoo *Cuculus canorus*. *Oecologia*, **118,** 265-276.

Soler, M. 2001. Begging behaviour of nestlings and food delivery by parents: the importance of the breeding strategy. *Acta ethologica*, **4,** 59-63.

Soler, M. 2002. Breeding strategy and begging intensity: influences on food delivery by parents and host selection by parasitic cuckoos. In: *The evolution of begging. Competition, cooperation and communication* (Ed. by Wright, J. & Leonard, M. L.), pp. 413-427. Dordrecht (The Netherlands): Kluwer Academic.

Soler, M. & Soler, J. J. 1999. The cuckoo chick tricks their reed warbler foster parents, but what about other host species? *Trends in Ecology and Evolution*, **14,** 296-297.

Soler, M., Soler, J. J., Martínez, J. G. & Møller, A. P. 1995. Chick recognition and acceptance. a weakness in magpies exploited by the parasitic great spotted cuckoo. *Behavioral Ecology and Sociobiology*, **37,** 243-248.

Wyllie, I. 1981. *The cuckoo*. London: Batsford.