Asimetría sexual de las estrategias vitales en aves

una aproximación comparada en Sturnidae, y experimental y teórica en el poligínico estornino negro

Juan Vicente Gallego Rubalcaba

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TESIS DOCTORAL

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Autor:
Juan Vicente Gallego Rubalcaba

Director:
Vicente Polo Sacristán

Programa de Doctorado en
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Vicente Polo Sacristán, Profesor Titular del Departamento de Biología y Geología, Física y Química Inorgánica de la Universidad Rey Juan Carlos,

CERTIFICA:

Que los trabajos de investigación desarollados en la memoria de Tesis Doctoral: “**Asimetría sexual de las estrategias vitales en aves: una aproximación comparada en Sturnidae, y experimental y teórica en el poligínico estornino negro”** son aptos para ser presentados por Juan Vicente Gallego Rubalcaba ante el Tribunal que en su día consigne, para aspirar al Grado de Doctor en Conservación del Recursos Naturales por la Universidad Rey Juan Carlos de Madrid.

VºBº Director de Tesis　　　　　　　　　　　El Doctorando

Vicente Polo Sacristán　　　　　　　　　　　Juan Vicente Gallego Rubalcaba

En Madrid, noviembre de 2016

A mi madre

Índice

Este trabajo es un recuerdo a Petri, mi madre, que fue quien más presente estuvo y quien mejor entendió mi motivación para desarrollarme personal y profesionalmente a través de esta carrera. Aunque no vaya a ver cómo concluye una de las etapas más importantes, seguirá siempre presente a lo largo de mi desarrollo personal y profesional. Con ello, agradezco también a mi padre su enorme apoyo durante este tiempo.

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Resumen

Antecedentes

La selección sexual la fuerza evolutiva que explica las diferencias entre los sexos y es causa última de los rasgos ornamentales utilizados para atraer a las parejas y de los armamentos implicados en la competencia intrasexual. Durante años de investigación, los biólogos se han preguntado por las consecuencias de la selección sexual sobre el fenotipo, la conducta y la estrategia reproductiva de machos y hembras en animales. Sin embargo, continúa habiendo muchas incógnitas acerca de cómo es la selección de rasgos en relación al sexo, puesto que a menudo se requieren estudios a largo plazo y enfoques comparados entre especies con información que suele ser difícil de obtener de poblaciones silvestres.

En esta Tesis se investigan las asimetrías sexuales en tamaño, conducta y estrategia reproductiva en estorninos, Sturnidae, y en el estornino negro, *Sturnus unicolor*. Muchas de las especies en la familia presentan cierto grado de dimorfismo sexual en tamaño, i.e. los machos son mayores que las hembras. Además, los machos de algunas de las especies desarrollan una conducta que tendría una función de cortejo: acarrean plantas verdes, flores y líquenes al nido al comienzo de la cría. Las hembras llevan a cabo conductas equivalentes, acarreando plumas ornamentales al nido durante la incubación, y además lo hacen en respuesta a la conducta del macho. Se piensa que la selección sexual está implicada en la evolución de estos dos rasgos: los machos acarrearían plantas para atraer a las hembras y éstas señalizarían su condición al macho o a hembras competidoras acarreando plumas tras el emparejamiento.

Objetivos y metodología

El objetivo de esta Tesis es entender los procesos evolutivos dependientes del sexo que modulan la morfología, conducta y estrategia reproductiva en Sturnidae. En concreto, se han investigado: (1) las causas últimas del uso de plantas y plumas, por machos y hembras respectivamente (Capítulos 1, 2 y 3); y (2) las causas últimas del dimorfismo sexual en tamaño y sus posibles consecuencias sobre la estrategia reproductiva de la madre en el estornino negro (Capítulo 4 y 5). Para ello, se ha utilizado una cuádruple aproximación observacional, experimental, comparada y teórica. La aproximación comparada en Sturnidae permite entender cómo evolucionó el uso de plantas verdes en relación al dimorfismo sexual en la familia. La aproximación experimental se utilizó para investigar los efectos del uso de plantas verdes y plumas sobre la eficacia biológica en el estornino negro. Haciendo uso de datos demográficos observacionales de 15 años en esta misma especie, se investigaron las presiones selectivas sobre el tamaño corporal de los machos y las hembras. Por último, la aproximación teórica se empleó para entender y especular acerca de cómo la selección sexual, modulando el tamaño y las expectativas de éxito de hijos e hijas, afectaría a la estrategia reproductiva de la madre en el estornino negro.

Resultados

Encontramos que el uso de plantas verdes en nidos de Sturnidae es más frecuente entre las especies con marcado dimorfismo sexual, i.e. mayor tamaño de los machos. Asimismo, esta conducta aparece ligada al anidamiento en cavidades, quizás desempeñando una función antiparasitaria (Capítulo 1). Al aumentar experimentalmente la cantidad de plantas verdes en nidos de estornino negro se encontraron efectos a corto plazo, afectando al tamaño corporal de los hijos al abandonar el nido; y a largo plazo, afectando a la conducta de dispersión de los hijos e hijas (Capítulo 3), y reduciendo las tasas de reclutamiento de los jóvenes de ambos sexos. Este efecto deletéreo de las plantas verdes sobre las tasas de reclutamiento de los hijos se debió a un aumento de la competencia intrasexual entre las madres por los machos más atractivos. Por su parte, el aumento experimental de la cantidad de plumas incrementó las tasas de reclutamiento de los hijos, probablemente debido a una disminución de la competencia intrasexual entre las madres en el momento de la cría (Capítulo 2).

En relación con el dimorfismo en tamaño, se encontró una correlación dependiente del sexo entre el peso corporal y el éxito reproductivo de por vida: positiva y significativa en los machos, pero no en las hembras. El peso que tienen los jóvenes de ambos sexos al abandonar el nido se relaciona con el que tendrán a lo largo de su vida adulta. Además, el peso de una madre se relaciona con el de sus hijos e hijas (Capítulo 4). Al proyectar con un modelo teórico (Capítulo 5) esta cadena de relaciones empíricas (i.e., entre el peso de la madre, el de sus hijos y el futuro éxito reproductivo éstos), se encuentra que las madres de mayor tamaño deben obtener mayor rendimiento reproductivo a través de sus hijos que de sus hijas, mientras que la relación será la opuesta para madres de menor tamaño. Aunque se espera que la madre produzca en mayor número el sexo que maximice su propio beneficio, los datos observacionales no muestran cambios en la proporción de sexos de la puesta en ninguno de los sentidos predichos por el modelo. Se discuten dos factores que podrían explicar la aparente contradicción entre la predicción teórica y los datos observacionales: la estocasiticidad ambiental (efecto apuntado ya en el modelo) y la competencia entre hermanos en el nido, agravada en el sexo de mayor tamaño, podrían ser variables importantes modulando la estrategia reproductiva de la madre.

Conclusiones

Los resultados de esta Tesis indican que la competencia intrasexual es la causa última más probable para el dimorfismo en tamaño en la familia Sturnidae. Asimismo, el acarreo de plantas verdes por parte de los machos y de plumas por parte de las hembras podría tener una función de señalización sexual (para atraer parejas reproductivas) o social (para señalizar la condición y capacidad competitiva a potenciales competidores) en Sturnidae. En ningún caso se descartaría el efecto de la selección natural en el origen y mantenimiento de estos rasgos, que podrían desempeñar también funciones sanitarias. La competencia entre las hembras por los machos atractivos o los recursos reproductivos sería importante en el

estornino negro, hasta el punto de que el uso de plantas verdes como señal de atractivo de los machos desencadena un conflicto sexual.

La selección sexual sobre el tamaño corporal podría tener efectos en la estrategia reproductiva de la madre, i.e. favoreciendo que ésta altere la proporción de sexos de las puestas, o la inversión de recursos en hijos e hijas. Un modelo teórico explorando esta idea muestra que debe haber variables adicionales involucradas en el proceso, modulando la estrategia reproductiva de la madre. Futuros modelos deberían tener en cuenta los múltiples componentes implicados en la optimización de las estrategias reproductivas de las aves.

Introducción General

En su obra *The Descent of Man and Selection in Relation to Sex*, Darwin (1871) define la selección sexual como la fuerza evolutiva que emerge de las diferencias en el éxito reproductivo causadas por la competencia por las parejas. Darwin se preguntó por el origen de las diferentes morfologías y conductas de los machos y las hembras, la presencia de rasgos sexuales secundarios implicados en el cortejo y la competencia intrasexual. Desde entonces, son muchos los trabajos que han continuado ahondando en las consecuencias de la selección sexual y las asimetrías en la inversión reproductiva (Bateman 1948; Trivers 1972; Dawkins 1976; Andersson 1994; Arnqvist y Rowe 2005). Un aspecto clave es que, aunque el macho y la hembra cooperan aportando la misma cantidad de información genética, la inversión del macho en el desarrollo de la descendencia generalmente es menor que la de la hembra (Bateman 1948; Trivers 1972). Esta asimetría, que estaría presente en la anisogamia, el desarrollo prenatal y en ocasiones durante el cuidado parental (Orians 1969; Trivers 1972; Hoekstra 1987), sería el resultado de una carrera evolutiva por obligar al sexo opuesto a invertir el esfuerzo y los recursos necesarios para sacar adelante a los hijos (Dawkins 1976). El resultado es, generalmente, un sexo masculino que trataría de maximizar el número de intentos reproductivos invirtiendo poca energía en cada uno de ellos, frente a un sexo femenino, que debería maximizar el éxito de cada intento reproductivo (Bateman 1948; Trivers 1972; Kokko *et al.* 2012).

Angus Bateman postuló en 1948 que (1) los machos competirían entre ellos para incrementar las oportunidades reproductivas y con ello su eficacia biológica mientras que (2) las hembras actuarían como sujeto pasivo seleccionando la calidad genética de los machos. Como consecuencia, (3) la eficacia biológica de los machos se relacionaría con el número de parejas que logran atraer, por lo que tendría mayor varianza que la de las hembras. Por tanto, (4) la selección sexual afectaría más intensamente al sexo masculino, favoreciendo la aparición de ornamentos para atraer a las hembras y armamentos implicados en la competencia con otros machos (Andersson 1994; Arnold 1994). El esquema originalmente propuesto por Bateman y ampliado por otros autores, ha servido para entender las asimetrías en las estrategias vitales de machos y hembras y en sus funciones reproductivas (e.g., Orians 1969; Trivers 1972). De los principios de Bateman se extrae que las presiones selectivas serían asimétricas sobre (1) los rasgos ornamentales y las conductas implicadas en la atracción de parejas; y (2) sobre los rasgos implicados en la competencia intrasexual por las parejas, como el tamaño corporal o los armamentos. En ambos casos la presión por desarrollar estos rasgos sería más fuerte sobre los machos que sobre las hembras. Sin embargo, existe un interés creciente acerca de la importancia y las consecuencias que tiene la competencia sexual y social entre las hembras. Se piensa que también las hembras podrían beneficiarse al utilizar ornamentos de señalización y desarrollar armamentos implicados en la competencia con otras hembras por los recursos y las parejas reproductivas (revisado en Clutton-Brock 2009; Clutton-Brock y Huchard 2013). Queda mucho por saber acerca de cómo la competencia entre hembras modularía su fenotipo y cómo afectaría esto a la expresión de los ornamentos sexuales de los machos.

Uno de los aspectos más debatidos acerca de las consecuencias de las asimetrías sexuales en las presiones selectivas es la teoría del reparto de sexos (i.e. *Sex-allocation theory*, Darwin 1871; Fisher 1930; Hamilton 1967; Trivers y Willard 1973; Charnov 1982). Esta teoría postula que una madre debería poder decidir qué sexo producir para maximizar su propia eficacia biológica. Diferentes predicciones se enmarcan en esta teoría general, por ejemplo, la hipótesis de Trivers y Willard (1973) que postula que una madre en buena condición, capaz de producir machos más grandes y poligínicos, debería producir más hijos que hijas. Sin embargo, continúa siendo una de las grandes preguntas no resueltas en Ecología Evolutiva de aves y mamíferos hasta qué punto es generalizable que la madre sea capaz de predecir diferencias en el éxito reproductivo de sus hijos e hijas y actúe en consecuencia controlando el sexo de su descendencia.

Esta Tesis aborda las asimetrías sexuales en la morfología, la conducta y la estrategia reproductiva de los estorninos, una familia donde se piensa que ha habido una radiación adaptativa ligada a procesos de selección sexual (Maia *et al.* 2013, 2016). Así, utilizando una cuádruple aproximación: comparada, con información bibliográfica de distintas especies de la familia; observacional, experimental y teórica, en una población silvestre de estornino negro *Sturnus unicolor*, se abordan dos grandes aspectos, que se tratarán en dos secciones en la Introducción y la Discusión General: (1) cómo evolucionan los ornamentos y conductas implicadas en la selección sexual. Concretamente, cuál es el origen evolutivo del uso de plantas verdes en los nidos por parte de los machos en la familia Sturnidae, y cuáles son sus efectos sobre la eficacia biológica de los machos y las hembras en el estornino negro; y (2) cómo evoluciona el dimorfismo sexual en tamaño en la familia Sturnidae, si está ligado a diferencias en el éxito reproductivo de los machos y las hembras en el estornino negro y, además, si las asimetrías en tamaño y éxito reproductivo futuro de la descendencia condicionarían la estrategia reproductiva de las madres.

Glosario

Asimetría sexual: diferencias sexuales en estrategias vitales y rasgos fenotípicos, resultado de un efecto de la selección favoreciendo rasgos para la competencia y atracción de parejas reproductivas.

Conflicto sexual: *sensu* Trivers 1972; Dawkins 1976; ocurre cuando los rasgos favorecidos en un sexo reducen la eficacia biológica del sexo contrario.

Dimorfismo sexual: diferencias en la morfología de uno y otro sexo, generalmente referido al tamaño corporal, i.e. peso y talla (e.g., Moore 1990).

Fenotipo extendido: *sensu* Dawkins 1982; rasgos externos al cuerpo del animal que han evolucionado por selección fenotípica.

Condición física: o condición fenotípica; estado fisiológico de un animal, que se relaciona con la cantidad de energía que puede invertir en sus actividades reproductivas, en la competencia y en su propia supervivencia.

Eficacia biológica: *fitness*; contribución media de los individuos con un fenotipo determinado a las generaciones siguientes en la población.

Éxito reproductivo: indicador de la eficacia biológica; número de hijos supervivientes producidos por un individuo con un fenotipo determinado.

Valor reproductivo: *sensu* Fisher (1930), indicador de la eficacia biológica; es la contribución relativa de un individuo a la tasa de crecimiento poblacional a largo plazo.

1. Ornamentos y conductas implicadas en la selección sexual

Darwin (1871) se preguntó por qué los machos son generalmente más conspicuos en su coloración, morfología y conducta que las hembras. Muchos de estos rasgos habrían evolucionado a través de una carrera evolutiva entre la preferencia de las hembras y el grado de exageración de un rasgo fenotípico en los machos (Fisher 1930). Los ornamentos masculinos podrían indicar la calidad de los machos como reproductores, sus habilidades competitivas, condición física o buenos genes, lo que iniciaría el proceso de selección sexual en favor de los machos que expresan el rasgo (Kokko *et al.* 2002). Pero no sólo los rasgos morfológicos cumplirían esta función; también rasgos de conducta, estructuras construidas por el macho, así como los objetos que puede encontrar y acarrear al nido, podrían haber evolucionado a través de la selección sexual. Estos componentes externos al cuerpo del animal constituyen el fenotipo extendido de un individuo (*sensu* Dawkins 1982).

Se conocen distintos ejemplos donde el fenotipo extendido de un animal está implicado en la señalización sexual: el macho del gasterósteo (*Gasterosteus aculeatus*) construye un nido e incorpora objetos brillantes y coloreados, con los que incrementa las posibilidades de atraer a una hembra reproductora (Östlund-Nilsson y Holmlund 2003; Rushbrook et al. 2007). Uno de los ejemplos más llamativos en aves se da en los pergoleros (Ptilonorhynchidae), donde los machos construyen una estructura sobre el suelo, i.e. la pérgola, a la que acarrean objetos de colores que después muestran a la hembra durante el cortejo (Borgia 1985 en *Ptilonorhynchus violaceus*). El macho del pergolero grande (*Chlamydera nuchalis*) acarrea conchas y otras piezas blancas que dispone a la entrada de la pérgola en orden creciente de tamaño conforme aumenta la distancia a la entrada, con lo que controla la perspectiva de la hembra desde el interior de la pérgola (Endler *et al.* 2010). Estos rasgos servirían para informar a la hembra acerca de la condición del macho o sus habilidades cognitivas y, en suma, su valor como una posible pareja reproductiva. En el estornino negro, el macho realiza el cortejo cerca del nido con un fragmento de liquen, hojas verdes o plantas aromáticas en el pico (Fig. 1). Después acarrean el material al nido, donde la hembra obtiene información acerca del estatus social y la condición de su propietario (Veiga *et al.* 2006). Los machos utilizan el material verde para atraer hembras a su nido (Fauth *et al.* 1991; Pinxten *et al.* 2003; Brouwer y Komdeur 2004; en el estornino pinto *Sturnus vulgaris*) y la cantidad de material verde acarreado por el macho se relaciona con su nivel de poliginia (Veiga *et al.* 2006 en el estornino negro).

Aunque se ha especulado acerca de cuál podría ser el origen evolutivo de estos rasgos de señalización, pocos trabajos han encontrado pistas sobre su origen y la forma en la que fueron modulados por la selección sexual. Se piensa que muchos de ellos se habrían originado por selección natural, e.g. la fabricación de un nido o el acarreo de elementos no estructurales como plantas o plumas con funciones antiparasitarias, podrían haber servido para mejorar la condición y supervivencia de los pollos. La selección sexual podría exagerar estos rasgos si, por ejemplo, las hembras prefieren a los machos que construyen nidos más grandes o a los que acarrean más cantidad de componentes no estructurales como plumas o plantas verdes al nido (Soler et al. 1998; Moreno 2012).

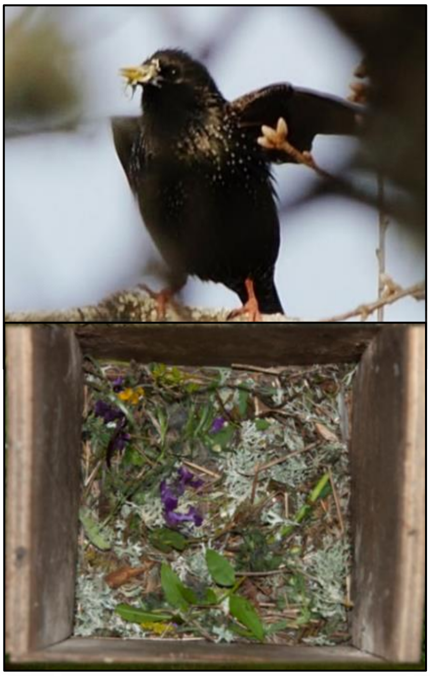

Fig 1. Acarreo de plantas verdes por machos de estornino negro. Arriba: macho joven realizando el cortejo con un fragmento de liquen en el pico. Abajo: aspecto del interior de una caja nido al comienzo del período reproductivo (J.G. Rubalcaba y V. Polo, en Manzanares el Real, Madrid).

El uso de plantas verdes en las especies de la familia Sturnidae, pudo haber sido favorecido en su origen por la selección natural. Existen ejemplos en distintas aves donde el uso de plantas verdes aromáticas por parte de ambos reproductores reduce la carga de ectoparásitos y mejora la condición de los pollos (e.g., Mennerat *et al.* 2009 en el herrerillo común *Cyanistes caeruleus*). Esto explicaría que el uso de plantas verdes en nidos se originara en aves que anidan en cavidades (Clark y Mason 1985), más proclives a ser infestadas por ectoparásitos (Marshall 1981; Collias y Collias 1984). También en estorninos existe evidencia de que las plantas acarreadas por los machos mejoran la salud de los pollos (e.g., Gwinner y Berger 2005). Sin embargo, la mayoría de los trabajos con estorninos negro y pinto sugieren que las plantas desempeñan sobre todo una función sexual (revisado en Scott-Baumann y Morgan 2015). Así, por ejemplo, en el estornino negro el uso de plantas verdes por los machos no confiere beneficio para la salud y supervivencia de los pollos y, de hecho, aumenta la competencia entre las hembras que tratan de emparejarse con los machos atractivos (Polo *et al*. 2010). Quizás como consecuencia de esto, las hembras retiran todo el material verde acarreado por el macho antes de comenzar la puesta, evitando que otras hembras traten de criar con su pareja (Veiga y Polo 2012).

La competencia entre las hembras por los machos y los recursos para la reproducción podría condicionar la expresión de los rasgos ornamentales, tanto morfológicos como de fenotipo extendido, de los machos. Además, en muchas especies, la propia morfología y conducta de las hembras habrían sido moduladas por la competencia intrasexual (revisado en Clutton-Brock 2007).

Señalización sexual en hembras y machos

Aunque los rasgos ornamentales y las conductas sexuales han sido típicamente estudiados en los machos, las hembras también se beneficiarían al señalizar su condición y su estatus social al macho o a potenciales competidores. Típicamente se pensó que la presencia de ornamentos y otros rasgos de señalización sexual estarían presentes en hembras por un efecto correlacionado de las presiones selectivas que actúan sobre el fenotipo masculino (Lande 1980; Halliday y Arnold 1987). Sin embargo, la evidencia reciente sugiere que muchos de estos rasgos habrían evolucionado de forma independiente en los dos sexos (revisado en Amundsen 2000).

En especies donde la competencia intrasexual durante la reproducción es alta en ambos sexos, la varianza en la eficacia biológica de las hembras puede llegar a ser tanto o más alta que la varianza en la eficacia biológica de los machos (Hodge *et al.* 2008; Clutton-Brock y Huchard 2013). En estos casos las hembras desarrollarían rasgos típicamente masculinos como es un gran tamaño corporal (e.g., en estorninos con cría cooperativa, Rubenstein y Lovette 2009), altos niveles de testosterona circulante (e.g. en el suricato, *Suricata suricata*, Carlson *et al.* 2004), o rasgos sexuales secundarios llamativos (e.g., en la jacana, *Jacana jacana*, Emlen and Wrege 2004). La evolución de ornamentos en ambos sexos, como señales mutuas de calidad genética o condición física, habría tenido lugar sobre todo en especies sociales, o especies que anidan en colonias con alta densidad de reproductores, donde la oferta de potenciales parejas y la competencia sexual es alta para ambos sexos (Johnstone *et al.* 1996; Amundsen 2000; ver también Velando *et al.* 2001).

La información transmitida por los ornamentos, tanto de machos como de hembras, podría ir dirigida al sexo contrario, y/o a potenciales competidores del mismo sexo. Por ejemplo, en el herrerillo común, donde tanto el macho como la hembra acarrean plantas al nido, el incremento experimental de la cantidad de plantas aumenta el esfuerzo reproductivo del macho. Esto sugiere que la conducta de acarreo en las hembras estaría en parte modulada por el hecho de que favorece la inversión de su pareja tras el emparejamiento (Tomás *et al.* 2013). En el gorrión chillón (*Petronial petronia*), la hembra incorpora plumas al nido. Aunque esto no incrementa la inversión de su pareja en el cuidado de los pollos, la presencia de plumas reduce las intrusiones al nido por parte de competidores (García-Navas *et al.* 2015). A menudo se denomina selección sexual al primer caso (comunicación intersexual) y selección social al segundo (comunicación intra-sexual; ver revisiones en Amundsen 2000 y Tobias *et al.* 2012). Se piensa que muchos de los rasgos ornamentales desarrollados por las hembras habrían emergido por selección social, para señalizar la condición a hembras competidoras (Clutton-Brock y Huchard 2013). Sin embargo, se sabe poco acerca de cómo la competencia entre hembras interactúa con la selección sexual, modulando los rasgos ornamentales y conductas de señalización de ambos sexos.

En el estornino negro, tanto hembras como machos poseen rasgos de señalización sexual propios, los cuales están mutuamente relacionados (Polo y Veiga 2006). Las hembras disponen plumas en el nido durante la incubación, sin estar necesariamente en contacto con los huevos, mostrando la cara que mejor refleja la luz ultravioleta, lo que maximiza su visibilidad en el interior del nido (Veiga y Polo 2005) (Fig. 2). La cantidad de plumas en el nido está relacionada con la condición de la hembra, quien además acarrea más plumas cuando está emparejada con un macho atractivo, i.e. aquellos que incorporan mayor cantidad de plantas verdes (Polo y Veiga 2006). Con esta conducta la hembra no parece lograr que el macho participe más en el cuidado de los pollos (Veiga y Polo 2011). Por el contrario, en un experimento reciente, se encontró que las hembras acarrean más plumas cuando crían en ambientes con alta densidad de reproductores, donde serían más frecuentes los encuentros agresivos y sabotajes por parte de conespecíficos (Rubalcaba *et al.* en prep). Existen además datos no publicados que indican que los nidos con mayor cantidad de plumas son menos afectados por el parasitismo de cría intraespecífico. Las hembras invertirían tiempo en buscar y acarrear plumas al nido durante la incubación para señalizar su condición y su capacidad para defender el nido. La presencia de plumas en el nido alertaría a posibles competidoras de la calidad de la hembra propietaria y frustraría el intento de sabotear o parasitar la puesta cuando la propietaria esta ausente.

Señalización del atractivo y eficacia biológica de machos y hembras

Son pocos los trabajos que han abordado el efecto de los rasgos de atractivo sobre la eficacia biológica de animales en poblaciones silvestres. En las aves, la mayor parte de los trabajos indican que la manipulación de un rasgo del atractivo del macho desencadena un mayor esfuerzo reproductivo en su pareja, quien puede incrementar el número o el tamaño de los huevos o la frecuencia de cebas (revisado en Horváthová *et al.* 2011). Cabría especular, por tanto, que la hembra obtiene un beneficio por el hecho de estar emparejada con un macho atractivo, quien aportaría una ventaja genética, mejores recursos para la cría o sería mejor padre. Por otro lado, serían frecuentes los casos en lo que las hembras compiten por los machos preferidos, y esto afecta a su éxito reproductivo (Clutton-Brock 2009; Clutton-Brock y Huchard 2013).

Fig 2. Interior de las cajas nido de estornino negro durante la incubación. La hembra acarrea al nido un número variable de plumas (de 0 a 40) desde el comienzo de la puesta hasta la eclosión de los huevos (V. Polo, Manzanares el Real, Madrid).

La evidencia previa en el estornino negro sugiere que existe un coste para las hembras que compiten por los machos más atractivos. En un experimento donde se aumentó la cantidad de plantas verdes en los nidos en los días previos al inicio de la puesta, se incrementaron los encuentros agresivos entre hembras, quienes duplicaron sus niveles de testosterona circulante durante la reproducción (Polo *et al.* 2010). La mayor competencia entre hembras podría tener efectos negativos sobre el cuidado parental y el éxito reproductivo (ver Veiga y Polo 2008 para el efecto negativo de la testosterona circulante sobre la eficacia biológica de la madre). Por lo tanto, aunque no podemos descartar que en el estornino negro las hembras se beneficien por el hecho de emparejarse con machos atractivos (como sugieren estudios con otras aves; Horváthová *et al.* 2011) también podría existir un coste debido a la competencia intrasexual.

Como corolario, los machos más atractivos en el estornino negro defienden varios sitios reproductivos, tienen mayor tamaño corporal, y acarrean más plantas verdes a cada una de sus cajas nido (Veiga *et al.* 2006). Con ello atraen a más hembras a sus territorios, quienes compiten agrediendo y saboteando las puestas de sus rivales (Polo *et al.* 2010). La hembra propietaria retira la totalidad de las plantas verdes acarreadas por el macho (Veiga y Polo 2012), y una vez iniciada la puesta, incorpora mayor cantidad de plumas al nido (Polo y Veiga 2006 y datos observacionales no publicados).

En este sistema de estudio, se abordan tres preguntas (1) si el uso de plantas verdes en la familia Sturnidae está modulado por la selección natural, la selección sexual o ambas; (2) si al aumentar la cantidad de plantas verdes en los nidos, la competencia entre las hembras impone un coste sobre su éxito reproductivo; y (3) si al aumentar la cantidad de plumas en los nidos, se frustran los intentos de sabotaje de hembras competidoras y esto mejora el éxito reproductivo de la hembra propietaria.

2. Evolución del dimorfismo sexual en tamaño y presiones selectivas sobre el control del sexo de la descendencia

En especies poligínicas y con dimorfismo sexual, la varianza en el éxito reproductivo de los machos excede a la de las hembras (Darwin 1871). Como consecuencia, la intensidad de la selección sexual sobre los rasgos implicados en la competencia por las parejas es más intensa entre los machos, quienes desarrollarían mayores tamaños corporales y otros rasgos fenotípicos para mejorar la competencia intrasexual (Andersson 1994). El tamaño corporal de los adultos, quienes compiten por las parejas y los recursos reproductivos, está relacionado con su tamaño al independizarse de sus padres. Por lo tanto, las presiones selectivas que actúan sobre el tamaño de los hijos e hijas condicionarían también las estrategias reproductivas de sus padres. Así, sería esperable encontrar un mayor efecto de los niveles de inversión parental sobre la eficacia biológica de los hijos que de las hijas (Trivers y Willard 1973).

Trivers y Willard (1973) plantearon que, si la eficacia biológica en los machos es más variable y depende más de su condición física que en las hembras, las madres en buena condición (capaces de invertir más energía en producir hijos de mayor tamaño), deberían producir machos; mientras que las madres en peor condición deberían producir hembras (el sexo con un rendimiento reproductivo menos variable).

Aunque se ha planteado que la madre podría ajustar la inversión reproductiva en sus hijos e hijas sin cambiar la proporción de sexos del nido (Carranza 2002; Pen y Weissing 2002), el mayor debate se encuentra en si los vertebrados tendrían la capacidad de controlar el sexo de los hijos (Ewen *et al.* 2004; Veller *et al.* 2016). Para que se cumpla la predicción anterior se deben satisfacer las siguientes asunciones: (1) la condición de la madre está positivamente relacionada con la de los hijos al final del periodo de cuidado parental; (2) las diferencias en condición de los hijos permanecen en su vida adulta; y (3) el éxito reproductivo de los machos depende más de su condición de adulto que el de las hembras. Si se cumplen estas tres condiciones, la mayor presión selectiva sobre la condición de los machos estaría presente desde el momento de su desarrollo temprano, y por tanto se esperaría que la madre ajustara su esfuerzo reproductivo en consecuencia (Kruuk *et al.* 1999).

Debido a la dificultad de abordar estas preguntas en poblaciones de vertebrados silvestres, son escasos los estudios empíricos que investigan si las presiones selectivas sobre el peso de los adultos se transfieren al peso de los jóvenes y a la estrategia reproductiva de la madre. En muchos casos se desconoce si las poblaciones objeto de estudio cumplen las condiciones descritas más arriba (pero ver Kruuk *et al.* 1999), o incluso si estas asunciones representan realmente la compleja demografía de los vertebrados (Schindler *et al*. 2015). A pesar de ello, son muchos los trabajos que han utilizado el dimorfismo sexual en tamaño como un correlato de la intensidad de la selección sexual (Moore 1990; Dunn *et al.* 2001; Bonduriansky and Rowe 2005; Emlen *et al.* 2012), y el peso de la madre durante la reproducción como un indicador de su estado físico, y su capacidad para producir hijos más grandes y con mayor éxito reproductivo (revisado en Ewen *et al.* 2004 para las aves). Esto ha generado un debate en torno a la idea de si el tamaño corporal (y el dimorfismo sexual en tamaño) es un indicador apropiado de la asimetría entre sexos en la intensidad de la competencia por las parejas, o si el efecto está oscurecido por factores ecológicos o diferencias en la fertilidad de las hembras (e.g., Dunn *et al.* 2015).

A pesar de que la idea de Trivers y Willard ha despertado el interés de numerosos estudios empíricos y algunos trabajos teóricos, la evidencia sigue siendo confusa y existe poco consenso acerca de si la predicción se cumple de manera general (Hewison y Gaillard 1999; Ewen *et al.* 2004; Komdeur y Pen 2002; Schindler *et al.* 2015). A menudo no encuentran cambios en la razón de sexos de los hijos en relación al peso corporal de la madre u otros indicadores de su condición física (Clutton-Brock 1986; West y Sheldon 2002; Ewen *et al.* 2004). Se piensa que las diferencias en la eficacia biológica de machos y hembras pueden no ser tan grandes en algunas especies de vertebrados como para alterar la estrategia reproductiva de la madre, en especial, si determinar el sexo de los hijos es costoso. Así, si no existe un mecanismo que permita ajustar facultativamente el sexo de los hijos, la manipulación de la razón de sexos en la puesta puede requerir matar selectivamente a uno de los dos sexos durante el desarrollo. Si el beneficio que revierte el sexo sobreproducido no excede ese coste, no existiría una presión selectiva para controlar el sexo de los hijos (Cockburn *et al.* 2002).

Las diferencias en la eficacia biológica esperada de hijos e hijas podrían atenuarse al tener en cuenta la complejidad de los procesos demográficos de los vertebrados (Schwanz *et al.* 2006; Schindler *et al.* 2015); y las diferentes decisiones reproductivas que puede tomar una madre, como cambiar el tamaño de puesta (Williams 1979; Carranza y Polo 2012). Por ejemplo, revisiones de la hipótesis de Trivers y Willard sugieren que que el solapamiento de generaciones (Schwanz *et al.* 2006), las diferencias en la mortalidad de cada sexo (Schindler *et al.* 2015) y la libertad de la madre a la hora de controlar el número de hijos/as en la puesta (Carranza y Polo 2012) pueden modificar, e incluso hacer desaparecer, las presiones selectivas sobre la proporción de sexos de la descendencia.

Un aspecto que puede ser clave en la demografía de los vertebrados es la incertidumbre y la estocasticidad en los procesos demográficos, que reduce la capacidad de la madre de predecir el valor reproductivo de sus hijas e hijos. Se piensa que en ambientes estocásticos desaparecería la presión selectiva para producir diferencialmente uno u otro sexo. West y Sheldon (2002) sugirieron que cuando los factores que determinan las diferencias de eficacia biológica de hijos e hijas son difíciles de predecir, sobreproducir uno de los dos sexos puede llevar, por azar, a producir el sexo con una eficacia biológica más baja, con el coste que esto conllevaría. Así, concluyen que la impredecibilidad ambiental debilitaría la selección sobre un posible mecanismo para determinar el sexo de los hijos. El argumento verbal de West y Sheldon contrasta con modelos previos (Proulx 2000; ver modelo general en Proulx 2004) que sugieren que la proporción de sexos evolutivamente estable en ambientes espacialmente estocásticos se sesgaría hacia el sexo cuya eficacia biológica tiene menor varianza, generalmente las hembras. Existen muy pocos trabajos que analicen los posibles efectos de la estocasticidad ambiental sobre el cuidado parental (Westneat *et al.* 2013) y, más allá, sobre las estrategias de control del sexo de los hijos (revisado en Proulx 2000) y, hasta donde sabemos, no existen trabajos empíricos que aborden esta cuestión.

En el facultativamente poligínico y dimórfico estornino negro, los machos más agresivos y de mayor tamaño defienden territorios más grandes e incrementan el número de parejas sociales (Veiga *et al.* 2001 y datos no publicados). Asmismo, existe evidencia en esta especie de que las hembras modifican la proporción de sexos de las puestas en relación a diferentes facores, como el atractivo de su pareja (Polo *et al.* 2004), su nivel de testosterona circulante (Veiga *et al.* 2004), o estacionalmente, sesgando la razón de sexos en favor de las hembras conforme avanza la fecha de incio de la puesta (datos no publicados). Por todo ello, el estornino negro sería un candidato para explorar cómo actúan las presiones selectivas sobre el peso corporal de adultos y jóvenes, y más allá, sobre la estrategia reproductiva de la madre en el contexto de la hipótesis de Trivers y Willard. Así, utilizando datos observacionales de hasta 15 años en una población silvestre de estornino negro, se abordaron las siguientes cuestiones: (1) si el peso corporal de adultos afecta al éxito reproductivo más en los machos que en las hembras; si las diferencias en peso de adulto se relacionan con el peso al abandonar el nido y con el peso de la madre durante la reproducción; y si la madre controla la razón de sexos del nido como predice la hipótesis de Trivers y Willard. Por último, (2) si la estocasticidad ambiental durante la reproducción, aumentando la varianza en el peso corporal de los hijos, afectaría a su éxito reproductivo futuro y cómo debería la madre ajustar su inversión en consecuencia.

Organización general de la Tesis

Las consecuencias de los sistemas de señalización y el dimorfismo sexual en tamaño sobre la eficacia biológica y las estrategias vitales en estorninos se abordan en los siguientes cinco capítulos. Estos capítulos no reflejan un orden cronológico de ejecución, sino un esquema que aporta una visión global y facilita su discusión.

El **Capítulo 1** es un estudio comparado que analiza cómo evoluciona un rasgo del fenotipo extendido en los machos, i.e., las plantas verdes no estructurales añadidas al nido, en la familia Sturnidae por vía de selección natural (i.e., con una función antiparasitaria) y selección sexual (i.e., como señal sexual de los machos para atraer parejas). Así, se analiza si la presencia o ausencia de plantas verdes en los nidos está relacionada con el tipo de anidamiento, sistema social y tipo de hábitat, todos ellos correlatos de la presión de ectoparásitos en los nidos; o bien, si el uso de plantas se relaciona con el dimorfismo sexual en tamaño, un indicador de la intensidad de la selección sexual.

Los siguientes capítulos se centran en el estornino negro, una especie sexualmente dimórfica y donde los machos utilizan plantas con una función eminentemente sexual. En el **Capítulo 2** se investiga cómo la selección sexual habría modulado el uso de plantas verdes por los machos y el uso de plumas ornamentales por parte de las hembras. Este capítulo resulta de la fusión de dos manuscritos correspondientes a dos experimentos a largo plazo (i.e., 4 años de experimentación y 3 años adicionales para recapturar todos los reclutas) donde, respectivamente, se aumentó la cantidad de plantas verdes y la cantidad de plumas, para examinar su efecto sobre el éxito de las nidadas. La finalidad de presentar los resultados de ambos experimentos en un único capítulo es tratar de aportar una visión conjunta de cómo la selección sexual modularía dos rasgos del fenotipo extendido de hembras y machos que podrían haber coevolucionado como señales honestas de calidad.

En el **Capítulo 3** se analizó cómo es la movilidad de los reclutas nacidos en el experimento donde se añadieron plantas verdes a los nidos. Se especula que los hijos de padres atractivos y poligínicos tratarían de criar lejos de su territorio natal para evitar la competencia con el padre.

Los **Capítulos 4 y 5** analizan las presiones selectivas sobre el peso corporal de machos y hembras reproductores utilizando datos observacionales en el estornino negro. En el primero se investigó si existe una relación específica del sexo entre el éxito reproductivo de por vida y el peso corporal, i.e., una relación positiva y más acentuada en padres que en madres, como se predice para una especie poligínica y con dimorfismo sexual. Con base en esta idea, se especula acerca de qué estrategia reproductiva maximizaría la eficacia biológica de la madre en relación a su propio peso corporal durante la reproducción, i.e., si debería incrementar la proporción de machos en relación a su condición. Finalmente, en el **Capítulo 5**, se realiza una proyección teórica, utilizando datos del estornino negro, para entender cómo sería la estrategia de la madre en un ambiente estocástico donde existe varianza en el peso corporal y valor reproductivo de los hijos e hijas. Se analiza, posteriormente, los resultados de esta proyección a la hora de explicar los datos empíricos. En concreto, se pretende comprobar si las madres ajustan o no la proporción de sexos en base a su peso corporal o bien hay variables confundidoras que rompen esta predicción.

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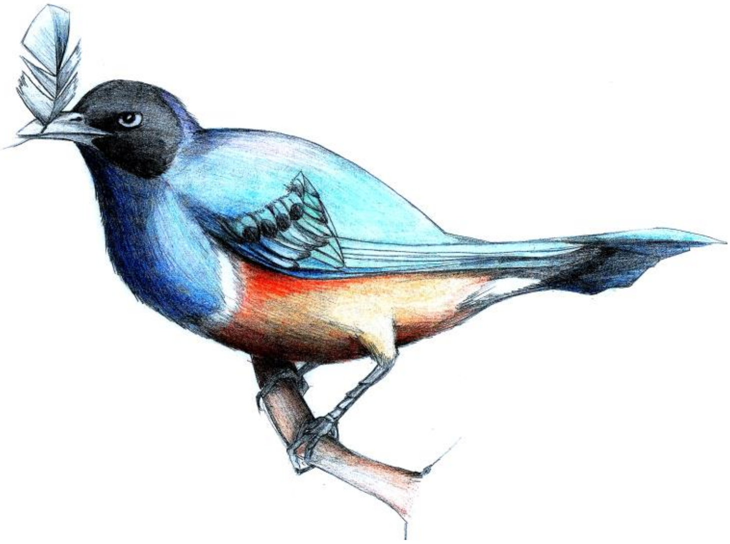
Capítulo 1

Sexual and natural selection in the evolution of extended phenotypes: the use of green nesting material in starlings



Rubalcaba, J. G., Polo, V., Maia, R., Rubenstein, D.R. and Veiga, J.P. 2016. *J Evol Biol*. **29:** 1585-92.

Abstract

Although sexual selection is typically considered the predominant force driving the evolution of ritualized sexual behaviors, natural selection may also play an important and often underappreciated role. The use of green aromatic plants among nesting birds has been interpreted as a component of extended phenotype that evolved either via natural selection due to potential sanitary functions, or via sexual selection as a signal of male attractiveness. Here we compared both hypotheses using comparative methods in starlings, a group where this behavior is widespread. We found that the use of green plants was positively related to male-biased size dimorphism, and that it was most likely to occur among cavity-nesting species. These results suggest that this behavior is likely favored by sexual selection, but also related to its sanitary use in response to higher parasite loads in cavities. We speculate that the use of green plants in starlings may be facilitated by cavity nesting, and was subsequently co-opted as a sexual signal by males. Our results represent an example of how an extended phenotypic component of males becomes sexually selected by females. Thus, both natural and sexual selection are necessary to fully understand the evolution of ritualized behaviors involved in courtship.

INTRODUCTION

Individuals exhibit a variety of morphological and behavioral traits in courtship that provide reliable information about the quality of the sender (Borgia and Gore 1986; Ryan 1998; Maynard-Smith and Harper 2003; Emlen *et al.* 2012), and can even constitute a non-morphological extension of the individual’s phenotype (i.e. extended phenotype, *sensu* Dawkins 1982). For example, males of several bird species collect materials and build complex structures, perform exaggerated exhibitions of nesting components, or incorporate odd materials into their nest structures (Collias and Collias 1984; Borgia and Gore 1986; Soler *et al.* 1998; Hansell 2000). These behaviors are often used to attract partners, or to incentivize reproductive effort in their partners after mating, through displays of a particular breeding activity related to their condition, breeding status, or experience (Hansell 2000; Veiga et al. 2006; Trnka and Prokop 2011; Moreno 2012; Tomás *et al.* 2013; García-Navas *et al.* 2015). Although sexual selection for improving mating and fertilization success is generally considered the predominant force driving the evolution of these ritualized behaviors, natural selection may also play an important role in their origin and maintenance (e.g. Candolin and Tukiainen 2015).

One of the best-studied examples of these extended phenotypic signaling behaviors is the presence of green plants, flowers, and fresh aromatic herbs in bird nests, which are incorporated by breeders and do not form part of the nest structure. A variety of naturally and sexually selected non-mutually exclusive hypotheses have been suggested to explain the evolution of the use of green nesting material in birds. First, the volatile compounds of green plants could positively affect nestling health, either because they reduce ectoparasite and bacterial loads (the ‘nest protection hypothesis'), or because they directly favor nestling condition and immune response (the 'drug hypothesis') (Clark 1991; Gwinner and Berger 2005; Mennerat *et al.* 2009a, 2009b). Both of these hypotheses posit that green nesting material improves offspring condition and fitness, and therefore the behavior would be favored by natural selection. Second, the 'sexual selection hypothesis' suggests that the green plant-carrying behavior is related to male quality, condition, or social status, and is therefore favored by sexual selection (e.g. Fauth *et al.* 1991; Pinxten *et al.* 2003; Brouwer and Komdeur 2004; Veiga *et al.* 2006; Polo *et al.* 2015).

Supporting the idea that natural selection favored the use of green nesting material in birds, a review of the natural history literature on nesting behavior and composition of nesting material revealed that passerines nesting in cavities, which typically reuse old nest sites, were more likely to incorporate green plants into their nests, whereas passerines nesting in open nests were less likely to use green nesting material (Clark and Mason 1985; Scott-Baumann and Morgan 2015). For example, female blue tits, *Cyanistes caeruleus*, incorporate plants in the nest from the end of nest construction until fledgling, favoring nestling condition and immune response (Mennerat *et al.* 2009b; Tomás *et al.* 2013). In contrast, most studies of starlings suggest that males carry green plants to nests to attract females during courtship, rather than to control ectoparasite and bacterial loads during the nesting period (Dubiec *et al.* 2013; Scott-Baumann and Morgan 2015). Moreover, male starlings generally incorporate green plants only during the beginning of the breeding period (European starling, *Sturnus vulgaris*, Fauth *et al.* 1991; and spotless starling, *Sturnus unicolor*, Veiga *et al.* 2006) and then females remove green plants from nests after beginning egg-laying (Veiga and Polo 2012). These observations contradict what would be expected if these plants provided a beneficial function to their offspring (Polo *et al.* 2015; Scott-Baumann and Morgan 2015). Moreover, males carry more plants when females are close to the nest box (Brouwer and Komdeur 2004), and polygynous males incorporate more green plants to each of their nests than monogamous males (Veiga *et al.* 2006); both of which further support a sexually-selected function for the use of green plants.

Here we attempt to understand the relative importance of natural and sexual selection in modulating the evolution of an extended phenotypic trait involved in sexual signaling, the presence of green nesting material among starling species. Based upon the assumption that sexual size dimorphism reflects the intensity of sexual selection and sex-specific selective pressures (Moore 1990; Dunn *et al.* 2001; Cotton *et al.* 2004; Ford 1994; Bonduriansky and Rowe 2005; Rubenstein and Lovette 2009), and that cavity nests are more prone to be infested with ectoparasites and bacteria (Collias and Collias 1984; Marshall 1981; see also Clark and Mason 1985; Scott-Baumann and Morgan 2015), we tested the predictions that (1) the use of non-structural green nesting material is more frequent among sexually-dimorphic species, and that (2) green nesting material evolved as a sanitary behavior, thus being more prevalent among cavity-nesting species, group-living species and/or species

Table 1. Phylogenetic Principal Component Analysis for morphological variables of sexual dimorphism: The phylogenetic signal (lambda), standard deviation, proportion of variance and factor loadings are presented for pPCA1 (with body mass, wing length, tail length and tarsus length dimorphism; n =29 species) and pPCA2 (in which body mass was excluded to increase the sample size; n = 44 species).

	pPCA1			**pPCA2**		
	lambda = 6.67e-5			lambda = 0.26		
Phylogenetic PCA	PC1.1	PC1.2	PC1.3	PC2.1	PC2.2	PC2.3
Standard deviation	1.361	1.076	0.818	1.363	0.847	0.651
Proportion of variance	0.463	0.289	0.167	0.619	0.239	0.141
Factor loadings						
MassD	0.649	-0.465	0.579	-	-	-
WingD	0.873	0.259	0.012	0.856	0.154	0.493
TailD	0.087	0.935	0.281	0.696	-0.703	-0.146
TarsusD	0.813	-0.007	-0.51	0.800	0.446	-0.400

living in humid habitats, which typically are more susceptible to contagion and transmission of parasites (Rózsa *et al.* 1996; Piersma 1997; Heeb *et al.* 2000; Moyer *et al.* 2002; Tella 2002).

MATERIAL AND METHODS

Comparative data

We collected information on nesting materials, other life-history traits (i.e. nest type, social system, and habitat type; Urban *et al.* 1997, Feare and Craig 1999 and Craig *et al.* 2009); and adult morphology (Feare and Craig 1999, Lislevand *et al.* 2007; Dunning 2007) for up to 47 starling species, roughly half of the Sturnidae in the world. A species was considered to use green nesting material (n = 28 species) only when one of the sources explicitly reported the presence of non-structural green leaves, flowers, fresh aromatic plants, or small green branches. We only categorized a species as not using green plants when there was no mention of the presence of these materials, but there was information about other nesting components (e.g. dry grass and twigs, feathers, hairs, snakeskin, or plastic). Species with incomplete descriptions of nesting materials were excluded, as well as those where information was reported from only a single nest. Life-history variables were considered as discrete characters: nest type (open or cavity), social behavior (group-living or solitary), and habitat type (grassland, scrubland, or closed forest).

Sexual dimorphism was determined as the standardized residuals of the phylogenetically-controlled linear regression of male over female morphological measurements (log-transformed) for the following traits: body mass (n = 31 species), wing length (n = 47 species), tail length (n = 46 species), and tarsus length (n = 45 species). Considering all of the available information, we obtained complete morphological data for 29 species. When dimorphism in body mass was excluded, the number of species with complete data increased to 44.

Statistical analyses

We controlled for relatedness among starling species using a subset of the phylogenetic reconstruction of Maia *et al.* (2013). The phylogeny was initially built for all 113 starling species and five outgroups using sequences from up to five mitochondrial coding genes and four nuclear introns (Lovette and Rubenstein 2007; Lovette *et al.* 2008). The topology and branch lengths were inferred using Bayesian methods in BEAST (Drummond and Rambaut 2007; see details in Maia *et al.* 2013).

We characterized sexual size dimorphism as the linear combination of the morphological traits outlined above using phylogenetic Principal Component Analyses (pPCA, Revell, 2009). In a first pPCA we conducted, all four traits (body mass, wing, tail and tarsus lengths) were considered (pPCA1; n = 29 species). However, to increase the sample size, we performed a second pPCA excluding body mass (pPCA2; n = 44 species). The pPCA1 described 75% of total variance through its first two components (PC1.1, 46% and PC1.2, 29%), and the pPCA2 described 86% of variance (PC2.1, 62% and PC2.2, 24%). Both first components (PC1.1 and PC2.1) had positive and high loadings for the dimorphism in wing length and tarsus length (and, in the case of PC1.1, also body mass; Table 1), whereas the second components (PC1.2 and PC2.2) were mostly defined by dimorphism in tail length (Table 1). Thus, the first principal components were interpreted as general descriptors of dimorphism in body size, whereas the second principal components described dimorphism in tail length relative to body size.

We first explored the use of green nesting material in relation to each morphological trait (body mass, wing, tail and tarsus length) using phylogenetic logistic regressions. We then analyzed the use of green plants in relation to the components of dimorphism, with the presence or absence of green nesting material as the response variable and the first two principal components as predictor variables. We also analyzed the phylogenetic principal components in relation to life-history traits (nest type, social system, habitat type, use of green nesting material, and use of other odd nesting material) to identify potential confusing covariations between sexual dimorphism and other predictor variables. Analyses were conducted using the packages phylolm (Ho and Ane 2014) and phytools (Revell 2012) in R v3.2.2 (R Core Team 2015).

To explore the evolution of green plant use in relation to nest type (open or cavity), social behavior (group-living or solitary), and habitat type (grassland, scrubland, or closed forest), we examined the transition rates between states of green plant use conditioned on each of those traits in separate models of ancestral state reconstruction (Pagel and Meade 2006). In a first model, rates of gain of the behavior were considered to be the same in (i.e. not influenced by) the presence or the absence of the other trait, and therefore four transitions were estimated (gains and losses of adding greenery and gains and losses of the ecological trait examined). This model was then compared to a full model in which transitions in the same direction (i.e. all gains or all losses) were allowed to have different rates when occurring in the presence or absence of the conditional trait.

This model therefore describes a scenario in which transition rates for the evolution of adding green nesting material are conditional on the ecological trait being examined, and was described by eight parameters (gains and losses of greenery in the presence and in the absence of the secondary trait, as well as gains and losses of the secondary trait in the presence and in the absence of greenery addition). We compared the maximum likelihood of both models, and considered the evolution of adding nest greenery to be associated to the ecological trait in question when the 8-parameter model was preferred over the 4-parameter model (i.e. when different transition rates for gain and loss of greenery depending on the state of the secondary trait improved model fit). The relationship between the use of plants and habitat type (with three states corresponding to the three classes of habitat) was analyzed as a multistate character, and therefore an eight-parameter model of independent evolution was compared to the full 18-parameter model in which transition rates were allowed to vary among types of habitat. Model parameters were estimated using BayesTraits v2 (Pagel and Meade 2006).

RESULTS

Sexual selection

The use of green leaves, flowers, and aromatic plants in nests occurred more frequently among sexually dimorphic starlings (Fig. 1). In particular, species that carry green plants were more dimorphic in body mass (phylogenetic logistic regression: t = 2.42, df = 29, *P* = 0.02) and wing length dimorphism (t = 2.86, df = 45, *P* = 0.01). There were similar but non-significant trends in tail length dimorphism (t = 1.07, df = 44, *P* = 0.29) and tarsus length dimorphism (t = 1.62, df = 43, *P* = 0.11) (Fig. 1). The probability of using green nesting material increased with the degree of male-biased size dimorphism (Fig. 2). Specifically, the use of green plants was positively and significantly associated with both first components PC1.1 and PC2.1, which described dimorphism in body mass, wing length, and tarsus length (Table 2). However, there were no significant relationships with the second components (describing tail length dimorphism, Table 2). Sexual size dimorphism (PC2.1) was not related to habitat type (t = 1.07, *P* = 0.29), nest type (t = -1.01, *P* = 0.32), social system (t = -1.21, *P* = 0.23), nor to other odd types of nesting materials (green plants: t = 2.39, *P*

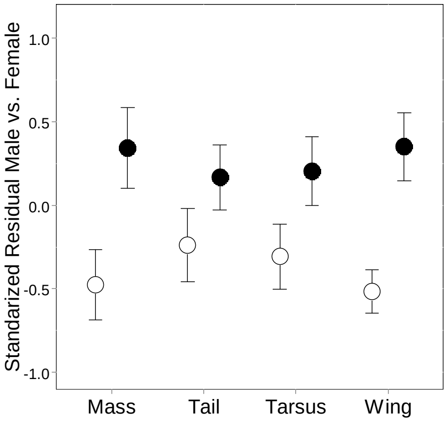


Fig 1. Differences in sexual dimorphism (mean standardized residuals ± SE) between starling species that use green nesting material (black circles) and species with no evidence of the use of green plants (white circles). Sample sizes are presented for each group and morphological variable.

= 0.02; other odd materials: t = -1.39, *P* = 0.17). When all of these variables were considered together in a logistic model, the use of green nesting material maintained a positive and significant relationship with sexual size dimorphism (PC2.1) and nest type, but there was no relationship with habitat type or social system (Table 3).

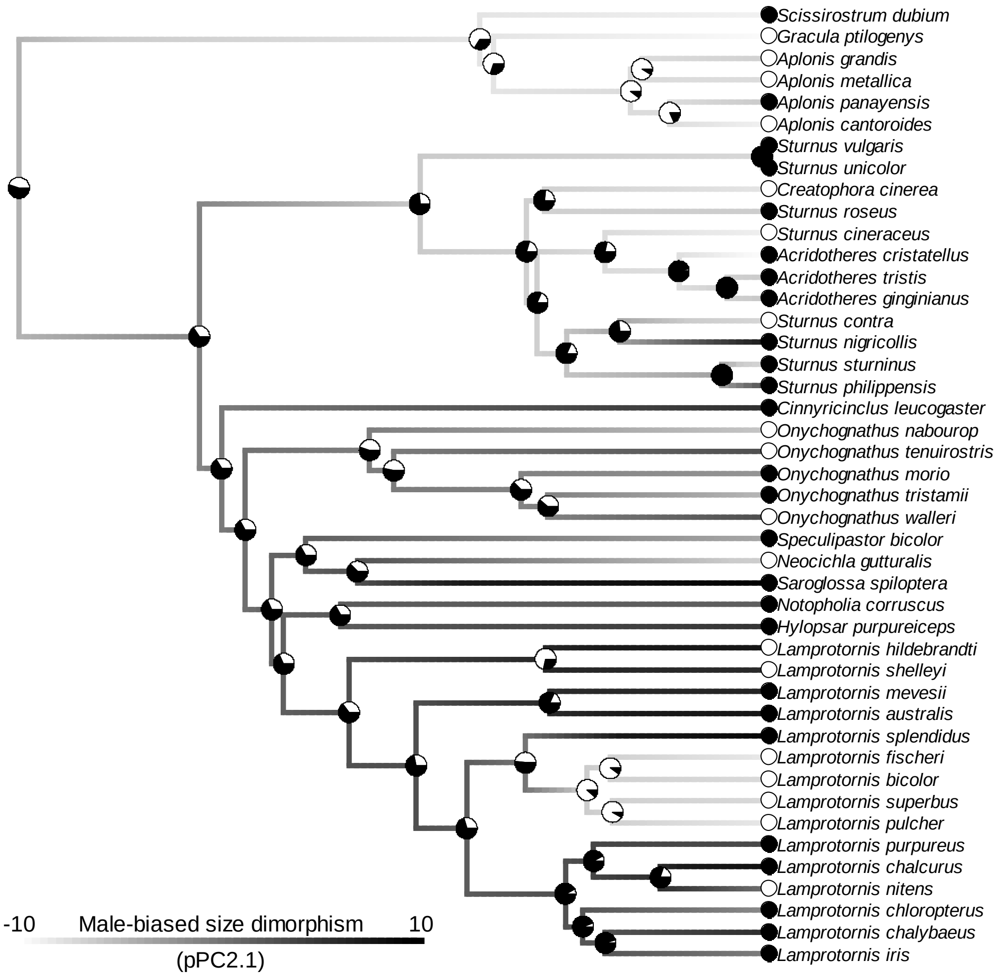


Fig 2. Relationship between the use of green plants in nests and sexual dimorphism in starlings. The color of the branches represents the first component of variation in the pPCA2 (n = 44 species) (white: low dimorphism, to black: male-biased size dimorphism). Terminal points represent the use green nesting material (black: yes, white: no). The points at nodes represent ancestral character estimation for the green plants-adding behavior.

Table 2. Use of green nesting material in relation to the principal components of sexual dimorphism (pPCA1, n = 29 spp and pPCA2, n = 44 spp). Results are from the phylogenetic logistic regressions. Bold indicates significant predictor variables.

	Alpha	Estim	SE	*z*	*P-val*
pPCA1	0.104				
PC1.1		**0.250**	**0.108**	**2.326**	**0.020**
PC1.2		0.069	0.095	-0.73	0.465
pPCA2	0.137				
PC2.1		**0.158**	**0.069**	**2.277**	**0.023**
PC2.2		0.065	0.118	0.547	0.584

Table 3. Relationship between the use of green nesting material and predictor variables of sexu selection (sexual size dimorphism) and natural selection (habitat type, nest type and social system Results from the phylogenetic logistic regression (alpha = 0.26). Bold indicates significant predict variables.

	Beta	SE	z	*P-value*
Habitat type	-0.24	0.57	-0.43	0.667
Social system	1.35	0.97	1.38	0.164
Nest type	**3.04**	**1.39**	**2.19**	**0.028**
Sexual dimorphism (PC2.1)	**0.16**	**0.08**	**2.15**	**0.031**

Natural selection

The model that considered independent transition rates for the evolution of the use of green nesting material when in cavities and open nests was preferred over the model with equal rates (LRT = 13.37, df = 4, *P* = 0.01; Fig. 3). This model suggests that gains in the use of green plants are about nine times more likely in cavity-nesting species, while loss of the behavior is about four times more likely in open nesting species. Transitions to the use of green nesting material were not correlated to transitions in social system (χ^2 = 3.15, df = 4, *P* = 0.53) nor in habitat type (χ^2 = 4.12, df = 10, *P* = 0.94).

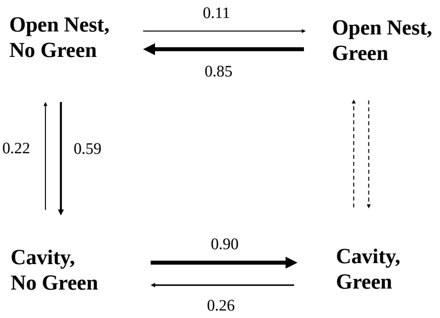


Fig 3. Flow diagram for the most probable transitions between the use of green plants in nest and the type of nest (i.e., cavity *vs.* open nest) in starlings. Parameters refer to the state transitions estimated via Bayesian procedures using a uniform prior between 0 and 1. Decreasing robustness of arrows indicates decreasing magnitude of transition rates; dashed lines represent the transition rates estimated in zero.

DISCUSSION

We found that the use of green plants, leaves, flowers, and aromatic herbs in nests is positively related to both sexual size dimorphism and cavity nesting in starlings. The use of green nesting material among sexually dimorphic species supports the idea that this behavior is involved in sexual selection (Moore 1990; Dunn *et al.* 2001; Cotton *et al.* 2004; Ford 1994; Bonduriansky and Rowe, 2005; Emlen *et al.* 2012). This result is consistent with experimental studies in two species of starlings showing that the amount of plants carried by males plays a role in mate attraction (Brouwer and Komdeur 2004) and is positively related to male mating status (Veiga *et al.* 2006). Female preference for males that carry more green plants to nests may have exaggerated this trait, especially in species under stronger and more asymmetric sexual selection (i.e. those with marked size dimorphism) (Fig. 2). Thus, this result is consistent with the hypothesis that green nesting material played a major role in sexual signaling within the Sturnidae (Fauth *et al.* 1991; Pinxten *et al.* 2003; Brouwer and Komdeur 2004; Veiga *et al.* 2006).

Our results also show that the use of green plants was more likely to evolve among cavity-nesting species. That is, gains in the use of green plants were more common in cavity-nesting species while losses were more common in open-nesting species. It is worth noting, however, that nest type distribution in this group is unbalanced (with 39 cavity-nesting species and only 8 open nesting species) and that both traits were evolutionarily labile, which made it difficult to determine their ancestral states. However, this result is consistent across two different types of comparative analyses (i.e. PGLS and analysis of transition rates) and also with previous studies (Clark and Manson 1985). Because cavities might be more susceptible to be colonized by parasites (Collias and Collias 1984; Marshall 1981), the use of green plants in cavity-nesting species suggests that this behavior likely has a sanitary function in this group (Scott-Baumann and Morgan 2015). In support of this idea, Gwinner and Berger (2005) found fewer bacteria in nests treated with green plants in the European starling. However, most experiments with starlings have not found that green nesting material reduces ectoparasite loads in nests (Fauth *et al.* 1991; Gwinner *et al.* 2000; Brouwer and Komdeur 2004; reviewed in Scott-Baumann and Morgan 2015). Furthermore, males did not carry more green plants when nests were experimentally contaminated with ectoparasites (Brouwer and Komdeur 2004). This mixed evidence on the sanitary function of green nesting material in starlings could be attributed to the fact that most experimental studies have focused on only specific best-studied groups of ectoparasites. Our results are consistent with the hypothesis that green plants in nests may play a sanitary function, likely affecting some groups of parasites or inducing particular changes in microbial communities of cavity nests. However, the antiparasitic effects of green plants in starling nests remains unclear (Dubiec *et al.* 2013, Scott-Baumann and Morgan 2015), suggesting this original function might have been lost, at least in the extant species in which it has been experimentally tested.

Experimental studies have shown that the use of green nesting material affects female breeding activity and can have negative effects upon reproductive success, as opposed to what would be expected if only anti-parasitic functions are invoked (Polo *et al.* 2010, 2015; Veiga and Polo 2012). Thus, the experimental addition of green plants to nests attracted competing females (Polo *et al.* 2010) and reduced offspring recruitment rates (Polo *et al.* 2015). Female-owners also remove green nesting material from nests (Veiga and Polo 2012), and therefore, recent reviews have argued that this male extended phenotype likely evolved via sexual selection in starlings (Scott-Baumann and Morgan 2015). Therefore, green plants may have originally provided a sanitary function in cavity nests and had a positive effect on nestlings' health (Gwinner 2013), becoming a reliable signal to females, which could have favored the exaggeration of this trait via sexual selection (Veiga *et al.* 2006). In species with high rates of polygyny and marked size dimorphism like the spotless starling, where polygynous males carry more plants to attract partners (Veiga *et al.* 2006), the ornamental function of this behavior may have been exaggerated to the point where it actually incurs fitness costs on the female (Polo *et al.* 2015), and its sanitary function may have been lost entirely. Hence, in some cases green nesting material may be involved in sexual conflict, where the potential positive effects on nestling health might be exceeded by the costs that this male behavior imposes upon the female during the breeding period (Polo *et al.* 2015). Thus, we speculate that the maintenance of this trait might depend upon experienced and high-quality females being capable of coping with the increased rates of intrasexual competition when paired with attractive males (Polo and Veiga 2006). Future studies in other species within this clade should elucidate how pervasive are sanitary functions, or loss thereof, among extant starling species.

Finally, the use of green plants at nests had multiple gains and losses across the starling phylogeny (Fig. 2). The disappearance of this behavior is particularly interesting given that they are more likely among species with open nests and lower sexual dimorphism, and could be related to the fitness costs associated with nest greenery. One possibility is that these costs are incurred through competitive interactions among breeding females (Polo *et al.* 2010, 2015). In other words, the use of plants may be lost at the same time that sexual size dimorphism decreases, as this secondary reduction of sexual dimorphism has also been observed in highly social, cooperatively breeding species of African starlings (Rubenstein and Lovette 2009). The strength of sexual selection in cooperatively breeding species is similar in males and females, which may have eroded differences between the sexes in traits used for competition for mates and/or breeding resources (Rubenstein and Lovette 2009). The loss of the use of green nesting material in some species was likely related to this reduction in sexual size dimorphism in highly social species that experience intense female-female competition. Although we did not find a relationship between the use of greenery and social system (Table 3), sexual dimorphism was significantly lower in cooperatively breeding species, consistent with other studies in this group (Rubenstein and Lovette 2009, Maia *et al.* 2016). Another possibility is that these fitness costs are higher for species that have open nests, potentially because the presence of aromatic plants in open nests increase their detectability by predators (see Cresswell 1997; Weidinger 2002, for predation as a selective force acting on nesting behavior of birds). This would explain why the loss of green-plant adding behavior is many times more likely for open nesters than for cavity nesters. Additional research is required to understand the potential costs associated to the evolution of these extended phenotypes.

In summary, this is the first comparative study exploring the function of green plants in nests of different bird species. Experimental and observational studies have provided detailed, but often conflicting, information on the function of plants in some avian species. Our results suggest that both natural and sexual selection were involved in the evolution of this behavior in starlings. Our results also show that the evolution of this extended phenotype is favored by cavity nesting, and is associated with sexual selection. In the light of previous experiments and observational studies, we speculate that the green plant-carrying behavior originated in cavity nests with a sanitary function and was subsequently favored by females via sexual selection. This hypothesis provides an evolutionary framework to explain previous mixed experimental results in some starling species, and the rapid and complex evolution of this behavior highlights the need for studies on its functional role across species. Our study adds to the growing literature suggesting that that both natural and sexual selection may be necessary to provide a complete picture of the evolution of sexual signals in animals. Although sexual selection is typically considered the predominant force driving the evolution of ritualized behaviors and exaggerated phenotypic traits involved in courtship, natural selection may also play an important and often underappreciated role.

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Capítulo 2

Fitness consequences of two extended phenotypes of male and female starlings: the use of green plants and feathers in nests

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Abstract

Both males and females may benefit from signaling their condition and quality as breeders to conspecifics, either to elicit reproductive effort in their partner or to frustrate intrusions by potential competitors during breeding. In the spotless starling, males and females signal their attractiveness and condition using odd nesting materials: males incorporate green plants to nests at the beginning of the breeding period, and females add feathers during the incubation. Such male and female behaviors are mutually related, as the addition of green plants by the male promotes the addition of more feathers by the female. However, little is known about their consequences for fitness and whether selection favors those male and female behaviors to covary in nature. We conducted two long-term and separated experiments: adding green plants, and adding feathers to nests, to analyze their consequences on fitness, measured as the rate of recruitment of the offspring. The experimental addition of feathers increased offspring recruitment rates. However, the addition of green plants reduced offspring recruitment, because the experimental manipulation of male attractiveness attracted competing females to nests and increased the number of aggressive encounters with the female-owner. The use of green plants might therefore be involved in a sexual conflict, i.e. it increases the mating opportunities of the male, but has fitness costs for the female. Females in good condition, i.e. those that incorporate larger amount of feathers to nests, might be able to cope with the cost of an increased intra-sexual competition for preferred males, which might explain the positive relationship between the male and female signaling behaviors. This study demonstrates that sexual selection acting on both male and female phenotypes is important to understand the evolution of signals involved in mating choice.

INTRODUCTION

Although males are typically the sex that exhibits ornaments and perform displays during courtship, females frequently possess morphological traits and behaviors for sexual or social signaling (Burns 1998; Amundsen 2000; Jawor *et al.* 2004; Veiga and Polo 2006; Clutton-Brock and Huchard 2013; Tomás *et al.* 2013). A distinctive sort of these signaling behaviors is the use of odd nesting materials, which can be seen as extensions of their phenotype (i.e. extended phenotypic traits, *sensu* Dawkins *et al.* 1982). Males in several avian groups collect and incorporate materials such as plants, stones, prominent decorations, or accomplish complex nest constructions, which provide reliable signals of mating status or genetic quality (Borgia and Gore 1986; Gwinner 1997; Brouwer and Komdeur 2004; Veiga *et al.* 2006; Moreno *et al.* 2012). Although the use of these materials among females has received much less attention, recent evidence is giving consistence to the idea that they may have evolved independently in both sexes (Clutton-Brock and Huchard 2013; Tomás *et al.* 2013, García-Navas *et al.* 2015).

In the spotless starling (*Sturnus unicolor*) males and females develop two causally-related signaling behaviors (Polo and Veiga 2006): during the pre-mating phase, males incorporate green plants to nests until laying (Veiga *et al.* 2006); after mating, females add flight feathers of different bird species until hatching (Veiga and Polo 2005; Polo and Veiga 2006). Our observations in the spotless starling show that the amount of feathers and the amount of green plants in nests are positively related in natural conditions (unpublished data). Furthermore, when the amount of green plants was experimentally increased in nests, females responded adding more feathers during the incubation period (Polo and Veiga 2006). Females place feathers in the nest showing the side with the higher UV reflectance to maximize their conspicuousness (Veiga and Polo 2005). The amount of feathers incorporated by the female is related to her condition (Polo and Veiga 2006), and also females carry more feathers when they breed in areas with high density of conspecifics, where the aggressive interactions among breeding females are more frequent (Rubalcaba *et al.* in prep).

Male starlings use green plants to attract females (Brouwer and Komdeur 2004; Veiga *et al*. 2006, with the European and the spotless starling respectively). In these facultatively colonial species, the competitive interactions among breeding females are important and could determine their behavior and, potentially, the way they choose their partners. For example, the experimental addition of green plants to nests of spotless starlings attracted females and increased the number of aggressive encounters with the female-owner (Polo *et al.* 2010). In this species, the female-owner removes all the green nesting material added by the male and also that experimentally incorporated, probably to reduce the intrasexual competition and the potential negative effects on their own reproductive success (Veiga and Polo 2012).

The transmission of honest information between partners on their quality and reproductive competence might be especially important when intrasexual competition for mates is costly. Thus, attractive males might benefit by transferring honest information on their quality if females have differing abilities to cope with the costs of mate choice. High-quality females might target attractive males if they are able to overcome higher intrasexual competition, while poor competitors may avoid the highest-quality partners and instead targeting the less preferred low-quality partners to minimize the costs they incur (Fawcett and Johnstone 2003). Moreover, females might benefit signaling their competitive abilities either to the male or to competing females to frustrate attempts of aggressions, nest sabotages or infanticide. Given this framework, we speculate that female spotless starling in better condition, capable to cope with the costs of mating competition, might choose attractive males and also incorporate more feathers to signal their status to potential competitors. This might explain why the amount of green plants and the amount of feathers are related in natural conditions, and also the absence of a significant relationship between the amount green plants and the reproductive success of the female in natural conditions (unpublished results). The experimental manipulation of the male and the female signals would decouple the way they perceive the attractiveness or quality of their potential partners, and therefore: (1) the experimental increase of the male signal of attractiveness might increase the cost of mate competition among females, i.e. reducing her fitness returns; (2) the experimental increase of the female signal of quality might reduce the number of aggressions from competing females, i.e. increasing her fitness returns.

Here we present the results of two experiments in which (1) we increased the amount of green plants in nests during four years; and (2) we increased the amount of feathers during four additional years. The experiments were performed separately. We compared the treatment with green plants and the treatment with feathers with their respective control nests each year, to analyze the consequences of both treatments on fitness, measured as the offspring rate of recruitment. Finally, we discuss the importance of sexual selection acting on both male and female phenotypes.

MATERIAL AND METHODS

Study area and species

The spotless starling is a medium-sized, facultatively polygynous passerine (Cramp *et al.* 1983). Females commonly lay 2 clutches per season; the first by mid-April and the second near the end of May. At the beginning of both reproductive periods, 7–10 days before laying, male spotless starlings carry fragments lichens, green twigs and flowers of the species *Lavandula stoechas*, *Santolina rosmarinifolia*, *Geranium robertianum*, *Lamium purpureum* and also ash leaves, *Fraxinus angustifolia*, and oak flowers, *Quercus ilex.* Males generally stop adding green plants at the onset of laying (Veiga *et al.* 2006). The female incorporates feathers every day since the incubation starts until hatching. The average number of feathers at hatching is 7 in a rank from 0 to 40 (Polo and Veiga 2006).

This study was conducted in a colony of spotless starlings breeding in 54 nest boxes in Manzanares el Real (Madrid), during the years 2002, 2004, 2005 and 2006 (addition of green plants) and during the years 2003, 2007, 2008 and 2009 (addition of feathers).

Experimental addition of plants

We increased the amount of green plants in a total of 27 nest-boxes every year. The same amount of nest-boxes was used as controls every year. Experimental and control nest-boxes were randomly allocated each year, and the assignment of a given nest-box was maintained over consecutive clutches within the same year. For first clutches, we started adding plants approximately 10 days before the onset of laying (mean 8.2 days, range 4–12 days before laying initiation), and for second clutches, immediately after fledgling of the first brood (mean 6.3 days, range 3–10 days). We ceased adding green plants when the first egg appeared in the nest box, mimicking the natural behavior of male starlings.

We incorporated daily ca. 10g of shredded fresh aromatic herbs of the species added naturally by starlings in the study colony. The amount of plants was similar to the highest amount recorded in nests. Therefore, we simulated the stimulus within the natural range experienced by starlings in natural conditions, which may prevent complications such as overstimulation of the immune system (e.g., allergic reactions). Control nest-boxes were checked daily and the natural greenery added by the male was not removed. We spent the same time daily in the inspection and the manipulation of the control and experimental nests to prevent differential stress that could affect prelaying females during the period of the experimental manipulation.

We manipulated the amount of green plants in 165 nests, maintaining 160 nests as controls. The proportion of polygynous males and the proportion of secondary females was balanced in the experimental and control groups (rate of polygyny: 1.21 vs. 1.23 females per male (Control vs. Experimental); total number of secondary females: 17 vs. 18).

Experimental addition of feathers

We increased the amount of feathers in 27 randomly-selected nest boxes, maintaining the same number as controls every year. We incorporated flight feathers of wood pigeon (90%) and other species mimicking the pattern observed in unmanipulated nests: we added a single feather the day when the second egg was laid (i.e., on day 2), 6 feathers on day 5 after the start of laying, 9 feathers on day 8, 15 feathers on day 11, 6 feathers on day 14 and finally 3 feathers on day 17 (approximately the day of hatching). Thus, we added 40 feathers to each experimental nest, the maximum number of feathers found in unmanipulated nests. The experimentally added feathers were summed up to those carried by the starlings. Although either in experimental or control nests part of the feathers disappeared during incubation, our experiment succeeded in establishing a marked difference between the number of feathers at hatching in experimental and control nests (experimental nests: average 31 feathers, range 25–40; control nests: average 8 feathers, range 0–30). At the end of the experiment, the amount of feathers was manipulated in 191 nest boxes, maintaining 202 nests as controls.

Monitoring individuals

Breeders were captured by means of custom-made traps fitted into the nest-boxes. Most males were captured in March, during the beginning of their breeding activity associated with the acquisition and defense of nest-boxes against other males. Females were captured during feeding 5-days-old nestlings to minimize the probability of nest abandonment by human disturbance. Nestlings were marked with a numbered aluminum ring for future individual identification.

Nestlings were marked when 5 days old using numbered rings for individual identification. We recaptured ca. 5–10% of all fledged chicks (experimental addition of plants: 832 fledglings, of which 75 were recaptured, Table 1; experimental addition of feathers: 1073 fledglings, of which 103 were recaptured, Table 2). Recruits from both experiments were recaptured when breeding in the nests boxes of the study area (ca. 70%) and two adjacent colonies locate at 1 and 2km apart from the study area. The same recapture program was followed until 2016, but no individual was recaptured beyond the spring of 2014.

Most of the recruited females were 1-year-old, while it took 2 or more years to recruit males. All of the recruited birds, in the natal or in the surrounding colonies, were

Table 1. Experimental addition of green plants: number of male (M) and female (F) fledglings, number of recruits and recruitment rate by treatment (control, CTR y experimental, EXP) and year.

		CTR			**EXP**		
		Fledglings	**Recruits**	**Rate**	**Fledglings**	**Recruits**	**Rate**
2002	**M**	55	2	0.04	57	0	0.00
	F	65	11	0.17	69	5	0.07
2004	**M**	30	3	0.10	39	6	0.15
	F	52	10	0.19	34	4	0.12
2005	**M**	57	7	0.12	58	2	0.03
	F	55	6	0.11	47	5	0.11
2006	**M**	47	4	0.09	55	1	0.02
	F	57	5	0.09	55	4	0.07
Total	**M**	189	16	0.08	209	9	0.04
	F	229	32	0.14	205	18	0.09

Table 2. Experimental addition of feathers: number of male (M) and female (F) fledglings, number of recruits and recruitment rate by treatment (control, CTR y experimental, EXP) and year.

		CTR			**EXP**		
		Fledglings	**Recruits**	**Rate**	**Fledglings**	**Recruits**	**Rate**
2003	**M**	69	1	0.01	65	8	0.12
	F	65	7	0.11	73	8	0.11
2007	**M**	66	5	0.08	81	9	0.11
	F	79	11	0.14	89	13	0.15
2008	**M**	58	2	0.03	71	8	0.11
	F	60	5	0.08	71	11	0.15
2009	**M**	65	1	0.02	59	5	0.08
	F	67	6	0.09	39	3	0.09
Total	**M**	258	9	0.03	276	30	0.11
	F	271	29	0.11	268	35	0.13

used in the analyses. We did not consider juvenile birds that were captured in the boxes but were not observed as breeders until the present day (15 individuals from the experiment with green pants and 21 from the experiment with feathers). Results did not differ when these individuals were included in the analyses (see Polo *et al.* 2015).

Data analyses

We analyzed each experiments separately: i.e. experimental and control nests (hereafter EXP and CTR, respectively) from years 2002, 2004, 2005 y 2006 were compared to analyze the effect of the addition of green plants; and the EXP and CTR nests from years 2003, 2007, 2008 y 2009 were compared to analyze the effect of the addition of feathers. We analyzed the effect of both treatments on the number of fledglings and their probability of recruitment. We used generalized linear mixed models including as fixed factors: the treatment (EXP vs. CTR), sex, clutch order (first vs. second clutches) and year. The mother and the clutch IDs were considered as random factors to control the non-independence of fledglings from the same clutch or from different clutches of the same mother. We used a log link assuming Poisson distribution to analyze the effect of treatment on the number of fledglings and a logistic regression assuming binomial distribution and using logit link to analyze the probability of recruitment. Models were fitted using “lme4” (Bates *et al.* 2012) in R 3.02.0 (R Development Core Team 2012).

RESULTS

Addition of green plants

The experimental addition of green plants did not significantly affect the number of fledglings (mean ± SE: 2.50 ± 0.15 vs. 2.58 ± 0.16 fledglings, EXP vs. CTR; $z = -0.9$, $P = 0.367$).

The probability of local recruitment was significantly lower in the experimental group (0.065 ± 0.016 vs. 0.118 ± 0.01; experimental vs. control; $\chi^2 = 5.99$, df = 1, $P = 0.014$; Fig. 1a and Table 1). Although the probability of recruitment differed significantly between males and females (0.063 ± 0.012 vs. 0.115 ± 0.015; males vs. females; $\chi^2 = 6.05$, df = 1, $P = 0.014$), there was no sex-specific effect of treatment on the offspring recruitment rates (Interaction Treatment × Sex: $\chi^2 = 0.16$, df = 1, $P = 0.68$).

Addition of feathers

The experimental addition of feathers did not significantly affect the number of fledglings in nests (mean ± SE: 2.80 ± 0.14 vs. 2.64 ± 0.12 fledglings, EXP vs. CTR; $z = 1.29$, $P = 0.198$).

The addition of feathers significantly increased the probability of recruitment of fledglings (11.9% ± 1.4 vs. 7.18% ± 1.1; EXP vs. CTR; $\chi^2 = 6.91$, df = 1, $P = 0.009$; Fig 1b and Table 2). The effect of treatment on the probability of recruitment differed between sexes (Interaction Treatment × Sex: $\chi^2 = 4.84$, df = 1, $P = 0.027$). Thus, the addition of feathers increased the probability of recruitment of males more than that of females (Fig. 2). However, the main effect of treatment, i.e. in absence of the interaction term, remained significant.

DISCUSSION

Fitness consequences of adding plants

Our experiment revealed a negative effect of the use of green plants on the rate of offspring recruitment that was maintained across years (Fig. 1a). This result is suprising, because the plant-carrying behaviour is related to the attractiveness and level of polygyny of male spotless starlings and the fitness of polygynous males is higher in this species (unpublished result. Thus, this striking result needs an explanation. The green nesting material is a component of male attractiveness in starlings (Brouwer and Komdeur 2004; Veiga *et al.* 2006), and increasing the amount of plants in nests increases the level of intrasexual competition among females for the preferred males, and also their levels of circulating testosterone during the breeding period (see Polo *et al.* 2010 with the same females of this study). Female spotless starlings that were treated

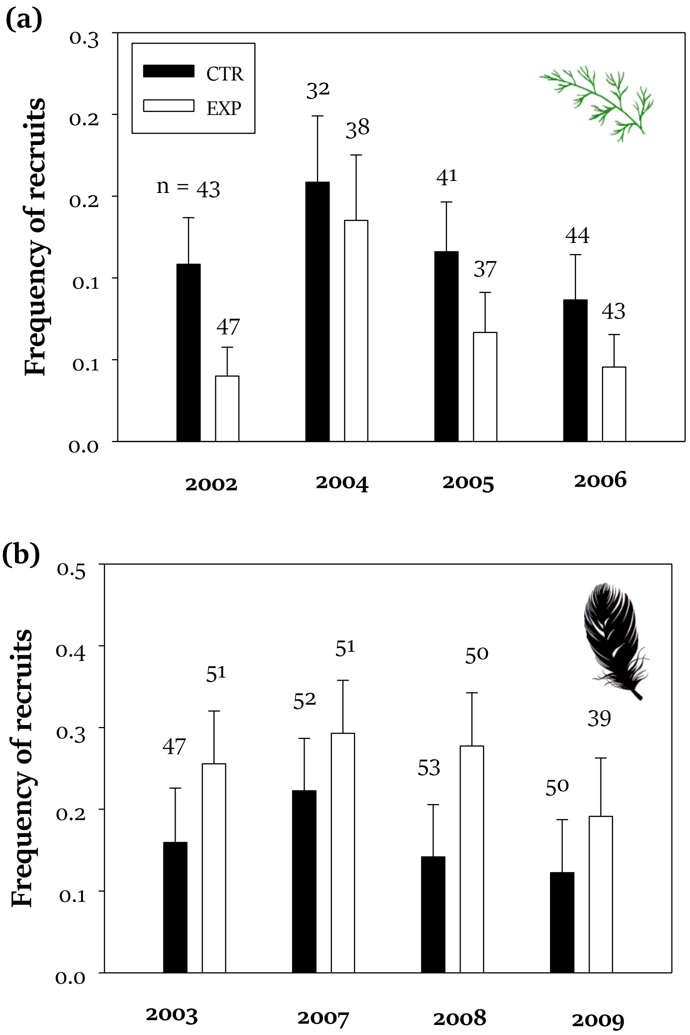

Fig 1. Frequency of nests with recruits in the green plants addition experiment (a) and feathers addition experiment (b). Sample size (number of nests) is shown for the experimental (EXP) and (CTR) groups of each year.

with exogenous testosterone reduced feeding rates, and also produced less recruits than controls (Veiga and Polo 2008), which might provide a proximate cause for the reduction of the offspring recruitment rates found in this study.

By experimentally increasing the amount of plants, we probably decoupled the female perception of the attractiveness of their mates. Thus, the negative effect of treatment on fitness may have resulted from the fact that most females were unable to cope with the increased levels of competition, thereby reducing their reproductive success. Another possibility is that the experimental addition of green plants allowed low-quality males to breed, which in absence of the treatment would not be able to attract a female and produce offspring. Thus, our results support that the use of green plants is involved in mating choice in starlings (Fauth *et al.* 1991; Brouwer and Komdeur 2004; Veiga *et al.* 2006; Polo *et al.* 2010).

Although this experiment was not designed to discard a possible sanitary effect of the addition of green plants in starlings, our results contradict the hypothesis that parents incorporate plants to favor nestling health and fitness (Wimberger 1984; Clark and Mason 1985; Mennerat *et al.* 2009). Evidence about the potential sanitary use of plants in starlings is confusing (Dubiec *et al.* 2013; Scott-Baumann and Morgan 2015). Thus, previous experimental studies showed that (1) the addition of greenery did not reduce the load of ectoparasites in the nest and (2) the addition of parasites did not increase the male carrying behavior (Fauth *et al.* 1991; Brouwer and Komdeur 2004). Nevertheless, they suggested that the fresh plants in the nests of European starlings mainly served a function in mate attraction (Fauth *et al.* 1991; Pinxten *et al.* 2003; Brouwer and Komdeur 2004).

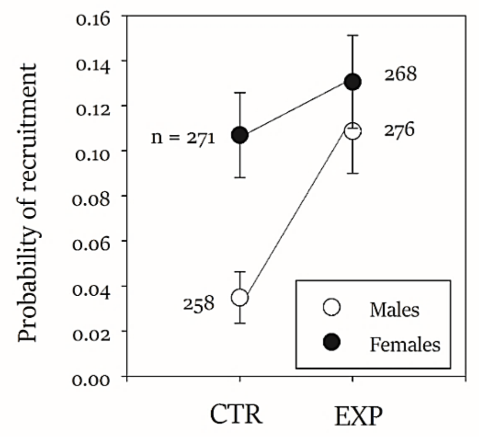

Fig 2. Probability of recruitment of males and females from the feathers-addition experiment. The sample size (number of male and female fledglings) is shown for both the experimental (EXP) and control (CTR) groups.

Our results suggest that the use of green plants by male starlings involve fitness costs for the female due to mating competition (Polo *et al.* 2010). However, the negative effect on the reproductive success was only evidenced when the amount of green plants was experimentally manipulated. By contrast, offspring recruitment rates were not significantly related to the amount of green plants naturally carried by the male (unpublished data). This suggests that females in natural conditions rely on the information provided by the green nesting material to choose their mates, while the experimental manipulation decoupled the system of mating choice.

The amount of plants that a male incorporate to his nests might provide honest information about the potential cost for the female to mate with him. A good-quality female might choose this partner if she can cope with the costs due to the increased competition with other females, while poor-quality females might instead target less attractive partners, so that they minimize the costs they incur (Fawcett and Johnstone 2003).

Fitness consequences of adding feathers

The experimental addition of feathers increased the offspring probability of recruitment consistently across years (Fig 1b). The use of feathers in nests of spotless starling is a female behavior that is related to female condition and breeding experience (Veiga and Polo 2005; Polo and Veiga 2006). Here we provide experimental evidence that this behavior had consequences for fitness. Recent evidence is given consistence to the idea that some signaling behaviors evolved independently in males and females favored by intra-sexual competition, as processes involved in sexual and/or social selection (Tobias *et al.* 2011; Clutton-Brock y Huchard 2013; Tomás *et al.* 2013; García-Navas *et al.* 2015).

Females might benefit, as males do, from signaling their phenotypic or genetic attributes to males through ornamental traits (Amundsen 2000; Veiga and Polo, 2006; Tobias *et al.* 2011). By signaling her quality and breeding competence, the female could either elicit differential allocation in the male (i.e., inter-sexual communication) or frustrate aggressions from competing females (i.e., intrasexual communication). In a previous experiment, the addition of feathers did not affect feeding rates or the removal of fecal sacs by male or female spotless starlings, although it increased the clutch size and a reduced of nestling mortality (Veiga and Polo 2011). The increase in clutch size (on average 0.5 eggs) is surprising because the female might not be expected to adjust her investment in response to the manipulation of her own trait. In this sense, we cannot discard that male starlings also gather feathers to the territory and this behaviour could increase in response to the experimental addition of feathers. However, the filmations suggest that is the female who introduces and disposes feathers into the nest box (Veiga and Polo 2005).

Recent studies have found that some female signaling behaviors are involved in intrasexual communication (e.g., García-Navas 2015 *et al.* 2015 in *Petronia petronia*; Heinrich 2015 in *Tachycineta bicolor*). For example, nests with experimentally-added feathers in the rock sparrow (*Pretronia petronia*) had fewer intrusions by floater individuals and were defended with greater intensity by both parental than control nests (García-Navas *et al.* 2015). In a recent experiment with the spotless starling, we found that females breeding in plots with an increased density of nest boxes carried more feathers to their nests than females breeding apart from conspecific nests (Rubalcaba *et al.*, in prep). Breeding females might be tempted to signal their presence in the territory, especially when the density of conspecifics is high, to frustrate attempts of sabotage by competing females (e.g. Polo *et al.* 2010) or to reduce the risk of intraspecific parasitism (Calvo *et al.* 2000; Heinrich 2015; authors' unpublished data). Thus, the experimental addition of feathers during laying and incubation periods could have reduced the frequency of intruders and potential competitors, which might have favored breeding activities of parents during the nestling period and subsequently increased offspring recruitment rates.

In the light of our results, one can also speculate that feathers incorporated to nest exert, *per se*, sanitary effects on the load of ectoparasites, which might improve nestling condition and survival (Lombardo *et al.* 1995; Peralta-Sánchez *et al.* 2010). Thus, some chemical compounds present on feathers, as waxes and other substances from the uropygial gland could reduce bacteria, fungi or ectoparasites of avian nests (e.g., Haribal *et al.* 2005; Reneerkens *et al.* 2008). For example, it has been recently shown that lining feathers incorporated to nests in the spotless starling have antibacterial proprieties (Ruiz-Castellano *et al.* 2016). However, the effect of lining feathers on pathogens affecting nestling condition after hatching remains equivocal (Stephenson *et al.* 2009), and the importance of pathogens present on eggshells on the subsequent condition and mortality of young has not yet been studied. It is possible that bacterial infection through the eggshell also affect nestling condition and survival during the growth phase. By corollary, further research might be necessary to understand the proximate mechanisms that relate the presence of feathers in the nest and their deferred effect on offspring probability of recruitment. Thus, we cannot discard potential sanitary effects of feathers in nests of spotless starling.

The effect of feathers addition was unexpectedly sex-specific, i.e. it increased especially the rate of recruitment of male fledglings (Fig. 2). It is worth noting that, although the effect was stronger on males, both males and females increased their probability of recruitment in the experimental treatment, which maintained significant differences in the overall mean (Fig. 1b; and see Results). This sex-specific effect might be consequence of a greater sensitivity of sons, the bigger sex in the spotless starling, to the rearing conditions (Anderson *et al.* 1993; Benito and González-Solís 2007; Bowers *et al.* 2015). The rate of recruitment is generally female-biased in our population of starlings (males: mean 5% rank 1-12% *vs*. females: mean 12% rank 8-17%; Tables 1 and 2; and unpublished data), likely because competition among males for breeding sites and mates exceeds that among females. Consequently, males generally require more time than females to settle in a breeding territory (Rubalcaba *et al.* 2016). Males from the experimental group increased their rate of recruitment close to the rate of control females (Fig. 2), which suggests that they benefited more than their sisters from the positive effects of the presence of feathers in the nest. This result, however, must be taken cautiously because, although there was no effect of treatment on dispersal distances within the study area (unpublished data), we do not know the extent to which the sex-specific effect on recruitment rates was affected by the sex-specific patterns of dispersal in the population.

Conclusions

This study provides experimental evidence on the deferred consequences for fitness of two sex-specific extended phenotypic traits. Our results suggest that both the use of green plants by males and the use of feathers by females might be modulated by selection. Thus, both males and females might benefit from signalling their condition or competitive habilities to conespecifics. The theory of sexual selection cannot be considered complete ignoring the importance of female intrsexual competition and its consequences for the evolution of signalling processes of females (Amundsen 2000; Clutton-Brock and Huchard 2013).

Sexual selection might be an ultimate cause to explain the evolution of green nesting material by males in the spotless starling. The use of plants likely provides honest information about the quality of the male and the potential cost that his partner might incur due to mating competition. On the other hand, the use of feathers can ultimately be explained as a social signal of females, i.e. a trait intended to inform to competitors about the quality of the female nest-owner. In both the male and female behaviors, we cannot discard the importance of natural selection, as it might potentially explain their origin as sanitary components of nests.

When the amount of green plants was experimentally increased, females carried more feathers to their nests (Polo and Veiga 2006), probably because they required investing more time signaling their condition to competing females. Our results suggest that such behaviours might covary in natural conditions. However, a future experiment combining both treatments, i.e. the addition of green plants and feathers, might inform about the potential fitness benefits of ornamental feathers for a female paired with an attractive male. The transmission of honest information between partners and with intrasexual competitors might be important for the evolutionary persistence of costly mating systems.

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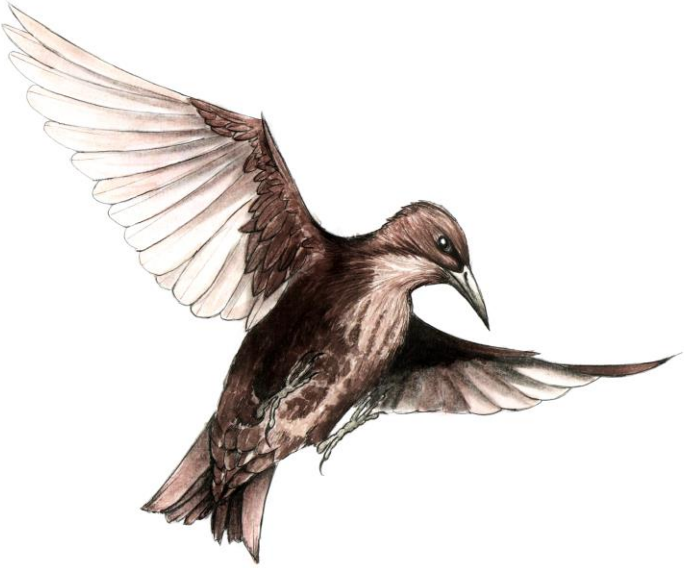
Capítulo 3

Is offspring dispersal related to male mating status? An experiment with the facultatively polygynous spotless starling



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Abstract

Patterns of natal dispersal are generally sex-biased in vertebrates, i.e., female-biased in birds and male-biased in mammals. Interphyletic comparisons in mammals suggest that male-biased dispersal occurs in polygynous and promiscuous species where local mate competition among males exceeds local resource competition among females. However, few studies have analysed sex-biased patterns of dispersal at the individual level, and facultatively polygynous species might offer this opportunity. In the spotless starling, polygynous males exhibit their mating status during courtship carrying higher amounts of green plants to nests than monogamous males. We experimentally incorporated green plants to nests during four years to analyse long-term consequences on breeding success and offspring recruitment rates. We unexpectedly found that experimental sons recruited farther than experimental daughters, while control daughters recruited farther than control sons. A similar pattern was found using observational information from eight years. We discuss this result in the context of local competition hypothesis and speculate that sons dispersed farther from nests controlled by polygynous males to avoid competition with relatives. The amount of green plants in nests affects female perception of male attractiveness and degree of polygyny, although little is known about proximate mechanisms linking this process with the offspring dispersal behaviour. Our results support the idea that male-biased dispersal is related to polygyny in a facultatively polygynous bird.

INTRODUCTION

Natal dispersal, i.e. the distance from the site of birth to the site of first reproduction, differs between sexes in most bird and mammals (Greenwood 1980; Greenwood and Harvey 1982; Pusey 1987; Sutherland *et al.* 2000). In birds, males are generally the more philopatric sex while females are more philopatric in mammals. Male-biased patterns of dispersal might evolve when intrasexual competition among males (e.g., for mates) exceeds that among females (e.g., for breeding resources; Perrin and Mazalov 1999, 2000; Mabry *et al.* 2013). This is more common in mammals because most of them are polygynous and males disperse to reduce levels of local competition for mating opportunities (Greenwood 1980; Greenwood and Harvey 1982; Mabry *et al.* 2013). In birds, males generally defend breeding territories. Accordingly, they might increase their chances of acquiring and keeping breeding territories when they have prior knowledge of the breeding area. Thus, males might benefit of remaining in the vicinity of their natal territories, while females disperse by being able to choose among potential territory holders (Greenwood and Harvey 1982).

At the individual level, variations in dispersal distances result from ecological and social factors such as local competition for mates or resources and the individual body condition (Pärt 1990, Negro *et al.* 1997; Verhulst *et al.* 1997; Pasinelli *et al.* 2004, 2007; Michler *et al.* 2011). When the costs and benefits of dispersal differ between males and females, these factors are expected to determine dispersal decisions in a sex-specific way (Greenwood 1980; Perrin and Mazalov 1999, 2000; Michler *et al.* 2011). In birds, local competition for breeding territories mainly affect dispersal behaviour of males (Eikennar *et al.* 2008; Wilson and Arcese 2008; Richardson *et al.* 2010), which might also depend more on their body condition to acquire and defend breeding sites close to their natal territories (Forero *et al.* 2002; Nevoux *et al.* 2013). In species where males monopolize breeding territories and mates (i.e., polygynous and facultative polygynous species), local competition for breeding territories may be high, especially among polygynous males that control larger territories (Emlen and Oring 1977; Dyrcz 1986; de Ridder *et al.* 2000; Veiga *et al.* 2001, 2002). Therefore, males are expected to disperse from areas controlled by polygynous males avoiding competition for mates or breeding sites.

The spotless starling (*Sturnus unicolor*) is a facultatively polygynous bird in which sexual roles are markedly different during reproduction (Moreno *et al.* 1999; Veiga *et al.* 2002). Polygynous males in this species control larger territories and spend more time in their defence (Veiga *et al.* 2001, 2002). Furthermore, male starlings carry green plants to nests to signal their mating status and attract partners during courtship. The amount of green plants incorporated by males is related to their level of polygyny: polygynous males carry larger amount of green material to each of their nests than monogamous males (Veiga *et al.* 2006). The experimental addition of green plants to nests of spotless starling attracted competing females, increased circulating testosterone levels of the female-owner during the breeding period (Polo *et al.* 2010) and had negative effects on female fitness, reducing offspring recruitment rates (Polo *et al.* 2015).

Here, we analyse natal dispersal distance of recruits from the experiment in Polo *et al.* (2015). In this experiment we manipulated the attractiveness of male spotless starlings by adding green plants to their nests during four years. We then analyse offspring recruitment rates in subsequent years. Here we aimed to explore the extent to which the effect of treatment on offspring recruitment rates was affected by variations in dispersal distance from natal nests (e.g., Tinbergen 2005; Doligez and Pärt 2008). We tested the hypothesis that sons disperse farther from nests controlled by attractive and polygynous fathers to avoid competition for nesting sites or mates. Using observational data, we also explored the relationship between the amount of green plants carried by males and the offspring patterns of dispersal under non-manipulative conditions.

MATERIAL AND METHODS

General procedures

This study was conducted in a colony of spotless starlings breeding in 54 nest boxes in Manzanares el Real (Madrid province, central Spain). From 2002 to 2009, we recorded the amount of green plants incorporated to nests and analysed its relationship with offspring natal dispersal distances (observational study). During the years 2002, 2004, 2005 and 2006, we experimentally incorporated green plants to 27 randomly selected nests each year (experimental study). Individuals were recaptured until 2011 in three breeding colonies: the original natal colony and two surrounding areas (located ca. 1000 m and 2000 m apart from the study plot; see Fig S1 in Supplementary Material).

Observational study

Male starlings display the green plant-carrying activity during the 7-10 days that immediately precede the beginning of laying. They mainly incorporate fragments lichens, green twigs and flowers of the species *Lavandula stoechas*, *Santolina rosmarinifolia*, *Geranium robertianum Lamium purpureum* and also ash leaves, *Fraxinus angustifolia*, and oak flowers, *Quercus rotundifolia*. Males generally stop adding green plants at the onset of laying (Veiga *et al.* 2006). To measure the amount of green plant material carried by male starlings we used a classification with six categories (similar to that used in Veiga *et al.* 2006): (1) Absence of green plants in the nest; (2) traces of green plants: a single fragment less than 2-4 cm or two fragment until 2 cm; (3) small amount of plants: a single fragment of 4-5 cm or two fragments between than 2-4 cm; (4) intermediate amount of plants: two or three fragments between 2 - 5 cm or three to five fragments about 2 cm; (5) high amount of plants: four or five fragments between 2 -5 cm or six to eight fragments less than 2 cm; and (6) very high amount of plants: more than five fragments between 2 - 5 cm or more than eight fragments less than 2 cm. These classes represent an exponential scale in which each level approximately duplicates the amount of material of the previous class (i.e., 0, 0.4, 0.8, 1.6, 3.2 and 6.4 g for the classes (1) to (6) respectively). We recorded the amount of green plants several times per nest, from two weeks before the onset of lying until the first egg appeared (for an average of 5.3 times per nest and range of 3 - 7 times per nest and reproductive attempt). The average of all these measures was log-transformed and considered as a linear continuous predictor.

Experimental study

Green plants of the same species added by male starlings in our colony were incorporated to nests approximately 10 days before the onset of laying (average 8.2 days; range 4 - 12 days). In second clutches, green plants were added immediately after fledging of the first brood (average 6.3 days before the onset of the second laying; range 3 - 10 days). The amount of plants added experimentally to nests was similar to the highest amount recorded in nests (corresponding to a class 6), so the stimulus created by the manipulation was within the range of natural levels experienced by starlings under natural conditions. Control nest boxes were checked daily, but no plants were added or removed. We spent the same time in the inspection and manipulation of experimental and control nests to prevent differential stress that could affect pre-laying females.

We experimentally manipulated the amount of green plants in 27 nest boxes each year in 2002, 2004, 2005 and 2006. The same number of nest boxes was used as control. Experimental (EXP) and control (CTR) nest boxes were randomly determined each year, therefore, treatments were not spatially structured but randomly distributed within the colony. The assignment to one or the other group was maintained in both the first and second clutches. At the end of the study, 328 nests (165 EXP and 163 CTR) produced fledglings, and 73 individuals (27 EXP and 46 CTR) were recaptured as breeders in the following years (Table S1). Control recruits were also considered in the observational study.

Recapture of individuals

Individual nestlings were marked when 5 days old using numbered rings for individual identification, and they were weighed (to the nearest 0.1 g) and sized (tarsus–metatarsus length, to the nearest 0.01 mm) before emancipation. Breeders were captured within their nest boxes during the breeding period; most males were captured while displaying the plants-carrying behaviour, and most females while feeding 5-days-old nestlings. Although male and female breeders were identified in different phases of the breeding cycle, the frequency of nests with un-identified breeders was low (between 1-3 nests each year) and did not differ between sexes. We recaptured 5-10% of all fledged chicks (Table S1) when they started breeding in the nest boxes of the natal colony and two surrounding areas at 1 and 2 km apart from the natal colony. The same recapture program was followed in the three study colonies until the spring of 2016, but no individual from the experimental or the observational study was recaptured beyond the spring of 2012. Natal dispersal distance was determined as the linear distance from the nest box of birth to the nest box of first reproduction (e.g., Greenwood 1980; Fitze *et al.* 2004; Tinbergen 2005; Tschirren *et al.* 2007).

Data processing and statistics

The procedure to determine natal dispersal distances has limitations when making inferences about the distribution of dispersal distances because it in part depends on the spatial distribution of the nest boxes (Baker *et al.* 1995; Koenig *et al.* 1996; Doligez and Pärt 2008). Because the study was carried with a constant distribution of nest boxes, we can assume that the error due to the detectability of each distance is similar for all groups (i.e., EXP daughters, CTR daughters, EXP sons and CTR sons). However, to estimate the extent of this assumption we randomized the spatial distribution of the nest boxes through computer simulations (based on Baker *et al.* 1995). Analyses using observed and corrected means yielded similar results (Table S2 and Fig. S2). The following analyses were conducted using observed dispersal distances.

Natal dispersal distances were analysed using general linear mixed models considering the amount of green plants, sex, year and the interaction between the amount of plants and sex as fixed factors. Additionally, dispersal distances of sons and daughters were compared through separate analyses for control and experimental nests. We also analysed the effect of the amount of green plants on the body mass and tarsus-metatarsus length of fledglings using general linear mixed models. The identity of fathers was included as a random factor to avoid problems of pseudoreplication. We used Box-Cox transformations for the

Table 1. Model for natal dispersal distance in relation to the amount of green plants in nests (n = 73 recruits in the experiment and n = 86 recruits in the observational study). Models included the year as a fixed factor and fatherID as a random factor to avoid problems of pseudoreplication. Bold indicates significant predictor variables.

(a) Experimental study

	F	df	*P*
Treatment	0.01	1, 58	0.93
Sex	0.23	1, 58	0.63
Year	2.47	3, 58	0.07
Treatment:Sex	8.21	1, 58	**0.01**

(b) Observational study

	F	df	*P*
Greenery	1.37	1, 48.2	0.25
Sex	9.39	1, 58.2	<0.01
Year	3.86	7, 57.1	**0.05**
Green:Sex	5.49	1, 58.7	**0.02**

dispersal distances, body masses and tarsus lengths to normalize the residuals of the models (Box-Cox power parameter was estimated using likelihood-based methods in R 3.02.0 software, R Development Core Team 2015).

RESULTS

Most recruits from the experimental study were captured in the colony where the experiment was conducted (58 of 73 total recruits). Only 1 individual from the experimental study was captured in the colony 1000 m away from the study area and 14 individuals recruited in the colony at 2000 m. For the observational study, 86 recruits were recaptured (27 males and 59 females; Table S1), 55 of them in the natal colony, 17 recruits in the colony at 1000 m from the study colony and 14 recruits in the colony at 2000 m. The age of first recapture of females was 1.50 ± 0.07 years old (mean ± SE) while most males were recaptured when 2 years old (1.96 ± 0.11, mean ± SE). Recaptures were female-biased in both CTR and EXP groups (Table S1) and both sexes had lower recruitment rates in the EXP treatment (Polo *et al.* 2015; Table S1).

Dispersal distances of recruits did not significantly differ between EXP and CTR nests (529 ± 127 m, n = 27 *vs.* 603 ± 97 m, n = 46; EXP *vs.* CTR observed means ± SEs; Table 1) and there was no significant effect of sex (Females: 570 ± 95 m, n = 48 *vs.* Males: 588 ± 132 m, n = 25; Table 1). However, the addition of green plants to nests affected dispersal distance in a sex-specific way, with a significant interaction between treatment and sex (Fig. 1 and Table 1). Thus, daughters dispersed farther than sons in control nests (Females: 741 ± 147 m, n = 30 *vs* Males: 394 ± 115 m, n = 16 (mean ± SE); $F_{1,\ 23} = 5.20$, $P = 0.027$), while the opposite pattern was found in experimental nests, where sons dispersed farther than daughters (Females: 292 ± 102 m *vs* Males: 1053 ± 310 m; $F_{1,\ 38} = 4.83$, $P = 0.037$; Fig. 1).

The addition of green plants to nests had no significant effect on fledgling body mass ($F_{1,\ 828} = 1.61$, $P = 0.20$) or tarsus-metatarsus length ($F_{1,\ 758} = 2.57$, $P = 0.11$). However, there was a significant interaction between treatment and sex affecting fledgling body mass and tarsus-metatarsus length. Thus, males from the EXP group had lower body mass than CTR (82.2 ± 0.52 g vs. 84.1 ± 0.45 g; EXP vs CTR); although female body mass did not

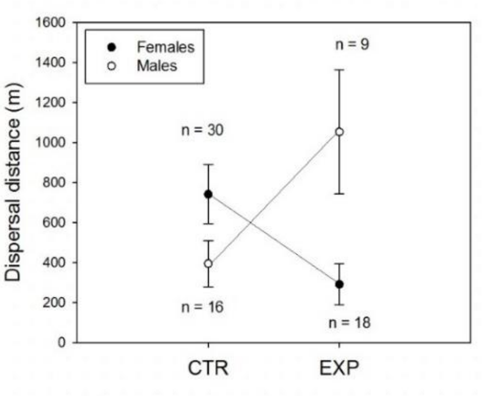

Fig 1. Natal dispersal distances of males and females (means ± SEs of recruits from years 2002, 2004, 2005 and 2006) in response to the experimental addition of green plants to their natal nest experimental: EXP versus control: CTR.

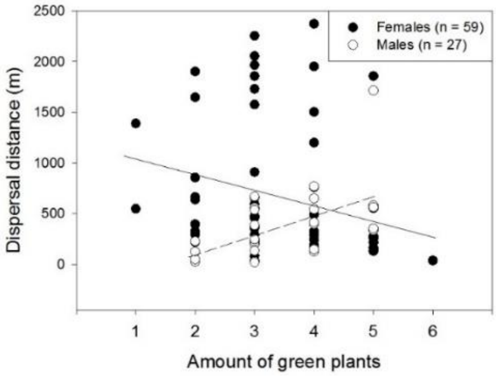

Fig 2. Natal dispersal distances of males (dashed line) and females (solid line; years 2002–2009) in relation to the amount of green plants recorded in their natal nests (categorized in six classes, see Methods). Statistical analyses were conducted using Box–Cox transformed dispersal distances to normalize model residuals.

differ between treatments (79.5 ± 0.49 g vs. 79.3 ± 0.44 g; EXP vs. CTR; Interaction Treatment × Sex: $F_{1,\,828} = 4.49$, $P = 0.034$). Conversely, EXP females had longer tarsus-metatarsus lengths (30.0 ± 0.06 mm vs. 29.87 ± 0.05 mm; EXP vs. CTR) although tarsus–metatarsus lengths did not differ in males in relation to treatment (30.4 ± 0.05 mm vs. 30.4 ± 0.05 mm; EXP vs. CTR; Interaction Treatment × Sex: $F_{1,\,758} = 4.52$, $P = 0.033$).

In the observational study, the amount of green plants recorded in nests had no overall effect on dispersal distance of recruits; however, the interaction between sex and the amount of plants was significant (Table 1), showing a sex-specific effect on dispersal distances of recruits (Fig. 2).

DISCUSSION

Dispersal distances differed between sexes in control nests similarly to the general pattern of birds, i.e. females dispersed further than males from their natal territories (Greenwood and Harvey 1982; Mabry *et al.* 2013). The experimental addition of green plants to nests differentially affected dispersal distances of sons and daughters. Thus, experimental sons dispersed farther than controls, while experimental daughters dispersed closer. A similar pattern was found observationally, where dispersal distances were positively related in sons and negatively related in daughters to the amount of green material in their natal nests. This surprising result suggests that male and female natal dispersal distances were oppositely related to the mating status of the father (Veiga *et al.* 2006). Thus, this result is consistent with the idea that male-biased dispersal emerges in polygynous systems to reduce levels of local competition for mates and resources among relatives (Perrin and Mazalov 2000). However, the result showed here was an unexpected finding of an experiment originally designed to analyse long-term consequences of green nesting material on fitness (Polo *et al.* 2015). Therefore, we have not identified the mechanism that caused this phenomenon, but we speculate in the light of previous studies in the spotless starling (Polo *et al.* 2004; Polo *et al.* 2010, 2015; López-Rull and Gil 2009a, 2009b).

Male starlings carry green plants to nests to attract females during courtship (Brouwer and Komdeur 2004 in the European starling). Furthermore, polygynous males carried larger amount of green material to each of their nests (Veiga *et al.* 2006 in the spotless starling). In both European and spotless starling, males that are bigger and more aggressive defend larger territories and attract more females to their nests (de Ridder *et al.* 2000; Veiga *et al.* 2001; Veiga *et al.* 2006). Therefore, competition among males for breeding territories and mates may be high in starlings. Thus, generally more females than males are recaptured as breeders in the colony and also males require more time to acquire their first breeding site. Male starlings spend more time as floaters to acquire information on local breeding territories before settlement (Tobler and Smith 2004) and disperse from the breeding colony when they are not able to acquire a breeding vacancy.

The son-biased pattern of dispersal found in nests controlled by attractive fathers, i.e. those that carried larger amount of green material, probably resulted from a tendency of males to avoid competition for breeding sites and mates. However, we showed that sons dispersed from these nests almost 700m more than from nests of less attractive fathers, which far exceeds the radius controlled by polygynous males around their breeding territories in this species, ca. 200m (see Veiga *et al.* 2001; unpublished data). One possible explanation for this is that polygynous males explored larger territories at the beginning of the breeding period, when they compete for breeding sites, although they finally settled in a subset of nest boxes within these territories. Thus, the territory that males control at the moment of reproduction might be smaller than the area that they originally explored. Therefore, sons might have dispersed larger distances than expected in relation to the size of the territory of their fathers during the breeding period. Another possible explanation is that the tendency of sons to disperse from nests controlled by attractive fathers evolved in a context where these males were spatially aggregated, for example, in areas with better conditions for reproduction or higher availability of breeding sites. Thus, sons might be tempted to disperse from larger plots to avoid, not only local competition with the father, but also with neighbouring males. Unfortunately, our experimental design does not allow testing this hypothesis because treatments were not spatially structured in sub colonies, but randomly distributed in the study area.

Dispersal distance was female-biased in control nests and male-biased in nests treated with green plants. We have previously shown that the addition of green plants had negative effect on female breeding success, reducing recruitment rates of both sons and daughters (Polo *et al.* 2015). Therefore, we cannot discard that lower recruitment rates of males in part resulted from their increased dispersal distance. However, experimental females also had lower recruitment rates although they were recaptured closer to their natal nests. Thus, treatment reduced offspring recruitment rates of both sons and daughters but the effect on dispersal distance was sex-specific. This suggests that the addition of green plants affected not only natal dispersal distance but also post-fledgling survival (Polo *et al.* 2015). The addition of plants to nests attracted competing females; thus, female-owners experienced more aggressive encounters and increased their circulating testosterone levels during the breeding period (Polo *et al.* 2010). Female starlings with increased testosterone level fed nestlings at a lower rate (Veiga and Polo 2008). Possibly as a result of this reduction in feeding rates, sons from nests treated with green plants had lower body mass at fledgling. Sons in worse condition might also have lower ability to acquire breeding vacancies close to their natal territory (Verhulst *et al.* 1997; Dufty and Belthoff 2001; Forero *et al.* 2002; Tinbergen 2005; Nevoux *et al.* 2013). However, we also found that the experimental addition of green plants increased tarsus-metatarsus length of daughters. This sex-specific effect on nestling body mass and structural size suggests that there is a complex influence of mothers on the phenotype of their offspring, potentially driven by maternal effects (Dufty and Belthoff 2001; see Muriel *et al.* 2013 for an experiment with spotless starlings).

Although avoiding local mate competition could be an ultimate cause for the sex-specific pattern of dispersal in relation to the mating status of the father (Greenwood 1980; Greenwood and Harvey 1982; Perrin and Mazalov 1999, 2000; Mabry *et al.* 2013), it is unlikely that son-biased dispersal was only caused by direct aggressions by the father. Thus, the experimental manipulation of parental attractiveness increased dispersal distance of sons in a similar way to that observed in non-manipulated conditions. In relation to this, recent studies have shown that maternal effects may play an important role affecting offspring dispersal behaviour (Dufty and Belthoff 2001; Tschirren *et al.* 2004, 2007; Meylan and Clobert 2004). They suggest that mothers adaptively modify offspring dispersal distances in relation to environmental and social conditions perceived during reproduction via maternal effects (Dufty and Belthoff 2001; Tschirren *et al.* 2004, 2007). In a first attempt to find a proximate mechanism to explain the consequences on dispersal of our experimental manipulation, we speculate that green plants added to nests, which inform females about the attractiveness of their mates, might promote mothers to increase the amount of androgens transferred to the egg yolk affecting offspring dispersal behaviour. Supporting this idea, Gwinner *et al.* (2013) observed higher concentrations of androgens in eggs from nests with greater amounts of green material in the European starling. In the spotless starling, female owners from nests treated with green plants increased circulating testosterone levels during the breeding period (Polo *et al.* 2010), and females with higher levels of testosterone also transfer more androgens into the egg yolk (López-Rull and Gil 2009a; but see experiment in López-Rull and Gil 2009b). Yolk androgens have been shown to affect nestling growth in a sex-specific way in the spotless starling (Muriel *et al.* 2013): the injection of testosterone and androstenedione to the eggs had an antagonistic effect on the different sexes by increasing the tarsus length of daughters and reducing that of sons, during the first 7 days of growth (in our study the effect remained until the 16th day). This lasting sex-specific effect on chicks suggests a complex long-term influence of mothers on the phenotype and performance of their offspring in relation to a phenotypic trait of paternal attractiveness. Further research is required to understand the proximate mechanisms involved in the facultative determination of offspring dispersal behavior via maternal effects.

In summary, we experimentally showed that a behavioural trait of male starlings related to their mating status differentially affected dispersal distances of sons and daughters. This result was an unexpected finding and little is known about the mechanisms linking these processes. However, few studies have analysed sex-specific patterns of dispersal in wild populations and our results support the idea that male-biased dispersal is related to polygyny in a facultatively polygynous bird. Further research is required to understand the mechanisms involved in the adaptive determination of the offspring patterns of natal dispersal.

Acknowledgements

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Supplementary material

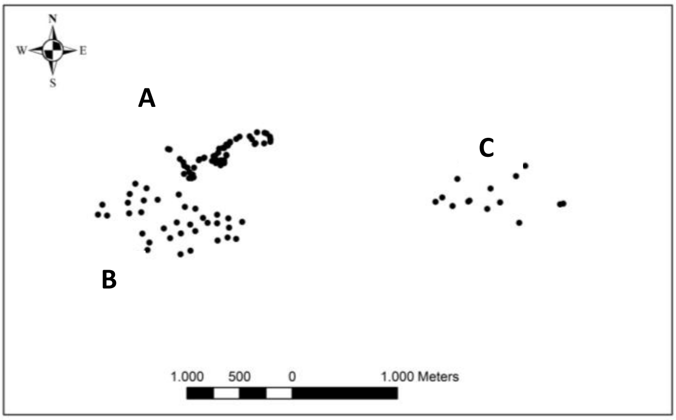

Fig S1. Spatial location of nest-boxes in the study area. All individuals used in this study (both the experimental and the observational) were born in the colony A. Recruits were recaptured in subsequent years in colonies A, B and C.

Processing dispersal distances

The probability of detecting dispersal events differs throughout the spectrum of distances depending on the shape of the study area (e.g., Baker *et al.* 1995, Condor 97: 663-674). Generally, as the dispersal distance of an individual becomes higher it is less likely for its breeding site to fall within the study area. Furthermore, the frequency of the observed dispersal distances within the study area may depend on the spatial distribution of the nest-boxes. Therefore, studies with nest boxes in limited plots may incur the risk that the frequency of long-distance dispersal events is underestimated, while the short and medium distances are overestimated (Baker *et al.* 1995 op cit.).

Table S1. Total number of nests and number of fledglings and recruits from experimental (EXP), control (CTR) and observational nests (Observ) each year of study, including both first and second clutches. The sex ratio of fledglings and recruits (i.e., proportion of males from total fledglings or recruits per year) is presented for each year and for the total sample (means ± SEs).

Year	Treatment	n nests	n fledglings	n recruits	Fledgling SR	Recruits SR
2002	**EXP**	48	126	5	0.45	0.00
	CTR	49	120	13	0.46	0.15
2004	**EXP**	36	73	10	0.53	0.60
	CTR	31	82	13	0.37	0.23
2005	**EXP**	38	105	7	0.55	0.29
	CTR	41	112	11	0.51	0.64
2006	**EXP**	43	110	5	0.50	0.20
	CTR	42	104	8	0.45	0.44
2003	**Observ**	45	119	8	0.52	0.13
2007		48	136	17	0.42	0.35
2008		43	113	7	0.46	0.29
2009		49	119	9	0.52	0.33
Total	**Observ**	185	487	41	0.46 ± 0.02	0.27 ± 0.06
	EXP	165	414	27	0.51 ± 0.02	0.27 ± 0.13
	CTR	163	418	45	0.45 ± 0.03	0.37 ± 0.11

We estimated the detectability of each interval of distances between our nest boxes. Our aim was to correct the observed frequency of dispersers at a given distance by the probability of sampling this distance. Thus, the probability of detection was estimated as Nr / Br (i.e., Nr: number of dispersal events / Br: number of nest boxes at a distance r). Then, we randomized the distribution of distances and estimated the number of dispersal events expected at a distance r in the randomized plot. Thus, we compared the observed and corrected mean ± SEs of the experimental and control groups (Fig S2). The method penalized the frequency of dispersers at the most sampled distances of the study area, and increased the frequency of dispersers in long- and intermediate distances. However, the difference between observed and corrected means was nearly the same in both EXP and CTR groups. This is reasonable because both experimental and control individuals were born and recruited in the same study area (i.e., with a constant distribution of nest-boxes) and therefore all dispersal distances had similar sampling errors. Results were similar using either corrected or observed data, with a significant interaction between greenery and sex in both observational and experimental studies (Table S2).

Table S2. Experimental data: Results of the General Linear Model of the observed and corrected dispersal distances in relation to treatment and sex. Distances were Box-Cox transformed to normalize the residuals of the model.

	Observed		Corrected	
	$F_{1,69}$	P	$F_{1,69}$	P
Treatment	0.556	0.458	0.057	0.812
Sex	0.136	0.714	0.282	0.597
Treatment:Sex	10.069	0.002 (*)	16.596	<0.001 (*)

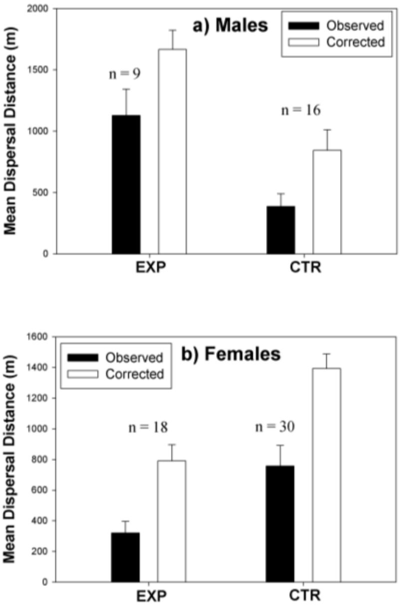

Fig S2. Experimental data: Observed and corrected dispersal distances (means ± SEs) of males (a) and females (b) from experimental and control nests.

Capítulo 4

Sex differences in early determinants of lifetime reproductive success in the sexually dimorphic spotless starling: should mothers adjust offspring sex ratios?

Rubalcaba J.G. and Polo, V. 2016. *Unpublished manuscript*.

Abstract

Trivers and Willard proposed that a mother should control offspring sex ratio when she has sex-specific influences on the condition and fitness of her offspring. In particular, a mother in good condition should overproduce sons if they have more chances to become polygynous thereby increasing her fitness expectancies. Despite the elegance of the theory, support for it has been limited. Here, we explored this prediction using long-term data from a wild population of the facultatively polygynous and sexually dimorphic spotless starling *Sturnus unicolor*. We analyzed whether (1) male and female lifetime reproductive success is related to their body mass at independence, (2) whether maternal body mass is related to that of their offspring, and (3) whether the mother adjust offspring sex ratio according to the expected reproductive success of her male and female offspring. We found that lifetime reproductive success was significantly related in males but not in females to their body mass at fledging. The body mass of the mother at reproduction was positively related to the body mass of her offspring; moreover, the body mass of sons increased faster in relation to the maternal body mass than that of their sisters. However, clutch sex ratio did not vary in relation to maternal body mass and there was no significant departure from parity. Our results suggest that the selective pressure acting on body mass at independence is higher for males in the facultatively polygynous spotless starling. Thus, selection might favour males to grow faster hoarding parental resources. Mothers in better condition produced bigger male offspring but maintained even sex ratios, probably to avoid increasing sibling competition reducing the average condition and fitness of the brood. Our study reinforces the idea that it is necessary to take into account the complexities of vertebrate sex determination and life histories to make further predictions on sex allocation theory.

INTRODUCTION

Trivers and Willard (1973) proposed that when the fitness of males is more variable and depends more on their condition at independence, mothers in better condition should bias offspring sex ratio towards sons, whereas mothers in poor condition should bias offspring sex ratio towards daughters. This prediction relies upon the assumptions that (1) mothers in good condition produce offspring in good condition; (2) differences in condition at the end of the period of parental care endure into adulthood; and (3) slight variations in body condition produce greater variations in the fitness of males than females (Trivers and Willard 1973; Hewison and Gaillard 1999; Kruuk *et al.* 1999; Carranza 2002; Cockburn *et al.* 2002; Pryke *et al.* 2011; Schindler *et al.* 2015). Although Trivers and Willard's hypothesis was referred to polygynous and sexually dimorphic big mammals that produce a single offspring at a birth, it has also been tested in a wide variety of birds and mammals with different life histories. Most of such studies have provided equivocal results, with variations in maternal condition, age or social rank failing to produce facultative variations in offspring sex ratio (e.g., Sheldon 1998; Hewison and Gaillard 1999; Hardy 2002; Ewen *et al.* 2004; Komdeur and Pen 2002;

MacLeod and Clutton-Brock 2013). There is little consensus on whether the Trivers-Willard prediction is generally fulfilled or not, and few studies, especially of birds, have investigated whether subject populations satisfy conditions necessary for the Trivers and Willard effect to emerge.

The discrepancy between theoretical predictions and empirical observations has led to multiple explanations falling into three broad categories. First, applying the original argument of Trivers and Willard to different species might require a better understanding on the complexities of their life histories, and the interplay between different allocation decisions, e.g. the clutch size and sex ratio, or the amount of resources provided per individual son and daughters (Williams 1979; Pen and Weissing 2000; Jones 2009; Carranza and Polo 2012; Schindler *et al.* 2015). For example, parents could bias their investment towards sons or daughters by controlling the sex ratio or by biasing the amount of resources provided per individual offspring: i.e., a mother can keep the allocation to each individual offspring fixed and manipulate the sex ratio; or, conversely, she can keep the sex ratio fixed, but vary the allocation to individual sons and daughters (Pen and Weissing 2002; Veller *et al.* 2016). Second, even if selective pressures favoring biased sex ratio are present, they could be insufficient to overcome the constraint imposed by the random segregation of sex chromosomes (Krackow 1995; West and Sheldon 2002; Pike and Petrie 2003; Ewen *et al.* 2004). Thus, in absence of a specific mechanism to control the sex of the offspring, sex ratio manipulation might require killing selectively the offspring of one of the two sexes at some point during development, which might potentially make the cost to exceed the benefit of overproducing the fitter sex (Myers 1978; Cockburn *et al.* 2002; Pryke *et al.* 2011; Komdeur 2012). Third, irrespectively to the costs of sex determination, selection for biased sex ratios could be weak or favor sex ratios close to parity (Cockburn *et al.* 2002; Uller 2006; Bowers *et al.* 2015). This might occur, among other possibilities, if differences in condition between male and female offspring at the end of the period of parental investment are weakely related to differences in their future reproductive success.

In this study, we used long-term data from a wild population of spotless starlings, *Sturnus unicolor*, to investigate whether male and female lifetime reproductive success is related to their body mass at the end of the period of parental investment, and whether the mother adjusts offspring sex ratio accordingly. The spotless starling is a facultative polygynous and sexually dimorphic passerine in which males compete to increase the number of breeding territories and mates within a breeding season. The polygynous strategy might be favored in males over parental care activities, as it allows them to increase their annual reproductive success (Veiga *et al.* 2002). Thus, larger and more aggressive males generally control more breeding territories and increase the number of mates (Veiga *et al.* 2001 and unpublished data), while females generally care for the offspring, especially if they are secondary females paired with a polygynous male (Veiga *et al.* 2002; Moreno *et al.* 2002).

Experimental studies have also shown that females facultatively increase the proportion of sons in the brood in response to the manipulation of the attractiveness of their partners (Polo *et al.* 2004), and also when their own social rank is experimentally increased with the administration of exogenous testosterone (Veiga *et al.* 2004). Thus, if a mother in good condition is able to raise bigger male offspring, with higher chances of becoming polygynous, they might be expected to overproduce sons to maximize her fitness returns.

Adult body mass at reproduction and offspring body mass at the end of the period of parental investment have been typically used in birds as correlates of individual body condition as they are related, respectively, to the ability of parents to provide care, and the offspring probability of survival and recruitment (Westerdahl *et al.* 2000; Cordero *et al.* 2001; Bowers *et al.* 2015). Although many studies have considered the body mass corrected for structural size as a proxy for body condition, the body mass itself (i.e. uncorrected by size) is a phenotypic trait related to the competitive abilities of individuals for parental resources (Oddie 2000; Uller 2006; Bowers *et al.* 2015) and, among adults, for breeding resources and mates (e.g., Nelson-Flower *et al.* 2013; Young and Bennett 2013).

Here, we investigate (1) whether maternal body mass at reproduction is related to male and female fledging body mass; (2) whether variations in body mass at fledging are translated into adulthood and affects male and female survival and lifetime reproductive success; and finally, (3) whether mothers adjust offspring sex ratio according to the variations in offspring lifetime reproductive success.

MATERIAL AND METHODS

Data collection

This study was conducted in a colony of spotless starling breeding in 54 nests boxes in Manzanares el Real, Madrid (central Spain). Nest boxes were visited daily during the breeding period in order to capture and mark breeding individuals and nestlings with numbered rings for individual identification. Most males were captured in March, during the intrasexual competition activity, while females were captured five days after hatching, when feeding chicks, to minimize the probability of nest desertion. All breeding birds were weighed (to the nearest 0.1g) and sized (i.e. tarsus metatarsus length; to the nearest 0.01mm). Nestlings were sexed from blood taken at day five following Griffiths *et al.* (1998), and marked with aluminium numbered rings. Body mass at the end of the period of parental investment was determined when fledglings were 16 days old, approximately 2-4 days before emancipation.

The breeding colony have been monitored continuously from 2002 to the present, but for this study we only considered broods that did not participated in previous experiments (i.e., Polo *et al.* 2015 and unpublished data). Thus, we analysed the relationship between maternal and fledging body mass in 270 broods that produced 498 female and 435 male fledglings. To analyse variations in primary sex ratio we used 149 broods (363 female and 314 male nestlings) in which all eggs hatched and all nestlings survived until their fifth day of life, i.e. when we determined nestling sex ratio.

The total sample of breeding individuals consists of 126 females and 57 males that were born and recruited in the nest boxes of the study colony. The average number of breeding years of males and females was, respectively, 2.86 ± 0.24 years and 1.92 ± 0.12 years in a range from 1 to 10 years. Most individuals were captured every year of their breeding life. Thus, 3.3% of males and 11.4% of females registered gaps of one year throughout their history of recaptures, while the frequency of gaps of two or more years was lower than 1% in both males and females. Each year, the rate of polygyny varied between 1.0 and 1.4 females per breeding male, average 1.23. A male was considered to mate polygamously if he was captured in more than one nest boxes during the same breeding attempt. Because males can potentially breed in natural cavities as well as in our nest boxes, there is a possibility that a polygynous male was wrongly labelled as monogamous. To minimize this error, we considered that a male was polygynous if he mated polygamously at least one year in his life.

Analysis of recruitment success

We analysed the effect of fledging body mass at the end of the period of parental care on the probability of post-fledging recruitment, i.e. the probability that an individual was captured in subsequent years when trying to breed in the nest boxes of the study colony. The sex and the interaction between sex and fledgling body mass were considered as predictor variables to explore potential sex-specific effects of body mass on the probablity of post-fledging recruitment. Additionally, we analysed the effect of fledging body mass on the age of first reproduction of males and females. In order to control pseudorreplication due to non-independency of individuals from the same brood or from different broods of the same parents, we considered the identity of the brood and the identity of their parents as random factors in the mixed-effect linear models.

Lifetime reproductive success

Using those birds that were born and recruited in the study colony, we explored whether differences in fledgling body mass endured into adulthood, and whether adult and fledgling body mass determine lifetime reproductive success. To do so, we analysed the relationship between fledgling body mass and the average body mass during the adulthood. We then explored the relationship between lifetime reproductive success and adult body mass.

Lifetime reproductive success of each individual was measured considering the following components: (1) total number of recruits produced throughout their life; (2) total number of fledglings; (3) ratio of offspring survival, i.e. number of recruits / number of fledglings; and (4) the probability of polygyny, i.e. whether a male bred in more than one nest boxes during a breeding attempt at least one year in their life.

We explored the relationship between the components of lifetime reproductive success and the body mass of the individual, using as a predictor variable either (1) the average of all measurements of adult body mass, or (2) the body mass of this individual at fledging. The sex and the interaction between sex and body mass were included as predictor variables to analyse sex-specific relationship with the lifetime reproductive success. Analyses were conducted using generalized lineal models, using log link assuming Poisson error distribution for the number of fledglings, recruits and offspring survival, and logit link assuming binomial error distribution for the probability of polygyny. In this case no random effects were considered as the risk of pseudoreplication due to the relatedness between individuals was low.

Maternal body mass and sex ratio variations

Fledging body mass was related to the body mass of the mother at reproduction using general mixed-effects models with the sex of fledglings and its interaction with maternal body mass as predictor variables. The identity of the brood and its parents were included as random factors to control pseudorreplication. As female starlings generally carry out most of the parental investment, we only considered the relationship with maternal and not paternal body mass. Males can influence offspring body size and also primary sex ratio by indirectly affecting female physiology during the breeding period (Polo *et al.* 2004, Veiga *et al.* 2004, 2008). However, the mother, which is the heterogametic sex, might in principle be able to render her partner's preference of offspring sex ineffective and carry out the sex-allocation strategy that maximizes her own fitness returns (Krackow 1995; Pike and Petrie 2003).

Primary sex ratio variations were analysed using those broods that did not registered early egg or nestling mortality. We estimated the significance of overall deviation from an equal primary sex ratio comparing the observed distribution of sex ratios (i.e., proportion of males) for each clutch size with the expected distribution given an even sex ratio. We simulated 100 000 new data sets using the observed clutch sizes, generating each time a random number of males drawn from a binomial distribution with probability ½. We then calculated the goodness-of-fit statistic, associated to a chi-square distribution, to compare the observed and expected number of clutches of each size with each possible number of males. The proportion of simulated distributions with sex ratio variance greater than that observed allows an estimation of the significance of the deviation of the observed from the expected variance in clutch sex ratios if they were even.

Primary sex ratio was fitted as a response variable in relation to maternal body mass at reproduction in a generalized mixed-effect model using a logit link function and assuming binomial error distribution. Clutch size was also included as a predictor variable and the random factors listed above were used to control pseudorreplication. All statistical analyses were conducted using R 3.2.2 (R Core Team 2015).

RESULTS

Recruitment success

The probability of recruitment was positively related to the body mass at the end of the period of parental care in both sons and daughters. However, there was no significant relationship between fledging body mass and the age of first reproduction in either males or females (Table 1). Both the probability of recruitment and the age of first reproduction differed between sexes. Thus, the rate of recruitment of males was significantly lower than that of their sisters (0.09 ± 0.14 *vs.* 0.13 ± 0.15, males *vs.* females, mean ± SE), and also males started breeding later than females (1.82 ± 0.14 *vs.*1.38 ± 0.06 years) (Table 1).

Table 1. Results of the generalized linear mixed-effect models for the effect of fledgling body mass on recruitment success and age of first reproduction.

Probability of recruitment

	Estimate (SE)	χ^2	P
Fledgling body mass	0.54 (0.19)	10.67	**0.005**
Sex	-0.53 (0.24)	7.39	**0.025**
Body mass × Sex	-0.26 (0.27)	0.83	0.36

Age of first reproduction

	Estimate (SE)	$F_{1, 179}$	P
Fledgling body mass	-0.77 (1.50)	2.27	0.13
Sex	14.98 (10.49)	13.63	**<0.001**
Body mass × Sex	-3.27 (2.38)	1.89	0.17

Lifetime reproductive success

Differences in body mass at fledging endured into adulthood, as indicates the positive and significant relationship between fledging body mass and the average adult body mass (Beta = 0.26 ± 0.04, $F_{1, 179} = 90.18$, $P < 0.001$); and this relationship did not differ between sexes (interaction sex × fledging body mass, $F_{1, 179} = 0.13$, $P = 0.713$).

Adult body mass was significantly and sex-specifically related to two of the components of lifetime reproductive success: i.e., the lifetime production of recruits, and the rate of offspring survival (Fig. 1). The sex-specific effect of body mass on lifetime production of recruits remained significant when the predictor variable was fledging body mass instead of adult body mass, while the sex-specific effect on offspring survival became marginally significant (Table 2). Finally, the lifetime production of fledglings was higher in males than females (Table 2). This difference resulted from the fact that a proportion of males mated polygynously and multiplied the number of fledglings produced per breeding attempt.

Males that were heavier during adulthood had greater probability of being polygynous at least one year in their life ($\chi^2 = 6.87$, df = 1, $P = 0.01$), although the probability of polygyny was not significantly related to their body mass at fledging ($\chi^2 = 3.14$, df = 1, $P = 0.076$).

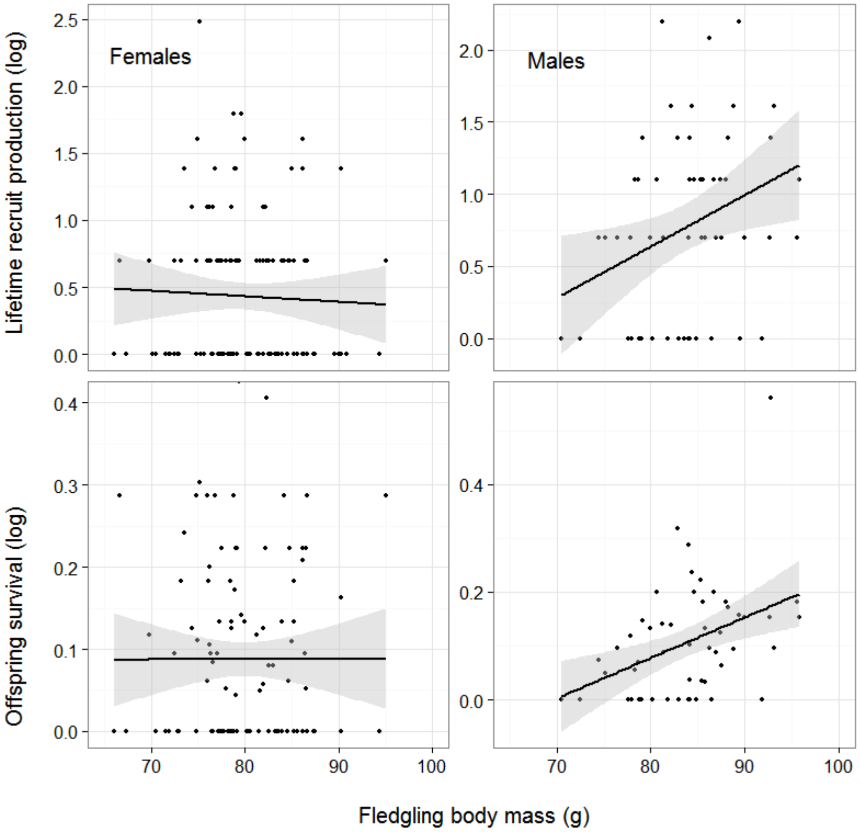

Fig 1. Components of lifetime reproductive success of males and females in relation to their own body mass at fleging: (1) Total number of recruits produced throughout their life; (2) ratio of offspring survival: n recruits / n fledglings.

Table 2. Analysis of the components of lifetime reproductive success of recruits in relation to (a) their own body mass at fledging and (b) their average body mass during the adulthood. Bold indicates interpretable significant relationships.

	(a) Fledgling body mass			**(b) Adult body mass**		
Total number of recruits						
	Estimate (SE)	$F_{1, 179}$	*P*	Estimate (SE)	$F_{1, 179}$	*P*
Body mass	0.1 (0.59)	6.23	0.013	-0.001 (0.012)	8.18	0.004
Sex	-10.21 (4.68)	9.79	0.002	-5.81 (2.12)	3.37	0.067
BM×Sex	2.38 (1.06)	5.14	**0.025**	0.07 (0.02)	5.72	**0.017**
Total number of fledglings						
	Estimate (SE)	$F_{1, 179}$	*P*	Estimate (SE)	$F_{1, 179}$	*P*
Body mass	-0.01 (0.99)	1.14	0.28	0.017 (0.02)	13.31	**<0.001**
Sex	-0.38 (7.92)	20.13	**< 0.001**	-1.07 (3.6)	9.97	**0.002**
BM×Sex	0.25 (1.79)	0.019	0.89	0.018 (0.04)	0.21	0.657
Offspring survival						
	Estimate (SE)	$F_{1, 179}$	*P*	Estimate (SE)	$F_{1, 179}$	*P*
Body mass	0.05 (0.11)	3.23	0.07	-0.001 (0.002)	3.439	0.065
Sex	-0.33 (0.19)	0.99	0.32	-0.91 (0.36)	0.21	0.65
BM×Sex	0.01 (0.002)	3.34	**0.063**	0.01 (0.004)	6.18	**0.013**

Maternal body mass and sex allocation strategy

Maternal body mass at reproduction was positively related to the body mass of both male and female fledglings. Moreover, there was a significant interaction between maternal body mass and sex (Table 3), showing that the body mass of sons increased faster than that of their sisters in relation to the body mass of the mother (Fig. 2).

The proportion of male offspring in broods was 0.463 ± 0.5 (mean ± SD). Analyses of the difference from equal sex ratio in each clutch size class showed no significant departure from parity (Table 4). Clutch sex ratio did not significantly vary in relation to maternal body mass at reproduction (Beta = 0.09 ± 0.06, $\chi^2 = 1.30$, df = 1, *P* = 0.25, Fig. 3), and it was not significantly related to clutch size (Beta = 0.01 ± 0.12, $\chi^2 = 0.01$, df = 1, *P* = 0.91).

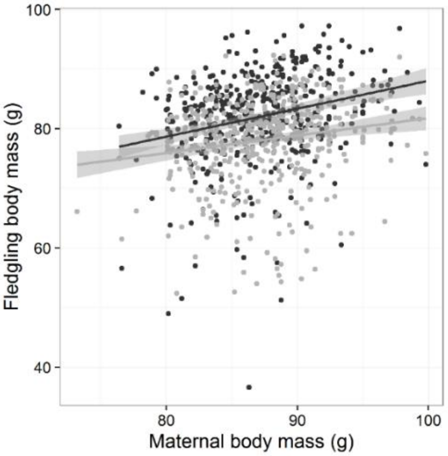

Fig 2. Relationship between male (black) and female (grey) fledgling body mass and the body mass of their mother at reproduction.

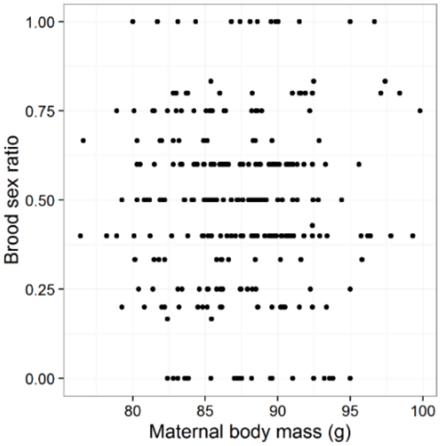

Fig 3. Clutch sex ratio (only completely-sexed broods) *vs* maternal body mass at reproduction.

Table 3. Relationship between fledgling and maternal body mass. Results of the mixed models considering brood ID and mother ID as random factors.

	Estimate (SE)	*F*	df	*P*
Maternal body mass	0.21 (0.06)	23.2	1, 170.9	< 0.001
Sex	0.58 (0.04)	194.8	1, 712.4	< 0.001
Body mass x Sex	0.09 (0.04)	4.24	1, 708.1	**0.034**

Table 4. Analysis of sex ratio deviations in each clutch size class. The Chi-squared test and the associated P-value indicates the significance of the deviation from an expected even sex ratio.

Clutch size	Simple size	Mean Sex Ratio (SD)	χ^2	d.f.	P
3	12	0.38 (0.23)	0.186	3	0.980
4	50	0.48 (0.24)	0.017	4	0.999
5	77	0.45 (0.26)	0.180	5	0.999
6	9	0.57 (0.22)	0.339	6	0.999

DISCUSSION

In this study we used 9 years of observational information of the lifetime reproductive success, which correspond to the lifespan of a longlived spotless starling in the wild. We analysed the selective pressures acting on male and female body mass, a trait related to the competitive abilities and condition of individuals. Ultimately, we intend to understand the evolutionary pressures acting on breeding strategies.

The body mass of fledglings at the end of the period of parental investment was related to the lifetime reproductive success of males, but not to that of females (Fig. 1); suggesting that differences in offspring condition at emancipation endured into adulthood and affected the fitness returns of males. Moreover, offspring body mass was related to that of their mother at reproduction, and there was a significant interaction with sex, i.e., the body mass of sons increased faster than that of their sisters in relation to the body mass of the mother at reproduction (Fig. 2). Therefore, larger mothers produced broods where sons were bigger than daughters and also had greater lifetime reproductive success. However, clutch sex ratio did not increase in relation to maternal body mass (Fig. 3) and did not differ from parity (Table 4). Therefore, although our population of starlings might fulfil the conditions of the prediction of Trivers and Willard (see Introduction section), we did not find facultative variations in clutch sex ratios. A possible explanation for this result is that mothers in better condition invested more resources in their sons, thereby producing bigger male fledglings, or male fledglings grew faster by hoarding parental resources (see models in Pen and Weissing 2002; Veller *et al*. 2016). Thus, mothers in good condition could prevent increasing sibling competition by producing even sex ratios, instead of male-biased broods (Uller 2006; Bowers *et al.* 2015). Our results support recent empirical and theoretical studies that evidence the need to take in consideration the multidimensional nature of sex-allocation decisions in vertebrates (Williams 1975; Uller 2006; Carranza and Polo 2012; Bowers *et al.* 2015; Schindler *et al.* 2015).

Understanding divergences in male and female breeding strategies in populations is important to generate appropriate predictions and models that sufficiently take into account the complexities of vertebrate sex determination and life histories (Pen and Weising 2002). The polygynous and sexually size-dimorphic nature of the spotless starling make this species an appropriated model for testing sexual differences in life-history traits and sex-allocation strategies (Veiga *et al.* 2001, 2002; Cordero *et al.* 2001; Moreno *et al.* 2002; Polo *et al.* 2004; Veiga *et al.* 2008). Thus, previous studies evidenced that the polygynous strategy might be favored in males over caring for the offspring, as males that were more aggressive and better competitors increased the number of social mates and their annual reproductive success (Veiga *et al.* 2001, 2002). Our results support this idea and demonstrate that variations in lifetime reproductive success can be anticipated since the end of the period of parental investment. Thus, if the fitness expectancy of sons and daughters is approximated through their probability of recruitment and subsequent lifetime production of recruits (Fig. 4), a son might increase his fitness in relation to his body mass to the point that he might outreproduce a sister with a similar body mass. Bigger males produced more recruits during their life, not only because they had more social mates, but also because their fledglings had greater recruitment success (Table 2). This suggests that males with higher body size increased the number of mates, but also acquired better breeding territories, or they were paired with better quality and experienced females.

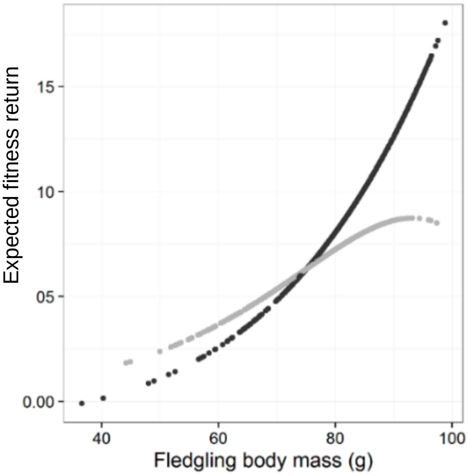

Fig 4. Expected fitness of males (black) and females (grey) in relation to their body mass at fledging. Values were obtained by multiplying the expected probability of recruitment (fitted values of the logistic model) by expected lifetime production of recruits (fitted values of the linear model) in relation to the body mass at fledging.

Our estimation of lifetime reproductive success must be taken with caution as cases of extra-pair paternity have not been controlled, and they might be more frequent among polgynous males. The frequency of extra-pair fertilizations in the spotless starling varies between 10-20%, and it was higher in males whose rates of polygyny were experimentally augmented using implants of exogenous testosterone (Cordero *et al.* 2003). In our study, the average lifetime production of recruits was 2.94 for polygynous males and 1.09 for monogamous males. Thus, although the risk of losing paternity could be higher in polygynous males, they probably accrued higher reproductive success irrespective of their risk of being cuckolded because they had more social mates and also registered greater survival in each individual brood. However, our results do not provide an exact measure on the contribution of males to next generations.

There is a common notion that the larger sex should be more sensitive (in terms of, e.g., growth speed, physiological condition or body mass at the end of the period of parental investment) to the conditions of their rearing environment

(Clutton brock 1985; Velando 2002; Råberg *et al.* 2005; Rowland *et al.* 2007; Jones *et al.* 2009). However, a number of studies, especially of birds, have found results that are contrary to this expectation either showing the opposite pattern (Nager *et al.* 2000; Hornfeldt *et al.* 2000; Kalmbach *et al.* 2005; Rowland *et al.* 2007) or no sex-specific sensitivity in growth rates or body mass (Smith *et al.* 1989; Westerdahl *et al.* 2000; Råberg *et al.* 2005; see review in Jones *et al.* 2009; Rosivall *et al.* 2010). Our results show that variations in maternal body mass produce higher variations in the body mass of sons than daughters, i.e. sons might be more sensitive to the condition of their mother at reproduction (Fig. 2). In line with this idea, previous experimental studies with the spotless starling showed greater sensitivity of sons in terms of body mass (see, e.g., Polo *et al.* 2015) and recruitment rates (V. Polo, J.G. Rubalcaba and J.P. Viega; unpublished results). The result of our observational study must be taken with caution because they do not allow distinguishing whether male fledglings grew faster than their sisters (thereby being more sensitive to variations in their rearing environment), or whether the mother fed more her sons when she was in better condition. Ultimately, both the strategy of biasing parental care toward sons and the strategy of increasing growth rates of sons share a common ultimate cause, i.e. the sex-specific relationship between fledging body mass and their lifetime reproductive success. Further research is necessary to understand how mothers can split resources between sons and daughters by controlling multiple allocation components.

Our results suggest that offspring fitness could be indirectly affected by the mother as her body mass at reproduction is related to fledgling body size, which is in turn related to offspring reproductive success. However, we have found no significant relationship between the components of lifetime reproductive success and the body mass of the mother at reproduction, probably due to a lack of statistical power (unpublished results). Although, understanding the relationship between maternal body mass and offspring fitness is necessary to fully understand why mothers did not adjust primary sex ratio in relation to her body mass, our results allow speculating about the apparent lack of fit of empirical data to theoretical predictions. Thus, a key result is that the body mass of sons increased faster than that of daughters in relation to maternal size (Fig. 2). If sons are bigger, producing male-biased broods might increase the level of sibling competition, which could be negative for daughters if they cannot compete with their brothers for parental resources (Cordero *et al.* 2001). An experiment with house wrens, *Troglodytes aedon*, showed that parents rearing even sex ratios were more productive than those that overproduced one of the two sexes, because sons were more sensitive to the increased rivalry with their brothers and had lower survival in male-biased broods (Bowers *et al.* 2015). Therefore, male-biased sex ratio might reduce the overall condition and fitness of sons, while a female-biased sex ratio might not maximize the fitness of a mother in good condition. Therefore, selection might favour producing even sex ratios or small variations around parity (Oddie 2000;

Råberg *et al.* 2005; Uller 2006; Bowers *et al.* 2015).

In summary, this is an observational study used to speculate about the breeding strategy that might maximize the fitness of a mother who produces sons and daughters with different fitness expectancies. The main value of our results is that we used long-term information where individuals' reproductive success was monitored each breeding year during their entire lifespan. Thus, we showed that male and female might obtain different fitness returns in relation to their body mass at fleging, suggesting that the evolutionary pressures acting on male body size are stronger than those on female size. The evolution of sexual size dimorphism is important in starlings, and our results support the idea that it emerges via sexual selection. Under these circumstances, the breeding strategy of a mother is expected to be affected, i.e. heavier mothers should produce more sons. Among the different hypotheses that have been proposed to explain the lack of empirical support for this hypothesis, recent studies are giving consistence to the idea that sex ratios are stabilized by selection due to the combined effect of sex-specific fitness expectancies and the sex-specific sensitivities to the rearing conditions. Thus, even in presence of sex-specific selective pressures on fledgling body mass, it is dificult to make predictions about the strategy that maximizes the fitness of a mother given the complexity of the life history of vertebrates. Our study reinforces the idea that it is necessary to take into account the complexities of vertebrate sex determination and life histories to continue making predictions on sex allocation theory.

Acknowledgements

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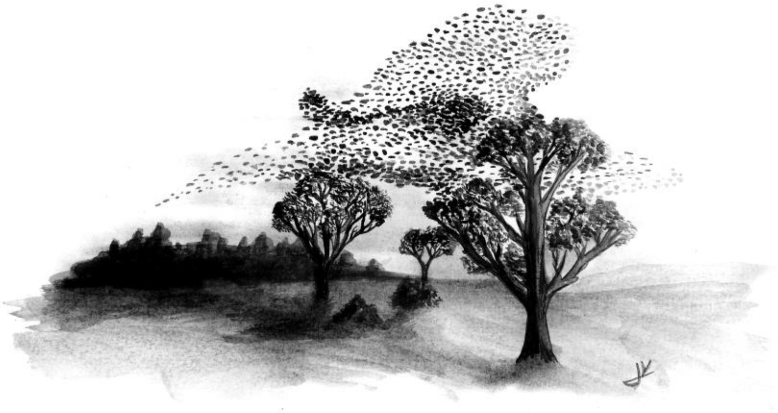
Capítulo 5

Sex-specific fitness consequences of variance in offspring phenotypic condition and its implications for the Trivers-Willard hypothesis



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Abstract

When the condition of a mother has different effects on the condition and future fitness of her sons and daughters, she is expected to produce the sex that maximizes her benefits. However, few studies have addressed how the uncertainty about the condition and fitness of the offspring can affect the strength of selection for a facultative sex determination. We analyzed the effect of increasing the residual variance in offspring condition in relation to that of their mother at reproduction on the reproductive value of sons and daughters. Based upon previous arguments, we expected to find two possible responses: (1) a reduction in the difference between male and female reproductive values, i.e. weaker selection of sex ratio adjustments; or (2) greater reduction of the male reproductive value, i.e. weaker selection for male-biased sex ratios. We developed an Integral Projection Model parameterized with long-term data from a wild population of a polygynous bird, to study the effect of maternal condition at reproduction on the reproductive values of her sons and daughters. We showed that increasing offspring phenotypic variance might reduce the reproductive value of sons more strongly than the reproductive value of daughters. Consequently, factors that increase the variance in offspring condition might weaken selection acting on mothers in good condition to overproduce sons and strengthen selection on mothers in poor condition to overproduce daughters. Our results support the idea that mothers might benefit by producing the less variable sex in stochastic environments, i.e. when the stochasticity affects the variance in offspring condition at independence. This study demonstrates that not only the change in the mean offspring condition, but also its variance in relation to the condition of their mother, is important to understand the selective pressures acting on a mother to adjust the sex of her offspring.

INTRODUCTION

When the benefit to a mother from producing sons and daughters is unequal, she is expected to produce the sex that maximizes her fitness (Hamilton 1967; Trivers and Willard 1973; Charnov 1982; Frank 1990). Trivers and Willard (1973) proposed that when the fitness of males is more variable than the fitness of females, mothers in good condition would benefit from producing a son, whereas mothers in poor condition would benefit from producing a daughter, the sex with more stable fitness returns. This hypothesis might apply to those polygynous and sexually dimorphic species whose life cycle satisfies the following criteria: (1) females in good condition produce offspring in good condition; (2) differences in offspring condition at the end of the period of parental investment endure into adulthood; and (3) males have more variable reproductive success and this depends more on their body condition than for females (Trivers and Willard 1973; Hewison and Gaillard 1999). However, although the hypothesis of Trivers and Willard has been tested in many different vertebrate species, the results are often equivocal and there is little consensus on whether the prediction is generally supported (e.g., Sheldon 1998; Hewison and Gaillard 1999; Hardy 2002; Ewen *et* *al.* 2004; Komdeur and Pen 2002; MacLeod and Clutton-Brock 2013; Schindler *et al.* 2015).

The controversy is compounded by the fact that little is known about the potential mechanism in birds and mammals capable of overcoming the constraints imposed by the random segregation of sex chromosomes (but see Pike and Petrie 2003; Cameron 2004; Rutkowska and Badyaev 2008). In the absence of a mechanism to control the sex of the offspring at conception, sex-ratio manipulation is likely to be costly if it requires, for example, selective killing of offspring at some point during development. Such costs might then outweigh the benefits of sex-ratio manipulation, especially if selection for sex ratio adjustments is weak (Myers 1978; Cockburn *et al.* 2002; Pryke, Rollins and Griffith 2011; Komdeur 2012). A factor that might weaken selection on offspring sex ratios is the unpredictability of the environmental conditions. It has been argued that lower environmental predictability might lead to weaker selection for sex-ratio adjustment, because strongly skewed sex ratios increase the risk of producing offspring of the sex with the lower reproductive value (West and Sheldon 2002). On the other hand, some theoretical models addressing the consequences for sex allocation of different sources of stochasticity (e.g., variance in mating efficiency, Charnov 1986; brood mortality, Freedberg 2002; or variance in the individual mortality of each sex, Proulx 2000; see a general model in Proulx 2004) suggest that parents might benefit by biasing the sex ratio towards the sex with less variable fitness returns, which might indeed represent the evolutionarily stable strategy in small population living in spatially variable environments (Proulx 2000, 2004). Little is known, however, about the consequences of environmental unpredictability for the reproductive value of sons and daughters and, ultimately, for the sex-allocation strategy of the mother in relation to her condition. Furthermore, as far as we know, there is no empirical study addressing the effect of environmental unpredictability on the direction and strength of selection on offspring sex ratios in the context of the Trivers–Willard hypothesis. Here we address this gap, using a demographic model to study the effect of maternal condition on the reproductive values of sons and daughters and applying this to long-term data from a wild population of spotless starlings, *Sturnus unicolor*.

Introducing stochasticity might relax the assumptions of the original Trivers–Willard hypothesis outlined above: (1) Offspring condition is determined not only by the condition of the mother, but also by environmental factors unrelated to maternal quality. Thus, while the condition of the mother might determine the mean phenotypic quality of the offspring, environmental variability determines their phenotypic variance. (2) Adult body condition depends not only on the condition at the end of the period of parental investment, but also on environmental conditions unrelated to individual quality. Finally, (3) adult reproductive success might depend not only on their condition, but also on the variability in environmental conditions. It is possible to distinguish here between two sources of variation: the phenotypic variance (i.e. variance in offspring and adult condition) and the stochasticity affecting vital rates (i.e. variations in survival, mating success or fecundity, i.e. demographic stochasticity, see Engen *et al.* 1998). The variance in phenotypic condition might not only be determined by unpredictable environmental conditions, but also by the transmission from parents to offspring of genetic and non-genetic variability and by developmental plasticity during growth (e.g., Danchin *et al.* 2001). The distribution of phenotypes of males and females in a population will be affected by these sources of variability and by selection acting on the mean and variance of the phenotypic trait. In particular, the variance of a given trait is most important when selection acts nonlinearly on that trait (Ruel and Ayres 1999; Plard *et al.* 2016). For the sake of simplicity, we focus here on how selection affects the variance in offspring condition at the end of the period of parental investment (point 1 above). Thus, variations in the environmental conditions, e.g. in the abundance and quality of resources to feed offspring, might alter the condition and future fitness of fledglings. If sons have greater energy requirements than their sisters during the period of parental investment, i.e. because they are bigger, grow faster or depend more on their condition to improve their reproductive success, variations in their condition might be more important in sons than daughters. Ultimately, this can alter the sex-allocation strategy of the mother in relation to her condition.

We developed a two-sex Integral Projection Model (IPM) (Easterling *et al.* 2000; Schindler *et al.* 2013, 2015) to analyse how increased variance in fledgling body mass affects the relationship between the reproductive values of sons and daughters, i.e. their relative contribution to the population growth rate, and the body mass of their mother at reproduction. IPMs are discrete-time models that describe the dynamics of a population structured by a continuous phenotypic trait (Easterling *et al.* 2000). The relative frequency of individuals with a given body mass can be projected in time knowing their probability of survival and their contribution to subsequent generations:

$$n(z,t)=\int\left[S(z_1,z)+R(z_1,z)\right]n(z_1,t-1)dz_1 \tag{1}$$

where $n(z, t)$ is the probability density of individuals with mass z at time t, which depends on the probability that an individual with mass z_1 at time $t-1$ survives to time t with a mass z [survival kernel $S(z_1, z)$] and produces offspring that are alive at time t with mass z [reproduction kernel $R(z_1, z)$]. The model can be then split into its contributions from the female sex $n_♀(z_♀, t)$ and from the male sex $n_♂(z_♂, t)$, where $n_♀(z_♀, t) + n_♂(z_♂, t) = n(z, t)$ (Schindler *et al.* 2013) (see General description of the model).

We parameterized the model using data from 15-years study of a wild population of spotless starlings, *Sturnus unicolor*. The spotless starling is a facultatively polygynous and sexually dimorphic passerine bird (Veiga *et al.* 2001). Male starlings compete to increase the number of breeding territories and mates within a breeding season; those that are bigger and more aggressive control more breeding territories, thereby increasing their number of social mates (Veiga *et al.* 2001). By contrast, females incubate the eggs and they are generally who provide most care for the offspring, especially if they are secondary females paired with a polygynous male (Veiga *et al.* 2002; Moreno *et al.* 2002). Females with higher body mass produce bigger offspring which have greater chances to survive and breed in subsequent years. Moreover, the lifetime reproductive success of males is more strongly related than that of females to their body mass at fledging (unpublished data). Thus, we used here the body mass as a proxy of the condition and competitive abilities of adults and post-fledging recruits for breeding resources and mates.

We used the IPM to explore the effect of increasing the variance in offspring body mass at fledging on the reproductive values of sons and daughters. We discuss our results in the context of the Trivers-Willard hypothesis, and therefore, interpreted the difference between the reproductive value of sons and daughters as a selective pressure acting on the mother to overproduce the fitter sex in relation to her own condition. Based upon previous arguments (West and Sheldon 2002; Proulx 2000, 2004), we explored whether (1) the variance in the condition of sons and daughters at fledging reduces the strength of selection on sex ratio adjustments; or (2) the variance in condition at fledging reduces more the reproductive value of sons, i.e. the sex whose fitness depends more on their condition at fledging, thereby favouring the production of daughters.

MATERIAL AND METHODS

The Model

General description

We modelled the dynamics of the size distributions and associated reproductive values of males and females in the population. Consider the functions $S_{♂}(z_{1♂}, z_{♂})$ and $S_{♀}(z_{1♀}, z_{♀})$ (i.e. the survival kernels), which denote the probability that an individual has body mass $z_{♂}$ (if it is a male) or $z_{♀}$ (if it is a female) at time t given it had body mass $z_{1♂}$ or $z_{1♀}$, respectively, at time $t – 1$. Males and females that survived to the current time step will breed and produce male and female local recruits. The size distributions of male and female recruits are obtained through $R_{♂}(z_{♂}| z_{1♂}, z_{1♀})$ and $R_{♀}(z_{♀}| z_{1♂}, z_{1♀})$, (i.e. the reproduction kernels), respectively, which give the frequency of local recruits with size $z_{♂}$ or $z_{♀}$ (for a son or a daughter, respectively) produced by a mother and a father with sizes $z_{1♂}$ and $z_{1♀}$. The iterative model equations take the form:

$$n_{♂}(z_{♂}, t) = \int S_{♂}(z_{1♂}, z_{♂}) n_{♂}(z_{1♂}, t-1) dz_{1♂} + \int R_{♂}(z_{♂} \mid z_{1♂}, z_{1♀}) n_{♂}(z_{1♂}, t-1) n_{♀}(z_{1♀}, t-1) dz_{1♂} dz_{1♀} \tag{2.1}$$

and

$$n_{♀}(z_{♀}, t) = \int S_{♀}(z_{1♀}, z_{♀}) n_{f}(z_{1♀}, t-1) dz_{1♀} + \int R_{♀}(z_{♀} \mid z_{1♂}, z_{1♀}) n_{♂}(z_{1♂}, t-1) n_{♀}(z_{1♀}, t-1) dz_{1♂} dz_{1♀} \tag{2.2}$$

where the male and female size distributions, i.e. $n_♂(z_♂, t)$ and $n_♀(z_♀, t)$, satisfy: $\int n(z,t)dz = \int n_♀(z_♀,t)dz_♀ + \int n_♂(z_♂,t)dz_♂ = 1$. Each time step represents a breeding period: the survival kernels, $S_♂(z_{1♂}, z_♂)$ and $S_♀(z_{1♀}, z_♀)$, first update the distribution of adult breeders that survived from the previous year, i.e. $s_♂(z_♂, t)$ and $s_♀(z_♀, t)$. Then the reproduction kernels, $R_♂(z_♂| z_{1♂}, z_{1♀})$ and $R_♀(z_♀| z_{1♂}, z_{1♀})$, give the contribution to local recruits produced by these breeders, i.e. $r_♂(z_♂, t)$ and $r_♀(z_♀, t)$. The population is then $n_♂(z_♂, t) = s_♂(z_♂, t) + r_♂(z_♂, t)$ and $n_♀(z_♀, t) = s_♀(z_♀, t) + r_♀(z_♀, t)$ for the male and female distributions respectively, which are normalized to satisfy that the integral of the whole population is equal to one.

The male and female survival kernels, $S_♂(z_{1♂}, z_♂)$ and $S_♀(z_{1♀}, z_♀)$, are the product of the survival, $\phi(z_1)$, and the recapture probability, $p(z_1)$, of an individual with body mass z_1, and the probability that it has body mass z at the next time step, $g(z_1, z)$ (see Appendix I for a detailed description of the model).

The reproduction kernels, $R_♂(z_♂| z_{1♂}, z_{1♀})$ and $R_♀(z_♀| z_{1♂}, z_{1♀})$, are the product of four functions: (1) the mating function, $m(z_♂, z_♀)$, i.e. the probability that mating occurs between a male and a female with sizes $z_♂$ and $z_♀$ respectively; (2) the fertility function, $f(z_♂, z_♀)$, i.e. the number of offspring that this pair is expected to produce; (3) the inheritance functions $c_♂(z_s|z_{1♂}, z_{1♀})$ and $c_♀(z_d| z_{1♂}, z_{1♀})$, i.e. the body mass of the offspring at fledging in relation to the body mass of their parents at reproduction; and (4) the post-fledgling recruitment functions $P_♂(z_s, z_♂)$ and $P_♀(z_d, z_♀)$, i.e. the probability of recruitment of males and females in relation to their body mass at fledging. This is then multiplied by the fledging sex ratio to estimate the number of male, x_1, and female recruits, $1 – x_1$ (Appendix I).

The mating function satisfies $\int m(z_♂, z_♀)dz_♂ = 1$ for all $z_♀$, which means that all females in the population (regardless of their own size) will find a partner, whereas we assume that the mating success of males is more variable and positively related to their size (Schindler *et al.* 2013, 2015). The inheritance functions give the distribution of male and female body masses at fledging, and they are defined as conditional probabilities on the body mass of the mother ($z_♀$) and the father ($z_♂$) at reproduction, i.e. $c_s(z_s|z_♂, z_♀)$ and $c_d(z_d |z_♂, z_♀)$, which satisfy $\int c_s(z_s \mid z_♂, z_♀)dz_s = \int c_d(z_d \mid z_♂, z_♀)dz_d = 1$ (Schindler *et al.* 2013, 2015).

Because reproduction depends on the proportion of male and female adults, we multiplied the functions of the reproduction kernels by the harmonic mean function, $K_{x,\ H}$, which constraints reproduction in relation to the adult sex ratio, x, and the average harem size, H (i.e. the number of social mates of a male), by multiplying offspring production by a factor smaller than one (Appendix I). Adult sex ratio at time t is calculated as $x = \int s_♂(z_♂, t)dz_♂$ and the average number of social mates is estimated from data as a function of male body mass. The harmonic mean function of the number of males and females in the population is considered to be the least flawed procedure to make reproduction dependent on the frequency of each sex (Caswell 1989; see also Lindström and Kokko 1998). Because the IPM considers the proportion of males and females rather than their absolute number, the function, $K_{x,\,H}$, is estimated using the adult sex ratio as follows:

$$K_{x,H} = 2\,\frac{x(1-x)}{x + (1-x)H^{-1}} \qquad \textbf{(3)}$$

The adult sex ratio that maximizes reproduction given by this function is $\hat{x} = 1/2$ when H = 1 and decreases (i.e. becomes more female-biased) as a function of H according to $\hat{x} = \frac{1}{\sqrt{H} + 1}$.

Stable body mass distributions and reproductive values

We used numerical procedures to solve the integrals (i.e. mid-point rule technique, see Appendix II for the Computational procedures) and, through iteration, obtained the stable distribution of body masses of males and females, their associated reproductive values and the long-term population growth rate. The iteration converges to a unique stable population distribution, i.e. $w(z) = w_{♂}(z_{♂}) + w_{♀}(z_{♀})$, and an asymptotic growth rate, λ:

$$\underset{t\to\infty}{Lim}\,\frac{n(z,t)}{\lambda_t} = w(z) \qquad \textbf{(4)}$$

where λ and $w(z)$ are, respectively, the dominant eigenvalue and right eigenvector of the population kernel (Ellner and Rees 2006). The reproductive value function is the dominant left eigenvector and can be computed by iterating the transpose kernels (Easterling *et al.* 2000; Ellner and Rees 2006), which converge to the stable functions $v_{♂}(z_{♂})$ and $v_{♀}(z_{♀})$ for males and females respectively. Using the inheritance functions $C_{s♀}(z_s|z_{♀})$ and $C_{d♀}(z_d|z_{♀})$ (see Appendix I), we can then calculate the reproductive values of male and female offspring as functions of maternal body mass (Schindler *et al.* 2015):

$$v_s(z_{♀}) = \int v_{♂}(z_{♂}) C_{s♂}(z_s \mid z_{♀})\,dz_{♀} \qquad \textbf{(5.1)}$$

$$v_d(z_{♀}) = \int v_{♀}(z_{♀}) C_{d♀}(z_d \mid z_{♀})\,dz_{♀} \qquad \textbf{(5.2)}$$

Selection acting on a mother to bias the offspring sex ratio towards males or females is the difference in reproductive value between her sons and daughters: when the difference $\Delta v(z_{♀}) = v_s(z_{♀}) - v_d(z_{♀})$ is negative, she is expected to overproduce daughters, whereas when it is positive she should overproduce sons. The absolute value of the difference $|\Delta v(z_{♀})|$ denotes the strength of selection acting on sex-ratio adjustment.

The mean reproductive values of sons and daughters in relation to the body mass of mothers in the population can then be estimated using the stable size distribution of adult females, $w_{♀}(z)$, as follows:

$$\mathrm{E}[v_s] = \frac{\int v_s(z) w_{♀}(z)\,dz}{\int w_{♀}(z)\,dz} \qquad \textbf{(6.1)}$$

$$\mathrm{E}[v_d] = \frac{\int v_d(z) w_{♀}(z)\,dz}{\int w_{♀}(z)\,dz} \qquad \textbf{(6.2)}$$

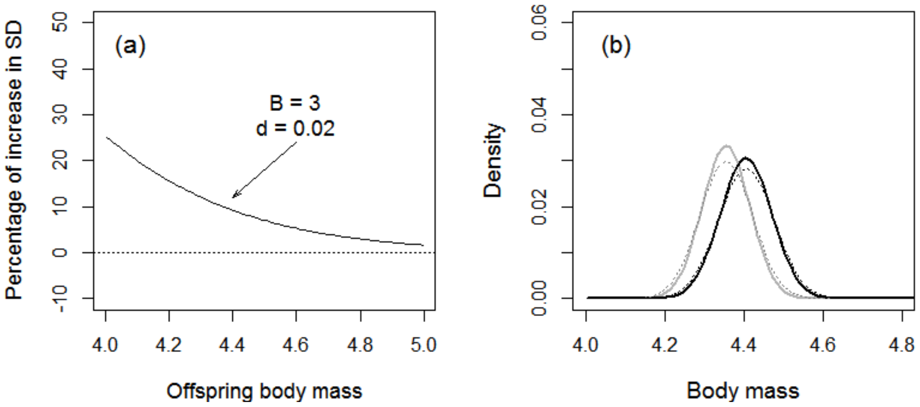

Fig 1. Effect of increasing standard deviation in offspring body mass (log-transformed) on the size distribution of fledglings: (a) Parameter *d* (eq. 7) was modified to simulate an unequal increase in standard deviation in relation to the mean. (b) The stable size distributions of male (black lines) and female (grey lines) fledglings was altered from those represented with solid lines (d = 0, i.e. observed standard deviation) to those represented with dashed lines (d = 0.02, i.e. increased standard deviation).

Modelling stochasticity

We aim to understand the effect of increasing variance in offspring condition at the end of the period of parental investment on both the sign and the strength of selection on offspring sex ratios. Thus, consider that the environmental conditions during the breeding period, e.g. the amount and the quality of the food available for the offspring, are spatially and temporary heterogeneous in the breeding colony. The variations in territory quality and environmental conditions might alter the body mass of her sons and daughters from the expected in relation to the condition of the mother at reproduction. That is, stochastic variations in offspring condition are the residual variance in the inheritance functions, which relate offspring body mass at fledging to the body mass of their mother and father.

We assume that environmental variance alters the condition of all fledglings from the same brood, i.e. it modifies the average condition of the brood, but not the within-brood variance. In natural conditions not only the average body mass, but also the within-brood variance could be affected by changes in environmental conditions (e.g., if offspring compete more under harsh environmental conditions favouring the bigger over the smaller fledglings). The variability in phenotypic condition could be hierarchized in within- and between-brood variance to explore how stochasticity might affect each individual fledgling in relation to its sex and rank position within the brood. However, we simplified the model assuming that all fledglings of the same brood are affected in the same way by the environmental conditions. Thus, both males and females will be affected to the same extent by a simulated increase in phenotypic variance, which facilitates the interpretation of possible sex-specific effects on their reproductive values.

The mean and the variance in offspring body mass are related variables. Thus, small and intermediate-sized offspring might be more strongly affected by random variations in the quality of the rearing environment than larger offspring, as fledglings might grow asymptotically to a maximum size, which constrains possible variations in body mass. The increase in the standard deviation, σ, as the mean offspring body mass, μ, increases was modelled as:

$$\Delta\sigma = de^{-B(\mu - L)} \quad \textbf{(7)}$$

where L is the lowest offspring body size, parameter d controls the strength of the increase in offspring phenotypic variance, and B controls the slope of the relationship between the variance and the mean (modifications in the unknown parameter B did not alter the output of the model or the interpretation of the results) (Fig. 1).

We first ran the model considering the observed standard deviation in offspring body mass (i.e. parameter $d = 0$ in eq. 7), and then repeated the simulation using a value of $d = 0.02$, which generates an increase in the standard deviation (Fig. 1). The value of d was arbitrarily selected after comparing the simulations in a range from $d = 0.01$ to 0.1 (Fig. A1 and see Results). We studied the effect of increasing the standard deviation in fledging body mass on: (1) the reproductive values of sons and daughters: $v_{♂}(z_{♀})^{d=0.02} - v_{♂}(z_{♀})^{d=0.00}$ and $v_{♀}(z_{♀})^{d=0.02} - v_{♀}(z_{♀})^{d=0.00}$; (2) the sign of selection acting on the mother to bias the offspring sex ratio toward sons or daughters: $\Delta v(z_{♀})^{d=0.02} - \Delta v(z_{♀})^{d=0.00}$; and finally (3) the strength of selection on offspring sex ratios: $|\Delta v(z_{♀})^{d=0.02}| - |\Delta v(z_{♀})^{d=0.00}|$.

Parameterization of the model

Field procedures

We monitored a colony of spotless starlings breeding in nest boxes in Manzanares el Real, Madrid (Spain) every breeding season from 2001 to the present. Nest boxes were visited regularly from the beginning of March to mid-July to capture and mark breeding individuals and nestlings with numbered rings for individual identification. We captured the mother and father when they were breeding, using traps fitted into the nest boxes. Most males were captured at the beginning of the period when they start settling in the nest boxes, whereas females were captured when feeding chicks, generally five days after hatching. All breeding birds were weighed (to the nearest 0.1g) and sized (i.e. tarsus and metatarsus length, to the nearest 0.01mm). Nestlings were sexed from blood taken at day 5 following Griffiths *et al.* (1998) and marked with aluminium numbered rings. Fledglings were weighed and sized when 16 days old, approximately 2-4 days before independence.

Adult and post-fledgling survival

The survival probability for adults [necessary to define survival kernels $S_{♂}(z_s, z_{♂})$ and $S_{♀}(z_d, z_{♀})$] was estimated as the combined probability that individuals survive and breed in the nest boxes of the study colony. We estimated the probability that a male or female with body mass z

survives between times $t-1$ and t [function $\phi(z)$] and the probability that it is captured at time t [function $p(z)$] in relation to their adult body mass. Because adult individuals were captured when breeding in the nest boxes of the study colony, the product of probabilities $\phi(z)p(z)$ denotes the probability that an individual with body mass z became part of the breeding population during a breeding period. We fitted Cormack-Jolly-Seber (CJS) capture-mark-recapture models in the R package 'marked' (Laake *et al.* 2013) to estimate the logistic functions $\phi(z)$ and $p(z)$. We used 362 capture histories of adult males and 520 capture histories of adult females between years 2001 and 2015, including their body mass as a static covariable (i.e. individuals were characterized by the average of all weight measurements taken since their first year of life) (Fig. 2 and see Table A1 in the Appendix for model parameters).

Post-fledging survival [necessary to define functions $P_♂(z_s, z_♂)$ and $P_♀(z_d, z_♀)$] was estimated as the probability that a male or female was captured in subsequent years in the nest boxes of the study colony as a function of their body mass at fledging. All body mass measurements were log-transformed prior to analysis. We used a sample of 270 broods (498 female and 435 male fledglings) between years 2002 and 2009 that did not participate in any experiment. From 2003 to 2015, we recaptured 56 females and 52 males from these broods in the study colony. We conducted separate analyses for males and females using generalized (logistic) linear mixed-effects models with the body mass at fledging as a predictor variable and the identity of the brood and its parents as three crossed random factors (Fig. 2).

Growth functions

Growth functions relate the body mass of an individual at time t to its body mass at time $t-1$. The change in body mass is necessary to define the functions for post-fledging survival, $P_♂(z_s, z_♂)$ and $P_♀(z_d, z_♀)$, and the survival kernels, $S_♂(z_s, z_♂)$ and $S_♀(z_d, z_♀)$.

Starlings grow during their first year of life and thereafter do not significantly vary in body mass throughout their reproductive life: we did not found a significant increase from yearling to adult size (i.e. the average size after the second year of life) in either males or females. Thus, for simplicity, we parameterized the growth component in both P (post-fledging survival and growth) and S functions (adult survival and growth) by estimating the linear regression of adult body mass (i.e. the average of all weight measurements obtained from an individual after its first year of life) on fledgling body mass (Fig. 2)

Reproduction

The contribution of new recruits is defined as the product of the mating probability, $m(z_♂, z_♀)$, fertility, $f(z_♂, z_♀)$, inheritance functions, $c_s(z_s|z_♂, z_♀)$ and $c_d(z_d|z_♂, z_♀)$, and probability of post-fledgling recruitment (see above).

The function $m(z_♂, z_♀)$ denotes the mating probability between a male and female with body masses $z_♂$ and $z_♀$ respectively. We assume that all females in the population breed and that their mating

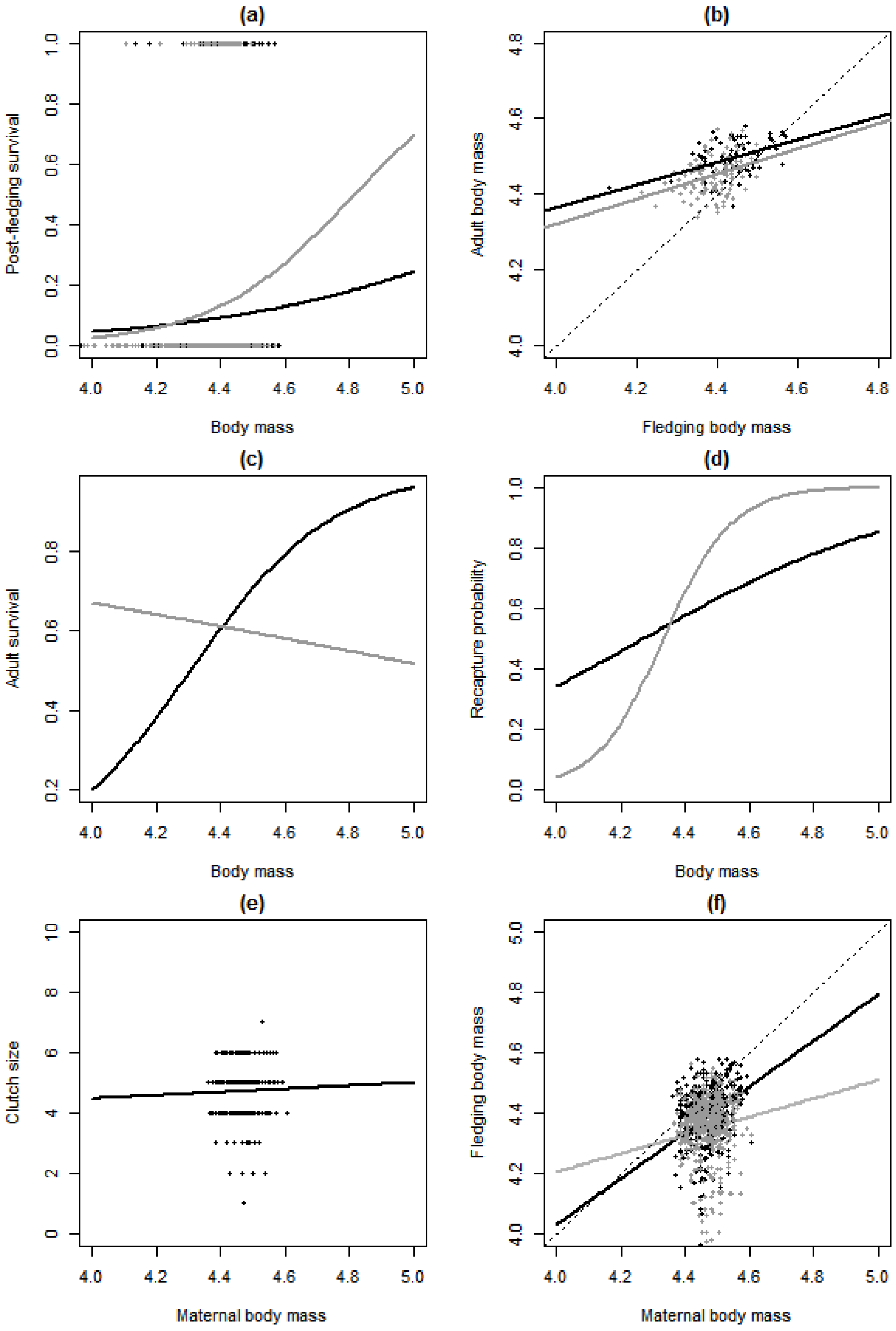

Fig 2. Demographic functions (black: males, grey: females) parameterized with data from a wild population of spotless starlings: (a) Post-fledging survival functions; (b) Growth functions; (c) Adult survival; (d) Recapture probability; (e) Fertility functions; (f) Inheritance functions.

success does not depend on their body mass, whereas the mating probability of males is more variable and increases with their body mass. We estimated the extent to which a male increases his mating success in relation to his body mass by relating the rate of polygyny (i.e. the probability that a male breeds with more than one female during a given breeding season) to his adult body mass (i.e. the average body mass after his first year of life) using a logistic regression. A male was considered polygynous if in at least one year in his life he was captured breeding in more than one nest box; otherwise he was classified as monogamous. The fertility function, $f(z_{♂}, z_{♀})$, is the expected number of offspring produced by a breeding pair in relation to their body masses. Using the sample of 270 broods we analysed the relationship between clutch size and the body mass of the mother and father at reproduction. We used a linear mixed-effects model including the identities of the mother and father as two crossed random factors (Fig. 2).

The inheritance functions, $c_s(z_s|z_{♂}, z_{♀})$ and $c_d(z_d|z_{♂}, z_{♀})$, give the probability that, respectively, a son is produced with body mass z_s at fledging and a daughter is produced with body mass z_d at fledgling, given that their parents have body masses $z_{♂}$ and $z_{♀}$. The distribution of body sizes of fledglings is obtained using the regression parameters α and β, which determine the mean, and $\varepsilon \sim N(0, \sigma)$, which determine the phenotypic variance. We then simulated the increase in offspring phenotypic variance by modifying parameter σ (see eq. 7). Parameters were estimated using the sample of 270 broods; male and female fledglings were analysed separately using linear mixed-effect models with the fledgling body mass as a response variable and the body masses of the mother and father as predictor variables, including the identities of the brood and their parents as three crossed random factors (Fig. 2).

RESULTS

Stable distributions and reproductive values

Male and female body sizes converged on the stable distributions shown in, Fig. 3a with associated reproductive values shown in Fig. 3b.

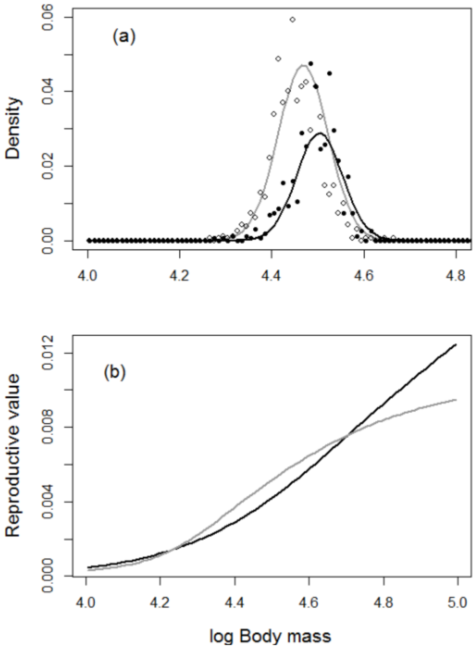

Fig 3. (a) Stable size distributions and (b) reproductive values of males (black lines) and females (grey lines) predicted by the model for the population of spotless starlings. Points in panel (a) represent the observed proportion of males (black points) and females (white points) for each class of body mass.

We simulated an increase of ca. 15% in the standard deviation in fledgling body mass by modifying parameter *d* in eq. 7 (i.e., observed standard deviation when *d* = 0, and increased standard deviation when *d* = 0.02), which simulates an unequal change in phenotypic variance in relation to the mean (as shown in Fig. 1).

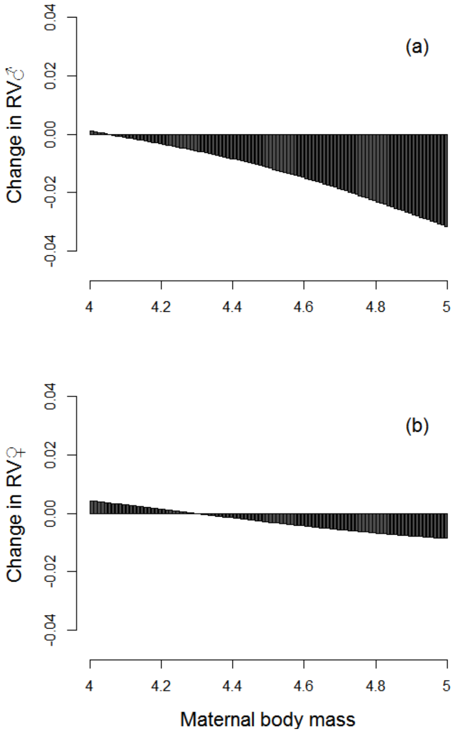

Fig 4. Change in the reproductive value (RV) of (a) sons, i.e. $v_{♂}^{d=0.02} - v_{♂}^{d=0.00}$, and (b) daughters, i.e. $v_{♀}^{d=0.02} - v_{♀}^{d=0.00}$, between the simulation with the observed and the increased standard deviation in offspring body mass. That is, negative values of the difference mean that the increase in offspring phenotypic variance reduces their RV, whereas positive values mean that increasing variance improves their RV.

Effect of stochasticity

The increase in offspring phenotypic variance produced slight changes in the reproductive values of sons and daughters in relation to the body mass of their mother (Fig. 4). In particular, the change in the mean reproductive value of sons (i.e., $E[v_{♂}^{d=0.02}] - E[v_{♂}^{d=0.00}]$) was -1.0 % and was higher than the change in the mean reproductive value of daughters (i.e., $E[v_{♀}^{d=0.02}] - E[v_{♀}^{d=0.00}]$), which was -0.23%. Increasing offspring phenotypic variance reduced the reproductive value of sons, especially those with higher body sizes (Fig. 4a). The reproductive value of daughters also decreased for mothers at the highest classes of body mass, but increased for mothers with lower body mass (Fig. 4b). The model showed the same behaviour when we used *d* values different from 0.02, being the effect on the reproductive values directly proportional to the increase of the parameter *d* (Fig. A1).

The difference between the male and female reproductive values, i.e. the effect of selection acting on a mother to bias the offspring sex ratio, is an increasing function with respect to maternal body size and crosses zero. That is, small mothers have negative values (selection might favour biases towards daughters) and big mothers have positive values (selection might favour to overproduce sons). As the variance in offspring body mass increases, the reproductive value of sons decreases more than that of daughters (Fig. 4). Consequently, the average reproductive value of male-biased broods might decrease more than the average reproductive value of female-biased broods.

As offspring phenotypic variance increases, selection acting on big mothers to overproduce male-biased broods becomes weaker (i.e., the difference in reproductive values is less positive). By contrast, selection acting on small mothers to overproduce daughters becomes stronger (i.e., the difference in reproductive values is more negative) (Fig. 5).

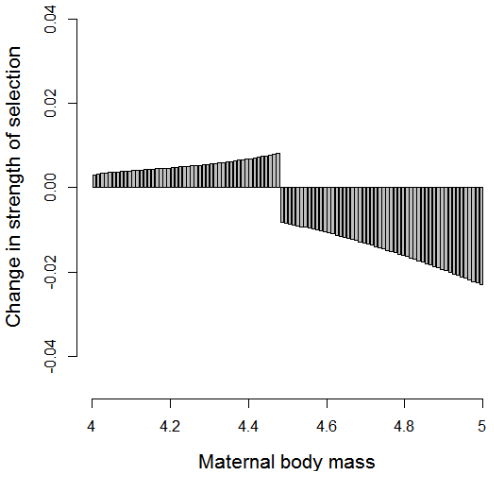

Fig 5. Effect of increasing standard deviation in offspring body mass on the strength of selection acting on a mother to bias sex ratio from equality, i.e. $|\Delta v|^{d\,=\,0.02} - |\Delta v|^{d\,=\,0.00}$, where $\Delta v = v_{♂} - v_{♀}$. That is, positive values of the difference mean that selection becomes stronger when increasing offspring phenotypic variance, whereas negative values mean that selection becomes weaker.

DISCUSSION

Our goal is to understand how environmental stochasticity during the breeding period, i.e., as a source of variance in offspring phenotypic condition, determines the sign and the strength of selection on offspring sex ratios.

We showed that increasing phenotypic variance might reduce the reproductive value of sons more strongly than the reproductive value of daughters (Fig. 4). First, this is consistent with the idea that variations in offspring condition at the end of the period of parental investment will have a stronger effect on the fitness of males than females (Trivers and Willard 1973). Second, these variations might negatively affect fitness, which suggests that selection constrains the variance in phenotypic condition of sons more strongly than the variance in condition of daughters. Therefore, male-biased broods in stochastic environments might be more negatively selected than female-biased broods, thereby favouring the production of the less variable sex (Proulx 2000, 2004). Consequently, factors that increase the variance in offspring phenotypic condition might weaken selection acting on mothers in good condition to overproduce sons and strengthen selection on mothers in poor condition to overproduce daughters (Fig. 5). This study demonstrates that not only the change in the mean offspring condition, but also the residual variance in relation to the condition of their mother, matters in order to understand the selective pressures acting on a mother to adjust the sex of her offspring. Recent evidence is giving consistence to the idea that the complexity of vertebrate life history and demography lead to a wider variety of sex-allocation tactics than expected by the sole relationship between maternal condition, offspring condition and offspring reproductive success (Schindler *et al.* 2015).

Phenotypic variance due to stochastic environmental conditions, developmental plasticity, or genetic differences, can have a strong influence on the individual fitness and population dynamics (Kendall and Fox 2002; Vindenes *et al.* 2008; Nussey *et al.* 2011; Vindenes and Langangen 2015; Plard *et al.* 2016). However, the variance in body condition has been widely overlooked in studies addressing patterns of sex allocation in wild bird and mammal populations. The phenotypic variance of a given trait could be either favoured or constrained when the strength of selection increases or decreases nonlinearly with that trait (Ruel and Ayres 1999; Plard *et al.* 2016). Thus, the mean fitness return will depend on both the mean and the variance of the trait as predicts the Jensen's inequality acting on nonlinear fitness functions: the concavity of the function makes the mean fitness to decrease when the phenotypic variance increases, while the convexity produce the opposite effect (Ruel and Ayres 1999; Plard *et al.* 2016).

Because offspring fitness represents diminishing returns with increasing maternal investment (Clutton-Brock *et al.* 1982; Clutton-Brock 1988), offspring fitness returns can be seen as a nonlinear (concave) function in relation to the amount of resources or energy devoted by the mother (Smith and Fretwell 1974; Lloyd 1987; Lessells 1998; Charnov 1982; Carranza and Polo 2012; Kölliker *et al.* 2015). Thus, the level of maternal expenditure is optimal and evolutionary stable, i.e. it maximizes her marginal fitness benefits, when an increment in the investment in each individual offspring do not produce a significant increase in their fitness expectancy (Smith and Fretwell 1974; Lloyd 1987). Therefore, random variations around the optimal level of maternal expenditure will decrease the mean fitness return as a result of the Jensen's inequality acting on a concave function. If the fitness accrued through sons and daughters are different functions in relation to the level of maternal expenditure (e.g., Lessells 1998; Carranza and Polo 2012), the selective pressures against the variance in levels of care could also differ between sexes. Therefore, the mother should adaptively buffer the phenotypic variance of their sons and daughters either by adjusting their levels of care, i.e. providing more resources to her sons, or adjusting the proportion of sexes, i.e. producing less sons. Although literature on sex-allocation theory typically focused on how parents should control the mean phenotype of their offspring, e.g. proving sex-specific levels of care, the potential influence of stochasticity has not been yet incorporated into evolutionary theory about parental care and sex-biased maternal investment. Understanding how parents can adaptively manage stochastic environmental variance when feeding their offspring could provide further insights for both theoretical and empirical research (Westneat *et al.* 2013).

The body mass of the mother at reproduction have been typically used as a proxy of the amount of energy that she can invest in her male and female offspring (Hewison and Gaillard 1999; Sheldon and West 2004; Ewen *et al.* 2004; Schindler *et al.* 2015). In the spotless starling, the body mass of the mother is related to that of her fledglings and it is more steeply related to the body mass of sons (i.e. there is a significant interaction between maternal body mass and the sex of the fledgling on its body mass; Fig. 2f). Moreover, the body mass of fledglings at the end of the period of parental investment is significantly related to the lifetime reproductive success of males, but not to that of females (unpublished results). Thus, the spotless starling might *a priori* satisfy the conditions necessary for the Trivers-Willard effect to emerge when the body mass of the mother is considered as a predictor variable. However, although previous studies have found facultative variations of the clutch sex ratio in this species (i.e. in relation to maternal testosterone level, paternal attractiveness, and a seasonal shift toward female-biased clutch sex ratios; Veiga *et al.* 2004; Polo *et al*. 2004), females do not vary the offspring sex ratio in relation to their body mass at reproduction (unpublished results).

Multiple hypotheses have been suggested for the lack of facultative sex ratio variations in relation to the condition of the mother at reproduction in birds (Cockburn *et al.* 2002; Ewen *et al.* 2004; West and Sheldon 2002). One of them suggests that selection for sex ratio adjustments might be weak when environmental factors that produce differences in the reproductive values of sons and daughters are difficult to predict (West and Sheldon 2002). This environmental unpredictability can be interpreted either as stochastic variations in the vital rates or as variations in adult of offspring phenotypic variance. We showed here that lower environmental predicttability on the factors that determine the offspring condition at fledging might not necessarily lead to weaker selection for sex ratio adjustment. Consequently, we conclude that the stochasticity affecting the condition of fledgling at emancipation might not provide an explanation for the lack of sex ratio variations in relation to maternal body mass in this species. Additionally, we highlight that specifying what is the source of such unpredictability is important before making predictions on the sign and the strength of selection acting on sex ratio adjustments. Thus, while the stochasticity in vital rates might potentially lead to a weaker selection on offspring sex ratios (i.e. if the future matting success or fertility of the offspring are difficult to predict), the stochasticity affecting phenotypic condition interacts with the condition of the mother due to the nonlinear nature of some demographic processes, and requires detailed analyses and a more careful interpretation.

As far as we know, this is the first empirical study addressing the consequences of environmental stochasticity on the condition of offspring and the sex-allocation strategies of mothers in a vertebrate species. Understanding how stochastic processes drive the dynamics of complex systems is important to understand how some evolutionary processes operate. Focusing on a specific source of stochasticity, we showed that selection might interact with the condition of mothers and operate asymmetrically between sexes, acting against the variance in phenotypic condition of males more strongly than on females. Further research using different sources of stochasticity (i.e. in adult condition, mating success or fertility), might provide a more complete picture and allow to understand the relative importance of each of these sources for fitness and, ultimately, for the sex allocation strategy of mothers.

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Appendix I

Each time step in the iterative model (eq. 2.1 and 2.2) represent a breeding period (time lags of 1 year). Thus, we first estimate the distributions of adult breeders at time *t* given their distributions at time *t* – 1:

$$s_{♂}(z_{♂},t)=\int \phi_{♂}(z_{1♂})\,p_{♂}(z_{1♂})\,g_{♂}(z_{1♂},z_{♂})\,n_{♂}(z_{1♂},t-1)\,dz_{1♂} \qquad \textbf{(A1.1)}$$

$$s_{♀}(z_{♀},t)=\int \phi_{♀}(z_{1♀})\,p_{♀}(z_{1♀})\,g_{♀}(z_{1♀},z_{♀})\,n_{♀}(z_{1♀},t-1)\,dz_{1♀} \qquad \textbf{(A1.2)}$$

where $\phi(z_1)$ is the survival probability of adults, $p(z_1)$ is their recapture probability, and $g(z_1, z)$ is the probability that an adult has body mass z given it had body mass z_1 at the previous time step. Thus, we obtained $s_{♂}(z_{♂}, t)$ and $s_{♀}(z_{♀}, t)$, which are the distribution of adult breeders that survived from the previous year.

We then calculated the total production of sons (given offspring sex ratio x_1 = ½) and daughters ($1 - x_1$) by a male of size $z_{♂}$ by summing his expected reproductive output across the size distribution of female breeders, $s_{♀}(z_{♀}, t)$, as follows:

$$f_{s♂}(z_{♂},t)=CK_{x,H}\,x_1\int m(z_{♂},z_{♀})\,f(z_{♂},z_{♀})\,s_{♀}(z_{♀},t)\,dz_{♀} \qquad \textbf{(A2.1)}$$

$$f_{s♀}(z_{♀},t)=CK_{x,H}\,x_1\int m(z_{♂},z_{♀})\,f(z_{♂},z_{♀})\,s_{♂}(z_{♂},t)\,dz_{♂} \qquad \textbf{(A2.2)}$$

where, for example, $f_{s♂}(z_{♂}, t)$, is the number of male fledglings produced by a male of size $z_{♂}$ at time t given the current size distribution of females and the probability of mating with those females. The normalization constant, $C=\left[\int m(z_{♂},z_{♀})\,f(z_{♂},z_{♀})\,s_{♂}(z_{♂},t)\,s_{♀}(z_{♀},t)\,dz_{♂}dz_{♀}\right]^{-1}$, is used to correct the production of new offspring by the number of mated individuals. The numerator of the constant is 1, which means that all adult males and females are potentially able to breed after recruitment and there is no minimum body size to start reproducing (cf. Schindler *et al.* 2013, 2015). Similarly, we calculated the total production of sons and daughters by a female of size $z_{♀}$ by summing across all possible male sizes $z_{♂}$ in the population, $s_{♂}(z_{♂}, t)$:

$$f_{d♂}(z_{♂},t)=CK_{x,H}\,(1-x_1)\int m(z_{♂},z_{♀})\,f(z_{♂},z_{♀})\,s_{♀}(z_{♀},t)\,dz_{♀} \qquad \textbf{(A2.3)}$$

$$f_{d♀}(z_{♀},t)=CK_{x,H}\,(1-x_1)\int m(z_{♂},z_{♀})\,f(z_{♂},z_{♀})\,s_{♂}(z_{♂},t)\,dz_{♂} \qquad \textbf{(A2.4)}$$

To estimate the offspring body size distribution, eq. A2.1 – A2.4 were multiplied by the inheritance functions that relate the body mass of male and female fledglings to the body mass of their parents at reproduction. The inheritance functions are denoted by $C_{s♂}(z_s|z_{♂})$, $C_{s♀}(z_s|z_{♀})$, $C_{d♂}(z_d|z_{♂})$ and $C_{d♀}(z_d|z_{♀})$, where, for example, $C_{s♂}(z_s|z_{♂})$ is the probability that a son with body mass z_s at fledging is produced by a father with body mass $z_{♂}$, summed across the current distribution of female body masses in the population, weighted by their probability of mating with that male, $m(z_{♂}, z_{♀})$, and by the associated fertility of that pairing, $f(z_{♂}, z_{♀})$:

$$C_{s♂}(z_s \mid z_{♂}, t) = \frac{\int c(z_s \mid z_{♂}, z_{♀}) m(z_{♂}, z_{♀}) f(z_{♂}, z_{♀}) s_{♀}(z_{♀}, t) dz_{♀}}{\int m(z_{♂}, z_{♀}) f(z_{♂}, z_{♀}) s_{♀}(z_{♀}, t) dz_{♀}} \quad \textbf{(A3.1)}$$

$$C_{d♂}(z_d \mid z_{♂}, t) = \frac{\int c(z_d \mid z_{♂}, z_{♀}) m(z_{♂}, z_{♀}) f(z_{♂}, z_{♀}) s_{♀}(z_{♀}, t) dz_{♀}}{\int m(z_{♂}, z_{♀}) f(z_{♂}, z_{♀}) s_{♀}(z_{♀}, t) dz_{♀}} \quad \textbf{(A3.2)}$$

$$C_{s♀}(z_s \mid z_{♀}, t) = \frac{\int c(z_s \mid z_{♂}, z_{♀}) m(z_{♂}, z_{♀}) f(z_{♂}, z_{♀}) s_{♂}(z_{♂}, t) dz_{♂}}{\int m(z_{♂}, z_{♀}) f(z_{♂}, z_{♀}) s_{♂}(z_{♂}, t) dz_{♂}} \quad \textbf{(A3.3)}$$

$$C_{d♀}(z_d \mid z_{♀}, t) = \frac{\int c(z_d \mid z_{♂}, z_{♀}) m(z_{♂}, z_{♀}) f(z_{♂}, z_{♀}) s_{♂}(z_{♂}, t) dz_{♂}}{\int m(z_{♂}, z_{♀}) f(z_{♂}, z_{♀}) s_{♂}(z_{♂}, t) dz_{♂}} \quad \textbf{(A3.4)}$$

The product of eq. A2.1 – A2.4 with their corresponding eq. A3.1 – A3.4 gives the size distributions of male and female fledglings in relation to the body mass of male and female breeders. The overall size distribution of male and female recruits to the population is then calculated as:

$$r_{♂}(z_{♂}, t) = \left[\frac{1}{2} \int f_{s♂}(z_{♂}, t) C_{s♂}(z_s \mid z_{♂}, t) s_{♂}(z_{♂}, t) dz_{♂} + \frac{1}{2} \int f_{s♀}(z_{♀}, t) C_{s♀}(z_s \mid z_{♀}, t) s_{♀}(z_{♀}, t) dz_{♀} \right] P_{♂}(z_s, z_{♂})$$

(A4.1)

$$r_{♀}(z_{♀}, t) = \left[\frac{1}{2} \int f_{d♂}(z_{♂}, t) C_{d♂}(z_d \mid z_{♂}, t) s_{♂}(z_{♂}, t) dz_{♂} + \frac{1}{2} \int f_{d♀}(z_{♀}, t) C_{df}(z_d \mid z_{♀}, t) s_{♀}(z_{♀}, t) dz_f \right] P_{♀}(z_d, z_{♀})$$

(A4.2)

where $P_{♂}(z_s, z_{♂})$ and $P_{♀}(z_d, z_{♀})$ denote the probability of post-fledging recruitment and growth of males and females in relation to their body mass at fledging. The factor ½ is included to avoid double-accounting for the production of sons and daughters by males and females (given that each offspring has both a father and a mother). Thus, $r_{♂}(z_{♂}, t)$ and $r_{♀}(z_{♀}, t)$ are the distribution of body sizes of male and female recruits. Finally, by adding together adult survival and fledgling recruitment, $s_{♂}(z_{♂}, t) + r_{♂}(z_{♂}, t)$ and $s_{♀}(z_{♀}, t) + r_{♀}(z_{♀}, t)$, we obtain the size distributions of all males and females in the population.

Appendix II

Computational procedures

We used numerical procedures to obtain the stable distribution of body masses of males and females, their associated reproductive values and the long term population growth rate iteratively. Thus, using the mid-point rule for numerical integration, we divided the range of body masses, z $[L, U]$, into m artificial classes of width $h = (U - L) / m$, where z_i is the midpoint of the ith class of body mass. We then obtained the discretized survival and reproduction kernels, which are $m \times m$ matrices that describe, respectively, the dynamics of the adult size distribution and the production of new recruits. Each iteration represents a breeding period: first, the distribution of adults that survived from the previous year is obtained by matrix multiplication of the survival kernel by the vector of size distribution of adults from the last breeding period. Second, the contribution of new recruits to the population is obtained by matrix multiplication of the reproduction kernel by the distribution of survival adults. The population kernel is then the sum of both distributions, normalized by the population growth rate. Similarly, we obtained the reproductive values by iterating the transpose of the survival and the reproduction kernels.

The survival kernels of males and females are the $m \times m$ matrices $\mathbf{S_m}$ and $\mathbf{S_f}$, respectively, where the entry $\mathbf{S_m}[z_i, z_j]$ gives the probability that a male with body mass z_j is captured alive with body mass z_i at the next time step. They were obtained as the product $\phi_m(z_j)\, p_m\,(z_j)\, g_m\,(z_j)\, h$ and $\phi_f(z_j)\, p_f\,(z_j)\, g_f\,(z_j)\, h$, for the male and female matrices respectively (see eq. A1.1 and eq. A1.2). The product of the sex-specific survival matrices ($\mathbf{S_m}$ and $\mathbf{S_f}$) with the sex-specific vectors of body mass distribution [$\mathbf{u_m}(t - 1)$ and $\mathbf{u_f}(t - 1)$] give the distribution of males and females that survived from the previous time step and breed in the current breeding period [$\mathbf{s_m}(t)$ and $\mathbf{s_f}(t)$].

The reproduction kernels of males and females are the $m \times m$ matrices: $\mathbf{R_{sm}}$, $\mathbf{R_{sf}}$, $\mathbf{R_{dm}}$ and $\mathbf{R_{df}}$ where the entry $\mathbf{R_{sm}}[z_i, z_j]$ gives the number of male recruits with body mass z_i produced by a male with body mass z_j; the entry $\mathbf{R_{sf}}[z_i, z_j]$ is the number of male recruits z_i produced by a female z_j, and matrices $\mathbf{R_{dm}}$ and $\mathbf{R_{df}}$ have the corresponding entries for daughters. The distribution of new males incorporated to the population is given by $\mathbf{r_m}(t) = ½\, \mathbf{R_{sm}}\, \mathbf{s_m}(t) + ½\, \mathbf{R_{sf}}\, \mathbf{s_f}(t)$, where $\mathbf{r_m}(t)$ is the vector of body masses of male recruits, $\mathbf{s_m}(t)$ and $\mathbf{s_f}(t)$ are the vectors of body masses of adult males and females, where the ½ is added to avoid double accounting for the sons produced by males and females. Similarly, the distribution of female recruits is $\mathbf{r_f}(t) = ½\, \mathbf{R_{dm}}\, \mathbf{s_f}(t) + ½\, \mathbf{R_{df}}\, \mathbf{s_f}(t)$. Finally, the male and female size distributions are updated as $\mathbf{u_m}(t) = \mathbf{s_m}(t) + \mathbf{r_m}(t)$ and $\mathbf{u_f}(t) = \mathbf{s_f}\,(t) + \mathbf{r_f}(t)$, respectively.

Calculation of the reproduction kernels

To obtain the reproduction kernels we multiplied the probability of mating, fertility and the inheritance functions with the distribution of male and female adults that survive to time t [$\mathbf{s_m}(t)$ and $\mathbf{s_f}(t)$], see eq. A2.1-A2.4. We first multiplied the matrix $\mathbf{M}$ that describes the probability of mating between a male and a female with body masses z_i and z_j respectively, with the fecundity matrix **F**, which gives the clutch size that this pair is expected to produce, as an element-by-element product, **M · F**. This was then multiplied by the scalars $K_{x,H}$ and x_1, which represent respectively the harmonic mean function (eq. 3) and the proportion of sons. Then, the matrix product with the size distributions $\mathbf{s_m}(t)$ and $\mathbf{s_f}(t)$, gives the number of sons and daughters produced by each male and female. Thus, using equations A2.1 to A2.4 we generated four row vectors, respectively: $\mathbf{f_{sm}}$, number of male fledglings produced by a male with body mass z_i, $\mathbf{f_{dm}}$, number of male fledglings produced by a female with body mass z_i, and similarly for daughters, $\mathbf{f_{dm}}$ and $\mathbf{f_{df}}$), where, e.g., $\mathbf{f_{sm}} = CK_{s,H}\, x_1\, \mathbf{(M \cdot F)\, s_f}$ (eq. A2.1).

The inheritance matrices $\mathbf{C_{sm}}$, $\mathbf{C_{dm}}$, $\mathbf{C_{sf}}$ and $\mathbf{C_{df}}$ (eq. A3.1-A3.4) relate the body size distribution of sons with the body mass of their parents. For example, the entry $\mathbf{C_{sm}}[z_i, z_j]$ denotes the probability that a son with body mass z_i is produced by a father with body mass z_j. Therefore, the distribution of body sizes of sons is given by: $\mathbf{f_{sm}} \cdot \mathbf{C_{sm}}[z_i, \cdot]$, i.e. the element-by-element product of the vector $\mathbf{f_{sm}}$ with each row of the matrix $\mathbf{C_{sm}}$. To estimate the proportion of e fledglings that survive to the next time step, every column of the resultant matrix is multiplied by the matrix $\mathbf{P_m}$, which denotes the post-fledgling probability of recruitment and growth from body mass z_j at fledging to body mass z_i at recruitment. The resultant $m \times m$ matrix, $\mathbf{R_{sm}}$, represents the body mass distribution of male recruits produced by a male with body mass z_j. The same procedure is used to determine matrices $\mathbf{R_{sf}}$, $\mathbf{R_{dm}}$ and $\mathbf{R_{df}}$, i.e. respectively: the size distribution of male recruits produced by a female with body mass z_j, the distribution of female recruits produced by a male, and the distribution of female recruits produced by a female.

Iterations

For each iteration we estimated the number of males and females added with the summations: $\mathbf{n_m}(t) = \mathbf{s_m}(t) + \mathbf{r_m}(t)$ and $\mathbf{n_f}(t) = \mathbf{s_f}(t) + \mathbf{r_f}(t)$, for males and females, respectively. Then, the distribution of body masses of males in the population is $\mathbf{u_m}(t) = \mathbf{n_m}(t) / \|\mathbf{n_m}(t) + \mathbf{n_f}(t)\|$, where $\|\mathbf{n_m}(t) + \mathbf{n_f}(t)\|$ is the sum of all entries in the male and female vectors. Similarly, the distribution of females in the population is $\mathbf{u_f}(t) = \mathbf{n_f}(t) / \|\mathbf{n_m}(t) + \mathbf{n_f}(t)\|$, so that the whole population structure is $\mathbf{u}(t) = \mathbf{u_m}(t) + \mathbf{u_f}(t) = 1$. Thus, starting on any nonzero initial distributions, the iteration of the male and female size distributions, $\mathbf{u_m}(t)$ and $\mathbf{u_f}(t)$, and the population growth rate, λ, is:

$$\mathbf{s_m} = \mathbf{S_m u_m}(t-1) \qquad \textbf{(A5.1)}$$

$$\mathbf{s_f} = \mathbf{S_f u_f}(t-1) \qquad \textbf{(A5.2)}$$

$$\mathbf{n_m}(t) = \mathbf{s_m} + \frac{1}{2}\mathbf{R_{sm} s_m} + \frac{1}{2}\mathbf{R_{sf} s_f} \qquad \textbf{(A5.3)}$$

$$\mathbf{n_f}(t) = \mathbf{s_f} + \frac{1}{2}\mathbf{R_{dm}}\mathbf{s_m} + \frac{1}{2}\mathbf{R_{df}}\mathbf{s_f} \tag{A5.4}$$

$$\lambda_t = \left\|\mathbf{n_m}(t) + \mathbf{n_f}(t)\right\| \tag{A5.5}$$

$$\mathbf{u_m}(t) = \frac{\mathbf{n_m}(t)}{\lambda_t} \tag{A5.6}$$

$$\mathbf{u_f}(t) = \frac{\mathbf{n_f}(t)}{\lambda_t} \tag{A5.7}$$

The reproductive value function can be computed by iterating the transpose kernels that drives the dynamics of the male and female size distributions, as follows:

$$\mathbf{s_m} = \mathbf{S_m}^{\mathbf{T}}\mathbf{v_m}(t-1) \tag{A6.1}$$

$$\mathbf{s_f} = \mathbf{S_f}^{\mathbf{T}}\mathbf{v_f}(t-1) \tag{A6.2}$$

$$\mathbf{l_m}(t) = \mathbf{s_m} + \frac{1}{2}\mathbf{R_{sm}}^{\mathbf{T}}\mathbf{s_m} + \frac{1}{2}\mathbf{R_{sf}}^{\mathbf{T}}\mathbf{s_f} \tag{A6.3}$$

$$\mathbf{l_f}(t) = \mathbf{s_f} + \frac{1}{2}\mathbf{R_{dm}}^{\mathbf{T}}\mathbf{s_m} + \frac{1}{2}\mathbf{R_{df}}^{\mathbf{T}}\mathbf{s_f} \tag{A6.4}$$

$$\lambda_t = \left\|\mathbf{l_m}(t) + \mathbf{l_f}(t)\right\| \tag{A6.5}$$

$$\mathbf{v_m}(t) = \frac{\mathbf{l_m}(t)}{\lambda_t} \tag{A6.6}$$

$$\mathbf{v_f}(t) = \frac{\mathbf{l_f}(t)}{\lambda_t} \tag{A6.7}$$

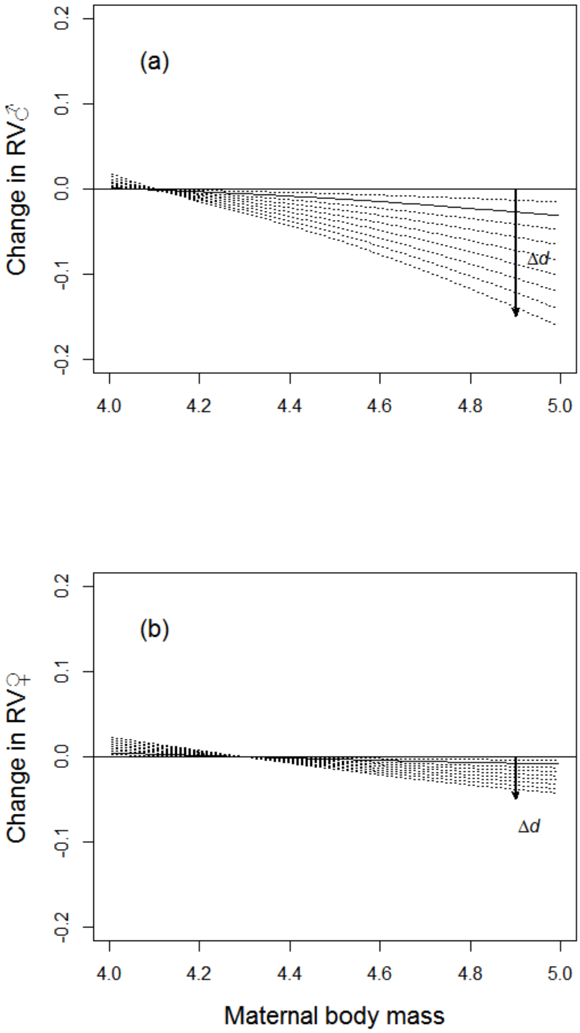

Fig A1. Change in the reproductive value (RV) of (a) sons and (b) daughters between the simulation with the observed and increased standard deviation in offspring body mass using *d* values (eq. 7) from 0.01 to 0.1 (the solid line represents *d* = 0.02, used to analyse the model).

Table A1. Demographic parameters obtained from the population of spotless starlings. Each variable was modelled using a generalised linear model (with either a logit or identity link function), where parameters α, β and ε denote the intercept, slope and residual standard deviation. Bold type indicates parameters that differ significantly from zero.

	Parameter	**Estimate**	**SE**
Post-fledging survival ♂	α	-10.910	8.180
	β	1.956	1.851
	ε	0.763	
Post-fledging survival ♀	α	**-21.809**	**8.039**
	β	**4.529**	**1.833**
	ε	0.822	
Growth ♂	α	**3.171**	**0.270**
	β	**0.298**	**0.061**
	ε	0.041	
Growth ♀	α	**2.905**	**0.397**
	β	**0.352**	**0.090**
	ε	0.049	
Adult survival ♂	α	-19.527	26.281
	β	4.536	5.838
	ε		
Adult survival ♀	α	3.241	5.722
	β	-0.636	1.282
	ε		
Recapture probability ♂	α	-10.186	18.558
	β	2.386	4.117
	ε		
Recapture probability ♀	α	-41.108	18.558
	β	9.489	4.117
	ε		
Mating success	α	**-42.261**	16.452
	β	**9.155**	3.640
	ε	1.075	
Fertility function	α	2.368	9.104
	β_1 (mother)	0.536	1.271
	β_2 (father)	-0.010	1.513
	ε	0.625	
Inheritance function ♂	α	0.996	1.014
	β_1 (mother)	**0.452**	**0.126**
	β_2 (father)	0.307	0.176
	ε	0.047	
Inheritance function ♀	α	**2.994**	**1.009**
	β_1 (mother)	0.148	0.141
	β_2 (father)	0.155	0.165
	ε	0.049	

Discusión General

Esta Tesis aborda la evolución de las asimetrías sexuales en el tamaño corporal, rasgos del fenotipo extendido y estrategias reproductivas en una familia, Sturnidae, donde se piensa que la selección sexual ha jugado un papel clave en su radiación adaptativa y el origen de nuevos ornamentos y conductas de señalización sexual (ver, e.g., Maia *et al.* 2013). Muchos estorninos presentan dimorfismo sexual en tamaño, i.e., los machos son más grandes que las hembras, en coloración y, además, desarrollan conductas de señalización durante el cortejo y la cría. Los estudios comparados nos indican que el dimorfismo sexual es un rasgo derivado en la familia (i.e., el dimorfismo en tamaño, Capítulo 1; o el dicromatismo, Maia *et al.* 2013), que además iría de la mano de algunos rasgos del fenotipo extendido, como es el acarreo de plantas verdes al nido por los machos con una función de cortejo. Los experimentos y estudios observacionales a largo plazo en una de las especies, el estornino negro, nos permiten entender cómo son estos procesos a nivel demográfico. En esta especie, donde los machos compiten por incrementar el tamaño de sus territorios y número de parejas sociales, la presión selectiva sobre el tamaño y rasgos ornamentales de los machos es más fuerte que para las hembras. Sin embargo, al analizar en detalle la estrategia reproductiva en esta especie, encontramos que la competencia entre hembras también jugaría un papel clave en la evolución de su conducta, modulando también algunos de sus rasgos de conducta, i.e. el uso de plumas ornamentales en los nidos.

Para discutir y aportar una visión general a la Tesis se abordan dos principales aspectos: (1) cómo son las presiones selectivas sobre el fenotipo extendido de los machos implicado en el cortejo; y (2) cómo son las presiones selectivas sobre el tamaño corporal, implicado en la competencia por los recursos y parejas reproductivas.

1. Rasgos del fenotipo extendido de machos y hembras, ¿existe un conflicto sexual?

El uso de plantas verdes, flores, líquenes y plantas aromáticas es un rasgo del fenotipo extendido de algunos estorninos, más frecuente entre las especies que anidan en cavidades y con alto dimorfismo sexual (Capítulo 1). Los machos utilizan plantas verdes para atraer a las hembras (Brouwer y Komdeur 2004 en el estornino pinto) y la cantidad de plantas acarreadas al nido es un indicador de su nivel de poliginia (Veiga *et al.* 2006 en el estornino negro). También se piensa que las plantas podrían desempeñar una función antiparasitaria en el nido (Gwinner y Berger 2005; Scott-Baumann y Morgan 2015). Si bien el estudio comparado no informa acerca de qué función evolucionó primero, i.e. la función sanitaria o la sexual, se puede especular que la selección natural favoreció el origen del rasgo y la selección sexual moduló su evolución en las especies de la familia. Esta idea es más parsimoniosa que el hecho de que ambas funciones evolucionaran por separado, o siguiendo el proceso contrario, con un origen sexual y posteriormente sanitario. Así, si el uso de plantas verdes en los nidos incrementaba inicialmente la supervivencia de los pollos (i.e., porque mejoraba su salud o reducía la carga parasitaria en el nido, e.g. Gwinner y

Berger 2005), las hembras habrían seleccionado a los machos que acarreaban más plantas iniciando una carrera evolutiva que habría exagerado el rasgo en los machos (Fisher 1930; Holland y Rice 1998). El rasgo se mantendría en la población incluso aunque en su forma exagerada no aporte el beneficioque inicialmente suponía sobre la supervivencia de los pollos (Kokko *et al.* 2002), o incluso resulte costoso.

Al aumentar experimentalmente la cantidad de plantas verdes en nidos de estornino negro encontramos una reducción de las tasas de reclutamiento de los hijos (Capítulo 2). Este resultado es sorprendente, puesto que *a priori* se esperaría el efecto contrario: un aumento del esfuerzo de la hembra en el cuidado de la nidada al estar emparejada con un macho atractivo (e.g., Horváthová *et al.* 2011), o una mejora directa de la condición y supervivencia de los pollos al añadir plantas con un efecto antiparasitario (e.g., Gwinner y Berger 2005). En el Capítulo 2 se discute el mecanismo próximo que explica la reducción del éxito reproductivo al añadir plantas verdes: i.e., el incremento de la competencia entre hembras aumentó su nivel de testosterona circulante durante la reproducción (Polo *et al.* 2010) y esto pudo tener efectos deletéreos sobre su éxito reproductivo (Veiga y Polo 2008). No podemos descartar que el menor éxito del grupo experimental se deba a que al añadir plantas verdes a los nidos se facilitara que machos de baja calidad, que en condiciones naturales no habrían logrado atraer a una hembra, lograran producir descendencia. Sin embargo, aquí se discute la hipótesis de que el proceso fue mediado por la competencia entre hembras, tal y como indican resultados previos (ver Polo *et al.* 2010 con las mismas hembras del experimento presentado en el Capítulo 2).

La competencia entre las hembras por los recursos para la reproducción es importante en esta especie, donde a menudo las parejas anidan en altas densidades e interfieren durante la cría. Por lo tanto, la evolución del uso de plantas verdes habría tenido lugar bajo una situación de conflicto sexual: los machos utilizarían plantas para atraer a las hembras, pero la competencia entre ellas reduciría su éxito reproductivo. En esta situación de conflicto de intereses, las hembras podrían verse tentadas a reducir el atractivo del macho retirando las plantas del nido (Veiga y Polo 2012). La resolución del conflicto podría consistir en lograr un fenotipo medio en la población entre los óptimos de machos y hembras. Es decir, los machos acarrearían menor cantidad de plantas para no incrementar en exceso el coste para las hembras, i.e. el rasgo se expresaría fuera de los niveles que son óptimos para ambos sexos (Arnqvist y Rowe 2005). Sin embargo, los modelos sugieren que generalmente uno de los dos sexos pierde en el conflicto sexual, ya que a menudo los costes que enfrentan son asimétricos (e.g., Holland y Rice 1998). Por ejemplo, en la carrera evolutiva de Fisher (1930), las hembras podrían exagerar un rasgo conspicuo en los machos, lo que incrementaría el riesgo de ser depredado y/o su coste metabólico. Los machos reducirían así su esperanza de vida, perdiendo en el conflicto sexual (Holland y Rice 1998; ver también Kokko *et al*. 2002).

En la situación anterior el sexo masculino es quien acarrea con los costes de expresar el rasgo; e.g., la larga cola de los machos en *Euplectes ardens* es preferida por las hembras, pero impone en el macho un coste sobre su supervivencia (Pryke *et al*. 2001). Sin embargo, aunque desconocemos si el acarreo de plantas verdes supone un coste para el macho en el estornino, sí sabemos que la competencia por los machos atractivos es costosa para las hembras (Capítulo 2). No son muchos los modelos que han incorporado la competencia entre las hembras en la selección sexual (e.g., Clutton-Brock 2007). Por ejemplo, Fawcett y Johnstone (2003) sugirieron que cuando las hembras (o en general, los individuos del sexo que selecciona un rasgo fenotípico en el sexo contrario) difieren en su condición física o habilidades competitivas, hembras en baja condición deberían elegir machos poco atractivos y las hembras en buena condición, capaces de soportar niveles más altos de competencia, seleccionan a los machos más atractivos.

Los resultados del Capítulo 2 y de experimentos previos (i.e., donde se encontró una relación entre la cantidad de verde y la cantidad de plumas incorporada por la hembra, Polo y Veiga 2006) podrían explicarse en el contexto de la hipótesis anterior. Así, las hembras en mejor condición, aquellas que acarrean mayor cantidad de plumas, preferirían a los machos atractivos y poligínicos, gracias a que soportarían los altos niveles de competencia intrasexual. Las hembras utilizarían las plumas para señalizar su condición o sus habilidades competitivas a hembras que trataran de emparejarse con el mismo macho (Polo y Veiga 2006). Apoyando esta idea vemos que, untilizando datos observacionales, no se encuentra una relación significativa entre la cantidad de plantas y el éxito reproductivo de la hembra, y sí una relación positiva entre la cantidad de plantas verdes y de plumas (datos no publicados). Probablemente las hembras en baja condición seleccionaron a machos menos atractivos; al aumentar la cantidad de plantas verdes experimentalmente en nidos seleccionados al azar, se habría impuesto un coste a las hembras en peor condición, disminuyendo el éxito reproductivo medio de los nidos del grupo experimental.

Con el objetivo de extraer una visión general de esta idea, se plantea a continuación un modelo que investiga (1) si exisitiría un conflicto asociado a la expresión del rasgo de atractivo del macho, y (2) la importancia de la competencia entre hembras sobre la expresión del fenotipo del macho.

Podemos extraer predicciones acerca de cuál será el efecto de expresar un rasgo de atractivo masculino sobre la eficacia biológica de hembras y machos cuando existe competencia por las parejas. Para ello, consideremos una población donde los machos señalizan honestamente su calidad expresando un rasgo x, que podría constituir un ornamento o un rasgo de su fenotipo extendido, e.g. el uso de plantas verdes en nidos de estornino negro. Las hembras obtienen un beneficio al estar emparejadas con machos atractivos, e.g., porque confieren una ventaja genética para sus hijos o porque aportan recursos reproductivos de mejor calidad (i.e., el rasgo evoluciona como una señal honesta de buenos genes, e.g. Kokko *et al.* 2002; Veiga *et al.* 2006). Asumamos, por simplicidad, que los machos no participan en el cuidado de los pollos. La competencia entre hembras por los machos preferidos supone un coste para ellas (Capítulo 2). Además, las hembras de mejor calidad, i.e. en mejor condición física o con mayor experiencia, son mejores competidoras y sufren menor coste por competencia intrasexual. El beneficio y el coste afectan, en última instancia, al número de hijos supervivientes que produce un individuo a lo largo de su vida.

Llamemos *b*(*x*) al beneficio que una hembra obtiene por estar emparejada con un macho de atractivo *x*, y *c*(*x*, *y*) al coste derivado de la competencia hembra-hembra, donde *y* es la condición de la hembra (entiéndase beneficio y coste como aumento o decremento, respectivamente, del número de hijos supervivientes). La función de coste es creciente respecto al atractivo del macho, *x*, y decreciente respecto a la calidad de la hembra, *y*. Así, la eficacia biológica (i.e., el número de descendientes que sobreviven) de una hembra es el balance beneficio - coste:

$$f(x,y) = b(x) - c(x,y) \qquad \textbf{(1)}$$

para *b*(*x*) > *c*(*x*), de forma que *f*(*x*, *y*) > 0 (i.e., valores negativos no tendrían un sentido biológico). La eficacia biológica de un grupo de hembras de distinta calidad que crían con un macho *x* es $\int f(x,y)dy$. Por lo tanto, la eficacia biológica del macho dependerá de la de sus parejas multiplicada por el número de hembras con las que se reproduce, *h*(*x*):

$$m(x,y) = -k(x) + h(x)\int f(x,y)dy \qquad \textbf{(2)}$$

donde *k*(*x*) es el coste que supone para el macho expresar el rasgo de atractivo *x*. La función de coste, *k*(*x*), refleja la pérdida en eficacia biológica que tiene un macho cuando es atractivo y poligínico, e.g., debido a la competencia con otros machos o a los costes energéticos de expresar el rasgo de atractivo. El caso extremo donde $k(x) = h(x)\int f(x,y)dy$ reflejaría la situación en la que el macho muere sin producir descendencia superviviente, no serían posibles valores de *k*(*x*) por encima de este valor.

A partir de (1) y (2) tenemos que la eficacia biológica del macho es:

$$m(x,y) = -k(x) + h(x)\int \left[b(x) - c(x,y)\right]dy \qquad \textbf{(3)}$$

Es decir, la expresión del rasgo *x* conlleva para el macho tanto costes directos, *k*(*x*), como indirectos, *c*(*x*, *y*), i.e. los derivados de aumentar la competencia entre las hembras de su harén (Fig. 1). Aunque no es el objetivo de este modelo, se puede extraer que estos costes garantizan la honestidad del rasgo *x*. Esto es, un mutante deshonesto nunca invadiría la población (e.g., Kokko *et al.* 2002).

El modelo (3) permite analizar si existiría un conflicto sexual en la expresión del rasgo de atractivo del macho, i.e., ¿machos y hembras maximizan su eficacia biológica para valores diferentes de *x*? Así, vemos que, desde el punto de vista de cada hembra del harén, podría existir un punto x_f que maximiza su balance de costes y beneficios (1). Si es así, se cumpliría que cuando $x = x_f$ se anula la primera derivada parcial de *f*(*x*, *y*) respecto a *x* (y la segunda derivada se hace negativa):

$$\frac{\partial}{\partial x} f(x, y)\Big|_{x=x_f} = b'(x) - c'(x) = 0 \quad \textbf{(4)}$$

donde *b*’(*x*) y *c*’(*x*) son las derivadas primeras de las funciones de beneficio y coste de la hembra que se empareja con un macho de atractivo *x*. Es decir, lo óptimo para la hembra es que el macho exprese $x = x_f$ de forma que maximice la diferencia beneficio – coste.

Podemos ahora evaluar la función de la eficacia biológica masculina, *m*(*x*, *y*), en el punto x_m, aplicando el mismo procedimiento que para las hembras, esta vez sobre la ecuación (3):

$$\frac{\partial}{\partial x} m(x, y)\Big|_{x=x_m} =$$

$$= -k'(x) + \frac{\partial}{\partial x}\left[h(x) f(x, y)\right] = 0 \quad \textbf{(5)}$$

Resolviendo (5) obtenemos:

$$\frac{h'(x)}{h(x)} - \frac{k'(x)}{h(x) f(x)} = -\frac{f'(x)}{f(x)} \qquad \textbf{(6)}$$

donde el lado izquierdo de la ecuación contiene los componentes de la eficacia biológica masculina y el lado derecho los de la femenina. En concreto, el primer término, *h*’(*x*) / *h*(*x*), es el incremento marginal en número de parejas reproductivas del macho al aumentar su atractivo. El segundo término, *k*’(*x*) / [*h*(*x*) *f*(*x*)], es el coste marginal del rasgo de atractivo respecto a la eficacia biológica del macho (nótese que *h*(*x*)*f*(*x*) es la eficacia biológica de un harén de hembras emparejadas con un macho *x*). De forma similar, el lado derecho, *f*’(*x*) / *f*(*x*), es el cambio marginal en la eficacia biológica de cada una de las hembras del harén al

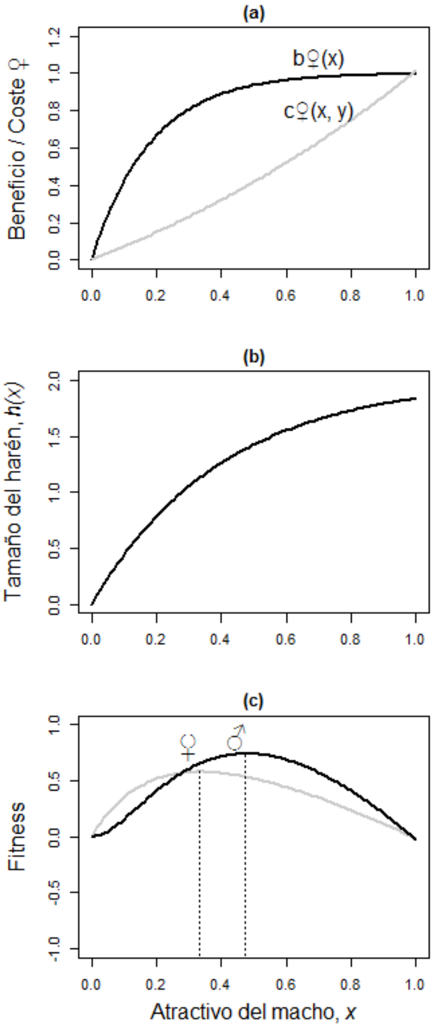

Fig 1. Efecto sobre la eficacia biológica, *fitness*, de la hembra de la expresión del atractivo del macho, *x*. (a) La hembra obtiene un beneficio al emparejarse con un macho atractivo (debido a sus buenos genes o calidad de sus recursos para la reproducción), pero sufre un coste debido a la competencia con otras hembras por los machos preferidos. (b) Los machos que expresan el rasgo *x* incrementan el número de oportunidades reproductivas. Finalmente, (c) la expresión del rasgo *x* en el macho afecta tanto a su eficacia biológica como a la eficacia biológica de sus parejas, lo que puede llevar a un conflicto de interés, i.e. la eficacia biológica de machos y hembras tiene distintos máximos en función de *x*.

cambiar ligeramente el atractivo del macho. Lo que dice la ecuación (6) es que el macho maximiza su propia eficacia biológica en el punto en el que el coste que impone a las hembras se iguala al beneficio de atraer a una nueva pareja, restando el coste que le supone expresar el rasgo. Así, el macho podría aumentar x por encima de lo que es óptimo para las hembras, x_f, a pesar de estar perjudicándolas individualmente, $f'(x) < 0$; lo que genera un conflicto sexual en la expresión del rasgo de atractivo del macho.

En la Figura 1c se aprecia, con un ejemplo numérico, que el óptimo de la función de eficacia biológica masculina se desplaza hacia valores más altos de x, alejándose del óptimo de sus parejas. Una hembra siempre se beneficiaría al emparejarse con un macho atractivo y de buena calidad. Sin embargo, cuando la competencia con otras hembras es costosa, éstas podrían tratar de reducir el atractivo del macho para que otras hembras no obtengan información sobre la calidad de su pareja. Así, en una situación de conflicto (Fig. 1c), la hembra podría, e.g., retirar las plantas verdes acarreadas por el macho (Veiga y Polo 2012).

Consideremos ahora que la calidad de la hembra y sus habilidades competitivas, están asociadas con un ornamento o un rasgo de su fenotipo extendido, e.g. el uso de plumas en nidos de estornino negro. El rasgo informa sobre la condición de su portadora y evita que hembras competidoras traten de atacarla o sabotear su puesta para emparejarse con su macho (e.g., Veiga y Polo 2005; García-Navas *et al*. 2015). Puesto que el rasgo femenino se relaciona directamente con su calidad y habilidad competitiva, diremos que y es el grado de expresión del mismo. Por tanto, la función $c(x, y)$ descrita previamente, que refleja el coste debido a la competencia intrasexual de una hembra, aumenta respecto al rasgo de atractivo del macho, x, pero disminuye respecto a su propio rasgo de calidad, y. El coste de expresar el rasgo femenino y viene dado por $k_f(y)$, y refleja, e.g., la pérdida en eficacia biológica de una hembra que tiene que desatender la puesta para acarrear plumas al nido. De nuevo, por simplicidad, supondremos que el macho no responde al atractivo de la hembra cebando o cuidando más a los pollos (Veiga y Polo 2011). La eficacia biológica de una hembra de atractivo y emparejada con un macho de atractivo x es:

$$f(x, y) = b(x) - c(x, y) - k_f(y) \qquad \textbf{(7)}$$

Por lo que la eficacia biológica del macho pasa a ser:

$$m(x, y) = -k(x) + \\ + h(x)\int\left[b(x) - c(x, y) - k_f(y)\right]dy \qquad \textbf{(8)}$$

Vemos así que el rasgo y de la hembra tiene un efecto positivo sobre la eficacia biológica de macho (Fig 2c). Es decir, si y es grande, el coste de la competencia entre hembras $c(x, y)$ se reduce. En este caso, a diferencia del anterior, ambos sexos se benefician al aumentar y. Por lo tanto, el macho podría tratar de exagerar el rasgo de sus parejas, e.g., acarreando plumas al nido de alguna de sus hembras. Aunque en el estornino negro es la hembra quien introduce y dispone las plumas en el nido, se ha especulado que el macho podría desarrollar parte del trabajo, por ejemplo,

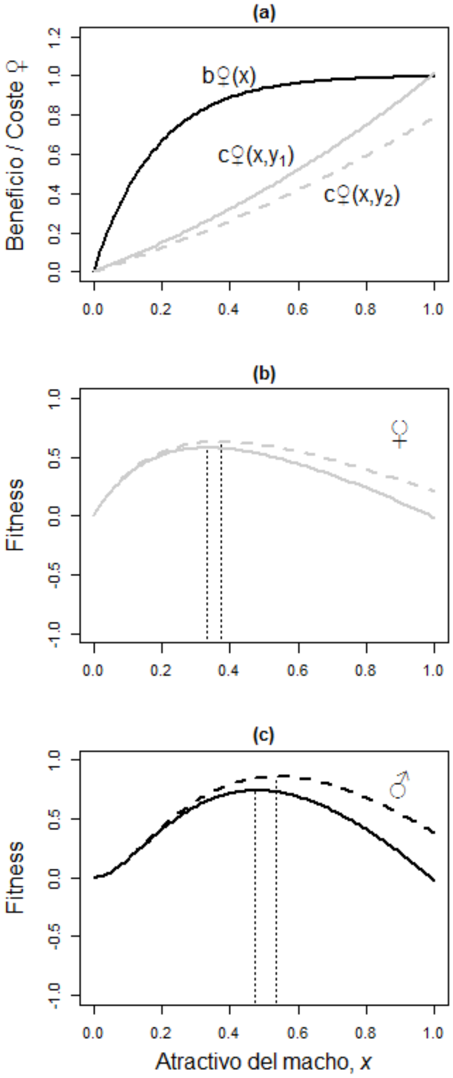

Fig 2. Efecto sobre la relación de coste y beneficio y la eficacia biológica, *fitness*, de expresar un rasgo *y* relacionado con la calidad de la hembra. (a) Beneficio y coste para la hembra al emparejarse con un macho de atractivo *x* cuando la hembra es de alta calidad y_1 (línea continua) y cuando es de baja calidad y_2 (línea discontinua). (b) y (c) muestran el cambio en la eficacia biológica de la hembra y el macho, respectivamente, al modificar el rasgo *y* de la hembra.

acarreando plumas a la cercanía del nido de su hembra primaria (Veiga y Polo 2011). Finalmente, al aumentar *y* también aumenta el óptimo de expresión del atractivo del macho, *x* (Fig. 2c). Esto significa que hembras de buena calidad incrementarían su éxito al emparejarse con machos más atractivos, por lo que se esperaría que ambos rasgos se relacionen positivamente en la naturaleza (Polo y Veiga 2006).

El modelo anterior indica que podrían existir asimetrías en los intereses de machos y hembras respecto al uso de ornamentos implicados en la señalización sexual: i.e. existiría un conflicto sexual ligado al ornamento masculino, puesto que los machos maximizarían su eficacia biológica atrayendo a más hembras, a pesar de que esto perjudique a sus parejas; sin embargo, el conflicto no está presente en el rasgo femenino. Un modelo más complejo podría examinar cómo evolucionaría el rasgo del macho y el grado de preferencia de la hembra simulando una carrera evolutiva (Fisher 1930; ver también Kokko *et al.* 2002). De tal manera, no tendría por qué existir un beneficio derivado de los buenos genes o la calidad del macho en relación a su atractivo. Por el contrario, las hembras tendrían una preferencia direccional por los machos atractivos que podría cambiar en función de cómo de intensa es la competencia con otras hembras (en preparación). Este modelo parte de la interpretación que se ha hecho de los resultados de esta Tesis. Por lo tanto, no pretende explicar cómo ha sido la evolución del uso del verde y las plumas en el estornino, sino establecer un punto de partida para futuros trabajos teóricos que incorporen la competencia entre hembras en los sistemas de selección sexual.

Sobre la información que aportan los ornamentos

Tanto las plantas verdes acarreadas al nido por los machos, como las plumas depositadas por la hembra durante la incubación, transmiten una información pública a conespecíficos que acceden al interior del nido. La información pública juega un papel importante en el colonial estornino negro, donde los intrusos visitan los nidos para obtener información sobre la calidad del sitio (Parejo *et al.* 2007; Veiga *et al.* 2012). Los reproductores suelen atacar a los intrusos, probablemente porque existen costes, como que traten de arrebatarles el nido en años siguientes, o como el parasitismo de cría por parte de jóvenes intrusas (Calvo *et al.* 2000). El uso de plumas cumpliría una función informando a los intrusos que acceden al nido acerca de la calidad de la hembra propietaria o la frecuencia con la que visita su nido para defenderlo (Veiga y Polo 2005). En este sentido, cabría preguntarse por qué las plumas aparecen en el interior y no en el exterior de la caja nido. Si el intrusismo por parte de conespecíficos es costoso, la hembra podría evitar que otros individuos tuvieran que acceder siquiera al interior de la caja para informarse sobre de la calidad de la propietaria.

Un ejemplo interesante de esto es el búho real (*Bubo bubo*), donde los reproductores marcan su territorio con grandes cantidades de heces y plumas de sus presas. Con esto, los búhos señalizan su estatus reproductivo a potenciales intrusos, como otros búhos territoriales o flotantes no reproductores (Penteriani y Delgado 2008). Del mismo modo las hembras de estornino negro podrían hacer más conspicuo su territorio depositando las plumas en el exterior de la caja nido, o incluso los machos, podrían atraer a más hembras colocando fragmentos de líquenes o plantas verdes esparcidas por el territorio. Podrían darse dos razones por las que esto no ocurre: primero, del mismo modo que el territorio sería más conspicuo para conespecíficos, lo sería para los depredadores. Así, a diferencia del búho real, que no tiene depredadores naturales, los huevos y los pollos de estornino negro son atacados por urracas, jinetas y distintas serpientes, como la culebra bastarda, quienes aprenderían a identificar los rastros de plumas depositados por los estorninos alrededor de sus nidos. La evitación de depredadores es una de las causas más importantes que modulan la conducta de anidamiento de las aves (e.g., Weidinger 2002). Una segunda razón es que exista determinismo filogenético, ya que tanto las plumas como las plantas se originaron como elementos no estructurales del nido. Así, aunque la única función de las plantas y las plumas en las especies actuales fuera transmitir información pública, podría no haber evolucionado nunca la conducta de esparcirlas por el territorio, por lo que seguirían siendo acarreados al interior del nido, donde originalmente cumplían sus funciones sanitarias.

La información pública aportada por las plumas y las plantas verdes tiene diferentes receptores en el estornino negro. Si bien las plumas acarreadas por la hembra aportan información potencialmente para cualquier individuo de la colonia (i.e., tanto hembras como machos acceden como intrusos a los nidos; Veiga *et al.* 2012), las plantas aportan información honesta para las hembras sobre la calidad del macho (Veiga *et al.* 2006). La función de las plumas se maximiza al aumentar el número de individuos que acceden a la información, y esto es igual para el macho que para la hembra propietarios. Es decir, si una hembra tiene que realizar el esfuerzo de acarrear y colocar las plumas en el nido durante la incubación, éste se compensará al aumentar el número de individuos que obtienen la información. Tanto el macho como la hembra propietarios se beneficiarán si gracias a esto el nido recibe menos intrusos o tiene menos posibilidad de ser parasitado. Sin embargo, en el caso de las plantas verdes, el macho tratará de conseguir que aporten una información pública, pero la hembra intentará que esa información sea privada. La conducta de las hembras de retirar las plantas del nido se ha interpretado como una vía para hacer que la información sobre el atractivo del macho no se haga pública (Veiga y Polo 2012). Sin embargo, también se podría interpretar como un mecanismo de la hembra para saber con qué frecuencia el macho visita el nido y aporta material verde. Así, a menudo es posible encontrar nidos, avanzada la época de cría, con gran cantidad de plantas que el macho propietario ha acarreado a lo largo de varias semanas sin lograr atraer a una pareja. En este sentido, la fuente de información para la hembra no sería la cantidad de plantas verdes, sino la frecuencia con la que el macho las acarrea al nido.

Es sorprendente el alcance de la información aportada por las plantas verdes en el estornino negro, donde se han encontrado efectos tanto a corto como a largo plazo, sobre la condición, supervivencia y patrones de dispersión de los hijos e hijas (Capítulo 3). Un resultado inesperado fue que al incrementar experimentalmente la cantidad de plantas verdes en los nidos se aumentara la distancia de dispersión de los hijos y se redujera la de las hijas. El efecto fue similar al analizarlo con datos observacionales (Capítulo 3). Resulta complicado explicar este resultado puesto que se desconocen los mecanismos próximos implicados en la determinación facultativa de la conducta dispersiva de los hijos. Atendiendo sólo a los resultados observacionales podríamos especular que los machos más atractivos y poligínicos expulsarían a los hijos de sus territorios reproductivos. Por el contrario, los machos menos atractivos y menos territoriales no harían esto, por lo que los hijos lograrían criar cerca de sus sitios natales, favoreciéndose de la familiaridad con el territorio. Las hijas reflejaron el patrón opuesto; quizás, machos atractivos defienden también territorios de buena calidad, por lo que las hijas tratarían de criar cerca de sus sitios natales. Sin embargo, al manipular experimentalmente la cantidad de plantas verdes se obtuvo un resultado similar, lo que indica que este rasgo en sí mismo media en un proceso de manipulación facultativa de la distancia de dispersión de los hijos. Por tanto, no sería la acción directa de los machos territoriales expulsando a sus hijos, sino que estos se marcharían por su propia voluntad. Las plantas verdes en el nido informarían a la madre acerca de la futura competencia local por los sitios de cría para sus hijos y podrían modular, vía efectos maternos, su conducta dispersiva (Dufty y Belthoff 2001; Tschirren *et al.* 2004, 2007).

Trabajos recientes sugieren que las madres podrían anticipar el éxito futuro de sus hijos e hijas utilizando pistas en el momento de la reproducción, tales como la calidad del territorio, el atractivo de su pareja, su propia condición física o la condición física de sus hijos al abandonar el nido. Se espera que la madre utilice esta información para modificar su estrategia reproductiva, el fenotipo y la conducta de sus hijos e hijas. Por ejemplo, cuando las madres de carbonero común *Parus major* crían en zonas propensas a ser infestadas por ectoparásitos, transfieren niveles más altos de testosterona a los huevos, lo que promueve que los pollos se dispersen a mayor distancia, mejorando su éxito reproductivo en el primer año de cría (Tschirren *et al.* 2004, 2007). Cuando las hembras jóvenes de lagartija de turbera *Lacerta vivípara* están en mala condición, incrementan los niveles de testosterona de sus hijas haciendo que se dispersen a mayor distancia, lo que reduce la competencia futura con su madre (Meylan *et al.* 2002). En última instancia, estos mecanismos habrían emergido porque la selección actúa sobre el fenotipo de la madre, quien se beneficia al alterar determinados rasgos en sus hijos e hijas (Starrfelt y Kokko 2010).

Se piensa que la dispersión de los hijos habría evolucionado para reducir la competencia local entre parientes (Hamilton y May 1977). Algunos de los modelos que abordan la cuestión predicen que la madre debería producir en menor número el sexo más filopátrico para no incrementar la competencia local por los recursos (Leturque y Rousset 2004; Guillon y Bottein 2011; Gros *et al.* 2008). Abordar esta hipótesis experimentalmente es complicado, porque requeriría manipular los patrones de dispersión, y analizar posibles cambios en la razón de sexos de las puestas; o bien hacer que la madre manipulara la razón de sexos y explorar el efecto sobre los patrones de dispersión. En este sentido, el experimento añadiendo plantas verdes a nidos de estornino negro, no sólo incrementó la distancia de dispersión de los machos, sino también sesgó la razón de sexos en favor de los hijos (Polo *et al.* 2004 y datos no publicados). La madre podría beneficiarse produciendo más hijos al emparejarse con un macho atractivo, y reduciría la competencia local entre machos haciendo que estos se dispersaran a mayor distancia. Aunque este experimento no permite saber si la razón de sexos de la puesta y los patrones de dispersión de los hijos guardan una relación causal, se apunta a que ambos podrían ser estrategias de la madre para relajar la competencia local por las parejas. Futuros trabajos deberían dirigirse a entender los efectos maternos implicados en la dispersión natal de machos y hembras en el estornino negro.

2. Evolución del dimorfismo sexual y presiones selectivas sobre el sexo de la descendencia

Existiría una tendencia general en la familia Sturnidae a aumentar el dimorfismo sexual en tamaño, i.e. a que los machos sean más grandes que las hembras (Capítulo 1). De nuevo, la competencia entre hembras tendría un papel importante en la evolución de su morfología en la familia. Por ejemplo, la competencia por los recursos para la reproducción es más alta en especies sociales y con cría cooperativa. En ellas, las hembras habrían desarrollado mayores tamaños corporales y coloración similar a la de los machos (Rubenstein y Lovette 2009). Vemos así que la tendencia general a incrementar el tamaño de los machos que se da en la familia Sturnidae se rompe en algunos grupos, donde las hembras aumentaron su tamaño reduciendo secundariamente el dimorfismo sexual (Capítulo 1).

El dimorfismo sexual en tamaño se ha utilizado en muchos estudios comparados como un correlato de la intensidad de la selección sexual, o de la asimetría en los niveles de competencia intrasexual por las parejas (Moore 1990; Dunn *et al.* 2001; Bonduriansky and Rowe 2005; Emlen *et al.* 2012). Sin embargo, existe un debate acerca de cómo de general es esta afirmación, puesto que a menudo el tamaño corporal no sólo está implicado en la competencia por las parejas, sino en la fertilidad de la hembra (ver, e.g., Dunn *et al.* 2015). Así, no siempre está claro en qué dirección evoluciona el dimorfismo en tamaño y a qué causas responde. Por esta razón es necesario abordar con estudios demográficos si existen y cuál es la causa de las asimetrías en la presión selectiva sobre el tamaño corporal de machos y hembras.

Al investigar cómo son las presiones selectivas sobre el peso corporal de machos y hembras en el estornino negro (Capítulos 4 y 5) encontramos que el valor reproductivo de los machos aumenta más deprisa que en las hembras en relación a su peso. Como consecuencia, exisitiría una presión más fuerte en los machos por aumentar el peso medio y también reducir su varianza (Capítulo 5 y ver más adelante). En este sentido, se podría especular que el peso corporal se comporta en el estornino negro como un rasgo de condición física, cuya fuente de varianza está sobre todo relacionada con la variabilidad ambiental y la inversión reproductiva de la madre. Aunque en ningún caso se ha investigado la heredabilidad del carácter, si ésta fuera alta podría no haberse encontrado una relación clara con el valor reproductivo (Kruuk *et al.* 2000).

En esta discusión se especula sobre la relación entre el resultado del estudio comparado (i.e., la evolución del dimorfismo sexual en tamaño en Sturnidae) y del estudio demográfico (i.e., la relación asimétrica entre peso corporal y el valor reproductivo de machos y hembras en el estornino negro). Así, la presión selectiva podría ser mayor sobre diferentes rasgos estructurales relacionados con el tamaño de los machos, e.g. el peso, la talla o el desarrollo muscular, los cuales incrementan el éxito en la competencia por los territorios y las parejas (Veiga *et al.* 2001, 2002). Si esto es así, el tamaño de los machos podría haber estado sometido a una mayor presión selectiva en algunas especies de la familia Sturnidae llevando los óptimos fenotípicos de machos y hembras a diferentes valoes, i.e. tamaños corporales superiores en machos. Es posible que la relación positiva que se ha encontrado en el estornino negro entre el peso corporal de los machos y su valor reproductivo indique que existe una presión selectiva sobre su tamaño. Este razonamiento debe tomarse como una especulación, ya que sólo se ha investigado un rasgo relacionado con el tamaño: el peso corporal, el cual estaría sobre todo afectado por la varianza ambiental (varianza compartida por hermanos 13.0% IC: 4.3 - 22.9%, no significativa; datos no publicados de un experimento de intercambio de pollos). Sin embargo, la interpretación de los resultados comparados y demográficos, lleva a afirmar que la causa última más probable para el dimorfismo en tamaño en la familia Sturnidae es la mayor competencia intrasexual en los machos.

Presiones selectivas sobre la estrategia reproductiva

Al explorar la relación entre el peso y el éxito reproductivo de machos y hembras se encontró que no sólo es asimétrica la selección sobre el peso de adulto, sino sobre el peso del pollo al abandonar el nido (Capítulo 4). Esto es, los machos que volaron con mayor peso corporal tuvieron mayor éxito reproductivo; mientras que el peso en las hembras no tuvo un efecto importante sobre su éxito reproductivo. Además de esto, el peso corporal de los pollos está relacionado con el de la madre durante la reproducción. Como consecuencia, la actividad reproductiva de la madre podría tener efectos asimétricos sobre la eficacia biológica de sus hijos e hijas. La transferencia del peso corporal y otros rasgos de condición (i.e., la relación positiva y significativa entre el peso de los pollos y su madre; ver Capítulo 4), debe entenderse como un proceso de transferencia no genética (o herencia en el sentido de, e.g., Mesoudi *et al.* 2013) diferente a la heredabilidad (i.e., proporción de varianza genética aditiva de un rasgo). Estudios demográficos sugieren que la transferencia no genética de la condición física dirige el cambio fenotípico de rasgos como el peso corporal (e.g., Schindler *et al.*2015). Por tanto, a lo largo de esta discusión se hace referencia a las presiones selectivas sobre el peso corporal y la estrategia reproductiva de la madre como procesos dirigido por la herencia no genetica (ver Mesoudi *et al.* 2013 y sus referencias).

De los Capítulos 4 y 5 se extrae que, en el estornino negro, las madres de mayor tamaño obtuvieron mayor rendimiento reproductivo a través de sus hijos que a través de sus hijas, mientras que en madres de pequeño tamaño la relación fue la opuesta. Así, se cumple que, (1) el peso corporal de la madre durante la reproducción se relaciona con el de sus pollos al abandonar el nido; (2) las diferencias en el peso de los pollos permanecen durante su vida adulta; y (3) tanto el peso de adulto como el peso de los pollos guarda una relación significativa en los machos con su éxito reproductivo de por vida (Capítulo 4). El Capítulo 5 sugiere, tras proyectar mediante iteración esta cadena de procesos, el valor reproductivo de los hijos aumentaría más deprisa que el de sus hermanas en relación al peso de la madre (Trivers y Willard 1973; ver también Schindler *et al.* 2015). Bajo estas condiciones, se esperaría que las hembras de mayor tamaño invirtieran sobre todo en el sexo masculino y las de menor tamaño en el sexo femenino (ver asunciones de la hipótesis Trivers-Willard en Introducción General). Sin embargo, no se encontró que las hembras con mayor peso corporal modificaran la proporción de sexos de la puesta; en lugar de esto, produjeron puestas más dimórficas, donde los hijos volaron con mayor peso que las hijas (Capítulo 4). Esto significa que el modelo planteado en el Capítulo 5, que representa el estado del conocimiento acerca del proceso de Trivers y Willard, no logra reunir la totalidad de procesos que determinan la estrategia reproductiva de la madre. Por el contrario, exisirían variables no consideradas en el modelo que, en última instancia, relajarían las asimetrías en valor reproductivo de hijos e hijas, y con ello debilitarían la presión selectiva sobre la razón de sexos de la puesta. Una posible interpretación es que las madres en buena condición, que produjeron machos de mayor tamaño, mantuvieran la razón de sexos equilibrada para no aumentar la competencia entre los pollos (Burley 1986; Clotfelter 1996; Uller 2006; Bowers *et al.* 2015).

La hipótesis de Trivers y Willard (1973) es una de las más referenciadas en Ecología Evolutiva (ver revisiones en Hewison y Gaillard 1999; Ewen *et al.* 2004; Alonso-Alvarez 2006; Schindler *et al.* 2015) y muchos de los trabajos se preguntan por qué los resultados no parecen apoyar la predicción de que las hembras ajusten la razón de sexos de la puesta en relación a su propia condición física (Cockburn *et al*. 2002; Carranza y Polo 2012; Schindler *et al.* 2015; Bowers *et al.* 2015; Veller *et al.* 2016). Se piensa que la selección sobre la estrategia de la madre podría ser débil y no compensar los posibles costes asociados al mecanismo de control del sexo de los hijos (Cockburn *et al*. 2002; Pike y Petrie 2003; Alonso-Álvarez 2006). Diferentes aspectos pueden debilitar la presión selectiva sobre la razón de sexos de la puesta: por ejemplo, si la madre tiene posibilidad de controlar varios componentes de su inversión, como el tamaño de puesta o la cantidad de recursos para los pollos (Pen y Weissing 2002; Carranza y Polo 2012). Esto es, una madre podría ajustar la inversión en sus hijos e hijas manteniendo fija la razón de sexos, pero sesgando la cantidad de recursos que aporta a uno u otro sexo (Pen y Weissing 2002; Veller *et al.* 2016). Además, se ha sugerido que la madre controlaría el número de hijos y, secundariamente, la razón de sexos en la puesta, por lo que variaciones en la razón de sexos sólo se detectarían para un tamaño de puesta constante y, especialmente, en puestas pequeñas (Carranza y Polo 2012). Estos trabajos han destacado que la predicción original de Trivers y Willard se complica al tener en cuenta la complejidad de las historias de vida (Williams 1979; Pen y Weissing 2002; Schwanz *et al.* 2006; Carranza y Polo 2012) y la demografía de los vertebrados (Schindler *et al.* 2015).

Un aspecto poco estudiado es el efecto de la incertidumbre sobre las estrategias reproductivas de la madre. Por ejemplo, se ha propuesto que la selección sobre la proporción de sexos de la puesta sería débil si la madre no puede predecir el éxito de sus hijos e hijas (West y Sheldon 2002). Así, si los factores que determinan las diferencias en el valor reproductivo de hijos e hijas son estocásticos, una madre podría sobreproducir por azar el sexo con menor valor reproductivo. Como consecuencia, tanto las puestas sesgadas a macho como las puestas sesgadas a hembra serían penalizadas en un ambiente estocástico (West y Sheldon 2002). Esto podría ocurrir si, por ejemplo, la mortalidad de hijos e hijas es alta e impredecible debido a las tasas de depredación. Una mortalidad juvenil alta y estocástica podría desincentivar las estrategias de inversión parental (Bonsall y Klug 2011). Sin embargo, la estocasticidad no sólo afecta a las tasas vitales (e.g., supervivencia, fertilidad, éxito de emparejamiento) sino también a los rasgos fenotípicos como la condición física (ver, e.g., Engen 1998 y Plard *et al.* 2016). El efecto de la varianza fenotípica en el valor reproductivo de los individuos puede ser poco intuituvo debido a la no linealidad de los procesos demográficos (Plard *et al.* 2016). Por tanto, los modelos verbales a menudo no son suficientes y se hace necesario aplicar los modelos matemáticos (ver revisión en Boyce *et al.* 2006).

Un modelo sencillo permite mostrar que la varianza en los niveles de inversión parental afectaría negativamente a la eficacia biológica de los hijos y con distinta intensidad a uno y otro sexo. Así, considérese que la eficacia biológica de los hijos y de las hijas de una nidada son dos funciones distintas en relación a la cantidad de energía invertida por la madre (*sensu* Trivers 1972), i.e. $F^{♀}(r)$ y $F^{♂}(r)$ (funciones de *fitness* de Carranza y Polo 2012; Fig. 3).

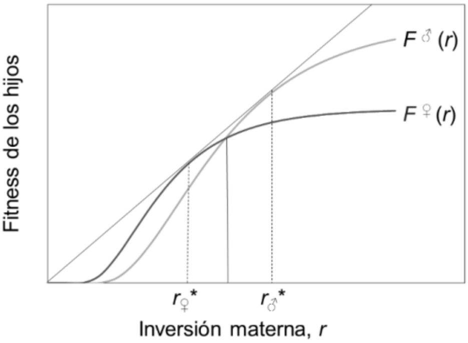

Fig 3. Funciones de eficacia biológica, *fitness*, de los hijos e hijas de una nidada en relación la cantidad de energía invertida por la madre. Los puntos $r_{♂}^{*}$ y $r_{♀}^{*}$ indican el nivel de inversión óptimo y evolutivamente estable en los hijos y en las hijas, respectivamente. La línea continua vertical entre ambos indica el punto sobre el cual pasa a ser más ventajoso producir machos.

La inversión materna tiene rendimientos decrecientes (Clutton-Brock *et al.* 1982; Clutton-Brock 1988; Kölliker *et al.* 2015). Por tanto, las funciones de eficacia biológica de los hijos e hijas deben ser no lineales (cóncavas) en relación a la inversión materna (Smith y Fretwell 1974; Lloyd 1987; Lessells 1998; Carranza y Polo 2012) (Fig. 3). Así, se espera que la madre trate de maximizar su beneficio marginal, i.e. la tasa de ganancia en eficacia biológica por unidad de recurso invertido. Esto sucede cuando la madre invierte una cantidad $r_{♂}^{*}$ de energía en sus hijos y $r_{♀}^{*}$ en sus hijas, que constituyen los niveles óptimos y evolutivamente estables de esfuerzo parental (Smith y Fretwell 1974; Lloyd 1987). Puesto que el coste de producir hijos es mayor que el de producir hijas ($r_{♂}^{*} > r_{♀}^{*}$), debe cumplirse que el beneficio marginal es igual en ambos sexos, para satisfacer el criterio de igualdad de Fisher (1930) (Lessells 1998). Es decir:

$$\frac{F^{♂}(r_{♂}^{*})}{r_{♂}^{*}} = \frac{F^{♀}(r_{♀}^{*})}{r_{♀}^{*}} \tag{9}$$

donde $F^{♀}(r_{♀}^{*}) / r_{♀}^{*}$ es la tasa marginal de beneficio de una madre que produce hijas, la cual debe ser igual al beneficio marginal de una madre que produce hijos (Carranza y Polo 2012). En este caso asumimos que el coste de invertir en la puesta aumenta linealmente con el nivel de inversión (ver Charnov 1982).

En un ambiente estocástico, la cantidad de recursos $r_{♂}^{*}$ y $r_{♀}^{*}$ que una madre invierte en sus hijos e hijas puede sufrir variaciones, e.g. si la calidad del territorio o su propia condición física varían durante la cría. Así, podemos definir $R_{♂}$ y $R_{♀}$ como funciones de distribución, en lugar de valores puntuales, pero cuya media se corresponde exactamente con $r_{♂}^{*}$ y $r_{♀}^{*}$ (Fig. 4).

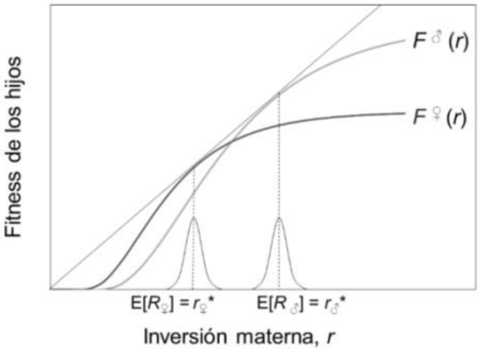

Fig 4. Funciones de eficacia biológica, *fitness*, de los hijos e hijas de una nidada en relación la cantidad de energía invertida por la madre en un ambiente estocástico. Las funciones de distribución $R_{♂}$ y $R_{♀}$ corresponden con los niveles de inversión óptimos $r_{♂}^{*}$ y $r_{♀}^{*}$ de la madre en un ambiente estocástico.

Puesto que las funciones de eficacia biológica son cóncavas en $r_{♂}^{*}$ y $r_{♀}^{*}$ se aplica la desigualdad de Jensen que establece que $E[F^{♂}(R_{♂})] < F^{♂}(r_{♂}^{*})$, y del mismo modo para las hijas. Esto significa que la eficacia biológica media de los hijos e hijas cuando se considera la varianza, $E[F^{♂}(R_{♂})]$, es más baja que la eficacia biológica que se obtendría en un ambiente sin estocasticidad, $F^{♂}(r_{♂}^{*})$. La intensidad de este efecto negativo será tanto mayor cuanto más deprisa decrezca el rendimiento, i.e. cuanto más cóncava sea la función de eficacia biológica. En este caso las funciones de machos y hembras difieren en su grado de concavidad, por lo que se deberían esperar diferencias en el efecto de la varianza sobre la eficacia biológica de uno y otro sexo.

El modelo anterior sirve para entender por qué la varianza ambiental en los recursos para la reproducción afectaría negativamente a los descendientes y, además, de forma asimétrica entre sexos. Sin embargo, es necesario investigar empíricamente qué sexo sería más sensible a estas variaciones. Resulta complicado abordar esta idea, entre otros motivos, por las dificultades de medir la eficacia biológica y el esfuerzo reproductivo en poblaciones naturales. El peso corporal ha sido en utilizado muchas ocasiones como un correlato de la capacidad de la madre de invertir en la reproducción (ver revisión en Ewen *et al.* 2004). En el caso del estornino negro, donde el peso corporal de los machos y de las hembras está sujeto a presiones selectivas asimétricas, se podría utilizar para simular las consecuencias de la varianza ambiental sobre la eficacia biológica.

Como se esperaba atendiendo a la hipótesis de la Fig. 4, el resultado del Capítulo 5 indica que la varianza en la condición física de los jóvenes reduciría levemente su valor reproductivo. Además, este efecto sería más acentuado en los machos que en las hembras. Como consecuencia de esta asimetría sexual, la diferencia en el valor reproductivo de hijos e hijas (i.e. un indicador de la presión selectiva que actúa sobre la madre para sobreproducir uno u otro sexo) se reduciría en valor absoluto para las madres de mayor tamaño, pero se incrementaría para las madres de pequeño tamaño. Es decir, la presión selectiva para sobreproducir hijos se debilitaría, pero la presión selectiva para sobreproducir hijas podría llegar a incrementarse. Este resultado vuelve a poner de manifiesto la importancia de considerar la complejidad demográfica para entender las presiones selectivas sobre las estrategias reproductivas de los padres. Sin embargo, queda mucho por saber acerca qué mecanismos podrían tamponar las variaciones en la condición y valor reproductivo de los hijos (ver, e.g., Westneat *et al.* 2013). En este sentido, el Capítulo 5 constituye una proyección teórica que abre la posibilidad de explorar con experimentos cuál debe ser la estrategia de una madre en un ambiente con incertidumbre.

Podría especularse que un aumento del esfuerzo reproductivo, i.e. aumentando la frecuencia de cebas a los hijos, aumentaría la media y disminuiría la varianza en tamaño corporal de los pollos. Es decir, la varianza ambiental favorecería que se cebara diferencialmente a los machos, más sensibles a cambios en las condiciones durante su desarrollo. Aunque se desconoce si un aumento en la tasa de cebas tamponaría las variaciones en la condición física de los hijos, se piensa que los padres ajustan su tasa de cebas en ambientes estocásticos (Westneat *et al.* 2013). También podría esperarse que las madres sobreprodujeran hijas en ambientes estocásticos. Modelos previos sugieren que la razón de sexos evolutivamente estable se sesgaría hacia el sexo menos variable cuando existe estocasticidad en diferentes fases del ciclo reproductivo (Proulx 2000, 2004). En especies poligínicas, una madre en buena condición física criando en un ambiente estocástico trataría de producir menos machos y, con ello, estaría reduciendo su eficacia biológica (en términos de media aritmética). Sin embargo, gracias a ello conseguiría reducir la varianza en la eficacia biológica. La estrategia de disminuir la eficacia biológica media para reducir la varianza se denomina inversión de cobertura (i.e., *bet hedging*; ver revisión en Starrfelt y Kokko 2012). Se piensa que las estrategias de *bet hedging* optimizarían el rendimiento reproductivo cuando el ambiente es impredecible. Abordar la hipótesis de Trivers y Willard en el marco de las teorías de *bet hedging* en ambientes estocásticos podría ser un reto de cara a futuros trabajos empíricos y teóricos.

Para concluir, esta Tesis investiga la importancia de la selección sexual modulando la morfología y el fenotipo extendido de machos y hembras. La combinación de un enfoque comparado y demográfico nos permite ahondar en las causas últimas del dimorfismo sexual en tamaño y conducta sexual de hembras y machos en estorninos. Se pone de manifiesto la importancia que tienen las asimetrías entre machos y hembras y su potencial conflicto sobre la evolución de sus estrategias reproductivas. Sin embargo, existen varios aspectos donde continúa siendo necesario construir tanto teoría como soporte empírico: la importancia de la competencia entre hembras en la selección sexual. La competencia entre hembras por los machos atractivos podría ser clave modulando rasgos de su fenotipo, pero también su preferencia por los rasgos de atractivo del macho. En estorninos, la competencia entre hembras es intensa, especialmente en especies sociales, y nuestros resultados sugieren que esta presión competitiva podría hacer desaparecer rasgos de los machos, como el uso de plantas verdes con función de señalización sexual. Existe un campo abierto de cara a trabajos teóricos para abordar cómo la competencia intrasexual en hembras alteraría los sistemas de elección de pareja y, a la larga, el propio fenotipo masculino.

Un segundo aspecto es tratar de entender cómo la selección sexual alteraría las estrategias reproductivas de la madre, i.e. en términos de proporción de sexos de los hijos o cantidad de inversión en sus hijos e hijas. Nuestros resultados sugieren que las presiones selectivas dependientes del sexo podrían estar presentes desde el momento de la emancipación. Así, el rendimiento reproductivo que una madre obtiene a través de sus hijos e hijas puede variar en relación a rasgos como su peso durante la reproducción (Capítulo 5). Sin embargo, la madre no parece sobreproducir el sexo que le reporta mayor valor reproductivo, lo que genera nuevas preguntas: la estrategia de la madre podría ser óptima de este modo, puesto que variar la razón de sexos podría implicar costes que no se han considerado en algunos modelos teóricos, e.g. la competencia entre los hijos en el nido. Por otra parte, podría existir una limitación filogenética en las aves y otros vertebrados, que impide que emerja un mecanismo de control facultativo del sexo. En este último caso, la estrategia de la madre podría ser subóptima. Entender las consecuencias adaptativas de esta posible limitación sobre la evolución del dimorfismo sexual y otros rasgos de la historia de vida de las aves es un reto para futuros trabajos teóricos y empíricos.

Por último, en esta Tesis se sugiere que no sólo existiría una presión selectiva asimétrica sobre el fenotipo medio de machos y hembras (i.e. su peso corporal) sino sobre su varianza. Diferentes factores pueden alterar la varianza fenotípica de un rasgo en la población, entre ellos, la impredecibilidad de las condiciones ambientales, i.e. la posible incapacidad de la madre de ajustar la inversión reproductiva para tamponar variaciones en la disponibilidad de recursos. Es necesario dedicar esfuerzo en la investigación de las estrategias reproductivas óptimas en ambientes estocásticos tanto desde una perspectiva teórica como empírica.

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Conclusiones

1. La selección sexual ha sido importante en la evolución de la familia Sturnidae, modulando tanto la morfología como el fenotipo extendido de machos y hembras: el uso de plantas verdes no estructurales en nidos está modulado por el doble efecto de la selección natural y la selección sexual en la familia. Esta conducta se habría originado entre especies que anidan en cavidades, con una función sanitaria, y habría pasado a cumplir una función sexual en las especies con mayor dimorfismo en tamaño.

2. El uso de plantas verdes tiene una función eminentemente sexual en el estornino negro. En esta especie la competencia entre las hembras por los machos atractivos puede llegar a ser costosa, por lo que la hembra selecciona la pareja de acuerdo al nivel de competencia que puede soportar. El aumento experimental de la cantidad de plantas desacopla el sistema de elección de pareja infligiendo un coste reproductivo a las hembras en peor condición.

3. La competencia entre las hembras modula rasgos de su fenotipo extendido en el estornino negro: i.e., el uso de plumas con función ornamental en los nidos. Esta conducta de las hembras estaría favorecida por la selección, probablemente como medio de señalización de la condición y habilidades competitivas a otras hembras, aunque no se puede descartar su efecto sanitario mejorando la condición y supervivencia de los hijos.

4. El uso de plantas verdes tiene efectos a corto plazo sobre el peso y crecimiento de los hijos, y a largo plazo, sobre su supervivencia y conducta de dispersión. Existe una cadena de mecanismos próximos, probablemente mediados por la testosterona materna y los andrógenos transferidos a los huevos. La determinación de la conducta de dispersión de los hijos podría resultar de un proceso adaptativo para reducir la competencia local entre machos por las parejas y territorios reproductivos.

5. La selección sexual es causa última del dimorfismo sexual en tamaño en la familia Sturnidae. En el estornino negro, la asimetría en tamaño es resultado de una mayor presión selectiva sobre el peso corporal de los machos. Esta presión selectiva desigual actúa tanto sobre el peso de los adultos como el de los jóvenes al abandonar el nido, puesto que las diferencias de peso en la emancipación permanecen a lo largo de la vida adulta y condicionan el éxito reproductivo de los machos.

6. Las hembras con mayor peso corporal obtienen más rendimiento reproductivo a través de sus hijos que de sus hijas, y la relación es la opuesta para hembras de menor peso. Las madres que pesaron más durante la reproducción produjeron machos que volaron con mayor peso, pero no aumentaron la proporción de machos en el nido. Así, aunque el estornino negro parece satisfacer las condiciones para que se dé una determinación facultativa del sexo en relación al peso materno, no se encontró este efecto, probablemente, porque la competencia entre los hijos en el nido condiciona la estrategia de la madre.

7. No sólo el peso medio de los hijos al abandonar el nido, sino su varianza, está modulada por la selección en el estornino negro. Así, variaciones estocásticas del peso corporal de los pollos al abandonar el nido reducen el valor reproductivo de los machos más que de las hembras. Esto podría condicionar la estrategia reproductiva de la madre en ambientes estocásticos: la selección penalizaría sobre todo las puestas sesgadas en favor de los machos.