



**UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO**  
**POSGRADO EN CIENCIAS BIOLÓGICAS**  
INSTITUTO DE ECOLOGÍA, UNAM  
Biología Evolutiva

**Funciones de la Promiscuidad en Hembras del Bobo de Patas Azules**  
**(*Sula nebouxii*)**

**TESIS**

QUE PARA OPTAR POR EL GRADO DE:  
**DOCTORA EN CIENCIAS**

PRESENTA:

**Lynna Marie Kiere**

**TUTOR PRINCIPAL DE TESIS: Dr. Hugh Drummond Durey**  
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Facultad de Ciencias, UNAM

**México D.F., Agosto, 2016**



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**Dr. Isidro Ávila Martínez**  
**Director General de Administración Escolar, UNAM**  
**Presente**

Me permito informar a usted, que el Subcomité de Biología Evolutiva y Sistemática, en su sesión ordinaria del día 13 de mayo de 2016, aprobó el jurado para la presentación de su examen para obtener el grado de **DOCTORA EN CIENCIAS**, del Posgrado en Ciencias Biológicas, de la alumna **KIERE LYNNA MARIE** con número de cuenta **510450627** con la tesis titulada: "**FUNCIONES DE LA PROSMICUIDAD EN HEMBRAS DEL BOBO DE PATAS AZULES (*Sula nebouxii*)**", bajo la dirección del **DR. HUGH DRUMMOND DUREY**:

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Sin otro particular, me es grato enviarle un cordial saludo.

**ATENTAMENTE**  
**"POR MI RAZA HABLARA EL ESPIRITU"**  
Cd. Universitaria, Cd. Mx., a 20 de junio de 2016



**DRA. MARÍA DEL CORO ARIZMENDI ARRIAGA**  
**COORDINADORA DEL PROGRAMA**

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## Resumen

La monogamia social es el sistema de apareamiento más común en las aves, pero evidencia conductual y genética han demostrado que la monogamia *sexual* y *genética* son raras debido a la participación de ambos sexos en conductas sexuales con otros individuos. Estas conductas- y los hijos que a veces resultan- se conocen como *extra-pareja* (EP). Las conductas EP benefician claramente a los machos, aumentando su número total de progenie y permitiéndoles parasitar el cuidado parental de otro macho. Por otro lado, en las hembras el número total de progenie aumenta poco al tener parejas sexuales adicionales, y para ambos sexos las conductas EP podrían implicar costos altos, incluyendo la represalia de la pareja social, tiempo y energía gastados en las interacciones EP, y exposición a infecciones de transmisión sexual. A pesar de más de 30 años de investigación, todavía no es claro que motiva las conductas EP de las hembras, y por qué su participación en conductas EP puede variar tanto a lo largo de la vida de un individuo, entre individuos y entre poblaciones y especies.

Al corazón de esta tesis está la pregunta: "¿por qué algunas hembras participan en conductas EP y otras no?". La abordamos evaluando dos hipótesis de la función adaptativa de la conducta EP femenina: *buenos genes* (Capítulo 1) y *compatibilidad genética* (Capítulo 2), además de evaluar si las condiciones ambientales adversas asociadas a El Niño Oscilación Sur reprimen la conducta EP de las hembras (Capítulo 3). Utilizamos una combinación de datos de historia de vida, observación directa de conducta sexual con el machos social y el macho EP, pruebas moleculares de paternidad, genotipaje de microsatélites, y datos ambientales, en los bobos de patas azules (*Sula nebouxii*) de la colonia reproductiva de Isla Isabel, Nayarit. Además de poseer varios rasgos que actualmente están sub-representados en la literatura (aves no passeriformes, que se reproducen en el trópico, con una historia de vida larga), los bobos son poco comunes en que sus cortejos y cópulas sociales y EP son relativamente fáciles de observar. Esto nos permitió utilizar la observación directa de conductas EP en lugar de, o en adición a, pruebas moleculares de paternidad, lo cual refleja más directamente las decisiones de las hembras acerca de su

reproducción EP, y permite considerar las interacciones entre los tres jugadores principales- hembra, macho social, y macho EP.

Como ocurre en muchos otros sistemas, encontramos apoyo mixto para la hipótesis de buenos genes; aunque las hembras tuvieron mayor probabilidad de cortejar a un macho EP entre más joven era su pareja social, ninguna característica del macho social afectó la probabilidad de que la hembra llegara a copular con su macho EP, y no encontramos ninguna diferencia en calidad entre machos sociales y sus rivales EP. Sin embargo, nuestros datos conductuales nos permitieron llevar a cabo análisis adicionales que revelaron que las hembras son más selectivas hacia sus machos EP entre más atractivo es su macho social, lo cual sugiere que las hembras podrían ganar padres genéticos más atractivos a través de las cópulas EP, aunque no siempre les rinde ser selectivas para lograrlo. También encontramos que los machos EP son selectivos hacia las hembras, apoyando la idea de que las cópulas EP son el resultado de una interacción compleja entre varios jugadores. No encontramos apoyo para la hipótesis de compatibilidad genética, la cual sugiere que las hembras se benefician al adquirir padres genéticos que generan hijos más heterocigotos (menos endogámicos), y por lo tanto de mayor calidad. No hubo correlación entre la heterocigocidad y la calidad individual en adultos o crías, ni aumento en la conducta o paternidad EP entre menos compatible o heterocigoto el macho social, ni diferencia en heterocigocidad o compatibilidad entre el macho social y el macho EP, ni diferencia en heterocigocidad entre crías sociales y EP o entre crías sociales con y sin medios-hermanos EP. Finalmente, encontramos que bajo las condiciones adversas de El Niño (menor disponibilidad de alimento, temporada reproductiva demorada, reducción en el éxito reproductivo) disminuye la probabilidad de que las hembras participen en conductas EP. Detalles de nuestros resultados sugieren que esto no es simplemente porque no tienen tiempo y energía inmediata para participar, sino probablemente una estrategia para evitar costos a largo plazo. En general, los trabajos contenidos en esta tesis demuestran la importancia de considerar la interacción compleja entre hembra, pareja social, macho EP, e incluso el ambiente, para entender las decisiones reproductivas de las hembras.

## Abstract

Social monogamy is the most common breeding system in birds, yet molecular and behavioral evidence have shown that *sexual* and *genetic* monogamy are rare due to the participation of both sexes in sexual behaviors with individuals outside the pairbond. These behaviors- and the offspring that sometimes result from them- are referred to as *extra-pair* (EP). EP behaviors clearly benefit males, increasing their total number of offspring and allowing them to parasitize another male's parental care. However, females' total number of young generally increases little with additional sexual partners, and EP behaviors have potentially high costs for both sexes, including retaliation by the social mate, time and energy spent interacting with EP mates, and exposure to sexually transmitted infections. Despite over 30 years of research, it is still not clear what drives females' EP behaviors, and why their participation varies so strikingly within and among individuals, populations, and species.

At the heart of this thesis is the question: "why do some females participate in EP behaviors while others do not?" which we addressed by evaluating the *good genes* (Chapter 1) and *genetic compatibility* (Chapter 2) hypotheses of the adaptive function of female EP behavior, as well as evaluating whether adverse environmental conditions associated with El Niño Southern Oscillation repress females' EP behaviors (Chapter 3). We used a combination of life history data, direct observation of EP behavior over four breeding seasons, molecular paternity testing, microsatellite genotyping, and sea surface temperature data in blue-footed boobies (*Sula nebouxii*) from the colony on Isla Isabel, Nayarit. In addition to possessing several traits that are currently under-represented in the literature (non-passerine, tropical-breeding birds with a long life-history), boobies are uncommon among birds in that their social and EP courtship and copulation behaviors are relatively easy to observe. As such, we were able to use direct observation of EP behaviors instead of, or in addition to, molecular paternity tests to more accurately reflect females' EP mating decisions and consider the behaviors and interactions of all three major players- the female, her social mate, and her EP mate.

As is common in the literature, we found mixed support for the good genes hypothesis; while females were more likely to court with an EP male the younger their social male, social males' quality did not predict whether females would go on to copulate with their EP courtship partners, and we found no differences in quality between social males and their EP rivals. However, our behavioral data allowed us to carry out additional analyses which revealed that females are choosier toward EP males the more attractive their social mate, suggesting that females may gain better genes for their offspring through EP behaviors, but it may not always pay to be choosy to do so. We also found evidence that EP males are choosy toward females, supporting the idea that EP copulations are the result of complex interactions among at least three players- female, social male, and EP male. We found no support for the genetic compatibility hypothesis, which suggests that females benefit by acquiring biological sires that yield more heterozygous (less inbred, more genetically diverse), and therefore higher quality, offspring than their social mates. There was no correlation between individuals' heterozygosity and their quality, no increase in females' participation in EP behaviors or EP young when their social mates were less compatible or heterozygous, no difference in heterozygosity or compatibility between social and EP males, and no difference in heterozygosity between within-pair and EP chicks or between within-pair chicks with and without EP half-sibs. Finally, we found that under the difficult conditions imposed by increased sea surface temperature (SSTA) associated with El Niño (decreased food availability, delayed breeding season, reduced reproductive success), females' probability of participating in EP behaviors is repressed. Details of our results suggest that this is likely because females strategically refrain from EP behaviors to avoid longer-term costs, rather than simply being unable to spare the time and energy necessary to interact with EP males. Overall, the works contained in this thesis demonstrate the importance of considering the complex interaction among females, their social mates, EP males, and even their environment, in understanding females' reproductive decisions.

## Introducción General

Aproximadamente el 90% de las aves tiene un sistema de apareamiento socialmente monógamo, donde un macho y una hembra mantienen un lazo social durante al menos un evento reproductivo y (por lo general) cooperan para criar a su progenie común (Lack 1968, Cockburn 2006). Esta aparente sencillez y armonía en la vida reproductiva de las aves fue generalmente aceptada por siglos, hasta que en los 1980s las pruebas moleculares de paternidad revelaron que frecuentemente una o más de las crías de un nido no son hijos biológicos del macho social (revisión en Griffith et al. 2002). Estas crías, que son producto de las cópulas de una hembra con un macho diferente a su pareja social, son conocidas como crías extra-pareja o EP. Desde ese entonces, se han detectado crías EP en más del 75% de las especies de aves socialmente monógamas (Griffith et al. 2002). Además, estos estudios han revelado una variación impresionante en la prevalencia de crías EP, tanto entre taxa (e.g. desde ~0% en especies genéticamente monógamas como *Rissa tridactyla*, hasta el 55% de crías en la especie socialmente monógama con mayor tasa de paternidad EP, *Emberiza schoeniclus*, Griffith et al. 2002) como entre poblaciones, entre individuos de la misma población, y dentro del mismo individuo a través del tiempo (Bennett y Owens 2002).

El confirmar que las crías EP no son la excepción, sino la regla, renovó el tema de la evolución de la selección sexual y sistemas de apareamiento en las aves porque comprobó que los sistemas genéticos de reproducción son más complejos- y por lo tanto interesantes- de lo que sugeriría el sistema social. Además, señala a la reproducción EP como un componente adicional de la adecuación total, y por lo tanto, a la elección de parejas EP como otro escenario importante para la selección sexual (e.g. Sheldon y Ellegren 1999). La reproducción EP en especies socialmente monógamas ha sido un tema de gran y creciente interés, sobre todo en las aves, donde la

monogamia social es mucho más común que en otros taxa (comparado, por ejemplo, con el 9% de mamíferos, Lukas y Clutton-Brock 2013).

Para los machos de especies socialmente monógamas, la participación en conductas EP tiene un beneficio claro; por lo general, su número total de progenie aumenta entre más parejas sexuales tienen (el *principio Darwin-Bateman*, revisión en Parker y Birkhead 2013). Cuando además los machos sociales proveen cuidado parental, el macho EP se libera de invertir en el cuidado sin sacrificar el éxito de su progenie EP, esencialmente parasitando el cuidado paterno de otro macho (Trivers 1972). Al contrario, las hembras generalmente *no* ganan en términos de su número total de progenie al copular con múltiples machos (o al menos, ganan poco comparado con los machos; revisión en Parker y Birkhead 2013). Al mismo tiempo, la participación en conductas EP puede tener costos directos potencialmente fuertes para ambos sexos, incluyendo la posibilidad de represalia de la pareja social (e.g. agresión, divorcio, reducción del cuidado parental, infanticidio), exposición a enfermedades, y riesgo de depredación y gasto de tiempo y energía durante la búsqueda e interacción con potenciales parejas EP (revisión en Arnqvist and Kirkpatrick, 2005).

La aparente paradoja de que las hembras frecuentemente participan en las conductas EP a pesar de sus potenciales costos y sin la ganancia clara de aumentar el número de crías que producen genera una pregunta básica: ¿Por qué las hembras participan en conductas EP? Además, la variación en las tasas de paternidad EP amplía esta pregunta a: ¿por qué *algunas* participan y *otras no*? Esta fue la pregunta central de la presente tesis, y la exploramos utilizando una combinación de datos de historia de vida, observación directa de conductas sociales y EP, pruebas de paternidad, genotipaje de microsátélites, y datos ambientales, en los bobos de patas azules (*Sula nebouxii*) de la colonia reproductiva de Isla Isabel, Nayarit, México.

### *Funciones de la conducta extra-pareja*

Un punto de partida para abordar por qué algunas hembras participan en conductas EP y otras no, es discernir la función (o funciones) de la conducta EP para ellas, ya que de eso dependerá cuáles se beneficiarán, y por lo tanto participarán, y cuáles no. Existe una variedad de hipótesis funcionales, que caen en dos categorías: a) beneficios directos, y b) beneficios indirectos (también conocidos como *beneficios genéticos*). Los *beneficios directos* proponen que la conducta EP es adaptativa para las hembras porque aumenta su propia supervivencia y/o éxito reproductivo, mientras los *beneficios indirectos* proponen que la conducta EP beneficia a las hembras al aumentar la calidad (supervivencia y/o éxito reproductivo) de sus *descendientes*.

Como alternativa, se ha propuesto que las hembras *no* se benefician de la conducta EP; esta tercera clase de hipótesis se llama *conflicto sexual*. La forma más obvia de conflicto sexual es cuando las cópulas EP de las hembras son forzadas por los machos (e.g. como ocurre en varias especies de patos; McKinney et al. 1983) o cuando las hembras participan solo para evitar el acoso. Sin embargo, hay otras formas más sutiles de conflicto sexual. Por un lado, si los mismos genes controlan la propensión a las cópulas EP en ambos sexos, la adecuación inclusiva podría llevar a que las hembras se vuelven más propensas a conductas EP a pesar de que disminuyan su propia adecuación porque sus parientes machos benefician fuertemente al tener cópulas EP (conocido como *conflicto sexual intralocus o pleiotropía antagónica intersexual*; Forstmeier et al. 2014, e.g. Forstmeier et al. 2011). Por otro lado, esto también podría ocurrir si los genes que aumenten la propensión a las conductas EP en las hembras (a costa de su adecuación) al mismo tiempo influyen positivamente otros rasgos que aumentan la adecuación, por ejemplo, la receptividad hacia el macho social (*pleiotropía antagónica intrasexual*; Forstmeier et al. 2014). Aquí no abordamos más el tema del conflicto sexual, pero se ha argumentado que esta hipótesis debería de recibir más atención (Forstmeier et al. 2014).



La clase de hipótesis adaptativas de *beneficios directos* abarca una gran variedad de propuestas, incluyendo 1) adquirir regalos nupciales (interactuar con machos EP a cambio de alimento u otros materiales útiles), 2) facilitar el parasitismo de nido (poder, con la cooperación del macho EP, poner un huevo en el nido social del macho EP y así parasitar la inversión parental de su hembra social), 3) desalentar el infanticidio por parte de los machos EP (evitar que otros machos maten a sus crías porque podrían ser hijos genéticos de ellos), 4) asegurar la fertilización (y así evitar invertir en huevos que nunca eclosionarán en caso de que el macho social sea temporalmente o permanentemente infértil), y 5) evaluar o adquirir futuras parejas sociales ("entrevistar" o adelantar la formación de lazos con un macho para tenerlo como pareja social en el futuro). Explicaciones más detalladas y discusión de las predicciones derivadas de cada una de estas hipótesis están disponibles en las revisiones de Kempenaers y Dhondt (1993), Griffith y colaboradores (2002), Westneat y Stewart (2003), y Forstmeier et al. (2014). En general, las hipótesis de beneficios directos siguen siendo poco exploradas, en parte porque es necesario observar las conductas previas a la fertilización para ponerlas a prueba, cosa que en la mayoría de las especies es logísticamente difícil (si no prácticamente imposible). Además, todas estas hipótesis menos el aseguramiento de la fertilización y evaluación/adquisición de futuras parejas sociales, requieren de situaciones específicas, por ejemplo que el cortejo incluya el intercambio de regalos nupciales, o que haya mortalidad de crías por infanticidio. Esto las hace explicaciones muy razonables para la conducta EP en algunas especies donde estas condiciones se cumplen, pero simplemente irrelevantes en muchas otras. En esta tesis no evaluamos ninguna hipótesis de beneficios directos, aunque los bobos de patas azules serían un buen modelo de estudio para abordar algunas de ellas- por ejemplo, la prevención de infanticidio, dado que el infanticidio por adultos puede llegar al 15% de la mortalidad de crías (Castillo-Guerrero et al. 2014), o la elección

de futuras parejas, dada la importancia de la aportación al cuidado parental del macho para la condición corporal de la hembra (Velandy y Alonso-Alvarez 2003).

Los beneficios indirectos difieren de los directos en que afectan a las hembras a través del aumento en la supervivencia y/o éxito reproductivo de sus hijos y descendientes subsecuentes, y por lo tanto *requieren de la fertilización EP*. De esta forma, las cópulas EP podrían ser un mecanismo a través del cual las hembras puedan corregir un error en su elección de la pareja social, o bien, una oportunidad para favorecer distintos rasgos en el padre genético que en el padre social de sus hijos. Por ejemplo, las hembras podrían elegir al padre social con base en su habilidad de proveer comida a las crías, mientras elige al macho EP en base a su atractivo o genotipo; ver Mays y Hill 2004). Dos tipos de beneficios indirectos han sido propuestos: *buenos genes*- donde la hembra consigue a un padre genético EP de mayor calidad genética que su pareja social, aumentando así la viabilidad y/o atractivo de su progenie- y *compatibilidad genética*- donde la *combinación* de los genotipos de la madre y el padre EP genera crías EP de mayor heterocigocidad (menor endogamia, mayor diversidad genética individual), y por lo tanto de mayor calidad. Todavía no es claro si para lograr esto es más eficiente que las hembras favorezcan mayor heterocigocidad en sí en potenciales parejas (como propone Brown 1997), o más bien favorezcan genotipos más distintos a los suyos (menor parentesco con ella, mayor "compatibilidad") para que a la hora de combinarse los dos genotipos se maximice la heterocigocidad de los hijos (como propone Tregenza y Wedell 2000), por lo que en esta tesis consideramos ambas posibilidades.

Generalmente las evaluaciones de estas hipótesis han consistido en poner a prueba una o varias predicciones sencillas e intuitivas derivadas de ellas. Primero, la probabilidad de que una hembra participe en conductas EP debe aumentar entre menor la calidad (o heterocigocidad/compatibilidad) de su macho social. Segundo, el macho EP debe ser de mayor calidad (o

heterocigocidad/compatibilidad) que el macho social. Para la hipótesis de compatibilidad genética, también se espera que las crías EP tengan mayor heterocigocidad que sus medios-hermanos sociales, y que las crías sociales cuyas madres fueron fieles al macho social (i.e. que no tuvieron motivo de recurrir al apareamiento EP) tengan mayor heterocigocidad que las crías sociales que tienen medios-hermanos EP. Tal vez la evidencia más contundente de que exista algún beneficio indirecto es si las crías EP tienen mayor supervivencia y/o éxito reproductivo que sus medios-hermanos sociales. Sin embargo, para evaluar esta predicción se necesitan datos de largo plazo, siguiendo a un gran número de medios-hermanos sociales y EP (Griffith et al. 2002). Además, debido a que aún diferencias sutiles en adecuación podrían ser relevantes en términos evolutivos al acumularse a lo largo de muchas generaciones, idealmente se tendría que hacer este seguimiento durante varias generaciones, y tomando en cuenta tanto los hijos sociales como los hijos EP de los individuos focales. Hasta la fecha, se ha evaluado esta pregunta usando datos de largo plazo en cuatro especies: *Periparus ater* (Schmoll et al. 2009), *Melospiza melodia* (Sardell et al. 2012), *Passer domesticus* (Hsu et al. 2014) y *Junco myemalis* (Gerlach et al. 2012), de los cuales solo *Junco myemalis* demostró mayor adecuación de por vida en crías EP que en crías sociales, principalmente debido a la mayor paternidad EP en hijos EP y mayor fecundidad en hijas EP. Es importante notar que en el estudio de *Periparus ater* no se tomaron en cuenta éxito reproductivo EP, que podría ser un componente importante. Por lo tanto, sigue sin resolverse esta pregunta básica, y hacen falta más estudios de este tipo, sobre todo en aves no-paseriformes.

Ahora que las herramientas moleculares para asignar la paternidad y conocer el genotipo de los individuos (y así poder cuantificar su heterocigocidad y parentesco) se han hecho cada vez más accesibles, estas dos hipótesis han recibido mucha atención. Actualmente, existen más de 70 estudios que evalúan la hipótesis de buenos genes (Hsu et al. 2015) y 40 que evalúan la de compatibilidad genética (Arct et al. 2015a) como explicación de la conducta EP en aves. Sin

embargo, a pesar de esta literatura relativamente abundante, existe todavía gran controversia alrededor de ambas hipótesis. Casi la mitad de los estudios han encontrado apoyo para cada una de estas hipótesis (Akçay y Roughgarden 2007), y a nivel meta-analítico (tomando en cuenta cuantitativamente toda la literatura existente, contemplando su tamaño de muestra y magnitud del efecto) a pesar de que hay cierta evidencia a favor de estas hipótesis, los efectos reportados no son suficientemente fuertes ni generalizados para considerarse contundentes (Akçay y Roughgarden 2007, Arct et al. 2015a, Hsu et al. 2015). Además, es común que alguna de estas predicciones se confirme en un estudio pero no otro de la misma especie, o que en el mismo estudio se confirmen algunas de las predicciones y no otras, lo que complica concluir si las hipótesis de buenos genes o de compatibilidad genética son apoyadas en una especie dada, sin mencionar en las aves en general.

Seguramente parte de esta falta de consenso se debe a que realmente *no existe* una sola explicación general para la conducta EP femenina en todas las aves, ni en todas las situaciones. Sin embargo, ciertas limitaciones de la literatura actual además restringen las conclusiones a las que se pueden llegar acerca de estas dos hipótesis, haciendo incluso más difícil evaluar su apoyo general. En principio, la gran diversidad taxonómica, geográfica y de historia de vida de las aves no se encuentra bien representada por la literatura actual, donde están desproporcionadamente estudiadas las aves canoras (orden Passeriformes) de zonas templadas, y de vida corta, dejando poco claro si las mismas tendencias se mantienen en aves con otras características.

Una cuestión más profunda todavía es que en la gran mayoría de los estudios de "conducta" EP jamás ven conducta directamente, sino infieren la conducta EP de la hembra con base en si tuvo o no *crías* EP (Westneat y Stewart 2003). Aunque esto es entendible dadas las dificultades prácticas de observar la conducta EP (después de todo, las hembras tienen motivos por no hacer conspicuas sus conductas EP), tiene dos desventajas importantes. Por un lado, la

proporción de hembras que tienen crías EP es solo un estimado *mínimo* de las que han tenido conductas EP- las hembras que tuvieron crías EP sin duda tuvieron al menos una cópula EP, pero no hay forma de saber cuáles de las hembras que *no* tuvieron crías EP *no* participaron en conductas EP. Dicho de otra forma, debido a que hay muchos factores que intervienen entre la cópula y la cría EP (muchos de ellos fuera del control de la hembra), la proporción de *crías EP* no necesariamente correlaciona con la incidencia de *conductas EP*, y por lo tanto podría no expresar bien los intereses y estrategias de las hembras (Griffith y Immler 2009). Por otro lado, el analizar exclusivamente la paternidad EP no permite conocer las conductas que llevaron a las fertilizaciones EP; no se puede distinguir a las hembras que tuvieron la oportunidad de participar en conductas EP y no lo hicieron de las que nunca tuvieron la oportunidad, ni entre hembras que copularon con un macho EP libremente versus las que fueron forzadas, ni entre los machos que una hembra evaluó y rechazó como macho EP de los que nunca interactuaron con ella. Como remarcan Westneat y Stewart (2003), las características y conductas de quienes *no* participan en conductas EP son igual de informativas que las características y conductas de quienes sí. En este campo, la disponibilidad de pruebas moleculares de paternidad se ha vuelto una "arma de doble filo". A la vez de que estas herramientas han permitido *conocer* los sistemas genéticos de apareamiento, también nos han frenado en *entenderlos*, al poder prescindir de la observación directa de la conducta (Westneat y Stewart 2003). Una de las metas más importantes de esta tesis fue aprovechar lo observables que son las conductas sexuales de los bobos de patas azules para poder no solo repetir el tipo de evaluaciones que se han hecho en otras especies (aunque esto también es valioso porque permite comparar al bobo con ellas), sino también para poner a prueba predicciones más matizadas, que consideran a mayor detalle el papel de cada uno de los participantes durante los varios puntos de decisión durante el proceso de la reproducción EP.

### *Beneficios dependientes del contexto*

Desde que comenzó el estudio de la conducta EP femenina se ha reconocido que muchas de las hipótesis propuestas no son mutuamente excluyentes; las hembras pueden ganar múltiples beneficios, beneficiarse de formas distintas en diferentes situaciones, o solo ser beneficiadas bajo ciertas condiciones. Sin embargo, fue hasta hace relativamente poco que se empezó a evaluar explícitamente esta posibilidad. Varios estudios más recientes consideran posibles beneficios dependientes del contexto, como por ejemplo, la elección de buenos genes por hembras en buena condición y de compatibilidad genética por hembras en mala condición en *Parus caeruleus* (Dreiss et al., 2008); elección de buenos genes por hembras apareadas con machos sociales de baja calidad y para asegurar la fertilización por hembras apareadas con machos de alta calidad en *Euplectes orix* (Friedl y Klump, 2005); o elección de buenos genes a principio de la temporada reproductiva y de compatibilidad genética más tarde en la temporada en *Carpodacus mexicanus* (Oh y Badyaev 2006).

Además de factores intrínsecos de la hembra o su pareja, una variedad de factores extrínsecos sociales y ambientales- como la densidad poblacional o de anidamiento, la sincronía reproductiva, la complejidad del hábitat, las condiciones climáticas, o la disponibilidad de alimento- pueden modificar el balance costo-beneficio de las conductas EP (Bennett y Owens 2002). Esto puede influir cuantas y cuales hembras participan, y por lo tanto ayudar a explicar la gran variación en tasas de conductas EP, sobre todo a niveles taxonómicos finos, i.e. desde variación en el tiempo del mismo individuo hasta entre especies cercanamente relacionadas (conocido como la *explicación jerárquica* de la conducta EP; ver Bennett y Owens 2002, Griffith et al. 2002), independientemente de la función de la conducta EP. El efecto de la densidad y sincronía sobre la reproducción EP han sido un tema casi tan amplia y controversial como su función, y sus hipótesis y predicciones se han tratado a detalle en las revisiones de Petrie y

Kempenaers (1998) y Griffith et al. (2002). En el bobo de patas azules en particular, se ha encontrado que la paternidad EP es mayor cuando hay menos obstáculos físicos que impidan el movimiento en la colonia y a densidades intermedias de anidamiento, probablemente porque estas condiciones maximizan tanto la disponibilidad como la accesibilidad de potenciales parejas EP (Ramos et al. 2014).

Por otro lado, las condiciones ambientales adversas como lluvia (ver Bouwman y Komdeur 2006), frío (e.g. Hoset et al. 2013) o baja disponibilidad de alimento (e.g. Korpimäki et al. 1996, Hoi-Leitner et al. 1999, Cameron et al. 2011) antes de la puesta podrían reprimir la conducta EP de las hembras al dejarlas sin tiempo y energía para interactuar con potenciales machos EP (Slagsvold y Lifjeld 1997) o más vulnerables al abandono de sus machos sociales al ser más dependientes de su ayuda para criar a su progenie (Gowaty 1996), o al aumentar costos particulares de la conducta EP como la exposición a patógenos de transmisión sexual al interactuar con más machos (Sheldon 1993). Este tema es relativamente poco explorado, y aunque algunos trabajos suplementan sus análisis con observaciones conductuales, ninguno está basado principalmente en la observación directa de conductas EP. Este fue la pregunta abordada en el tercer capítulo de esta tesis. En el caso de los bobos de la Isla Isabel y muchos otros depredadores marinos, la elevación de la temperatura superficial del mar asociado el fenómeno climático global de El Niño Oscilación Sur puede reducir drásticamente la disponibilidad de alimento y repercute negativamente en casi todo aspecto de la reproducción desde la puesta hasta el reclutamiento (Ancona Martínez 2012). En las cuatro temporadas de observaciones conductuales usadas para desarrollar esta tesis, los bobos fueron sujetos a un gran rango de temperatura superficial del mar- desde aproximadamente 3°C por debajo hasta 2°C por arriba de lo normal, incluyendo un evento moderado/severo de El Niño (cálido), un evento severo de La Niña (frío), y dos años de condiciones neutrales intermedias. Esto generó un experimento natural ideal para poner a prueba esta hipótesis.

*El bobo de patas azules (Sula nebouxii) como modelo de estudio*

En el bobo de patas azules, a diferencia de la mayoría de las especies estudiadas a la fecha, extensas observaciones de conducta han documentado la complicidad y control de las hembras en las interacciones EP, además del conflicto entre la hembra y su macho social sobre sus conductas EP. A pesar de que se requiere la participación de ambos padres para que sobrevivan las crías (*cuidado biparental obligado*, Nelson 2005), ambos sexos llegan a participar en cortejo y cópulas EP. Generalmente son los machos que inician el cortejo EP, y al encontrarse con un potencial macho EP dentro o cerca de su territorio, una hembra tiene tres opciones: puede a) ignorarlo, b) expulsarlo del territorio, o c) corresponder a su cortejo. Si la hembra responde al cortejo, luego pueden (o no) copular, y a veces sostener una relación de repetidos encuentros, cortejos, y cópulas durante varios días (Osorio-Beristain y Drummond 1998, Pérez-Staples y Drummond 2005). Durante el período de cortejo intra-pareja, que dura varias semanas, en diferentes años se observó entre 54 y 61% de machos, y entre 38 y 54% de hembras participando en conductas EP (Osorio-Beristain y Drummond 1998, Pérez-Staples y Drummond 2005), y en el período de observación de 4-5 días usado para los estudios de esta tesis en las temporadas del 2010 al 2013, un promedio del 25% de las hembras observadas (57 de 230) cortejaron con un macho EP, y 46% de ellas llegaron a copular con él (26 de 57). En el 2011, se detectaron crías EP en el 11% de los nidos (Ramos et al. 2014). Las hembras son 32% más pesadas y son notablemente más fuertes que los machos (Castillo y Chavez-Peón 1983), y permiten la cópula al pararse mientras el macho se balancea sobre su espalda. En miles de horas de observación nunca se han visto cópulas aparentemente forzadas, y las cópulas EP siempre han sido precedidos por cortejo recíproco (observaciones colectadas durante esta tesis, Osorio-Beristain y Drummond 1998, Pérez-Staples y



Drummond 2005). Esto sugiere fuertemente que las hembras están en control de sus conductas EP.

El conflicto entre la hembra y su macho social es evidenciado por el resguardo de pareja, inhibición de conducta EP tanto de machos como de hembras en presencia de la pareja social, y modificación de los horarios de asistencia en el territorio por hembras infieles (Pérez-Staples y Drummond 2005). Además, los machos a veces abandonan a sus parejas después de presenciar su infidelidad (Pérez-Staples et al. 2013), y destruyen el primer huevo si una manipulación experimental pone en duda su paternidad (Osorio-Beristain y Drummond 2001). Esto sugiere que hay potenciales costos para la hembra por participar en conductas EP.

Además, gracias al estudio de largo plazo que se lleva a cabo en la Isla Isabel desde el 1989 (detalles en Drummond, Torres & Krishnan 2003), los bobos cuentan con identificación individual en forma de anillos metálicos que se pueden leer a distancia, y contamos con datos de historia de vida completos (edad, historia reproductiva, identidad de los parientes, etc.) para la mayoría de las aves que se encuentran en la zona de estudio. Además, un ornamento sexual medible en ambos sexos que correlaciona con varios aspectos de salud y calidad- el color de las patas (revisión en Torres y Velando 2010)- es una variable que tiene un papel potencialmente importante en el contexto de la selección sexual EP. Esta colección de factores, y sobre todo el hecho de que se pueda observar la progresión de las conductas EP- del encuentro, al cortejo, a la cópula- además de contar con un protocolo para determinar la paternidad usando microsatélites, hace del bobo de patas azules una especie ideal para investigar la conducta EP de las hembras.

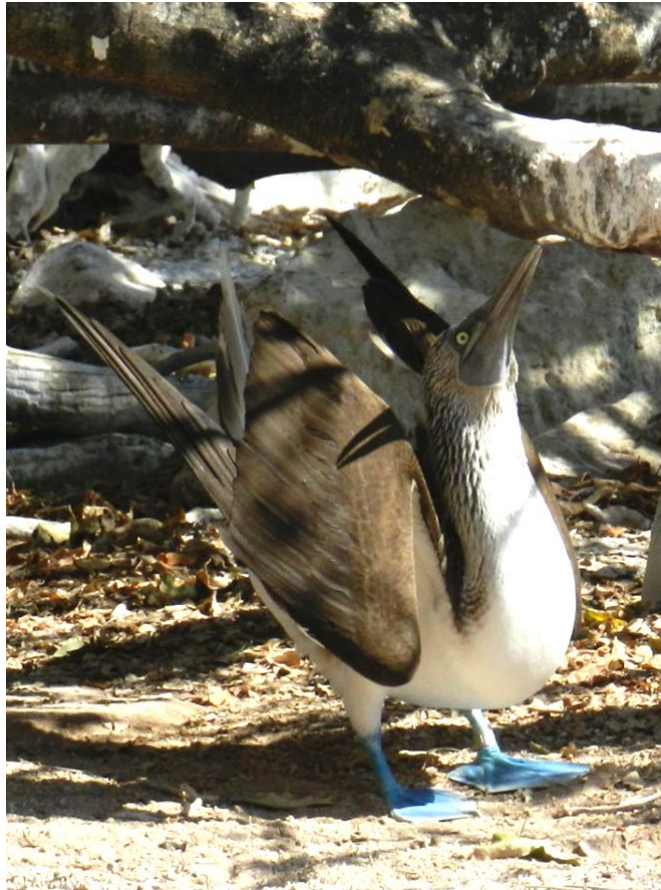
### *Objetivos*

El objetivo general de esta tesis fue explorar posibles funciones y fuentes de variación en la conducta extra-pareja (EP) en hembras del bobo de patas azules (*Sula nebouxii*) de la colonia de

Isla Isabel, Nayarit. Para esto, nos planteamos tres objetivos particulares. Primero, evaluamos las dos hipótesis funcionales más prevalentes en aves: la *hipótesis de buenos genes* (Capítulo 1: "Extrapair Behaviour Reveals Flexible Female Choosiness and Mixed Support for Classic Good Genes in Blue-footed Boobies") y la *hipótesis de compatibilidad genética* (Capítulo 2: "No Evidence that Genetic Compatibility Drives Extra-Pair Behavior in Female Blue-footed Boobies"). Además, evaluamos si las condiciones ambientales adversas en la forma de elevación en la temperatura superficial del mar asociadas a El Niño Oscilación Sur, restringen la participación de las hembras en conductas EP (Capítulo III: "Female Infidelity is Constrained by El Niño Conditions in a Long-lived Bird").

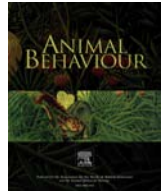
# Capítulo 1:

## Evaluación de la hipótesis de buenos genes



*"La conducta extra-pareja revela selectividad flexible de las hembras y apoyo mixto para la hipótesis clásica de buenos genes en bobos de patas azules"*

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## Extrapair behaviour reveals flexible female choosiness and mixed support for classic good genes in blue-footed boobies



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The good genes hypothesis has long been a major focus of research on the function of extrapair (EP) behaviour by socially monogamous females. It predicts that females should be less faithful when paired to low-quality males and, as EP males become increasingly superior to their social mates, that EP males should be of higher quality than the males they cuckold. Evaluations have yielded mixed results, perhaps in part due to an overly strict interpretation of these predictions. A recent theoretical model demonstrated that while females with high-quality mates should be selective, those with low-quality males may gain good genes benefits by random EP mating, provided EP choosiness has costs, phenotypic indicators are imperfect and sperm competition favours genetic quality. We tested these classic and modified behavioural predictions in the blue-footed booby, *Sula nebouxi*, at two critical steps of EP interactions: courtship and copulation. Support for the classic good genes predictions was limited: EP courtship was more common in females with younger mates, but EP copulation following courtship was not predicted by social mate or relative male age, body size, condition, foot colour (a dynamic ornament) or breeding success, and these characteristics did not differ consistently between social and EP males. However, EP copulation increased with relative attractiveness (greener feet) of the EP male only when the social male was attractive, supporting the model's prediction that female choosiness should increase with social male attractiveness. Moreover, EP copulation probability decreased as EP males became increasingly superior to unattractive females, suggesting that EP males can also be choosy and may constrain female behaviour. Our results confirm that EP interactions involve more complexity than originally envisioned, warranting further studies that focus on behaviour and include all three main players: female, social mate and EP male.

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The good genes hypothesis has been a major focus of research on the function of extrapair (EP) behaviour in female birds for nearly three decades (Griffith, 2007; Griffith, Owens, & Thuman, 2002). It proposes that females gain indirect (genetic) benefits through increased offspring viability and/or attractiveness by acquiring high-quality EP sires (Birkhead & Møller, 1992), and it generates three frequently tested predictions of the effect of male quality on EP behaviours. There should be a negative relationship between the quality of a female's social mate and her infidelity, females should be more likely to engage in EP behaviours as potential EP males become increasingly superior to their social mates, and EP males should be of higher quality than the males they cuckold (reviewed in Kempenaers & Dhont, 1993, among others).

Despite some positive findings, results of tests of classic good genes predictions can be difficult to interpret and evaluations have yielded mixed conclusions (Akçay & Roughgarden, 2007). Results frequently differ depending on which traits are used to represent genetic quality; EP males are generally larger and older than the males they cuckold, but not superior in secondary sexual characteristics (e.g. Foerster, Delhey, Johnsen, Lifjeld, & Kempenaers, 2003; Marshall, Buchanan, & Catchpole, 2007; see meta-analyses in: Akçay & Roughgarden, 2007; Cleasby & Nakagawa, 2012). Second, observed patterns can be variously attributed to female choice, easier subversion of young/small males' paternity guards, or difficulty identifying and measuring relevant ornamental traits (see Akçay & Roughgarden, 2007; Hasson & Stone, 2011). Third, when evaluating a single trait, results are often inconsistent between predictions; for example, even when the expected relationship between a given trait and EP paternity gain is found, paternity loss is frequently unrelated to male characteristics (e.g. Kleven, Jacobsen, Izadnegahdar, Robertson, & Lifjeld, 2006; Otter, Ratcliffe, Michaud, & Boag, 1998; Wetton, Burke, Parkin, & Cairns,

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1995). Finally, conclusions commonly differ among closely related species, between populations, or over time within a population (reviewed in [Petrie & Kempenaers, 1998](#)).

Alternative hypotheses for the function of EP behaviours have been proposed, including indirect benefits such as genetic compatibility or genetic diversity, and a variety of direct benefits, such as fertility assurance, provision of nesting materials or food, or facilitating switching to a higher-quality social male (reviewed in: [Griffith et al., 2002](#); [Jennions & Petrie, 2000](#)). Inconsistent results of evaluations of the good genes hypothesis may be due to different species obtaining different types of benefits, or to benefits being context dependent, such that there is no one general explanation for female EP behaviour. At the same time, however, it has been proposed that strict adherence to 'classic' predictions may lead to falsely dismissing good genes benefits as a main function of female EP behaviour, and thus underestimating its occurrence ([Hasson & Stone, 2011](#)).

In a theoretical model, [Hasson and Stone \(2011\)](#) recently demonstrated that while some females benefit most by conforming to good genes predictions, others obtain good genes benefits by copulating nonselectively with EP males. Their model shows that females can gain better genes by copulating with random males whenever (1) choosing among EP males is sufficiently costly or constrained, (2) male phenotype is an imperfect indicator of genetic quality and (3) sperm carrying higher-quality genes have some postcopulatory advantage. While even females paired to high-quality males may benefit from random EP pairings if the proportion of high-quality males in the population is sufficiently high, random EP interactions are expected to be most common in females paired to low-quality males. Females are expected to be more selective among potential EP mates based on phenotypic cues as the quality of their social males increases because random mating incurs a higher risk of replacing the sperm of their social mates with inferior EP sperm. This proposal is consistent with other evidence for conditional female choosiness among potential mates. In diverse taxa and a variety of mating systems females are less discriminating towards low-quality males when time is constrained, searching is costly, or competition is intense, or when they themselves are of low quality (reviewed in [Gibson & Langen, 1996](#)). Female selectivity has also been shown to vary in the more specific context of social mate choice (e.g. [Sheldon, Davidson, & Lindgren, 1999](#)).

Testing for conditional selectivity by females in the context of EP mate choice is constrained by the difficulty of systematically observing behaviour of all three major players: female, social mate and EP male. EP interactions are rarely observed, and the prevailing method of detecting EP pairings is molecular paternity testing ([Griffith, 2007](#); [Westneat & Stewart, 2003](#)). However, precopulatory interactions likely represent individual EP mating decisions more directly than EP paternity ([Forstmeier, Martin, Bolund, Schielzeth, & Kempenaers, 2011](#)) because postcopulatory mechanisms can bias paternity in nonstraightforward ways ([Pizzari, Løvlie, & Cornwallis, 2004](#); [Pryke, Rollins, & Griffith, 2010](#); [Thuman & Griffith, 2005](#)). Also, without systematic observation of EP courtship and copulation, mistaken inferences can be made; for example, confusing refusal to engage in EP copulations with lack of opportunity. This is an important interpretation problem even for results consistent with good genes predictions if higher-quality EP males are better at coercing females, if higher-quality social males are better at controlling their mates' behaviour, or if potential EP males are more reluctant to approach the mates of high-quality males in order to avoid agonistic interactions they are likely to lose ([Westneat & Stewart, 2003](#)).

We tested the behavioural predictions of the good genes hypothesis in the blue-footed booby, *Sula nebouxii*, including [Hasson](#)

and [Stone's \(2011\)](#) prediction of conditional female choosiness. In this species, unlike most studied to date, extensive observations of EP courtship and copulation in the wild have documented female complicity and control in EP interactions as well as conflict between females and their mates over female EP interactions. Despite obligate biparental care ([Nelson, 2005](#)), both sexes commonly engage in EP copulation during the weeks-long courtship period (54–61% of males, 38–54% of females; [Osorio-Beristain & Drummond, 1998](#); [Pérez-Staples & Drummond, 2005](#)) and EP young were detected in ~11% of nests ([Ramos et al., in press](#)). EP males are usually the initiators of courtship. Upon encountering a potential EP male on or near her territory, a female booby responds by ignoring him or expelling him from the territory, or by reciprocating courtship, after which they sometimes sustain a relationship of repeated encounters involving courtship and copulation over many days ([Osorio-Beristain & Drummond, 1998](#); [Pérez-Staples & Drummond, 2005](#)). Females are 32% heavier and notably stronger than males ([Castillo & Chavez-Peón, 1983](#)) and permit copulation by standing while males balance on their backs. Observations have never detected forced copulations, and EP copulation was always preceded by reciprocal courtship (L.M.K., personal observation; [Osorio-Beristain & Drummond, 1998](#); [Pérez-Staples & Drummond, 2005](#)). Sexual conflict is evidenced by mate guarding, inhibition of male and female EP behaviour by the social mate's presence and modification of daily attendance schedules by unfaithful females ([Pérez-Staples & Drummond, 2005](#)); males sometimes abandon partners after witnessing infidelity ([Pérez-Staples, Osorio-Beristain, Rodríguez, & Drummond, 2013](#)) and destroy their partner's first laid egg after paternity doubt is experimentally induced ([Osorio-Beristain & Drummond, 2001](#)).

Foot colour and age of blue-footed boobies are correlated with aspects of health and reproductive success and, therefore, are potential indicators of quality in this species. Foot colour is a dynamic carotenoid-based ornament in both sexes that is correlated with age, nutrition, immune function, reproductive history and, in older males, germ-line integrity, and it can change in the short term depending on food and carotenoid ingestion and immune activation (reviewed in [Torres & Velando, 2010](#)). In both sexes, greener feet elicit more intense courtship by social and EP partners (reviewed in [Torres & Velando, 2010](#)). In males and females, reproductive success increases with age up to 8–11 years, then declines ([Kim, Velando, Torres, & Drummond, 2011](#)), and the probability that fledglings will recruit increases up to age 5 years, plateaus through age 12 years, then declines sharply ([Torres, Drummond, & Velando, 2011](#)). Older males show damage in the DNA of their sperm ([Velando, Noguera, Drummond, & Torres, 2011](#)), and older females show a decline in quality of eggs and care of offspring ([Beamonte Barrientos, Velando, Drummond, & Torres, 2010](#)). In addition to these two variables, we included individuals' mean reproductive output relative to the other individuals in the colony during their previous reproductive attempts ('average standardized fledgling production', see [Methods](#)), which allowed us to examine correlates of reproductive success that are evaluated (indirectly) by females but are not themselves easily measured. Finally, we included body size and body condition to allow comparison with the many previous evaluations of the good genes hypothesis in other species that consider these variables (see review in [Akçay & Roughgarden, 2007](#)).

Using systematic observation of EP behaviour, we first asked whether EP courtship by females decreases with increasing social male quality and whether EP males that females court and copulate with are of higher quality than their social mates. Then, for females that courted EP males, we asked whether the probability of EP copulation increases with decreasing social male quality and increasing EP male superiority, as predicted by the classic good



genes hypothesis, or whether it depends on the interaction of these two terms, implying strategic variation in female choosiness towards EP males (sensu [Hasson & Stone, 2011](#)). As potential indices of genetic quality we used foot colour and age as well as body size, body condition and previous breeding success.

## METHODS

### *Study Colony and Long-term Database*

Fieldwork was carried out from December through February during the 2010, 2011 and 2012 breeding seasons of the booby colony on Isla Isabel, Nayarit (21°52'N, 105°54'W). Colony monitoring and banding of fledglings with stainless-steel leg bands engraved with a unique number have been carried out annually since 1988 (details in [Drummond, Torres, & Krishnan, 2003](#)). Ages and reproductive histories were thus known for more than 95% of focal individuals. We calculated individuals' average standardized fledgling production by first standardizing the number of fledglings produced for all birds that reproduced each year, such that a zero was assigned to pairs that produced the average number of fledglings, positive values corresponded to above-average fledgling output and negative values represented below-average success. In this way, the same number of fledglings produced confers a higher value in years of low colony reproductive success than in years in which breeders were generally successful. We then extracted this standardized fledgling production for each reproductive attempt by the focal individual (including multiple attempts in a single season) and divided by the total number of attempts to yield a lifetime average up to the year of observation. Focal males and females that had not reproduced previous to the observation year (124 individuals) were assigned a value of 0.

### *Behavioural Observations*

Two observers alternated 2 h shifts in each of three portable blinds in dense neighbourhoods of up to six social pairs with territories within 25 m of the blind. We observed pairs for 4–5 days during the daylight hours of maximal activity (0645–1145 hours and 1430–1745 hours), resulting in an average total observation time of 39.5 h/pair. We used binoculars to identify individuals by band number. Social pairs were identified as a male and female that jointly defended a territory and/or mutually allopreened, stood or slept within 1 m of each other ([Pérez-Staples & Drummond, 2005](#); [Torres & Velando, 2003](#)) but had not yet laid eggs. EP males were any other males with whom the female reciprocated courtship. Our full data set consisted of 219 social pairs, of which 55 females (25%) reciprocated the courtship of an EP male and 23 (41% of courters) went on to copulate with that male.

Observers recorded frequencies of twiglifting and skypointing courtship displays (described in [Nelson, 2005](#)) and copulations by focal individuals and, every 15 min, the presence/absence of focal individuals in the neighbourhood ([Osorio-Beristain & Drummond, 1998](#); [Pérez-Staples & Drummond, 2005](#)). Because females engage in EP behaviours more frequently when their social mates are absent, we quantified female time alone as the number of scans in which the female was present without her social mate divided by the total number of scans she was present during the 4–5-day observation period. Interobserver reliability was tested before observations until there was at least 90% agreement on individual identities and behaviour variables.

To take into account the changes in social and EP behaviour frequencies that occur as egg laying approaches ([Osorio-Beristain & Drummond, 1998](#)), we determined the date that each female laid her first egg from the annual colony monitoring data, or by

systematically searching observed neighbourhoods for new clutches every other day for 30 days after concluding neighbourhood observations. We did not detect a clutch for 41.1% of our 219 focal females. It is likely that these nonlaying females established social partnerships in order to reproduce but abandoned before laying due to factors such as unfavourable environmental conditions (e.g. sea surface temperature; see [Ancona, Sánchez-Colón, Rodríguez, & Drummond, 2011](#)). As long-lived birds, boobies often skip years or abandon an incipient reproductive effort in unfavourable circumstances in favour of future attempts under better conditions ([Velando, Drummond, & Torres, 2006](#)). Laying on Isla Isabel routinely occurs from December through June, during which time oceanic, colony and individual conditions frequently change ([Ancona et al., 2011](#)), and during their weeks-long courtship periods, pairs have ample opportunity to abandon reproduction before investing in laying and incubation.

We pooled laying and nonlaying females because there was no evidence of differences in social or EP behaviour. The rate of courtship (skypointing displays per time together) shown by females towards their social mates did not differ between laying and nonlaying females (Kruskal–Wallis rank-sum test:  $\chi_1^2 = 0.86$ ,  $P = 0.35$ ), nor did the courtship they received from their social mates ( $\chi_1^2 = 0.387$ ,  $P = 0.53$ ). The proportion of females that engaged in EP courtship did not differ between laying (24.8%, 32 of 129) and nonlaying females (25.6%, 23 of 90; Fisher's exact test:  $P \approx 1$ ), nor did the proportion of females that went on to copulate with their EP partners (39.1%, 9 of 23 nonlayers; 43.8%, 14 of 32 of layers; Fisher's exact test:  $P = 0.79$ ). For analysis, we expressed latency between the end of observations and onset of laying using three categories: females that laid within 5 days of observations (presumed fertile period; see [Osorio-Beristain & Drummond, 1998, 2001](#)), those that laid 6–30 days following observations and those that laid more than 30 days following observations, or that never laid.

### *Colour and Body Measurements*

We measured the foot colour, body mass and ulna length of females, social males and EP males at night within 48 h of observation. Foot colour was measured using a Coloreye XTH portable spectrometer (X-rite, Grand Rapids MI, U.S.A.) in the UV and visible spectrum (360–700 nm). Measurements from three nonoverlapping patches of the webs of the left foot were averaged to generate a single reflectance spectrum per individual. We calculated green chroma as the proportion of total reflectance falling within the 460–620 nm wavelength range (following [Torres & Velando, 2007](#)). This is the spectral region in which foot colour reflectance varies most between individuals and is the region of highest visual sensitivity in this species ([Velando, Beamonte-Barrientos, & Torres, 2006](#)). We measured ulna length to the nearest 1 mm with a tape and measured body mass to the nearest 10 g with a 2500 g capacity Pesola spring balance. We calculated body condition from the residuals of linear regressions of body mass on ulna length in each year (both log transformed, following [Torres & Velando, 2003](#)). We obtained full morphological data for the female and social male of 125 pairs and 28 trios of female, social mate and EP male.

### *Ethical Note*

Protocols were reviewed and approved by Mexican authorities including the administration of Isla Isabel National Park, the National Commission for Protected Natural Areas (CONANP) and the Secretary of Environment and Natural Resources (SEMARNAT, permit number SGPA/DGVS/08333/10). We captured birds nocturnally during moonless periods and processed each one within

10 min to minimize stress both for the individual and for colony neighbours. Birds were released as close as possible to the initial capture site, usually within 1–2 m. Similar observation and capture methods have been successfully used for numerous previous studies in this colony (e.g. [Osorio-Beristain & Drummond, 2001](#); [Torres & Velando, 2003](#); [Velando et al., 2011](#)), and we observed no apparent adverse effects on individuals after release.

### Statistical Analysis

#### Classic good genes predictions

We tested whether the probability of EP courtship by females increases as social male quality decreases using a binomial generalized linear model (GLM, response variable: 0 = no EP courtship; 1 = reciprocal courtship with at least one EP male) and logit link function ([Crawley, 2007](#)). Our sample included the 125 social pairs for which full morphological and life history data were available. Female and social male age, green chroma, ulna, body condition and average standardized fledgling production were included as main effects in the initial model, as well as quadratic ages and the female\*male age interaction. Male and female ages were centred on their medians to avoid collinearity between main, quadratic and interaction terms (following [Schielzeth, 2010](#)). We also included female time alone in our model to account for potential differences in mate-guarding intensity among social males, which may affect female opportunity to engage in EP behaviours. Observation year and latency to lay the first egg were also included as covariates to control for their effects.

We tested whether females are more likely to proceed from courtship to copulation with their EP partners (1) as social male quality decreases and (2) as the EP male becomes increasingly superior to the social male, using GLMs with binomial error distribution and logit link (response variable: 0 = no EP copulations; 1 = at least one EP copulation). We restricted analyses of EP copulations to females that had reciprocated EP courtship rather than analysing the probability of EP copulation independent of courtship because EP copulation is always preceded by reciprocal courtship ([Osorio-Beristain & Drummond, 1998](#); [Pérez-Staples & Drummond, 2005](#); this study). Relative male quality was calculated by subtracting the social male's value from the EP male's value for each quality variable. We constructed a separate model for each quality variable (age, ulna, body condition, green chroma, average standardized fledgling production), and thus, sample sizes vary depending on the data available for each trio (see [Results, Table 2](#)). Each measure of social male quality was centred on its median to avoid collinearity with the interaction term (following [Schielzeth, 2010](#)). Sample sizes of females that courted EP males did not allow for simultaneous analysis of multiple quality variables or inclusion of female quality and male\*female quality interactions.

We tested whether EP males that females court and copulate with are phenotypically superior to the females' social mates by comparing the age, ulna length, body condition, foot green chroma and average standardized fledgling production of each female's pair of males. We performed paired *t* tests for normally distributed variables (body condition, green chroma) and Wilcoxon matched-pairs signed-ranks tests for variables with non-normal distributions (age, ulna length, lifetime fledging success), as determined by the Shapiro test of normality. Again, sample sizes varied depending on data availability (EP courtship comparisons, see [Fig. 2](#); EP copulations, see [Table 2](#)).

#### Conditional female choosiness

We tested whether females are more selective of EP males as the quality of their social mate increases by including the interaction term social male quality\*relative male quality in models testing for

the relationship between social male quality and EP copulation (see above). This prediction would be supported if EP copulations by females paired to below-average social males are independent of relative male quality and EP copulations by females paired to above-average males increase with EP male superiority.

We simplified all initial models by stepwise removal of nonsignificant terms, using ANOVA to compare consecutive models until we arrived at the minimum adequate model ([Crawley, 2007](#)). It has been argued that this method may increase the chance of type I error, especially in models with correlation among explanatory variables and/or a low number of observations per predictor (i.e. multicollinearity and overparameterization; see simulation study by [Forstmeier & Schielzeth, 2011](#)). To minimize this possibility, we evaluated potential multicollinearity in all initial and minimum adequate models by examining the variance inflation factor (VIF) for each variable using the 'car' package for R software ([Fox & Weisberg, 2011](#)). The VIF quantifies the increase in variance of a particular variable in the presence of the other variables in the model relative to a model in which it is included alone ([Zuur, Ieno, & Elphick, 2010](#); [Zuur, Ieno, Walker, Saveliev, & Smith, 2009](#)). A threshold of 4 is considered a conservative rule of thumb, while values  $\geq 10$  suggest potentially serious collinearity ([O'Brien, 2007](#); [Zuur et al., 2009, 2010](#)). In all of our analyses VIFs were  $< 4$ , making it unlikely that our models were affected by collinearity. To reduce overparameterization in our most saturated model (probability of EP courtship among all focal females), we included quadratic and interaction terms only for age, since previous work has shown a nonlinear relationship between age and individual quality (e.g. [Kim et al., 2011](#); see Introduction). While our initial model followed the minimum guideline of three events per variable ([Crawley, 2007](#); [Forstmeier & Schielzeth, 2011](#)), it included more variables than the more conservative rules of thumb recommend for our sample size (e.g.  $\geq 10$  events per predictor; see [Forstmeier & Schielzeth, 2011](#); [Peduzzi, Concato, Kemper, Holford, & Feinstein, 1996](#)). Beginning with a less parameterized model including only social male age and foot colour (the variables most likely to signal individual quality in this species based on previous studies; see Introduction) and control variables yielded identical results (see [Supplementary Table S1](#)). As suggested by [Forstmeier and Schielzeth \(2011\)](#), we provide full parameter estimates, standard errors and test statistics for initial and minimum adequate models (see [Results, Tables 1, 2, Supplementary Table S1](#)).

We used post hoc model diagnostics for binomial GLMs outlined by [Crawley \(2007\)](#). We used the 'exactRankTests' package ([Hothorn & Hornik, 2011](#)) for Wilcoxon matched-pairs signed-ranks tests to compute exact *P* values including ties. All analyses were carried out with R software, version 2.15.0 ([R Development Core Team, 2012](#)) using the RStudio interface ([RStudio, 2012](#)).

## RESULTS

### Classic Good Genes Predictions

The probability that a female would court an EP male increased with the youth of her social partner but was not related to any other measure of his quality ([Table 1, Fig. 1](#)). Surprisingly, females that spent more of their time accompanied by their social mates were more likely to court an EP male ([Table 1](#)). To explain this result, we explored the relationship between social male age and territory attendance. Using the full data set ( $N = 219$  pairs), we evaluated the proportion of time the male was present as a function of his age (linear and quadratic) with a grouped binomial GLM (using a logit link and quasi-likelihood to compensate for overdispersion; dispersion parameter = 7.19), with observation year and latency to lay included as covariates. Controlling for significant differences

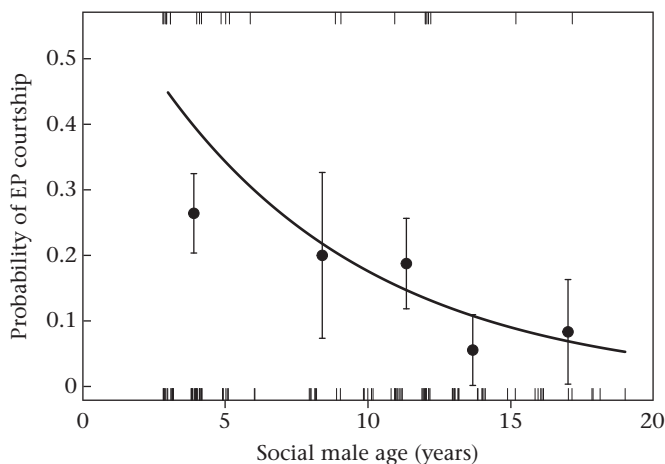
**Table 1**  
Results of a binomial generalized linear model (GLM) examining the effects of social male quality on the probability of female extrapair (EP) courtship in blue-footed boobies

	$\beta$	SE ( $\beta$ )	Z	P
<b>Initial model</b>				
<i>Social male</i>				
Age	-0.126	0.0764	-1.66	0.098
Age <sup>2</sup>	0.00577	0.0175	0.329	0.74
Ulna length	-0.0779	0.0546	-1.43	0.15
Body condition index	12.4	9.65	1.29	0.20
Green chroma	18.8	24.8	0.757	0.45
Avg. standardized fledgling production	0.0563	0.567	0.0990	0.92
<i>Female</i>				
Age <sup>2</sup>	0.0237	0.0158	1.50	0.13
Age	-0.0497	0.0797	-0.622	0.53
Ulna length	-0.0417	0.0601	-0.693	0.49
Body condition index	-21.3	11.1	-1.93	0.054
Green chroma	-2.31	16.1	-0.143	0.89
Avg. standardized fledgling production	-1.07	0.654	-1.63	0.10
Social male*female age interaction	-0.0235	0.0148	-1.59	0.11
% Female time alone	-5.68	3.02	-1.88	0.059
Observation year (2011)	2.10	1.31	1.61	0.11
Observation year (2012)	1.61	1.27	1.27	0.21
Latency to lay	-0.0894	0.554	-0.161	0.87
<b>Minimum adequate model</b>				
Age (social male)	-0.134	0.0571	-2.34	0.019*
% Female time alone	-4.57	2.33	-1.96	0.050*

Results of the initial saturated and minimum adequate model following backward stepwise simplification are given (see *Statistical Analyses* in *Methods*). Parameters for 2011 and 2012 observation years are with respect to 2010.  $N = 125$  focal pairs, in which 55 females engaged in EP courtship. Asterisks denote effects significant at an  $\alpha$  level of 0.05.

between observation years (GLM:  $F_{2,217} = 15.663$ ,  $P < 0.0001$ ), the likelihood that males would be on their territories during any given scan increased by 2.6% for each additional year of age ( $F_{1,216} = 5.374$ ,  $P = 0.021$ ), but was unrelated to the other covariates.

The probability that a female would copulate with the EP male that she courted was not significantly related to any measure of her social mate's quality or to the relative quality of her social and EP mates (main effects, *Table 2*).



**Figure 1.** Relationship between a male blue-footed booby's age and the probability of extrapair (EP) courtship by his social mate. Proportion of females that engaged in EP behaviour in 3-year bins of social male age is shown by black points  $\pm$  SE. Raw binomial data are shown as short vertical lines along the bottom (no EP behaviour) and top (female reciprocated courtship with at least one EP male) of the graph.  $P = 0.02$ ,  $N = 125$  focal pairs, of which 55 engaged in EP courtship. The curve shows predicted values, holding female time alone constant at its mean (0.19). Full model results in *Table 1*.

Females' social mates were not inferior in any measure of quality to the females' EP courtship (*Fig. 2*) or copulation partners ( $0.19 \leq P \leq 0.99$  for all five comparisons; data not shown). Indeed, average scores of EP males and social males on all five measures were very similar (*Fig. 2*). Inspection of individual scores suggests that similar average values were due to females reciprocating courtship and copulating with both superior and inferior EP males, rather than choosing EP males similar to their social mates (grey lines in *Fig. 2*).

### Conditional Female Choosiness

The probability of a female copulating with her EP courtship partner was related to the social male quality\*relative male quality interaction term for green chroma ( $P = 0.032$ ), but it was not related to the interaction term for any other measure of male quality (*Table 2*). EP copulation increased with the attractiveness of the EP partner relative to the social partner in females paired to social mates of above-average attractiveness (green chroma  $\geq 0.480$ : binomial GLM: deviance<sub>1,8</sub> = 8.11,  $P = 0.0044$ ), but not in females paired to social mates of below-average attractiveness (*Fig. 3*). Moreover, in females paired to unattractive males, the probability of EP copulation unexpectedly decreased as relative attractiveness of the EP male increased (green chroma  $< 0.480$ , binomial GLM: deviance<sub>1,16</sub> = 4.297,  $P = 0.038$ ). We hypothesized that this result could be due to assortative social mating and choosy EP males; females with unattractive partners could themselves be of below-average green chroma and therefore more likely to be rejected by increasingly attractive EP males. We tested this hypothesis using two additional analyses.

First, we tested for assortative mating by foot colour by examining the relationship between female and social male green chroma. Then, we constructed a GLM to test whether the interaction of female and relative EP male–female foot colour affected the probability of EP copulation. Results from both tests were consistent with our hypothesis: in the sample of 125 social pairs for which we scored foot colour for the males and females, a female's attractiveness was weakly correlated with that of her social mate (Pearson correlation:  $r_{123} = 0.356$ ,  $P < 0.001$ ), and among 27 females that reciprocated EP courtship, an unattractive female's (green chroma  $< 0.464$ , the average of all females captured) probability of copulating with him declined as his attractiveness exceeded hers, whereas an attractive female's probability of doing so increased with the EP male's attractiveness relative to her own (binomial GLM: deviance<sub>4,18</sub> = 4.235,  $P = 0.039$ ; *Fig. 4*).

## DISCUSSION

### Classic Good Genes Model

As is common in the literature, support for the classic good genes predictions was mixed. Females were more likely to court EP males if their social partners were young, but copulation by females with their EP courtship partners was unaffected by either social mate quality or EP male superiority, and no measure of quality differed consistently between social males and their EP rivals. Higher probability of EP courtship by partners of young males supports the good genes hypothesis if it is due to female preference for older sires, perhaps because their longevity indicates high genetic quality (Kokko, 1998; but see Hansen & Price, 1995). Females paired to young males could also experience a higher degree of uncertainty about their mate's quality if the reliability of sexual signals increases with age (Proulx, Day, & Rowe, 2002). This would seem particularly relevant for ornaments mediated by oxidative stress, where older individuals may have more accumulated oxidative damage or tissues that are more vulnerable to oxidation



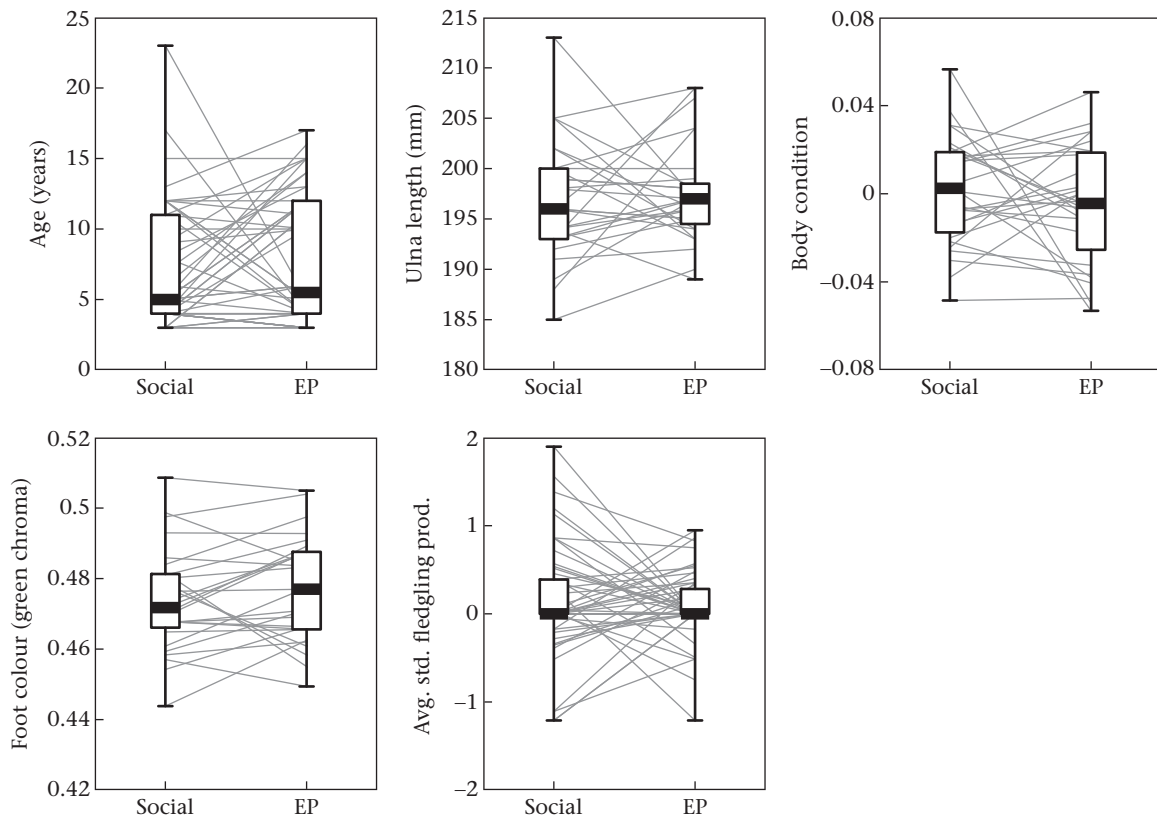
**Table 2**  
Results of five separate binomial generalized linear models (GLMs) examining the probability that a female blue-footed booby would copulate with her extrapair (EP) courtship partner as a function of each aspect of social male quality, relative quality (EP versus social male), and their interaction

N	EP copulation probability as a function of	Stepwise removal		Initial model results			
		$\Delta$ Deviation	$P(\chi^2)$	$\beta$	SE ( $\beta$ )	z	$P(z)$
50 (24)	<b>Age</b>						
	Social male	$3.01 \times 10^{-4}$	0.99	-0.011	0.0872	-0.129	0.90
	Relative	0.06	0.81	0.023	0.0737	0.315	0.75
	Interaction	0.09	0.76	$-2.47 \times 10^{-3}$	$8.14 \times 10^{-3}$	-0.303	0.76
28 (14)	<b>Ulna length</b>						
	Social male	0.08	0.77	0.044	0.105	0.419	0.68
	Relative	0.29	0.59	0.054	0.0915	0.596	0.55
	Interaction	3.77	0.053	-0.018	0.0128	-1.48	0.14
28 (14)	<b>Body condition</b>						
	Social male	2.42	0.12	40.1	24.8	1.620	0.11
	Relative	1.05	0.31	24.5	18.6	1.322	0.19
	Interaction	1.14	0.29	-473.3	476.6	-0.993	0.32
27 (13)	<b>Green chroma</b>						
	Social male	0.29	0.59	-42.8	41.0	-1.04	0.29
	Relative	0.02	0.88	-2.91	39.2	-0.074	0.94
	Interaction	4.60	0.032*	$7.28 \times 10^3$	$4.17 \times 10^3$	1.75	0.081
56 (26)	<b>Avg. standardized fledgling production</b>						
	Social male	0.67	0.41	-0.662	0.867	-0.764	0.45
	Relative	0.14	0.71	-0.272	0.737	-0.370	0.71
	Interaction	0.04	0.84	-0.0889	0.434	-0.205	0.84

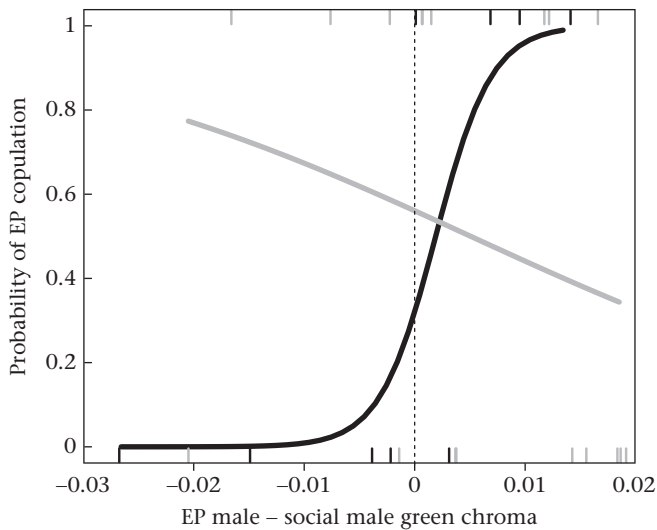
Sample sizes are given as the number of females with EP courtship included in each model, followed by the number of those females that went on to copulate with their EP partners in parentheses. Results of  $\chi^2$  tests between nested models following removal of each variable during backward stepwise model simplification are given ('stepwise removal'), as well as parameters from initial models including social male quality, relative male quality, and their interaction ('initial model results'). Asterisks denote effects significant at an  $\alpha$  level of 0.05.

(Monaghan, Metcalfe, & Torres, 2009). Consistent with this suggestion, low foot colour attractiveness in male blue-footed boobies predicted sperm DNA damage in senescent males but not in younger males (Velando et al., 2011). According to Hasson and

Stone's (2011) model, less reliable phenotypic indicators of quality would lead to females being less discriminating towards potential EP males, and therefore more likely to engage in EP behaviours.

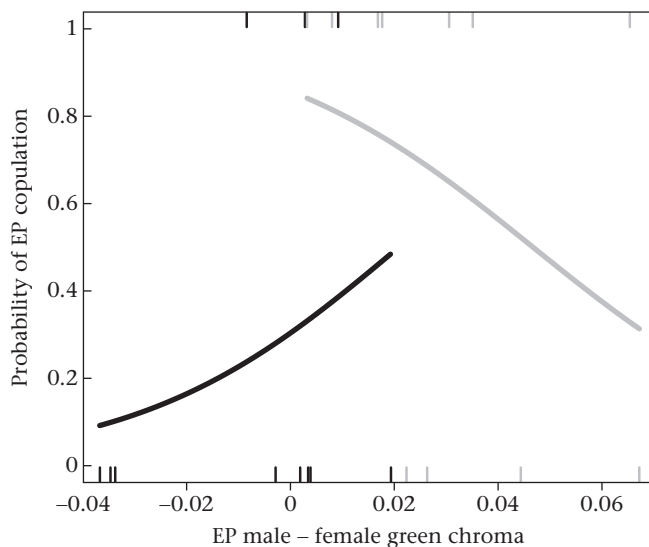


**Figure 2.** Comparisons of morphological and life history characteristics of social male blue-footed boobies and the extrapair (EP) males that their mates courted. Boxes show medians, quartiles and ranges of social and EP male characteristics; grey lines link the social and EP mates of each female (Wilcoxon exact signed-ranks tests: age:  $T = 293$ ,  $N = 50$ ,  $P = 0.18$ ; ulna length:  $T = 174$ ,  $N = 28$ ,  $P = 0.73$ ; average standardized fledgling production:  $T = 478.5$ ,  $N = 56$ ,  $P = 0.74$ ; paired  $t$  tests: body condition:  $t_{27} = 0.958$ ,  $P = 0.35$ ; green chroma:  $t_{26} = -1.160$ ,  $P = 0.26$ ).



**Figure 3.** Probability that female blue-footed boobies would copulate with the extrapair (EP) male that they courted as a function of the interaction between foot colour of the social male and foot colour of the EP male relative to the social male. Curves show predicted probability of EP copulation based on the difference between EP and social male green chroma when the social male had green chroma above ( $\geq 0.480$ , black) and below ( $< 0.480$ , grey) the average of all males captured (social + relative green chroma interaction:  $P = 0.032$ ; see Table 2 for full model results). Raw binomial data for these attractive (black) and unattractive (grey) social males are shown as short vertical lines along the bottom (EP courtship only) and top (EP copulations) of the graph. The dashed vertical line indicates social and EP males of equal green chroma.

Alternatively, higher probability of EP courtship by mates of young males could be due to poor paternity guards in young males (Arnqvist & Rowe, 2005). Reduced size, status or competitive ability of young males could weaken their ability to guard their mates (Wagner, Schug, & Morton, 1996) or discover and retaliate against infidelity. Indeed, younger males spent less time on their territories,



**Figure 4.** Probability that female blue-footed boobies would copulate with the extrapair (EP) male that they courted as a function of the interaction of female and relative (EP male/female) foot colour attractiveness. Curves show predicted probability of EP copulation based on the green chroma of females relative to the EP males that they courted when the female's green chroma was above ( $\geq 0.464$ , black) and below ( $< 0.464$ , grey) the average of all females captured (female + relative EP male – female green chroma interaction:  $P = 0.039$ ). Short vertical lines along the bottom (EP courtship only) and top (EP copulations) of the graph indicate raw binomial values for females of above- (black) and below-average (grey) green.

perhaps because inexperience makes them less efficient foragers, similar to royal terns, *Thalasseus maximus* (Buckley & Buckley, 1974), and little penguins, *Eudyptula minor* (Zimmer et al., 2011). Another (nonexclusive) possibility is that if older males' parenting experience makes them more valuable as social mates, their social partners may be less willing to risk a valuable pair bond by engaging in EP behaviour, despite potential genetic benefits (Gowaty, 1996).

Males that most often accompanied their partners on the territory were the most likely to be cuckolded, probably because cues of high infidelity risk or witnessing EP courtship elicit more mate guarding (Kokko & Morrell, 2005), as in blue tits, *Cyanistes caeruleus* (Kempnaers, Verheyen, & Dhondt, 1995), purple martins, *Progne subis* (Wagner et al., 1996), and bluethroats, *Luscinia svecica* (Johnsen, Lifjeld, & Krokene, 2003). In blue-footed boobies, unfaithful females have different territory attendance schedules than faithful ones, and their mates adjust their own attendance schedules to match, suggesting that mate guarding does occur (Pérez-Staples & Drummond, 2005). Even so, guarding appears ineffective at preventing females from establishing EP relationships (Pérez-Staples & Drummond, 2005), although unfaithful females do reciprocate 50% less EP courtship and engage in four to six times fewer EP copulations when their mates are present (Osorio-Beristain & Drummond, 1998; Pérez-Staples & Drummond, 2005). Mate guarding therefore probably functions to reduce the frequency of female EP behaviours to some degree, and may allow partners to monitor each other's behaviour in order to calibrate investment or switch mates (Osorio-Beristain & Drummond, 2001; Pérez-Staples & Drummond, 2005; Pérez-Staples et al., 2013).

#### Variation in Female EP Choosiness

The probability of a female copulating with her EP partner increased as he was increasingly superior to her social mate, but this selectivity was observed only when the social partner was attractive. To our knowledge, this result provides the first empirical support for diminishing female choosiness with respect to EP males as the attractiveness/quality of social males decreases, as outlined in Hasson and Stone's (2011) model. Moreover, at least two of the theoretical model's three main assumptions appear to be applicable to blue-footed boobies.

First, there are good grounds for inferring that female choosiness of EP males is costlier in terms of time, energy and risk than indiscriminate EP copulation. Female boobies court reciprocally with particular EP partners for up to 40 days before laying (Osorio-Beristain & Drummond, 1998), spend an average 8% of daylight hours actively courting with those partners (Pérez-Staples & Drummond, 2005), and on average perform 19 skypointing displays to (and receive 52 skypointing displays from) EP males per day (Kiere & Drummond, 2013), potentially imposing time and energy costs that may trade off against other important activities such as foraging, territory defence or within-pair interactions. In addition, since courting is more conspicuous than copulation, the choosier the female (the more she courts per copulation), the higher the risk of detection by her social mate, which may lead to dissolution of the pair before laying or egg infanticide by the social mate (Osorio-Beristain & Drummond, 2001; Pérez-Staples et al., 2013).

Second, although foot colour is a phenotypic cue used in assessment of booby mates, it may not always indicate individual quality precisely or reliably. Male foot colour is correlated with age, germ-line DNA damage, and recent nutrition and illness (reviewed in: Torres & Velando, 2010; Velando et al., 2011), but integration of these effects into one signal (green chroma) could lead to ambiguity, for example, between an individual's ability to acquire resources and allocation of those resources to specific fitness components, including ornaments (see Hunt, Bussière, Jennions, &

Brooks, 2004). It is also possible that dynamic ornaments like foot colour signal some specific attribute such as fertility (Hasson & Stone, 2009) or readiness to breed (S. C. Griffith, personal communication) rather than, or in addition to, overall individual quality. Unlike more stable traits such as adult body size, the dynamic nature of foot colour means that in the short term it could be misleading as a signal of genetic quality. This balance of information and uncertainty makes foot colour the type of imperfectly reliable ornament that is likely to generate flexible female choosiness in Hasson and Stone's (2011) model. The model's third assumption, that postcopulatory selection confers an advantage to sperm carrying better or more compatible genes, is increasingly endorsed by evolutionary theorists in the context of mixed reproductive strategies (Griffith & Immler, 2009; Kvarnemo & Simmons, 2013; Mays & Hill, 2004) but remains to be investigated in boobies.

#### *Female Constraint by EP Male Choosiness*

Unexpectedly, in females paired to unattractive (below-average green chroma) males, copulation probability tended to decrease with increasing EP male superiority to the social male. That this may be due to rejection of unattractive females by superior EP males was supported by two additional results: females paired to unattractive males tended to be unattractive, and the more an EP courtship partner outshone the unattractive female in green chroma, the less likely a copulation occurred (Fig. 4). There is experimental support for male blue-footed boobies discriminating against unattractive females: Torres and Velando (2005) found that females with artificially dulled feet received less courtship from both social mates and potential EP males. Our findings suggest that when females are attractive, they are the limiting, choosy sex in EP interactions, but when females are unattractive, EP males assume this role. Female foot colour is relevant to EP males because it is correlated with both immune activation and recent nutrition and may inform them on females' genetic quality, health status and ability to invest in offspring (Morales, Velando, & Torres, 2009). Selection among females by EP males is not contemplated within the classic good genes framework or in Hasson and Stone's (2011) model, but it is likely to occur when the probability of EP copulations leading to fertilization is low and gaining EP fertilizations is costly to males. Although 38–100% of female boobies court and 33–56% copulate with EP males at some point during the prelaying period (Osorio-Beristain & Drummond, 1998; Pérez-Staples & Drummond, 2005), only about 11% of female blue-footed boobies produce EP offspring (Ramos et al., in press), so males that dedicate many hours over a period of days or weeks to EP courtship could incur substantial costs for meagre average returns. In addition, depleting sperm by copulating with low-quality females may compromise both within-pair paternity and EP paternity with higher-quality females, and copulation with multiple females could increase exposure to sexually transmitted infections (Sheldon, 1993).

#### *Conclusions*

As has so often happened, analysing the effect of a sexual ornament on EP behaviour largely failed to support the classic good genes predictions, although females were more likely to court an EP male when their social mates were young. However, a more detailed behavioural analysis taking into account the quality of all three individuals involved (female, social mate, EP male), directly supported a conditional mating strategy in which a subset of females potentially gain good genes benefits by violating rather than following the classic predictions. While female blue-footed boobies that were socially paired with attractive males engaged in copulations only with EP partners that were more attractive than their

mates, females that were socially paired with unattractive males were relatively unselective, and may have been discriminated against by more attractive EP males. Similar patterns of conditional EP choosiness by both sexes could account for some of the inconsistencies in a field of inquiry that may have been hampered by excessive dependence on analyses of genetic outcomes rather than observation of behavioural processes. Interestingly, the factors influencing EP courtship apparently differ from those influencing copulation, the next step in EP behaviour in this species. Key issues awaiting resolution are whether the choices made by booby females (choosy and nonchoosy ones) lead to genetic benefits to offspring, and whether they do so through mate choice and postcopulatory sperm selection in the manner suggested by current models.

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#### **Supplementary Material**

Supplementary material for this article is available, in the online version, at <http://dx.doi.org/10.1016/j.anbehav.2014.07.007>.

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## Capítulo 2:

### Prueba de la hipótesis de compatibilidad genética



Foto: Esther Arce

*"No hay evidencia que la compatibilidad genética motiva la conducta extra-pareja en hembras del bobo de patas azules"*

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# No evidence that genetic compatibility drives extra-pair behavior in female blue-footed boobies

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The function of female birds' extra-pair (EP) behavior has remained an unresolved question in ornithology and behavioral ecology for > 30 yr. The genetic compatibility hypothesis (GCH) proposes that females benefit by acquiring biological sires that yield more heterozygous, and therefore fitter, offspring than their social mates. We used ten polymorphic microsatellite loci to test GCH predictions and its assumption that fitness increases with heterozygosity in blue-footed boobies *Sula nebouxii*, a long-lived tropical seabird. Our predictions were not supported. Heterozygosity was uncorrelated with quality indicators (fledging probability, fledgling or adult body size or mass, adult ornamentation, mean breeding success). Females were no more likely to have EP behavior or chicks when their social mates were less heterozygous or compatible, nor were EP males more heterozygous or compatible than the males they cuckolded. Finally, EP chicks were no more heterozygous than within-pair chicks overall or in half-sib comparisons, nor were within-pair chicks from all-within-pair nests more heterozygous than those with EP nest-mates. There are both methodological and biological explanations for these consistently negative results. Inadequate sample size is possible but unlikely, since our samples were comparable or larger than those of similar studies with significant findings. Lack of identity disequilibrium (within-individual heterozygosity correlation) among our marker loci could be responsible, and suggests either insufficient marker coverage or lack of inbreeding, bottleneck, and/or admixture. An independent social pedigree revealed infrequent inbreeding, suggesting that pressure on females to select sires that maximize offspring heterozygosity may be genuinely lax. Alternatively, it is possible that the GCH is only upheld when selection on young is strongest; this would not be detected in our sample, which was taken during an extremely productive year. Whatever their cause, our results expand the taxonomic breadth of avian EP behavior analyses and should be considered in future evaluations of the GCH.

Ever since molecular paternity assignment revealed the widespread occurrence of extra-pair (EP) paternity among socially monogamous birds over three decades ago, the function of female EP behavior has remained a major unresolved question in ornithology and behavioral ecology. Numerous explanations have been proposed (reviewed by Petrie and Kempenaers 1998, Griffith et al. 2002, among others) including some that are non-adaptive for females (reviewed by Forstmeier et al. 2014). Currently, the most investigated class of hypotheses suggests that females gain indirect (genetic) benefits, improving their offspring's (and ultimately their own) fitness by acquiring biological sires that are superior to their social mates (reviewed by Akçay and Roughgarden 2007), either because they contribute specific genes that increase offspring viability and/or attractiveness ('good genes'), or because their more diverse and/or compatible genomes increase offspring heterozygosity ('genetic compatibility') and fitness.

Research interest in the genetic compatibility hypothesis (hereafter, GCH) is considerable, and the increasing accessibility of molecular markers has led to a recent boom of empirical evaluations in birds (reviewed by Akçay and

Roughgarden 2007, Arct et al. 2015, Hsu et al. 2015). This increased attention has nonetheless yielded no clear consensus on whether (or under what circumstances) increasing offspring heterozygosity via EP paternity is an important driver of female birds' EP behavior. Individual empirical studies have yielded contrasting results (Akçay and Roughgarden 2007) and recent meta-analyses have been inconclusive. For example, one main GCH prediction is that the incidence of EP young should increase with increasing social pair relatedness (genetic similarity). Using the 23 relevant studies published at the time, Akçay and Roughgarden (2007) found the overall effect to be non-significant, while Arct et al. (2015), using an updated database of 39 studies, found a small but significant positive effect consistent with the GCH. Similar to Akçay and Roughgarden (2007), in a meta-analysis including 30 studies Hsu et al. (2015) found no significant difference between social and EP males' genetic similarity to the focal female. However, in view of high heterogeneity of effect sizes (Arct et al. 2015), potential sampling bias due to non-random offspring mortality prior to sampling (Reid 2015), inconsistent definitions of EP paternity (Griffith 2015), and a large number of studies and



species for which data are available but unreported (> 90%, according to Forstmeier 2015), it is still unclear how general the GCH is as an explanation of female birds' EP behavior. Furthermore, the current literature offers limited coverage of avian taxonomic, geographic, and life-history diversity. For example, 82% of the studies (32 of 39) reviewed by Arct et al. (2015) were of Passeriformes, with several families (and even species) represented by multiple studies, while the remaining seven studies included four avian orders, two of which were represented by a single study/species. A primary aim of this paper, using relatively large sample sizes for a long-lived tropical seabird, is thus to contribute to filling in such gaps; this is the first study we know of to evaluate GCH predictions in the family Sulidae (gannets and boobies), and only the second in the order Suliformes (see study of *Fregata minor* in Juola and Dearborn 2012).

An important assumption of the GCH is that increasing individual heterozygosity improves fitness (i.e. positive heterozygosity-fitness correlation due to 'general effects', reviewed by Chapman et al. 2009). This is an extension of the widely observed tendency for inbreeding over multiple generations to lead to decreased offspring fitness that then improves when distinct inbred lines are crossed. However, it is possible that inbreeding is only problematic beyond an extreme threshold value or only at particular loci; whether there is a continuous correlation between fitness and genome-wide heterozygosity as quantified by neutral molecular markers remains controversial (reviewed by Chapman et al. 2009, Szulkin et al. 2010). A main prediction of the GCH is that EP mating increases offspring heterozygosity. It is debated, though, whether this is best achieved by female preference for males that are themselves highly heterozygous ('heterozygosity theory', sensu Brown 1997) or males that are genetically dissimilar to the female ('compatibility' sensu Tregenza and Wedell 2000).

Here, we evaluate using putatively neutral markers whether the GCH (in its heterozygosity or compatibility variants) explains the EP behavior of females of the blue-footed booby *Sula nebouxi*, a long-lived seabird that breeds colonially in the Eastern Tropical Pacific. Socially monogamous pairs jointly raise broods of 1–3 chicks. Promiscuous females may be divorced (Pérez-Staples et al. 2013) or have their first egg destroyed by the social male (Osorio-Beristain and Drummond 2001) due to paternity doubt, and they reduce their participation in EP behaviors under adverse El Niño conditions (Kiere and Drummond 2016), all of which suggest that EP behaviors can be costly. Even so, females frequently sustain relationships with one or more EP partners before laying, and EP chicks are found in 11% of nests (Ramos et al. 2014). If heterozygosity is advantageous in this population, as assumed by the GCH, we expected a positive effect of individual heterozygosity on fitness-related traits (fledging probability, fledgling and adult size and mass, sexual ornament expression, and mean breeding success). If female choice of biological sires is based on males' heterozygosity, we expected the probability of EP behaviors and paternity to decrease with increasing social mate heterozygosity and for EP males to be more heterozygous than the social mates they cuckolded. If, alternatively, females choose biological sires on the basis of genetic dissimilarity, we

expected the probability of EP behaviors and paternity to increase with increasing relatedness between the female and her social mate, and for females to be more closely related to their social mates than their EP males. Finally, if females' EP pairings lead to higher chick heterozygosity, we expected EP chicks to have higher heterozygosity than within-pair chicks in overall and paired comparisons of maternal half-sibs, and for within-pair chicks from nests with no EP chicks to have higher heterozygosity than those from mixed-paternity nests.

## Methods

### Microsatellite genotype sampling

The study was carried out in the highly philopatric booby population of Isla Isabel, Mexico (21.85°N, 105.89°W), where annual monitoring of reproduction, including identifying parents, recording nest contents every 3–6 d, mapping nests, and measuring and banding fledglings, has been carried out since 1988 (hereafter, 'long-term study'; details in Drummond et al. 2003).

Blood samples for microsatellite genotyping and paternity assignment were obtained in 2011 from both putative parents and their 1–3 live chicks at all 478 nests in an 8450-m<sup>2</sup> plot within the study area where at least one chick reached 10 d of age between February and May (Ramos et al. 2014). Adults were captured at night, and their chicks during the day, when chicks were 10–40 d old. A 75 µl blood sample was taken by brachial venipuncture and stored immediately in a lysis buffer (1.0 M Tris: 0.5 M EDTA: 5.0 M NaCl: 10% SD) until DNA extraction. During capture, adults' bill and ulna lengths were measured to the nearest mm using a flexible tape and body mass to the nearest 10 g using a Pesola spring balance. We observed no apparent adverse effects on birds following observation, capture, or blood sampling (details in Kiere and Drummond 2014, Ramos et al. 2014).

DNA was extracted using Illustra Blood GenomicPrep Mini Spin Kits (GE Healthcare, Buckinghamshire, UK), amplified by PCR, and genotyped for ten polymorphic microsatellite loci (3–22 alleles each). Hardy–Weinberg equilibrium and absence of null alleles was verified for all ten loci (Raymond and Rousset 1995). We excluded from analyses individuals typed at < 6 loci (see Faircloth et al. 2009 for full genotyping details). Parentage was assigned using a likelihood-based approach in CERVUS 2.0 (Marshall et al. 1998), with a 95% strict assignment level. When assignment indicated that a chick was EP, we searched among the genotyped adult males for the biological sire (full details in Ramos et al. 2014).

In total, 440 adult males, 428 adult females, and 810 chicks from 463 nests were successfully genotyped at ≥ 6 loci, including 398 complete families (both social parents and all hatched chicks). Paternity was assigned as within-pair for 92.8% (675/810) and EP for 7.2% (52/810) of chicks; we detected at least one EP chick in 10.3% (41/398) of complete families, and the biological sire was identified for 30.8% (16/52) of EP chicks.

## Heterozygosity and relatedness variables

We used the R package GenHet (Coulon 2010) to calculate standardized heterozygosity (following Coltman et al. 1999, 'Hs\_exp' in GenHet, hereafter SH) for all genotyped individuals. This index was highly correlated with the other four common indices calculated by GenHet ( $0.95 < |r| < 0.99$  in all pairwise correlations). We also calculated mean  $d^2$ , an alternative heterozygosity index specific to microsatellites that quantifies as more distinct alleles which differ more in length, and thus is thought to express inbreeding events deeper in individuals' lineages than SH (Coulson et al. 1998). Mean  $d^2$  (Coulson et al. 1998) was calculated as the squared difference in number of microsatellite repeat units between the two alleles at each locus, divided by the number of loci typed. As expected, mean  $d^2$  was highly right-skewed with values ranging from 0 to 1200; it was log-transformed (Neff 2004; hereafter,  $Md^2$ ) for all analyses to condense this scale and improve model convergence. However, using untransformed mean  $d^2$  yielded similar results for all analyses. According to visual inspection of Q-Q plots, SH was normally distributed, with a global mean of 0.978 and standard deviation of 0.262. Log transformation left  $Md^2$  somewhat left-skewed, with a global median of 5.03 and inter-quartile range of 2.26. Neither heterozygosity measure differed between chicks and adults (SH: Welch's t-test;  $t = -0.35$ ,  $DF = 1658.2$ ,  $p = 0.70$ ;  $Md^2$ : Wilcoxon rank sum test;  $W = 341380$ ,  $p = 0.27$ ) or between adult males and females (SH: Welch's t-test;  $t = -0.047$ ,  $DF = 866.42$ ,  $p = 0.45$ ;  $Md^2$ : Wilcoxon rank sum test;  $W = 94920$ ,  $p = 0.93$ ).

Pairwise relatedness between females and their social and EP mates was calculated using COANCESTRY ver. 1.0.1.5 (Wang 2011). Because it is not clear a priori which of the many proposed relatedness indices is most suitable for a particular population (Rollins et al. 2012), we followed the methods of Rollins et al. (2012), selecting the index most similar to the pedigree-based inbreeding coefficient. From our genotyped sample we chose 119 pairs of individuals from four relatedness categories (confirmed by paternity assignment): parent/offspring, full sibling, maternal half-sibling, and unrelated individuals ( $n = 30, 30, 29$ , and  $30$ , respectively). We then calculated the correlation coefficient between the expected relatedness for each category (0.5, 0.5, 0.25, and 0, respectively) and each of the 14 indices calculated by COANCESTRY (7 distinct indices, each with and without inbreeding correction). To avoid erroneously classifying pairs of individuals as 'unrelated', we included in these pairs only individuals whose ancestry was known up to at least all four grandparents. We then constructed a social pedigree of all individuals from our long-term database for whom (at least) all grandparents were known (constructed using Pedigree Viewer ver. 6.5b, Kinghorn and Kinghorn 2010), and verified that our putatively unrelated pairs shared no relatives. The DyadML index (uncorrected for inbreeding) was the most highly correlated with the expected values ( $R = 0.754$ ,  $n = 119$ ), and thus only this index was calculated and used for subsequent analyses.

## Behavioral variables

Behavioral observations were carried out in the study area during the first peak of laying (December to March) during four breeding seasons (2010–2013). We observed neighborhoods of 2–6 females and their mates from a portable blind during the daylight hours of maximum activity (06:45–11:45 and 14:30–17:45 h) during 4–5 consecutive days (average 39.5 h per pair). We recorded the identity of the females, their social mates and any other males with whom the females reciprocated courtship or copulated (EP males), and the absolute frequency and sequence of those behaviors. Observations occurred after social pairs had been established but before they laid their first egg. In thousands of hours of observation, EP copulation was always preceded by reciprocal courtship; apparent forced copulations have never been observed (Osorio-Beristain and Drummond 1998, Kiere and Drummond 2014). Included in these behavioral observations were 101 of the males and 76 of the females genotyped in 2011. We were able to capture 155 of the observed (and genotyped) individuals at night within 48 h of observation to measure their bill and ulna length, body mass, and foot color (using an XTH Color-eye portable spectrometer; X-rite, Grand Rapids MI, USA). See Kiere and Drummond (2014) for details of behavioral observations and capture.

## Life-history variables

From the long-term study database (detailed in Drummond et al. 2003), we obtained data on fledging probability, fledging body size and mass, and mean breeding success for use as response variables, and age (years), proportional lay-date (a proportional rank from 0 to 1 expressing when the natal clutch was laid relative to all other clutches in that breeding season), brood size (number of chicks in the natal nest), and hatch order (whether an individual was the first, second, or third to hatch in its natal nest) for use as covariables, in heterozygosity-fitness correlation analyses. Specific samples used in each analysis are described along with statistical models in the following section.

## Statistical analyses

### Heterozygosity-fitness correlations

We evaluated the relationship between individual heterozygosity (linear and quadratic SH or  $Md^2$ ) and fledging probability, fledgling and adult body measurements, adult foot color, and adult mean breeding success using generalized linear mixed models (GLMMs).

We evaluated the effect of heterozygosity on chick survival using fledging probability, a binary variable expressing whether the chicks blood-sampled in 2011 died (0) or survived (1) between sampling (10–40 d old) and fledging (70 d old). These analyses included brood size and hatch order as covariables to account for possible effects of nest-mate competition and facultative brood reduction (Drummond et al. 2003), and proportional laydate to account for the decreased success of late-season broods (Peña Alvarez 2009). Nest identity was included as a random effect to account for



pseudoreplication from including multiple chicks per nest. Of the 810 chicks genotyped in 2011, 806 were included in this analysis (four were excluded because survival data were unavailable).

To test for effects of heterozygosity on body size at fledging, we used the body measurements taken at fledging (age 70 d) of the adults genotyped in 2011. Chicks genotyped in 2011 were not used for this analysis because their sex was not known and females are larger than males by fledging (Drummond et al. 1991), generating bimodally distributed response variables that could not be analyzed using GLMMs. Using fledging measurements of breeding adults could potentially bias the heterozygosity of our sample if, for example, low-heterozygosity individuals were less likely to survive or recruit and thus culled from the sample (or vice-versa). However, there was no evidence of that here, since individuals genotyped as chicks did not differ from those genotyped as adults in either heterozygosity index. Bill, ulna, and mass at fledging were standardized separately by sex to account for dimorphism and generate normally distributed response variables (assessed graphically using Q-Q plots). These models also included brood size, hatch order, and proportional laydate as covariables, and included birth cohort as a random effect to account for variation in individual quality and life history due to early life conditions (Ancona and Drummond 2013). Of the adults genotyped in 2011, full data were available for 750, 747, and 698 individuals, respectively, for analyses of bill, ulna, and mass at fledging.

We analyzed the effect of heterozygosity on adult body size and mass using measurements of the breeding adults genotyped in 2011; measurements were taken when they were captured for blood sampling in 2011 or following behavioral observations in 2010–2013. Adult bill, ulna, and mass were standardized separately by sex and observation year to account for dimorphism and possible inter-annual variation in the quality of individuals participating in breeding. Linear and quadratic age (at the time of measurement) were included as covariables to account for the possibility that body size continues to increase after recruitment, and may subsequently level off (bill and ulna) or decrease due to senescence (body mass; Beamonte-Barrientos et al. 2010). Birth cohort was included as a random factor. Of the adults genotyped in 2011, complete data were available for 642, 642, and 639 individuals for bill, ulna, and mass analyses, respectively.

The effect of heterozygosity on adult foot color was analyzed using measurements taken following behavioral observations in 2010–2013 from adults genotyped in 2011. Foot color was expressed as ‘green chroma’, the proportion of total foot reflectance (360–700 nm wavelength) occurring between 460 and 620 nm (following Torres and Velando 2010), and was standardized by sex and observation year. Linear and quadratic age (at the time of measurement) were included as covariables to account for possible increase followed by senescence (Torres and Velando 2010), and birth cohort was included as a random effect. Both foot color and age data were available for 124 of the adults genotyped in 2011.

Finally, we evaluated the effect of heterozygosity on reproductive performance by calculating the mean breeding success of adults genotyped in 2011. This index consisted of

summing the number of fledglings the individual produced every year (standardized by the mean number produced by pairs in that year) between birth and 2013 (the latest available year of data) and dividing this total by the adult’s total number of breeding attempts (including multiple and failed nests in a single year, as in Kiere and Drummond 2014). Mean breeding success was standardized by sex. Linear and quadratic age (at the time of the individual’s latest breeding attempt) were included as covariables, and birth cohort was included as a random effect. Full data were available for 749 of the adults genotyped in 2011.

#### ***Heterozygosity-based sire choice***

We generated six separate generalized linear models (GLM) and GLMMs to test whether increased social male SH and/or  $Md^2$  was associated with decreased probability of females engaging in EP courtship, escalating from EP courtship to EP copulation, or having EP chicks. EP copulation analyses were restricted to the 19 females that engaged in EP courtship, since courtship always preceded copulation. No covariables were included in these analyses. Observation year was included as a random term for EP courtship and copulation analyses because observations were carried out in four different years, but not for EP paternity analyses, since all paternity data were from 2011.

Since both maternal and paternal heterozygosity contribute to offspring heterozygosity (García-Navas et al. 2009), it is possible that females’ EP behaviors are driven by their own (rather than the social mate’s) low heterozygosity. Or, lower social male heterozygosity may only increase EP behavior when females’ own heterozygosity is also low (interaction effect). To test these two possibilities, we repeated the EP courtship and paternity models above using the heterozygosity of the female, her social mate, and their interaction as the independent variables of interest. Sample sizes for these analyses were smaller than for male-only analyses (Table 2) because heterozygosity data were not always available for both the female and social male of each pair; this prevented us from analyzing EP copulation probability.

For the 16 cases in which EP sires were identified, we carried out paired comparisons of the SH and  $Md^2$  of the chicks’ social and EP biological fathers to determine whether EP males were more heterozygous than the social males they cuckolded (see general statistical methods below).

#### ***Compatibility-based sire choice***

We constructed two separate GLM/GLMM models to examine whether greater female-social male relatedness was associated with greater probability of EP courtship and EP paternity. Because heterozygosity data were not always available for both members of the social pair, sample sizes were reduced for this analysis compared to analyses of heterozygosity-based sire choice, and were too small to analyze EP copulation probability. Again, observation year was included as a random effect in the EP courtship but not the EP paternity model, and no covariables were included.

To determine whether females were more closely related to their social than their EP mates, we directly compared females’ relatedness to their social and EP males using the 16 pairs for which EP sires were identified (see general statistical methods below).

### EP paternity and chick heterozygosity

We used paired and unpaired tests to compare the SH and Md<sup>2</sup> between within-pair and EP chicks and between within-pair chicks with and without EP half-sibs to determine whether EP paternity led to higher chick heterozygosity. In all comparisons, only the oldest chick of each type (within-pair or EP) was included from each nest to avoid pseudoreplication. For overall within-pair versus EP chick comparisons, we included all chicks for which paternity could be assigned, regardless of their nest-mates' paternity (444 chicks total; 396 within-pair, 47 EP). However, for the comparison of within-pair chicks from all-within-pair versus mixed paternity nests, only chicks from the 381 nests with within-pair chicks (24 from mixed-paternity and 357 from within-pair-only nests) in which all hatched chicks were successfully genotyped were included to avoid falsely classifying nests as within-pair-only.

### General statistical methods

When testing for effects of heterozygosity and relatedness using generalized linear (GLM) and mixed models (GLMM), we minimized the number of covariables included to avoid overparameterization and used a 'full model test' procedure to avoid cryptic multiple hypothesis testing (Forstmeier and Schielzeth 2011). This procedure consisted of using a  $\chi^2$  likelihood ratio test to compare a 'full' model containing 1) the effects of main interest, 2) other covariables previously shown (or strongly suspected) to influence the response variable, and 3) random effects when necessary, against a 'null' model excluding the effects of interest (Forstmeier and Schielzeth 2011). Both linear and quadratic heterozygosity or relatedness terms were included in models to account for the possibility of both inbreeding and outbreeding depression and/or threshold effects (reviewed by Chapman et al. 2009), and linear terms were median-centered to avoid collinearity with their quadratic terms (Schielzeth 2010). Continuous response variables were modeled using gaussian error distribution and identity link function, while probabilities were modeled using binomial error and logit link. Model assumptions were checked graphically following Crawley 2007.

Graphical examination of normal Q-Q plots showed that both in chicks and adults, SH was approximately normally distributed, while Md<sup>2</sup> and relatedness were not. Therefore, unpaired and paired Welch's t-tests (recommended when variance is uneven, Ruxton 2006) were used for all SH comparisons, while Wilcoxon rank sum and signed rank tests were used for all comparisons of Md<sup>2</sup> and relatedness.

To account for the multiple testing from using multiple measures of fitness and two heterozygosity indices, we adjusted p-values to account for the false discovery rate (rather than family-wise error rate, Nakagawa 2004) following Benjamini and Yekutieli (2001). We give both raw and corrected p-values in results, but our discussion is based on false discovery rate-corrected values only. Analyses were carried out in R software ver. 3.2.0 (R Core Team) using the RStudio interface (RStudio Team 2015) and the lme4 (Bates et al. 2014), glmmADMB (Skaug et al. 2014), exactRankTests (Hothorn and Hornik 2015), and plotrix (Lemon 2006) packages.

Data available from the Dryad Digital Repository: < <http://dx.doi.org/10.5061/dryad.02vs1> > (Kiere et al. 2016).

## Results

### Heterozygosity-fitness correlations

None of the measured variables reflecting quality was affected by linear or quadratic SH (Table 1). There was no effect of linear or quadratic Md<sup>2</sup> on fledgling survival or mass, or on adult characteristics. Raw p-values from full versus null model tests suggested that fledgling bill and ulna length might be affected by Md<sup>2</sup> (in both cases due to the quadratic component; upon removal of quadratic Md<sup>2</sup>, bill:  $\Delta$  deviance = 7.8,  $p = 0.0053$ ; ulna:  $\Delta$  deviance = 7.3,  $p = 0.0072$ ), but these effects were not significant after false discovery rate correction for multiple testing (Table 1). Full model summaries are included as supplementary material (Supplementary material Appendix 1, Table A1 and A2).

Table 1. Tests for correlations between heterozygosity and fitness variables. A separate model was constructed for each response variable for standardized heterozygosity and mean d<sup>2</sup>.  $\Delta$  dev refers to the change in deviance between a 'full' mixed model containing the independent variables of main interest (linear and squared heterozygosity), covariables, and random effects, versus a 'null' model excluding the variables of interest. Raw p refers to the result of the corresponding  $\chi^2$  likelihood ratio test, with values < 0.05 indicated in bold. In order to account for multiple testing (eight and ten tests, respectively, of fledgling and adult quality), we applied a conservative 'false detection rate' correction to p-values ('Adjusted p'). After this correction, no full model showed significant improvement over its null alternative. Sample sizes (n), covariables, and random effects (and associated variance in the null model,  $\sigma^2$ ) for each model are also listed. SH = standardized heterozygosity, Md<sup>2</sup> = mean d<sup>2</sup>, Nest = nest identity, Cohort = year of birth, Year = year of measurement, BS = brood size, HO = hatch order, PL = proportional laydate.

Response variable	n	Random effects ( $\sigma^2$ )	Covariables	$\Delta$ dev		Raw p		Adjusted p	
				SH	Md <sup>2</sup>	SH	Md <sup>2</sup>	SH	Md <sup>2</sup>
At fledgling:									
Fledging probability	806	Nest (195.8)	BS, HO, PL	-2.4	-0.6	0.30	0.75	1	1
Bill length	750	Cohort (0.053)	BS, HO, PL	-1.0	-9.6	0.60	<b>0.008</b>	1	0.108
Ulna length	747	Cohort (0.130)	BS, HO, PL	-1.8	-9.3	0.42	<b>0.01</b>	1	0.108
Body mass	698	Cohort (0.280)	BS, HO, PL	-0.1	-3.4	0.95	0.18	1	1
As adults:									
Bill length	642	Cohort (0.043)	Age	-0.8	-0.7	0.67	0.71	1	1
Ulna length	642	Cohort (0.033)	Age	-0.5	-0.1	0.79	0.98	1	1
Body mass	639	Cohort (0.013)	Age, Age <sup>2</sup>	-2.7	-0.7	0.26	0.71	1	1
Foot color	124	Cohort (0.00)	Age, Age <sup>2</sup>	-1.3	-2.6	0.51	0.28	1	1
Mean breeding success	749	Cohort (0.002)	Age, Age <sup>2</sup>	-1.9	-0.5	0.41	0.80	1	1

## Sire choice based on individual heterozygosity

Females' EP courtship and copulation probabilities were independent of their social mates' linear and quadratic SH and  $Md^2$ , and females' EP paternity probability was unaffected by their mates' linear or quadratic SH (Table 2). Raw p-values indicated a marginally significant effect of social males'  $Md^2$  on EP paternity probability due to the quadratic component (Table 2; upon removing squared  $Md^2$ :  $\Delta$  deviance = 6.23,  $p = 0.013$ ), but this effect was opposite of the predicted direction and was non-significant after multiple testing was accounted for (Table 2). Females' own heterozygosity and the interaction of female and social male heterozygosity, as expressed by either SH or  $Md^2$ , had no effect on females' probability of EP courtship or paternity (Table 2). See supplementary material Appendix 1 Table A3–A6 for full model details. Finally, paired comparisons did not show lower SH or  $Md^2$  in social males than in the EP males to whom they lost paternity (Fig. 1a).

## Sire choice based on female-social male relatedness

While the raw p-value suggested a negative effect of female-social male relatedness on EP courtship probability (opposite of the predicted direction;  $\Delta$  deviance = 6.66, raw  $p = 0.036$ ,  $n = 54$ ), this effect was not significant after accounting for multiple testing (adjusted  $p = 0.108$ ). EP paternity probability was independent of linear and quadratic female-social male relatedness ( $\Delta$  deviance = 2.03, raw  $p = 0.36$ , adjusted  $p = 0.55$ ,  $n = 384$ ; see Supplementary material Appendix 1, Table A7 for full model summaries). Finally, females were no more closely related to their social mates than to their EP mates (Wilcoxon signed rank test:  $V = 26$ ,  $p = 0.19$ , Fig. 1b).

## EP paternity and chick heterozygosity

Even before false discovery rate correction, EP chicks did not have higher SH or  $Md^2$  than within-pair chicks in overall or paired comparisons, nor did within-pair chicks have higher SH or  $Md^2$  when they came from within-pair-only rather than mixed-paternity nests (Fig. 2).

## Discussion

We found no evidence supporting the GCH's predictions or its assumption of positive heterozygosity-fitness correlation

Table 2. Tests of the effect of individual parental heterozygosity on probability of extra-pair (EP) behaviors and paternity. Probability of EP copulation was only analyzed among females that had EP courtship partners. Sample size was too small to analyze this variable for female  $\times$  social male heterozygosity.  $\Delta$  dev is the difference in deviance and raw  $p$  the result of a likelihood ratio test between full (including independent variables) and null (no independent variables) models. P-values were corrected for multiple testing using the false discovery rate (Adjusted  $p$ ), following which no full model was significantly better than its null alternative. SH = standardized heterozygosity,  $Md^2$  = mean  $d^2$ , Year = observation year.

Independent variables	Response variable	n (no/yes)	Random effects ( $\sigma^2$ )	$\Delta$ dev		Raw p		Adjusted p	
				SH	$Md^2$	SH	$Md^2$	SH	$Md^2$
Linear and quadratic	EP courtship	92 (73/19)	Year ( $3.5 \times 10^{-15}$ )	-0.999	-1.47	0.68	0.48	1	1
Social male heterozygosity	EP copulation	19 (11/8)	Year (0.00)	-0.742	-0.212	0.69	0.90	1	1
	EP paternity	384 (341/43)	–	-0.246	-6.25	0.88	<b>0.044</b>	1	0.64
Female $\times$ social male heterozygosity	EP courtship	54 (42/12)	Year ( $3.5 \times 10^{-15}$ )	-5.71	-2.76	0.13	0.43	1	1
	EP paternity	384 (341/43)	–	-0.658	-1.05	0.88	0.79	1	1

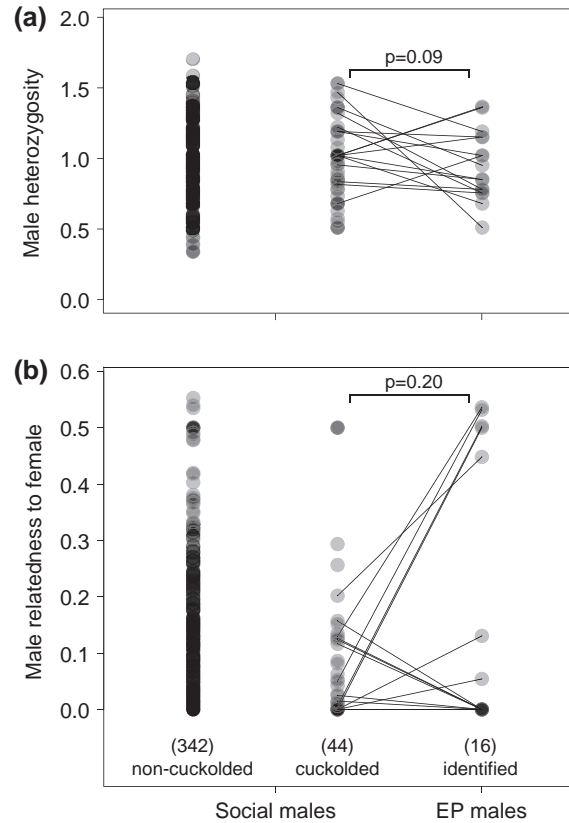


Figure 1. Males' heterozygosity and relatedness to the female. Standardized heterozygosity (a) and relatedness to the female (b) of social males from nests without ('non-cuckolded') and with ('cuckolded') extra-pair (EP) chicks, and of EP males identified as biological sires. Lines connect cuckolded social males with the EP males to whom they lost paternity; (uncorrected) p-values for these comparisons are given. Sample sizes are shown in parentheses above the bottom axis. Mean  $d^2$  is not shown, but results were similar.

in blue-footed boobies. Based on our panel of 10 polymorphic microsatellite markers, none of the aspects of chick or adult quality that we measured was positively correlated with individual heterozygosity; females' likelihood of engaging in EP behaviors or having EP chicks was not affected by their social mates' (or the females', or their interaction) heterozygosity or relatedness; EP sires were no more heterozygous or compatible than the social males they cuckolded; and EP pairings did not increase chick heterozygosity. Thus, our data yielded a consistent set of negative results.

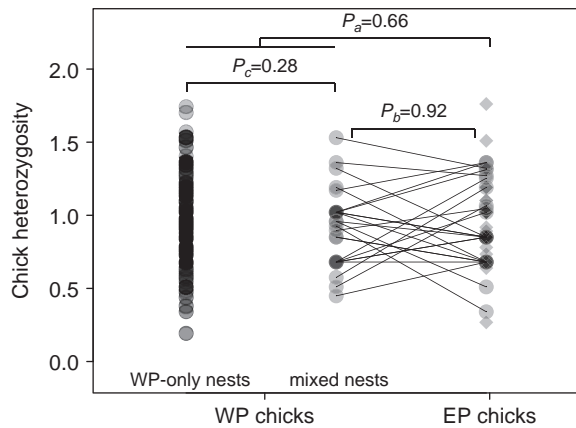


Figure 2. Chick heterozygosity in relation to individual and nest-mate paternity. Heterozygosity was compared between within-pair (WP;  $n = 396$ ) and extra-pair (EP;  $n = 47$ ) chicks overall ( $P_a$ ), between WP chicks and their EP half-sibs in mixed-paternity nests ( $P_b$ ), and between within-pair chicks from nests without ('WP-only nests',  $n = 357$ ) and with ('mixed nests',  $n = 24$ ) EP chicks ( $P_c$ ). Uncorrected p-values are given. Among EP chicks, circles indicate the 24 chicks from mixed paternity nests that were used for paired comparison with their within-pair half-sibs (connected by lines). Diamonds indicate EP chicks used in the overall comparison that did not have within-pair nest-mates (i.e. because all chicks in the nest were identified as EP, or because nest-mates died prior to sampling or were genotyped at  $< 6$  loci). Only standardized heterozygosity (SH) is shown; results were similar for mean  $d^2$ .

There are several possible explanations, both methodological and biological, for the lack of support for our predictions. While insufficient sample size is always a possible explanation for non-significant findings, it is unlikely that type II error alone accounted for our results since our sample sizes of 124–806 were similar or larger than those of comparable studies of the effect of heterozygosity/relatedness on paternity ( $n = 13$ –144 in Arct et al. 2015) and heterozygosity-fitness correlation ( $n = 7$ –1055 in Chapman et al. 2009), and our test results seldom approached significance, even before correction for multiple testing. Similarly, our use of 10 microsatellite loci is well within the range used in the studies with significant effects in Arct et al. (2015; 5 to 16 loci, mean = 8.25). However, factors other than number of markers can affect how well a set of molecular markers expresses genome-wide heterozygosity (Szulkin et al. 2010). For instance, for a set of microsatellite loci to be representative of the genome as a whole, the heterozygosity of the loci used should be correlated with that of other loci in the genome, a condition known as 'identity disequilibrium' (ID; reviewed by Szulkin et al. 2010). Under ID, correlation in heterozygosity among marker loci within individuals,  $g_2$ , should be significantly greater than zero, and it has been suggested that this parameter be routinely reported in microsatellite studies (Szulkin et al. 2010). In our sample, this condition was not upheld ( $g_2 = -0.001$ , and not different from 0,  $p = 0.64$ ; calculated using a 1000-iteration randomization procedure in RMES software, David et al. 2007), leaving uncertain whether the heterozygosity indices we calculated correlated closely with individuals' genome-wide heterozygosity. Lack of ID could be methodological, due to using too few microsatellite loci (up to several

hundred could be necessary; Miller et al. 2014, though see Forstmeier et al. 2012). However, lack of inbreeding, bottleneck, and/or admixture in our population can also lead to a genuine lack of ID, as well as null heterozygosity-fitness correlation (reviewed by Miller et al. 2014). Nonetheless, while lack of ID among our markers does reduce their power to reveal heterozygosity-fitness correlations or female choice for heterozygosity, it does not preclude significant findings, since individual traits are potentially sensitive to heterozygosity at more loci than were included as markers (Szulkin et al. 2010).

Assuming the lack of support for our predictions was not due to methodological or sample size constraints, there are two possible biological explanations for our negative results. First, both heterozygosity-fitness correlation and the selective pressure for females to be choosy with respect to males' heterozygosity and/or relatedness (compatibility) are expected to be low if the risk of inbreeding is low. For this reason, variation among study populations in the level of inbreeding is cited as a potential source of the substantial effect-size heterogeneity in Arct et al.'s (2015) meta-analysis. Interestingly, inbreeding is apparently quite rare in our study population. The social pedigree of 2488 individuals we constructed from our long-term database revealed that only 1.3% of individuals (32/2488) had non-zero inbreeding coefficients, and only 0.72% of individuals were the result of crosses between first-degree relatives (full sibling or parent/offspring pairs; offspring inbreeding coefficient = 0.25). This yielded a mean inbreeding coefficient ( $f$ ) of 0.002 ( $\sigma^2 = 0.0005$ ), very similar to that of a population of collared flycatchers *Ficedula albicollis* (mean  $f = 0.002$ ,  $\sigma^2_f = 0.0005$ ), and substantially lower than several other vertebrate populations (reviewed by Slate et al. 2004). Given that boobies from our study population are highly philopatric, with both males and females recruiting to nest sites within 40 m of their natal nests, selection for avoiding pairings between close relatives is expected to be strong (Kim et al. 2007), and the GCH is a reasonable candidate inbreeding avoidance mechanism, particularly because it does not necessarily assume pre-copulatory kin recognition (Griffith and Immler 2009). However, this pedigree based on social, rather than genetic relationships, implies that by the time social pairs form, inbreeding has already been largely avoided, and further inbreeding avoidance via EP behaviors or post-copulatory sperm selection is unlikely to yield substantial additional benefit.

A second possibility is that heterozygosity-fitness correlation and female mate choice to maximize offspring heterozygosity are context-dependent, and our sample did not capture the relevant context. If heterozygosity-fitness correlations are weak (as generally suspected; reviewed by Chapman et al. 2009, Szulkin et al. 2010), they may only be evident when selective pressures increase, for example when environmental conditions are poor (discussed in Chapman et al. 2009). And, if the advantage of high heterozygosity manifests primarily in early life (e.g. during the birth year), females may be selective of male heterozygosity or relatedness only when their offspring are likely to be exposed to bad conditions during this time. Both theoretical and empirical work (reviewed by Mays and Hill 2004) suggest it is realistic to expect females' mate choice to be flexible, both in degree



(e.g. increasing choosiness toward EP males when the social mate is attractive in this population; Kiere and Drummond 2014) and target (e.g. transition over the breeding season from favoring ornamentation to favoring compatibility in *Carpodacus mexicanus*; Oh and Badyaev 2006). Supposing the GCH is only upheld under adverse conditions, our sample was particularly unsuited to detect it; during the 2011 breeding season when paternity was sampled, our study colony experienced exceptional breeding success, likely due to a strong La Niña event (Ancona et al. 2011). In addition, our sample for analyzing the effect of chick heterozygosity on survival through fledging was not ideal because mortality between blood sampling (~ 10 d of age) and fledging (70 d of age) was minimal (6%), probably due both to the La Niña event and to delaying blood sampling of chicks until after their most vulnerable first days of life for ethical reasons (blood was not collected from dead chicks). Indeed, incompatibility effects are thought to be strongest early in development, perhaps even before hatching (Hemmings et al. 2012); and blood sampling of hatched chicks, rather than all fertilized eggs, is potentially a key limitation of GCH studies in general (Reid 2015).

Given that we found no evidence that heterozygosity (as we measured it) benefitted offspring or was boosted by EP mating, it is perhaps unsurprising that we found no evidence of female selection for genetic sires that would maximize it. Further analysis using either very large numbers of microsatellite loci or alternative techniques such as SNPs, as well as evaluating ID a priori, would go a long way toward ruling out insufficient marker coverage as a source of negative results. However, the low rate of inbreeding among social pairs in our study population, independent of genotype data, suggests that pressure for females to avoid inbreeding by obtaining EP sires may be low. Whatever the root cause of the lack of support for the GCH here, these results expand the taxonomic breadth of analyses of avian EP behavior and should be taken into account when addressing its generality as an explanation for female birds' EP behaviors, for example in future reviews and meta-analyses (Forstmeier 2015). At the same time, these negative results leave open the question of what does drive female boobies' EP behaviors. While there is some evidence of good genes benefits in this population (Kiere and Drummond 2014), long-term fitness differences between within-pair and EP offspring and alternative explanations such as sexual conflict or direct benefits remain to be explored in this species, as well as in the literature as a whole.

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Supplementary material (Appendix JAV-01061 at <[www.avianbiology.org/appendix/jav-01061](http://www.avianbiology.org/appendix/jav-01061)>). Appendix 1.

# Capítulo 3:

## Efecto de condiciones ambientales adversas sobre la conducta extra-pareja



Foto: Oscar Sánchez Macouzet

*"La infidelidad femenina es restringida por condiciones de  
El Niño en un ave de larga vida"*

(Publicado en Journal of Animal Ecology, DOI: 10.1111/1365-2656.12537)



# Female infidelity is constrained by El Niño conditions in a long-lived bird

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## Summary

**1.** Explaining the remarkable variation in socially monogamous females' extrapair (EP) behaviour revealed by decades of molecular paternity testing remains an important challenge. One hypothesis proposes that restrictive environmental conditions (e.g. extreme weather, food scarcity) limit females' resources and increase EP behaviour costs, forcing females to reduce EP reproductive behaviours.

**2.** For the first time, we tested this hypothesis by directly quantifying within-pair and EP behaviours rather than inferring behaviour from paternity. We evaluated whether warmer sea surface temperatures depress total pre-laying reproductive behaviours, and particularly EP behaviours, in socially paired female blue-footed boobies (*Sula nebouxi*). Warm waters in the Eastern Pacific are associated with El Niño Southern Oscillation and lead to decreased food availability and reproductive success in this and other marine predators.

**3.** With warmer waters, females decreased their neighbourhood attendance, total copulation frequency and laying probability, suggesting that they contend with restricted resources by prioritizing self-maintenance and committing less to reproduction, sometimes abandoning the attempt altogether. Females were also less likely to participate in EP courtship and copulations, but when they did, rates of these behaviours were unaffected by water temperature.

**4.** Females' neighbourhood attendance, total copulation frequency and EP courtship probability responded to temperature differences at the between-season scale, and neighbourhood attendance and EP copulation probability were affected by within-season fluctuations. Path analysis indicated that decreased EP participation was not attributable to reduced female time available for EP activities. Together, our results suggest that immediate time and energy constraints were not the main factors limiting females' infidelity.

**5.** Our study shows that El Niño conditions depress female boobies' EP participation and total reproductive activity. In addition to increasing general self-maintenance and reproductive costs, warm waters may increase costs specific to EP behaviours including divorce, reduced male parental care, or pathogen exposure. Our results suggest that female boobies strategically refrained from EP behaviours to avoid these or other longer-term costs, rather than being compelled by immediate constraints. This study demonstrates that current environmental conditions affect females' mating decisions, contributing to variation in EP behaviours, even in a long-lived, iteroparous species that can buffer against temporary adversity.

**Key-words:** adverse environmental conditions, El Niño Southern Oscillation, extrapair behaviour, mating system, reproduction vs. self-maintenance trade-off, reproductive investment, resource restriction

## Introduction

Over the past three decades, molecular paternity testing has revealed remarkable variation in the prevalence of extrapair (EP) paternity in socially monogamous animals,

particularly in birds, where this mating system is most prevalent and most research on EP behaviour has been carried out (reviewed in Griffith, Owens & Thuman 2002; Westneat & Stewart 2003). It is suspected that current ecological conditions explain some of this variation, potentially by modifying the costs of EP interactions (Bennett & Owens 2002; Griffith, Owens & Thuman 2002; Westneat &

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Stewart 2003). Specifically, restrictive environmental conditions such as low food availability or extreme weather could curtail females' EP behaviour by restricting the time and energy they have available to seek and interact with potential EP males (Slagsvold & Lifjeld 1997), increasing their dependence on their social mate's contribution to parental care (Gowaty 1996) and/or increasing their susceptibility to sexually transmitted pathogens (Sheldon 1993; Loehle 1995; reviewed in Petrie & Kempenaers 1998).

A handful of studies support this relationship between adverse conditions and EP behaviour by showing decreased EP paternity during cold weather (Hoset *et al.* 2013) and food scarcity (Korpimäki *et al.* 1996; Hoi-Leitner *et al.* 1999; Cameron, Berteaux & Dufresne 2011). However, the interpretation of correlations between environmental conditions and EP paternity can be complicated by potential confounding variables (e.g. spatial variation in individual quality; see Cameron, Berteaux & Dufresne 2011), differential effects of environmental conditions on females, social males and EP males (see Bouwman & Komdeur 2006), and inability to distinguish between effects of choice and opportunity and between forced and consensual copulations (see Westneat 1994; Václav, Hoi & Blomqvist 2003). Behavioural observation rather than paternity analysis alone is therefore needed to establish a more direct link between environmental conditions and female EP behaviour (reviewed in Griffith, Owens & Thuman 2002; Westneat & Stewart 2003; also see discussion of Bouwman & Komdeur 2006; Griffith & Immler 2009). Several existing studies buttress their conclusions with behavioural data (e.g. territory attendance and within-pair behaviours in Westneat 1994; anecdotal observations of EP copulations in Korpimäki *et al.* 1996; Hoi-Leitner *et al.* 1999; Václav, Hoi & Blomqvist 2003; paternal care in Cameron, Berteaux & Dufresne 2011), but none bases its main analyses on within-pair and EP behaviour rather than paternity.

Here, we tested whether unfavourable environmental conditions decrease females' reproductive behaviour in general, and their EP behaviour in particular, in a long-lived, socially monogamous seabird that nests colonially on islands in the Eastern Pacific Ocean. The blue-footed booby (*Sula nebouxii*; Milne-Edwards 1882) is characterized by highly observable within-pair and EP courtship and copulation, female complicity and control over EP interactions, and a strong negative correlation between a specific environmental variable – sea surface temperature – and reproductive activity and success. Extrapair copulation occurs in 54–61% of males and 38–54% of females (Osorio-Beristain & Drummond 1998; Pérez-Staples & Drummond 2005), and in one season, 11% of broods contained EP young (Ramos *et al.* 2014b). Though males usually initiate EP interactions, females are ~30% larger (Castillo & Chavez-Peón 1983) and their cooperation is necessary for males to balance on their backs to copulate. Moreover, EP copulations are always preceded by reciprocal courtship involving conspicuous skypointing

displays (Nelson 2005), and no attempts at forced copulation have been observed (Osorio-Beristain & Drummond 1998).

Reproductive success in the study population correlates strongly with the local seasonal sea surface temperature anomaly (SSTA, °C difference from the 30-year average temperature during the same month) associated with El Niño Southern Oscillation (ENSO), a continuously varying 2- to 7-year cycle. When warm 'El Niño' conditions prevail along the American coast, biotic productivity declines and members of marine food webs commonly face decreased food availability (see Schreiber 2002; Stenseth *et al.* 2002). Seabirds and other marine predators are particularly strongly affected (see Schreiber 2002; Stenseth *et al.* 2002). The study population shows increased circulating stress hormones (Wingfield *et al.* 1999), dietary shifts (Ancona, Calixto-Albarrán & Drummond 2012), delayed breeding, decreased breeding participation, small clutches and broods, and diminished hatching success, chick growth and nest success (Ancona *et al.* 2011). Most analyses of warm water effects consider seasonal (1–3 month) or yearly time-scales, but even daily warming reduces foraging success and chick growth in some seabirds, affecting time and energy trade-offs during chick rearing (e.g. Peck *et al.* 2004; Erwin & Congdon 2007). Here, we evaluate the effect of both prolonged seasonal SSTA conditions (between-season effects) and short-term SSTA fluctuations (within-season effects). To our knowledge, no previous study has analysed SSTA effects on females' pre-laying within-pair and EP mating behaviours at either of these scales.

We tested whether warm waters depress the pre-laying reproductive behaviour in general, and specifically the EP behaviours, of socially paired female blue-footed boobies. With warmer SSTA, we expected females to spend less time in the breeding colony (due to spending more time foraging), court and copulate less frequently, and be less likely to establish a clutch. In particular, we expected warmer SSTA to depress females' EP behaviours, decreasing their likelihood of participating in EP courtship and copulation, and reducing the rates of these behaviours when they did participate.

## Materials and methods

### STUDY COLONY AND YEARS

In the blue-footed booby colony on Isla Isabel, Nayarit (21–85°N, 105–89°W), where reproduction has been monitored and fledglings ringed every year since 1988 (Drummond, Torres & Krishnan 2003), we observed behaviour during the December–March laying peak of four successive breeding seasons. The prolonged moderate–strong El Niño (warmer than average) and La Niña (cooler than average) conditions during 2010 and 2011, respectively, were followed by intermediate, ENSO-neutral conditions during 2012 and 2013 (NOAA 2015), yielding short-term (4–5 day) SSTA values that varied from –3.62 to +2.49 °C over the four seasons (see Sea Surface Temperatures below).

## BEHAVIOURAL OBSERVATIONS

Neighbourhoods of two to six social pairs in dense nesting areas were observed from portable blinds during four to five consecutive days during the hours of maximum activity (0645–1145 h and 1430–1745 h), resulting in an average total observation time of 39.5 h/pair. Individuals' unique band numbers were read with binoculars (Kiere & Drummond 2014). Social pairs were a male and female still without a clutch that courted, jointly defended a territory, allopreened mutually, and consistently stood within one metre of each other; EP males were any other males with whom a focal female reciprocated courtship (Torres & Velando 2003; Pérez-Staples & Drummond 2005). Observations were completed for a total of 230 social pairs, comprising 212 unique females and 226 unique pairs; four females were observed in two different years with the same social mate, twelve females were observed in two different years and one female was observed in three years, each time with a different social mate. No females were observed twice in one year. Females from 57 of the 230 observed social pairs (24.7%) engaged in EP courtship, and 26 (45.6%) of these females copulated with their EP partners during our observations.

Absolute frequencies of focal females' within-pair and EP courtship displays (described in Nelson 2005) and copulations were recorded, and individuals' neighbourhood attendance (presence within the ~30 m radius visible from the blind) was scored by identifying the individuals present in the neighbourhood by band number every 15 min (scan sampling). For females that had multiple EP partners (15 of the 57 EP-courting females had two or three EP courtship partners, and five of these had two EP copulation partners) behavioural frequencies were summed across EP partners to generate a single score per female. To account for effects of mate guarding, we estimated the proportion of time each female was in solitary attendance as the number of scans present without her social mate divided by her total scans present during the observation period ('female time alone'). To account for the increase in social and EP courtship and copulation rates as egg laying approached (Osorio-Beristain & Drummond 1998), the laying date of each focal female's first egg was determined by searching neighbourhoods every other day for 30 days after observations ended there, or from routine monitoring of all nests in the study area every 3 days. Clutches were laid by 39% of the 230 focal females. Latency between the end of observations and laying of a female's first egg was expressed in three categories: within 5 days ('presumed fertile period'; see Osorio-Beristain & Drummond 1998, 2001), 6–30 days and >30 days (including females that did not lay).

This research was conducted in accordance with ABS/ASAB guidelines (Animal Behaviour Society 2012) and with approval from the administration of Isla Isabel National Park and the Secretary of the Environment and Natural Resources (SEMARNAT, permit #SGPA/DGVS/08333/10). Similar procedures have been used during earlier projects with no apparent adverse effects (e.g. Osorio-Beristain & Drummond 1998; Kiere & Drummond 2014).

## SEA SURFACE TEMPERATURES

The SSTA during the 4- to 5-day observation period of each focal pair was calculated using the NOAA Optimum Interpolation Sea Surface Temperature Analysis Dataset available through the IRI/LDEO climate library (NOAA 2014). This data set comprises weekly temperature estimates based on readings from

satellites, ships and buoys and uses the average monthly sea surface temperature from 1971 to 2000 in the same location as a baseline for anomaly calculation (Reynolds *et al.* 2002). Daily SSTA values were interpolated by assuming constant linear change between weekly readings for the 110 × 110 km square closest to Isla Isabel (centred 56 km to the southwest), then averaging the daily values over each pair's observation period.

## AGE OF FOCAL INDIVIDUALS

Male and female ages and their interaction can affect EP courtship and paternity (Kiere & Drummond 2014; Ramos *et al.* 2014a) and increasing SSTA may affect the representation of different-aged males and females in samples (e.g. under-representation of young individuals due to delayed recruitment Oro *et al.* 2010; over-representation of old individuals due to terminal investment Velando, Drummond & Torres 2006). Therefore, ages were included where sample size allowed – that is, in the analyses of all observed pairs ( $n = 230$ ) and of females with an EP courtship partner ( $n = 57$ ), but not in analyses of females with EP copulation partners ( $n = 26$ ). Male and female ages were median-centred to avoid collinearity with their interaction term (following Schielzeth 2010).

## GENERAL STATISTICAL METHODS

Generalized linear mixed models (GLMMs) were used, with year, neighbourhood and female identity included as crossed random (intercept) effects to avoid pseudoreplication and account for unmeasured factors which may be correlated within each of these grouping levels (Crawley 2007; Zuur *et al.* 2009). All count-based variables (female attendance, courtship frequencies and copulation frequencies) were overdispersed, so GLMMs with negative binomial error distribution and log link function were implemented using the glmmADMB package (Skaug *et al.* 2014). The total number of 15-min scans observed per pair was included as an 'offset' in these analyses to convert frequencies to rates per 15 min (Hilbe 2011). For binomial variables (probabilities of engaging in EP courtship, copulating EP and establishing a clutch), GLMMs with binomial error distribution and logit link function were constructed in the lme4 package (Bates *et al.* 2014). All analyses were carried out in R (R Development Core Team 2012) using the RStudio interface (RStudio 2012). Initial models were simplified using backwards-stepwise simplification, sequentially removing terms from highest to lowest order and from least to most significant, comparing nested models with ANOVA likelihood ratio tests until arriving at a minimum adequate model that included only terms whose removal worsened model fit ( $\alpha = 0.05$ , Crawley 2007). There were no likely multicollinearity problems (variance inflation factor  $\leq 3$  for all variable combinations in initial and final models; see Zuur, Ieno & Elphick 2010). Final models were evaluated using *post hoc* diagnostics following Crawley 2007; except that standardized model residuals  $< -3$  or  $> 3$  were used to diagnose influential points (i.e. outliers) because Cook's distance is not available for the glmmADMB package (see Brondeel *et al.* 2014).

## TOTAL REPRODUCTIVE BEHAVIOUR

Using all 230 focal females observed, the effect of SSTA on four aspects of females' general pre-laying behaviour was evaluated:

neighbourhood attendance (proportion of scans present), total courtship and copulation rates (within-pair + EP rates per 15 min) and whether a clutch was established that year (a binary variable). In each initial model, SSTA and SSTA<sup>2</sup> were included (to detect both linear and threshold effects; sensu Cairns 1987), as well as female and social male ages and their interaction, latency to lay category, and EP status (whether females exhibited EP courtship; a binary variable), as well as the interaction of EP status with SSTA and SSTA<sup>2</sup>, to account for a possible difference in SSTA effect between faithful and promiscuous females. For courtship rate, the total courtship the female received was also included to account for the call-and-response nature of booby courtship (description in Nelson 2005). For clutch establishment, latency to lay category was excluded because it contains nearly the same information as the response variable (by definition, all females in the <5 days or 6–30 days latency to lay categories established a clutch, while all females that did not establish a clutch were in the >30 days category).

#### EXTRAPAIR REPRODUCTIVE BEHAVIOUR

The occurrence of EP courtship (a binary variable) was analysed using the 230 focal females. The initial model included SSTA and SSTA<sup>2</sup>, female and social male ages and their interaction, latency to lay category, total observation time (total number of 15-min periods the pair was observed; to control for differences in observation time) and female time alone (to control for differences in females' opportunity to engage in EP behaviours). Since EP copulation only occurs following reciprocal courtship, the occurrence of EP copulation (a binary variable) was analysed using only the 57 females that showed EP courtship, including the same initial independent variables.

Analyses of female EP courtship and copulation rates included only the 57 females that courted with  $\geq 1$  EP male and 26 females that copulated with their EP courtship partners, respectively. One outlier was excluded from the EP courtship rate analysis (leaving a total sample size of 56) because the female emitted four times as many EP skypoints as the second-highest record and had a standardized residual of 4.95 in the initial model, implying an overly influential data point. The effect of SSTA on EP courtship rate was similar regardless of whether this outlier was included. EP courtship rate was the total frequency of skypointing displays to EP males over the 4- to 5-day period (offset by the number of scans per pair). The initial model included SSTA and SSTA<sup>2</sup>, female and social male ages and their interaction, female time alone, latency to lay category and total EP courtship received by the female. EP copulation rate was the total EP copulations over the 4- to 5-day period (offset by the total observation time), and the initial model included SSTA, SSTA<sup>2</sup>, female time alone and latency to lay.

#### WITHIN- AND BETWEEN-SEASON SSTA EFFECTS

Because each observation year contained only a portion of the overall range of SSTA observed, the effects of the short-term SSTA variable used in our main analyses may be due to SSTA effects within seasons, between seasons or a combination of both. Further, within- and between-season changes in SSTA could have different, even opposing effects (van de Pol & Wright 2009). We therefore used within-group centring (following van de Pol & Wright 2009) to distinguish within- from between-season effects,

as well as evaluate whether females' behaviour is affected similarly by SSTA at these two scales, for all analyses in which SSTA had a significant effect (Tables 1 and 2). To do so, we repeated each minimum adequate model, but expressed SSTA as two distinct terms for each female: the average SSTA during the season she was observed ('average SSTA', expressing only between-season variation) and her deviation from that average ('centred SSTA', expressing only within-season variation). Average SSTA was calculated by averaging weekly SSTA values from the first through the last date during which observations were carried out each year, and centred SSTA was calculated by subtracting average SSTA from the SSTA during each female's observation period. We followed up by testing whether the slopes of the between- and within-season effects differed from each other by including the original SSTA variable (combined between- and within-season effects) as well as centred SSTA; in this analysis, centred SSTA represents the difference between the within- and between-season effects (van de Pol & Wright 2009). When the within-group centring procedure was applied for the probability of EP copulation, models including all three random terms did not converge, so only the first observation of each female was included ( $n = 54$  females) and the female identity random factor was dropped to allow convergence.

#### PATH ANALYSIS

Upon finding that increasing SSTA depressed the probability of EP behaviours, we used generalized multilevel confirmatory path analysis ('d-sep' method; Shipley 2009, 2013) to test whether females' reduced participation in EP behaviours was due directly to SSTA, or to an indirect effect of SSTA on females' age (representation of different-aged females in the sample) or opportunity to participate (either because they spend less total time on the colony or because they are less able to avoid guarding by their social mates as SSTA increases). If reduced EP courtship is due to greater representation of older females as SSTA increases, the most supported model should show an increase in female age with increasing SSTA and a decrease in EP courtship participation with increasing age. If reduced EP courtship probability is due to decreased opportunity to participate as SSTA increases, the most supported model should show a negative effect of SSTA on females' time present and/or time alone and a positive effect of either or both of these variables on EP courtship probability. If, on the other hand, reduced EP courtship is not determined by female age or opportunity to participate, only a direct negative effect of SSTA on EP courtship probability should be supported. Note, though, that finding a 'direct' effect of SSTA on EP courtship probability does not suggest that there are no intervening factors mediating boobies' responses to SSTA conditions (e.g. food availability, wind patterns), only that the relevant intermediate steps are not included in the candidate models (Shipley 2002). The five *a priori* candidate models (Shipley 2002) proposed to test these predictions included SSTA, female neighbourhood attendance, female time alone, female age (modelled as an ordinal variable) and probability of EP courtship (Fig. 3a–e; Appendix S1, Supporting information), as well as latency to lay (because it was shown to affect neighbourhood attendance; Table 1a, Fig. 1a). These five alternative models were compared using AICc (Akaike Information Criterion, adjusted for small sample size) in an

**Table 1.** Minimum adequate models resulting from analyses of the effect of sea surface temperature anomaly during the 4- to 5-day observation period (SSTA) on females' a) attendance, b) total courtship rate, c) within-pair courtship rate and d) total copulation rate during the 4- to 5-day observation period, and e) probability of establishing a clutch during the year of observation ( $n = 230$ )

Fixed effects	$\beta \pm \text{SE}^*$	$Z^*$	$P_Z^*$	$P_Z^{\dagger}$
a) Neighbourhood attendance (scans present/total scans)				
SSTA	$-0.128 \pm 0.034$	-3.77	$1.7 \times 10^{-4}$	0.15
SSTA <sup>2</sup>	$-0.0323 \pm 0.012$	-2.76	0.0057	0.0075
Female age	$0.0184 \pm 0.0047$	3.91	$9.0 \times 10^{-5}$	$2.9 \times 10^{-4}$
Latency to lay				
$\leq 5$ vs. 6–30 days	$-0.183 \pm 0.071$	-2.58	0.0098	0.0023
6–30 vs. >30 days	$-0.0314 \pm 0.057$	-0.56	0.58	
Random effects: $\sigma_{\text{year}}^2 = 2.14 \times 10^{-9}$	$\sigma_{\text{neighbourhood}}^2 = 0.0182$		$\sigma_{\text{female id}}^2 = 1.29 \times 10^{-8}$	
b) Total courtship frequency (female-emitted within-pair + EP skypoints/15 min)				
Female age	$0.0462 \pm 0.011$	4.06	$4.8 \times 10^{-5}$	$7.04 \times 10^{-5}$
EP status	$0.291 \pm 0.13$	2.29	0.022	0.024
Total courtship received	$0.00415 \pm 4.7 \times 10^{-4}$	8.92	$<2.0 \times 10^{-16}$	$<2.2 \times 10^{-16}$
Random effects: $\sigma_{\text{year}}^2 = 2.061 \times 10^{-9}$	$\sigma_{\text{neighbourhood}}^2 = 0.122$		$\sigma_{\text{female id}}^2 = 0.196$	
c) Social courtship frequency (female-emitted within-pair skypoints/15 min)				
Female age	$0.0453 \pm 0.012$	3.88	$1.10 \times 10^{-4}$	$1.43 \times 10^{-4}$
EP status	$0.269 \pm 0.13$	2.11	0.035	0.038
Social courtship received	$0.00475 \pm 5.0 \times 10^{-4}$	9.41	$<2.0 \times 10^{-16}$	$<2.2 \times 10^{-16}$
Random effects: $\sigma_{\text{year}}^2 = 2.061 \times 10^{-9}$	$\sigma_{\text{neighbourhood}}^2 = 0.0814$		$\sigma_{\text{female id}}^2 = 0.246$	
d) Total copulation frequency (within + EP copulations/15 min)				
SSTA	$-0.102 \pm 0.038$	-2.66	0.0078	0.0092
Latency to lay				
$\leq 5$ vs. 6–30 days	$-0.180 \pm 0.150$	-1.20	0.23	0.0073
6–30 vs. >30 days	$-0.250 \pm 0.127$	-1.98	0.048	
Random effects: $\sigma_{\text{year}}^2 = 2.150 \times 10^{-8}$	$\sigma_{\text{neighbourhood}}^2 = 0.0537$		$\sigma_{\text{female id}}^2 = 1.567 \times 10^{-8}$	
e) Laying Probability				
SSTA	$-0.629 \pm 0.167$	-3.78	$1.6 \times 10^{-4}$	0.0016
Random effects: $\sigma_{\text{year}}^2 = 0.00$	$\sigma_{\text{neighbourhood}}^2 = 0.222$		$\sigma_{\text{female id}}^2 = 0.753$	

\*Parameters from minimum adequate model.

†P-value resulting from  $\chi^2$  nested model comparisons following removal of each variable during backwards-stepwise simplification.

information-theoretic approach (Shiple 2013), considering models that differed by less than 2 *AICc* units statistically equivalent (Johnson & Omland 2004). See Appendix S1 for details of path analysis methods.

## Results

### TOTAL REPRODUCTIVE BEHAVIOUR

Neighbourhood attendance by females decreased with increasing SSTA above  $-1.025$  °C (Table 1a; Fig. 1a), and the significant quadratic term was due to a threshold effect, rather than a true quadratic relationship (see Fig. 1 caption). Similarly, total copulation rate and probability of establishing a clutch declined with SSTA (Fig. 1c,d) and neither differed between faithful and promiscuous females (total copulation rate:  $\Delta$  deviance = 0.030,  $P = 0.87$ ; clutch establishment:  $\Delta$  deviance = 0.046,  $P = 0.83$ ).

Total courtship rate, on the other hand, was unrelated to SSTA (Fig. 1b), but increased with total courtship received (Table 1b) and was 33.8% higher in females that courted EP males than in faithful females (Table 1b; closed triangles vs. circles in Fig. 1b). Greater total courtship by promiscuous females was due mostly to within-pair, not EP courtship; when the analysis was confined to within-pair

courtship, promiscuous females courted their social mates 30.9% more than faithful females (Table 1c; open triangles vs. circles in Fig. 1b).

### EXTRAPAIR REPRODUCTIVE BEHAVIOUR

The probability of a female engaging in EP courtship decreased with SSTA (Table 2a; Fig. 2a), as did the probability of an EP-courting female copulating with her EP partner (Table 2b; Fig. 2b).

In contrast, rates of EP courtship and EP copulation by females that performed those behaviours were unrelated to SSTA (Table 2c and d). Females' EP courtship rates increased with the amount of EP courtship they received (Table 2c), and their EP copulation rates increased as they spent more time alone (Table 2d).

### WITHIN- AND BETWEEN-SEASON SSTA EFFECTS

Females' neighbourhood attendance, total copulation rate, laying probability and EP courtship probability were all significantly affected by between-season (average) SSTA. The effect of within-season (centred) SSTA was nearly significant for females' neighbourhood attendance (with a predicted slope very similar to those of the



**Table 2.** Minimum adequate models of the effect of sea surface temperature anomaly during observation (SSTA) on females' EP behaviours: a) probability of EP courtship ( $n = 230$ , 57 with EP courtship), b) probability of EP copulation ( $n = 57$ , 26 with EP copulation), and among participating females, c) EP courtship rate ( $n = 56$ ) and d) EP copulation rate ( $n = 26$ )

Fixed effects	$\beta \pm SE^*$	$Z^*$	$P_Z^*$	$P_{\chi^2}^\dagger$
a) EP courtship probability				
SSTA	$-0.198 \pm 0.101$	-1.96	0.050	0.050
Random effects: $\sigma_{year}^2 = 0.00$	$\sigma_{neighbourhood}^2 = 1.16 \times 10^{-47}$		$\sigma_{female\ id}^2 = 4.00 \times 10^{-14}$	
b) EP copulation probability (among EP-courtiers)				
SSTA	$-0.439 \pm 0.252$	-1.74	0.082	0.039
Female age	$0.164 \pm 0.079$	2.09	0.037	0.0094
Random effects: $\sigma_{year}^2 = 0.00$	$\sigma_{neighbourhood}^2 = 0.0509$		$\sigma_{female\ id}^2 = 0.0533$	
c) EP courtship rate (female-emitted EP skypoints/15 min)				
EP courtship received	$0.00751 \pm 0.0019$	3.9	$9.6 \times 10^{-5}$	$1.17 \times 10^{-4}$
Random effects: $\sigma_{year}^2 = 2.06 \times 10^{-9}$	$\sigma_{neighbourhood}^2 = 0.477$		$\sigma_{female\ id}^2 = 0.179$	
d) EP copulation rate (EP copulations/15 min)				
Female time alone	$1.924 \pm 0.831$	2.32	0.021	0.027
Latency to lay				
≤5 vs. 6–30 days	$-0.612 \pm 0.357$	-1.71	0.087	0.015
6–30 vs. >30 days	$-0.407 \pm 0.357$	-1.14	0.26	
Random effects: $\sigma_{year}^2 = 1.84 \times 10^{-7}$	$\sigma_{neighbourhood}^2 = 9.84 \times 10^{-9}$		$\sigma_{female\ id}^2 = 5.78 \times 10^{-9}$	

\*Parameters from minimum adequate model.

† $P$ -value resulting from  $\chi^2$  nested model comparisons following removal of each variable during backwards-stepwise simplification.

between-season and overall effects; Tables 1a and 3a) and non-significant for copulation rate, laying probability and EP courtship probability (Table 3a–d). Neither within- nor between-season effects reached significance on their own for EP copulation probability, though the within-season effect was close to significance and had a predicted slope similar to that of the overall effect (Tables 2b and 3e). This suggests that the significant overall effect is due to a combination of effects at both within- and between-season scales. The within-season effect was in the expected negative direction for all variables except EP courtship probability (the slightly positive predicted slope was far from significance), and the difference between within- and between-season effects was non-significant for all variables (Table 3). This suggests that effects at these two time-scales are generally consistent with each other and validates the use of the combined SSTA variable ('standard mixed models') in our other analyses (van de Pol & Wright 2009).

#### PATH ANALYSIS

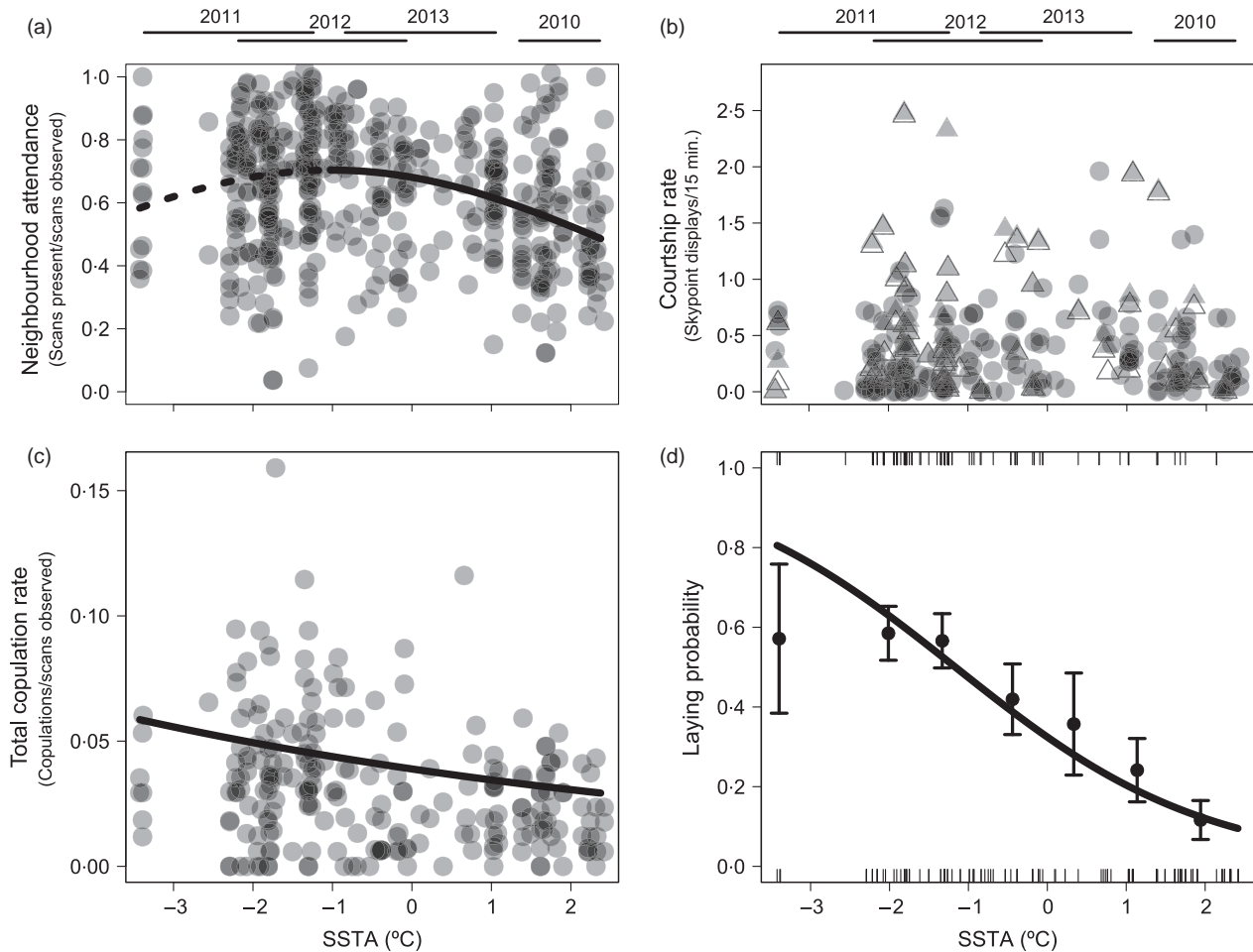
The most supported path model was the one proposing a direct negative effect of SSTA on EP courtship probability (Fig. 3e; Table 4), with an  $AICc$  weight of 73.07% and an  $AICc$  score nearly 3 units lower than the second-most supported model. The model proposing a mediating effect of female neighbourhood attendance ranked second ( $AICc$  weight = 16.58%), but the direction of this effect was negative, contrary to expectation if females' EP behaviour were limited by their time present (Fig. 3b). The three remaining models had similarly small  $AICc$  weights (1.27%, 3.07% and 6.34%). Complete statistics are provided in Tables 3 and S2.

## Discussion

As SSTA increased, females overall spent less time in their colony neighbourhoods, copulated less frequently, and were less likely to establish a clutch. Their probability of engaging in EP courtship and EP copulation also decreased, but females that did participate in EP behaviours did so at similar rates, regardless of temperature. Among the variables that were negatively correlated with SSTA, between-season effects were shown for all except EP copulation probability, and within-season effects were apparent for neighbourhood attendance and propensity to engage in EP copulation. Our main results confirm that adverse environmental conditions constrain females' total and EP reproductive behaviours, and path analysis further suggests that females reduced EP behaviour to avoid future costs, rather than being compelled by immediate temporal or energetic constraints (i.e. 'reproductive regulation'; sensu Goodman 1979; Stearns 1992).

#### TOTAL REPRODUCTIVE BEHAVIOUR

The decrease in females' total pre-laying reproductive activities in warm water conditions was consistent with reported effects of interannual SSTA variation on breeding variables of this species from laying through fledging (Ancona *et al.* 2011; see introduction), but reveals earlier effects of warm water on breeding than previously demonstrated. There is a convincing body of research showing negative effects of SSTA on marine predators' reproductive activity and success at the seasonal or longer time-scales (reviewed in Schreiber 2002; Stenseth *et al.* 2002; Congdon *et al.* 2007). Particularly in earlier work, ENSO is mostly considered at large spatial and temporal scales,

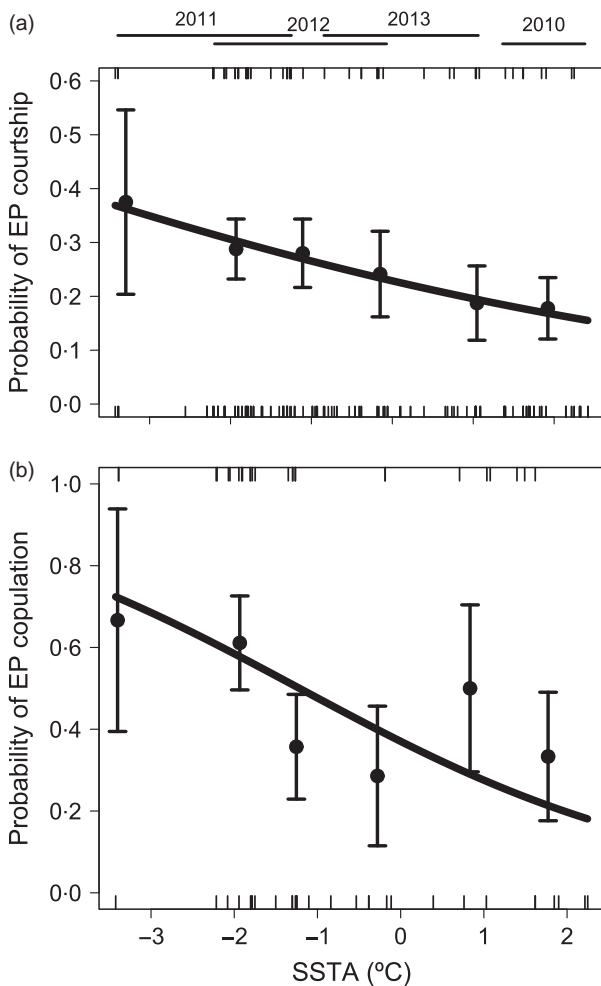


**Fig. 1.** Effect of sea surface temperature anomaly during the 4- to 5-day observation period (SSTA) on females' (a) neighbourhood attendance, (b) courtship rate, (c) total copulation rate and (d) probability of establishing a clutch. In (a) and (c), the predicted effect of SSTA is shown for females  $\leq 5$  days from laying. In (a), piecewise regression showed no SSTA effect below  $-1.025$  °C (dashed portion of line:  $\Delta$  deviance = 0.814,  $P = 0.37$ ,  $n = 105$ ), but a strong negative effect above this temperature (solid portion:  $\Delta$  deviance = 6.964,  $P = 0.0083$ ,  $n = 125$ ). In (b), courtship rate was not related to SSTA, but promiscuous females had higher total courtship (closed triangles) than faithful females (closed circles), due primarily to increased within-pair courtship (open triangles). In (d), raw binomial data are shown as short vertical lines along the top (laid) and bottom (did not lay) of the graph, while points and standard error bars represent the proportion of females that laid within 7 SSTA bins. Labelled horizontal lines along the top of the figure indicate SSTA ranges during each observation year. Complete model results in Table 1.

with its effects attributed to decreased prey species recruitment due to bottom-up trophic cascades (reviewed in Schreiber 2002; Stenseth *et al.* 2002; Congdon *et al.* 2007). However, more recent research has shown effects of SSTA and other weather and oceanographic fluctuations at the within-season, even day-to-day, scales (Peck *et al.* 2004; Erwin & Congdon 2007; Denhard *et al.* 2013). Such effects may be due to prey species moving out of seabirds' reach horizontally or vertically in the water column following shifts in the local thermocline or distribution of subsurface predators (reviewed in Congdon *et al.* 2007). In the blue-footed booby, these shorter-term effects may operate in addition to prevailing yearly or multiyear ENSO cycles.

We found that female neighbourhood attendance decreased when SSTA exceeded  $-1.025$  °C (Fig. 1a).

Furthermore, within-group centring showed both a clear effect of prolonged between-season differences and a marginally significant effect of short-term, within-season fluctuations in SSTA (of similar slope to between-season and overall effects; Table 3a). This apparent fine-scale responsiveness of females' neighbourhood attendance to short-term SSTA conditions could reflect increased foraging effort to compensate for decreased foraging success. That time budget is the most flexible of the behaviours observed is consistent with Cairns's (1987) general predictions, and similar decreases in neighbourhood attendance when SSTA is elevated have been found in other seabirds (e.g. Piatt *et al.* 2007) and mammals (Soto, Trites & Arias-Schreiber 2006) at the between-season scale, as well as within seasons in other seabirds while rearing young (Peck *et al.* 2004; Erwin & Congdon 2007; Denhard *et al.* 2013).



**Fig. 2.** Effect of sea surface temperature anomaly during observation (SSTA) on probability of (a) EP courtship and (b) EP copulation. SSTA ranges during each observation season are shown as labelled horizontal lines along the top of the figure. Short vertical lines near the top and bottom of each graph show raw binomial data of whether females engaged (top) or not (bottom) in each EP behaviour. Points and standard error bars show average proportions of females engaging in EP courtship and EP copulation, in 6 SSTA bins. Curves show the effect of SSTA predicted from each minimum adequate model (holding female age constant at its mean, 7 years, in b). Complete model results in Table 2.

Increasing SSTA had no effect on rates of within-pair or total courtship of social pairs, but it depressed total copulation rate slightly and probability of laying greatly (laying probability nearly halved with each °C increase; Table 1e, Fig. 1d). Thus, even though females apparently had the opportunity to copulate, they did so less frequently and often abandoned their reproductive attempt before laying. While there were clear effects of SSTA at the seasonal scale (Table 3b and c), within-season SSTA variation had only a non-significant effect on total copulation rate (though the predicted slope of within-season effects was very similar to both between-season and overall effects; Tables 1d and 3b), and no discernible effect on laying probability (Table 3c). The decision to establish a

clutch and commit to breeding is likely based on prospective breeders' accumulation of information (Frederiksen *et al.* 2004) and/or body condition (Weimerskirch 1992; Chastel, Weimerskirch & Jouventin 1995; Robinson, Chiaradia & Hindell 2005) over the weeks or months prior to laying, and is thus less likely to be influenced by immediate conditions than neighbourhood attendance is.

As long-lived, iteroparous birds with higher adult than juvenile survival (Oro *et al.* 2010; Ancona *et al.* 2015), life-history theory predicts that boobies should reduce their overall reproductive effort when conditions are poor and reproduction is relatively costly, in order to favour future reproduction under better conditions in the current or subsequent seasons (Goodman 1979; Stearns 1992). Given this booby's long pre-laying courtship period (Osorio-Beristain & Drummond 1998) and the decreased success of late-season clutches (Peña Alvarez 2009), females may form and maintain pair-bonds under adverse current conditions as a bet-hedging strategy. Should conditions improve, they can lay immediately; should conditions remain poor they can abandon the attempt before investing heavily in egg formation or committing to incubation and chick rearing.

#### EXTRAPAIR BEHAVIOUR

Warmer waters depressed females' probability of participating in EP courtship and copulation (Table 2a and b; Fig. 2), confirming the prediction that poor conditions depress female boobies' EP behaviour. Several of our results suggest that this cutback was not due to reduced immediate availability of time or energy for interacting with EP males (*sensu* Slagsvold & Lifjeld 1997). First, within-group centring showed that females' propensity to participate in EP courtship responded to between-season, but not to within-season, variations in SSTA (Table 3d); had females' participation been limited by immediate time or energy constraints we would have also expected a clear within-season effect, similar to SSTA's effect on their attendance. Secondly, path analysis showed that decreased participation in EP courtship was not due to females' reduced attendance in the neighbourhood or inability to escape guarding by their social mates (Fig. 3). Thirdly, rates of EP courtship and copulation were unaffected by water temperature, and while unfaithful females did invest more in courtship overall than their faithful counterparts, this was due to increased courtship with their social mates, not the addition of EP interactions (Table 1c; Fig. 1b). Thus, rather than being simply unable to engage in EP behaviours because of immediate time or energy restrictions, female boobies strategically refrained from EP behaviours, presumably to avoid incurring longer-term costs to their survival and reproduction (Goodman 1979; Stearns 1992).

The boobies' response to poor conditions differs from that of arctic-breeding passerines, which are thought to produce fewer EP chicks in cold weather because high

**Table 3.** Results of the within-subject centring procedure to separate within- from between-season effects of sea surface temperature anomaly (SSTA) on the aspects of females' pre-laying behaviour found to be affected by SSTA in main analyses. For a–d,  $n = 230$  females; for e,  $n = 54$  females. 'Within-between seasons' refers to the difference between the effects at these two scales. Models include additional covariates and random factors included in the minimum adequate models (Tables 1 and 2)

Effect of SSTA	$\beta \pm \text{SE}^*$	$Z^*$	$P_Z^*$	$P_{\chi^2}^\dagger$
a) Neighbourhood attendance <sup>‡</sup> (scans present/total scans)				
Within seasons	$-0.141 \pm 0.077$	-1.82	0.068	0.074
Between seasons	$-0.128 \pm 0.034$	-3.76	<b><math>1.7 \times 10^{-4}</math></b>	<b>0.019</b>
Within-between seasons	$-0.0134 \pm 0.071$	-0.19	0.85	0.85
b) Total copulation frequency (within + EP copulations/15 min)				
Within seasons	$-0.132 \pm 0.092$	-1.42	0.15	0.15
Between seasons	$-0.0941 \pm 0.044$	-2.16	<b>0.031</b>	<b>0.033</b>
Within-between seasons	$-0.0375 \pm 0.10$	-0.36	0.72	0.72
c) Laying probability				
Within seasons	$-0.170 \pm 0.28$	-0.61	0.54	0.54
Between seasons	$-0.726 \pm 0.18$	-4.01	<b><math>6.1 \times 10^{-5}</math></b>	<b><math>3.66 \times 10^{-4}</math></b>
Within-between seasons	$0.556 \pm 0.32$	1.74	0.082	0.078
d) EP courtship probability				
Within seasons	$0.0203 \pm 0.25$	0.082	0.93	0.93
Between seasons	$-0.255 \pm 0.12$	-2.14	<b>0.032</b>	<b>0.029</b>
Within-between seasons	$0.275 \pm 0.28$	0.974	0.33	0.336
e) EP copulation probability				
Within seasons	$-0.815 \pm 0.53$	-1.54	0.12	0.092
Between seasons	$-0.374 \pm 0.29$	-1.31	0.19	0.15
Within-between seasons	$-0.413 \pm 0.55$	-0.753	0.45	0.46

\*Parameters from model summary.

† $P$ -value resulting from  $\chi^2$  nested model comparisons following removal of each variable.

‡Using data above the  $-1.025$  °C SSTA threshold of the linear negative effect revealed by piecewise analysis in main models. Significant  $P$ -values are indicated in bold.

risk of adult mortality from exposure, starvation and predation severely constrain their capacity to engage in EP behaviours (Johnsen & Lifjeld 2003; Hoset *et al.* 2013). However, unlike these small birds breeding in extreme environments, adult seabirds are able to buffer against temporary adversity; they rarely starve except under extreme conditions (Schreiber 2002), and experimentally handicapped female blue-footed boobies were able to maintain constant investment in chick rearing by temporarily sacrificing their own body condition (Velando & Alonso-Alvarez 2003). Thus, a female's decision to engage in EP behaviours may be calibrated much like her decision to lay a clutch, mostly in response to the conditions experienced over the weeks or months before pairing and, ultimately, the potential long-term costs and benefits.

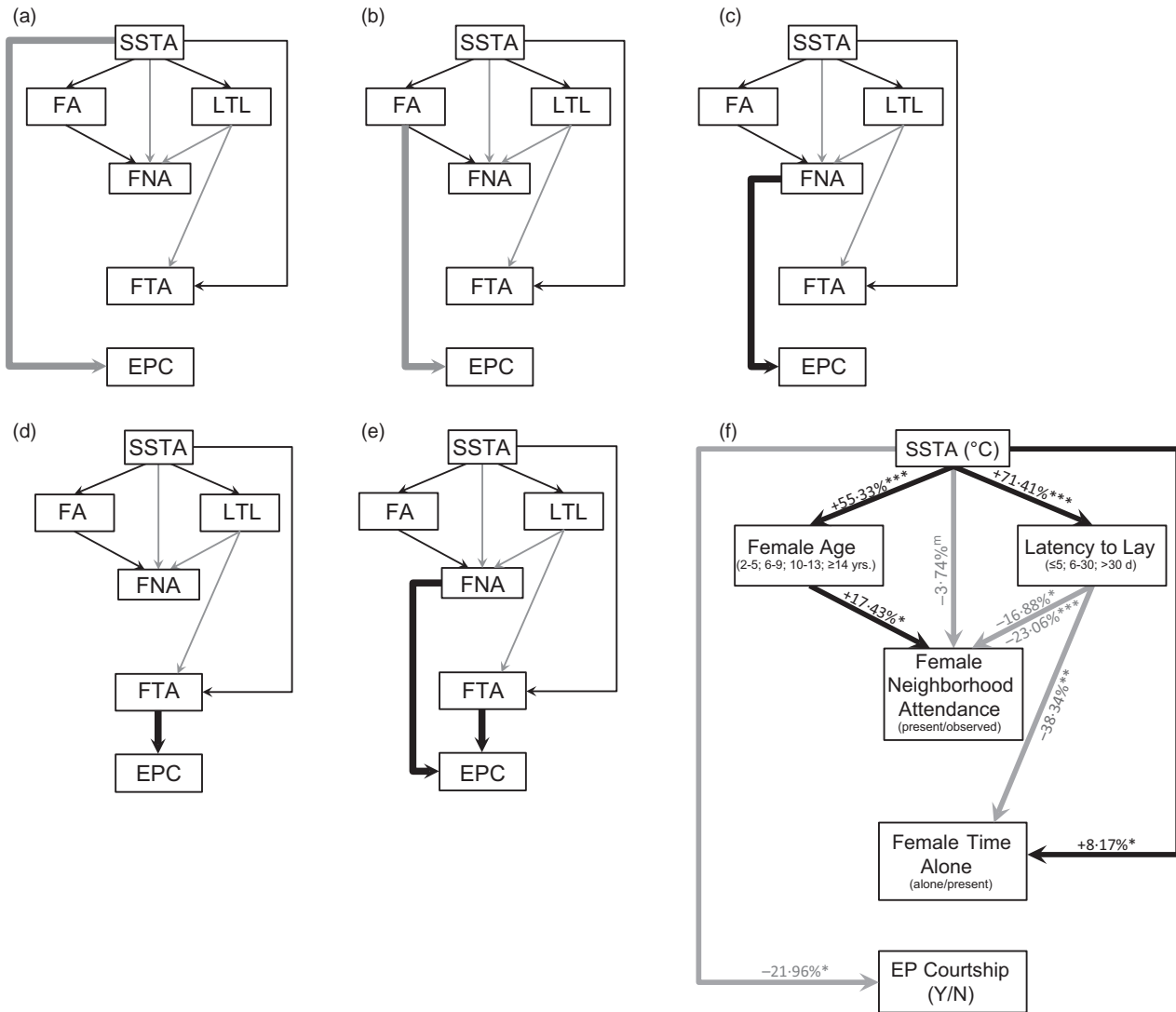
An effect of immediate time or energy constraints on females' EP behaviour rates might have gone undetected in our analyses if promiscuity is restricted to high quality females who are able to pay increased maintenance costs without sacrificing total courtship rate. However, this is unlikely because we found no evidence in our sample that promiscuous females were more efficient foragers, more successful breeders, or less affected by adverse conditions than faithful ones (EP status and EP status  $\times$  SSTA were non-significant and eliminated from minimum adequate models; see Table 1a and d).

An alternative explanation for the diminished incidence of female EP courtship in warm conditions is that this

was an outcome of females' potential EP partners engaging in fewer EP interactions due to energetic or temporal constraint. There are reasons to expect males to be more severely affected by adverse conditions than females. Handicapped males are less willing to increase parental effort than handicapped females (Velando & Alonso-Alvarez 2003), and in our path analysis the direct effect of SSTA on females' time alone was positive (i.e. as conditions worsened mate guarding by males declined; Fig. 3e). This could suggest that under adverse conditions males are less able than their mates to prioritize attendance in the colony over foraging. Decreased availability of EP partners due to male constraint would not, however, explain females' reduced EP copulation following courtship.

As SSTA increases, female boobies may cut back on EP activities because costs specific to EP activities increase under adverse conditions, for example loss of male parental care (i.e. 'constrained female hypothesis'; see Gowaty 1996; e.g. Hoi-Leitner *et al.* 1999; Cameron, Berteaux & Dufresne 2011) and exposure to sexually transmitted disease (Sheldon 1993; Loehle 1995; reviewed in Petrie & Kempenaers 1998). Unlike the immediate time and energy required for interacting with EP males, these costs are not expected to trade off directly against self-maintenance and within-pair reproductive behaviours. Female blue-footed boobies' social mates sometimes abandon them after witnessing their infidelity (Pérez-Staples *et al.* 2013), and since biparental care is obligate in





**Fig. 3.** Candidate (a–d) and final (e) path models testing whether the effect of SSTA on EP courtship probability is direct or mediated by females’ opportunity. Black arrows indicate positive and grey arrows negative effects. In a–d, thin arrows indicate paths that were previously resolved and therefore identical among models (see Appendix S1; Table S1), while thick arrows indicate paths that differed among models in order to test whether the overall negative effect of SSTA on EP courtship probability was (a) direct, or was mediated by (b) females’ neighbourhood attendance, (c) females’ time alone or (d) both. Variables are abbreviated as follows: SSTA, Sea surface temperature anomaly; FA, Female Age; LTL, Latency to lay; FNA, Female neighbourhood attendance; FTA, Female time alone; and EPC, Extrapair courtship probability. Model (a) was most supported (details in e). Numbers in (e) indicate effect coefficients (slopes) expressed as units of change in the response variable per unit increase in the explanatory variable (e.g. for each 1 °C increase in SSTA, females are 55.3% more likely to be in an older age class, spend 3.7% less time on their territories and are 71.4% more likely to be in a class further from laying). Significance of these slopes is indicated as: <sup>m</sup> =  $P < 0.06$  (marginal), \* $P < 0.05$ , \*\* =  $P < 0.01$  and \*\*\* =  $P < 0.001$ . For categorical explanatory variables, only significant contrasts are shown: neighbourhood attendance:  $\geq 14$ -year-old vs. 2- to 5-year-old females and females  $< 5$  vs. 6–30 (above line) and 6–30 vs.  $> 30$ d (below line) from laying. For female time alone,  $< 5$  days vs. 6–30 days from laying.

boobies (Nelson 2005), females should be less willing to risk the current social pair-bond during warm water conditions when a later start to breeding (with a new partner) would reduce success in a second breeding attempt (Ancona *et al.* 2011). Indeed, the elevated social courtship we observed in promiscuous females could be a tactic to reinforce the social pair-bond and avoid divorce (as suggested to explain increased social courtship immediately following EP courtship, Pérez-Staples & Drummond 2005). Short of divorce, even a partial reduction in male

parental care could be particularly costly when elevated SSTA makes food scarce, since reduced male effort during chick rearing decreases female body condition in this species (Velando & Alonso-Alvarez 2003).

Limiting the number of sexual partners when conditions are poor may be a behavioural mechanism to avoid exposure to sexually transmitted disease at a time when the immune system is weakened by stress (Loehle 1995). This could account for female boobies reducing their probability of EP copulation nearly twice as steeply as their

**Table 4.** Summary statistics for candidate path models proposed in Fig. 3. d.f. refers to the total degrees of freedom contained in all paths from each candidate model. Fisher's *C* and its corresponding *P* and *AICc* values for each model were calculated following (Gonzalez-Voyer & von Hardenberg 2014; Shipley 2013).  $\Delta AICc$  is with reference to model a, the lowest-AIC model. Details of path analysis methods are provided in supporting information

Model	d.f.	Fisher's <i>C</i>	<i>P<sub>C</sub></i>	<i>AICc</i>	$\Delta AICc$
a	32	14.262	0.4304	90.061	0
b	34	16.719	0.2715	98.165	8.104
c	32	17.254	0.2429	93.053	2.992
d	32	19.181	0.1516	96.425	6.364
e	33	16.370	0.1749	94.977	4.916

probability of EP courtship (35.5% vs. 18.0% per °C SSTA increase, respectively) as water temperature increases. There was evidence that this overall negative effect of warm waters on EP copulation comprised both within- and between-season components. Females could avoid exposure to sexually transmitted pathogens when immunologically vulnerable by discriminating against males of dubious health. In boobies, this could be achieved by being selective of foot colour, a sexual ornament correlated with immune status that shows short-term variation, declining within 48 h of food deprivation or immune challenge (Torres & Velando 2007; Kiere & Drummond 2014 discussion). Thus, variation in female willingness to copulate with EP partners could be due to within- or between-season variation in environmental conditions acting on them either directly or indirectly through effects on EP partners. While sexually transmitted disease has long been proposed as a selective pressure in avian mating systems (Sheldon 1993) and there is some evidence of sexual transmission of microbes in wild birds (White *et al.* 2010), it remains to be seen whether potential exposure to pathogens imposes a significant constraint on EP behaviour during environmentally induced stress.

## CONCLUSIONS

Our study demonstrates that adverse El Niño conditions depress female blue-footed boobies' participation in EP courtship and copulation, in addition to their pre-laying reproductive activities in general. To our knowledge, it is the first study to do so by quantifying within- and extrapair courtship and copulation rather than inferring behaviour from paternity. While SSTA effects were most apparent at the between-season scale, females' neighbourhood attendance and propensity to copulate with their EP partners appeared to be sensitive also to within-season SSTA fluctuations. Furthermore, our results suggest that females do not refrain from EP behaviours because they are unable to afford the immediate time or energy costs they impose, but rather as part of a strategy to avoid longer-term costs specific to EP behaviour. Thus, current conditions can influence EP behaviour of a long-lived

species that is ostensibly able to buffer against temporary adversity. Further investigation is needed to determine the relative importance of putative costs of EP behaviour to both males and females (e.g. time/energy budget, divorce, disease), and to uncover the proximate mechanisms that mediate their responses (e.g. stress hormones; see Wingfield *et al.* 1999). The ENSO cycle and other large-scale climate fluctuations have been extensively linked to population- and community-level phenomena (see review in Stenseth *et al.* 2002), but the behavioural mediation of these links, including life-history and intergenerational effects (e.g. Ancona & Drummond 2013; Ancona *et al.* 2015), also needs to be understood. As global climate change continues to modify environments, including ENSO cycles (Cai *et al.* 2014), we urgently need to come to grips with the effects of climate on behaviour at a variety of temporal and spatial scales.

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## Data accessibility

Data upon which this study is based are available through the Dryad Digital Repository <http://dx.doi.org/10.5061/dryad.41qh7> (Kiere & Drummond 2016).

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## Supporting Information

Additional Supporting Information may be found in the online version of this article.

**Appendix S1.** Details of confirmatory path analysis methods and results.

**Figure S1.** Candidate pre-models with non-significant  $P_C$  values.

**Table S1.** Summary statistics for candidate models with non-significant  $P_C$  values.

**Table S2.** Basis sets describing the four candidate path models proposed in Fig. 3.

## Discusión General

La aparente paradoja de que las hembras frecuentemente participan en las conductas EP a pesar de sus potenciales costos y sin la ganancia clara de aumentar su número total de progenie ha sido motivo de más de 30 años de investigación para resolver *¿por qué lo hacen?*; la impresionante variación entre y dentro de individuos, poblaciones, y especies ha cambiado esta pregunta a *¿por qué algunas lo hacen y otras no?* Estas fueron las preguntas centrales de esta tesis, que se concretaron en la evaluación de tres hipótesis específicas en hembras del bobo de patas azules: la primera y segunda acerca de posibles funciones adaptativas de las conductas EP para las hembras, y la tercera acerca de la variación individual en su participación debido a condiciones ambientales.

### *Funciones de la conducta EP en hembras del bobo de patas azules*

Durante los últimos 30 años las hipótesis de *buenos genes* y *compatibilidad genética* han recibido mucha atención, por lo que es evidente que las preguntas abordadas en los primeros dos capítulos de esta tesis no son nuevas. Sin embargo, dada la falta de consenso acerca de si (o bajo qué circunstancias) estas hipótesis explican los patrones de conducta EP observados en las aves, también es evidente que hace falta seguir explorando este tema. Revisiones prominentes durante casi dos tercios de ese tiempo han sugerido que para seguir avanzando nuestro entendimiento de la conducta EP en hembras, es necesario ampliar nuestra forma de investigarla (Griffith et al. 2002, Westneat y Stewart 2003, Akçay y Roughgarden 2007, Forstmeier et al. 2014). Siguiendo ese consejo, la aportación de esta tesis es novedosa en dos aspectos: 1) exploramos estas hipótesis de forma *comparable* con trabajos anteriores, pero en una especie con características taxonómicas, geográficas, y de historia de vida que están sub-representadas en la literatura actual, y 2) pusimos a prueba estas hipótesis considerando versiones más matizadas de sus predicciones, lo cual fue posible debido a la observación directa de conducta en lugar de (o en adición a) pruebas moleculares de paternidad.

En el primer capítulo, pusimos a prueba la hipótesis de *buenos genes*, que propone que las hembras utilizan las conductas EP para conseguir padres genéticos de mayor calidad que sus machos sociales, y así aumentar la calidad de su progenie. Al hacerlo usando las predicciones "clásicas," sencillas e intuitivas de 1) menor probabilidad de conductas EP entre mayor la calidad del macho social, y 2) mayor calidad del macho EP que el social, nos encontramos con la misma dificultad de interpretación que otros han señalado- el encontrar apoyo en una de las predicciones y aspectos de calidad, pero no en otras (Akçay y Roughgarden 2007). Entre más joven fue el macho social, fue más probable que la hembra cortejara con un macho EP, pero la edad del macho social *no* influyó en la probabilidad que la hembra llegara a copular con el macho EP (habiendo cortejado con él), y los machos EP *no* fueron más viejos que los sociales. Además, ningún otro aspecto de la calidad del macho social (tamaño, condición corporal, color de patas, o éxito reproductivo en el pasado) tuvo efecto sobre la probabilidad de participar en conductas EP, y ningún aspecto de calidad difirió entre machos sociales y sus rivales EP. En general, resultados mixtos como estos han puesto en duda cuantos de los resultados negativos en la literatura actual realmente se deben a que no haya selección por buenos genes, y cuantos se deben a que no se han medido las características más relevantes para la elección de las hembras o a que las reglas que siguen las hembras son más complejas que una simple comparación del macho social con el potencial macho EP (Hasson y Stone 2011). Incluso se ha propuesto que la tasa menor de paternidad EP en hembras apareadas con machos de mayor edad (una de las tendencias generales más fuertes; Akçay & Roughgarden 2007; Cleasby & Nakagawa 2012) se debe no a elección femenina, sino a que los machos mayores son más efectivos para resguardar a sus parejas (Cleasby y Nakagawa 2012), lo cual también es posible aquí.

En su modelo teórico, Hasson y Stone (2011) expandieron la hipótesis de buenos genes, considerando que bajo algunas condiciones, las hembras podrían ganar mayores beneficios netos de buenos genes al *romper* las predicciones clásicas que al seguirlas. Si el ser selectiva hacía machos EP

implica costos, si se favorecen pos-cópula los espermatozoides de machos de mayor calidad, y si los indicadores físicos de la calidad no son completamente confiables, las hembras que están apareadas con machos sociales de baja calidad se podrían beneficiar al copular con machos EP al azar, *sin* ser selectivas de su calidad (Hasson y Stone 2011). Esto genera la predicción que las hembras apareadas con machos de alta calidad deben ser selectivas (y por lo tanto cumplir con las predicciones clásicas), mientras que aquellas con machos sociales de baja calidad deben copular con machos EP al azar, aunque sean de *peor* calidad aparente que el macho social. Encontramos el primer apoyo empírico para esta predicción, encontrando que entre más atractivo (verde) el color de las patas del macho social, más superior tenía que ser el color del macho EP comparado con el social para que la hembra llegara a copular con él. Lo que nos permitió siquiera hacer esta comparación fue el poder cuantificar las características de los machos que tuvieron oportunidad de ser elegidos como parejas de cópula EP (i.e. recibieron cortejo EP recíproco de la hembra) pero no lo fueron. Esto no es posible al usar datos de paternidad, donde solo se identifican a los machos EP que fueron exitosos- no solo en lograr *cópulas* EP, sino también cuyos espermatozoides fueron exitosos en *ganar fertilización* EP de un huevo que se desarrolló exitosamente hasta ser muestreado como *cría* EP. Además, encontramos evidencia de que los machos EP son selectivos hacia las hembras, dejando claro que las cópulas EP ocurren solo cuando coinciden los intereses de la hembra y del macho EP, y contradiciendo el frecuente supuesto no hablado que cualquier macho debería aceptar cualquier oportunidad de cópula EP. Estos resultados, en el contexto de la literatura general de este tema, enfatizan que el considerar "escritas en piedra" un juego de predicciones, por muy intuitivas que sean, puede llevar a la subestimación del apoyo para una hipótesis. Sería, por lo tanto, muy informativo evaluar si existe similar selectividad flexible de las hembras y selectividad de los machos EP en otras especies donde no se ha encontrado apoyo para la hipótesis de buenos genes.



Cabe mencionar que después de obtener estos resultados, intentamos poner a prueba más explícitamente la selectividad EP flexible de las hembras a base de lo atractivo del macho social con un experimento en campo durante la temporada reproductiva del 2013. Después de un período de observación control de 3 días para cuantificar la conducta social y EP de hembras promiscuas, capturamos a la hembra, su pareja social, y el macho EP. Medimos el color de patas de los tres y manipulamos el color de patas del macho EP usando maquillaje azul que simula un color menos atractivo (siguiendo el método de Torres y Velando 2003), esperando que esta manipulación disminuyera más la frecuencia de cortejos y cópulas de la hembra hacia el macho EP entre más atractivo el macho social. Desafortunadamente, el experimento resultó no ser factible, debido en parte a las dificultades logísticas de primero identificar a hembras que tenían conductas EP dentro de un tiempo relativamente corto (que solo representan un ~30% de la población), y luego lograr capturar a los tres individuos. Por lo tanto, después de tres meses de esfuerzo intensivo, el tamaño de muestra fue demasiado restringido y se abandonó el experimento. Sin embargo, si se encontrara una forma más eficiente de identificar y cuantificar las interacciones entre hembras promiscuas y sus machos EP (e.g. vía monitoreo remoto), valdría la pena volver a intentar este experimento, ya que sería una valiosa prueba de esta versión más matizada de la hipótesis de buenos genes. Una alternativa sería hacer un experimento similar en una especie que demuestra una flexibilidad similar en la selectividad EP, pero que se puede mantener en cautiverio o semi-cautiverio para facilitar este tipo de manipulación.

En el segundo capítulo, evaluamos la hipótesis de *compatibilidad genética*, que propone que las hembras utilizan las conductas EP para conseguir padres genéticos que generarán crías de mayor heterocigocidad individual, y por lo tanto mayor adecuación. Aquí los resultados fueron más claros que en el Capítulo 1; no encontramos *ningún* apoyo para esta hipótesis. No hubo evidencia de que individuos con mayor heterocigocidad fueran de mayor calidad, ni hubo menor probabilidad de conductas o paternidad EP entre mayor heterocigocidad o compatibilidad del macho social, ni mayor heterocigocidad



o compatibilidad del macho EP que el social, ni mayor heterocigocidad de las crías EP que las sociales, ni mayor heterocigocidad de crías sociales en nidos sin crías EP que en nidos de paternidad mixta. Similar al primer capítulo, también exploramos la posibilidad de flexibilidad en la selectividad de las hembras con respecto a la heterocigocidad, y tampoco hubo evidencia de eso.

Aunque es posible que limitaciones metodológicas fueron la causa de estos resultados consistentemente negativos, nuestros métodos y tamaños de muestra fueron comparables a otros estudios que sí han encontrado apoyo (ver revisiones en Chapman et al. 2009, Arct et al. 2015). Claro, el aumentar el tamaño de muestra y usar otras técnicas moleculares que podrían cuantificar de forma más detallada la heterocigocidad individual, como los polimorfismos de un solo nucleótido ("single nucleotide polymorphisms", o SNPs, en inglés) serían útiles para descartar esta explicación. También es posible que la ventaja de mayor heterocigocidad (y por lo tanto la selección por machos que la maximicen en los hijos) es contexto-dependiente, y que nuestro muestreo no capturó el contexto relevante. Por ejemplo, muchos de los efectos de la endogamia se manifiestan temprano en la vida (discusión en Chapman et al. 2009), y es posible que las hembras solo favorecen a machos que generarán hijos más heterocigotos cuando es probable que los hijos confronten condiciones particularmente difíciles temprano en la vida, por ejemplo al nacer cuando las condiciones ambientales son pobres. Si esto es el caso, nuestro muestreo fue particularmente malo para detectarlo, ya que el año de muestreo para este estudio (2011) fue de los más productivos de la historia conocida de la colonia (probablemente debido a un evento fuerte de La Niña; ver Ancona et al. 2011). Finalmente, es posible que en esta población simplemente no hay un riesgo fuerte de endogamia, dejando poca presión por evitarla al seleccionar le padre genético con base en su heterocigocidad o compatibilidad. Cuando exploramos esta posibilidad generando un pedigrí basado en las identidades de las parejas sociales y sus hijos en la Isla Isabel, encontramos una tasa sorprendentemente baja de apareamiento social entre parientes- solo el 0.72% de individuos tuvieron padres sociales que fueron parientes de primer grado (madre-hijo/padre-hija, hermanos

completos), y la coeficiente de endogamia promedio ( $f$ ) fue de 0.002 ( $\sigma^2=0.0005$ ). Esto fue inesperado dada la filopatria de los bobos (Kim et al. 2007) y su reproducción insular. Estas tasas tan bajas de endogamia de parejas sociales- independiente de la paternidad EP- sugieren que al formarse la pareja social ya se evitó en gran medida la endogamia, por lo que el evitarlo a través de cópulas EP probablemente no brindaría mucho más beneficio. Sería interesante usar simulaciones estadísticas y cálculos de genética de poblaciones basados en los microsatélites utilizados en este estudio para evaluar si el nivel de endogamia está dentro del rango esperado por el azar dado el tamaño de la población, o si es probable que haya algún mecanismo que evite el apareamiento entre parientes cercanos.

#### *Efecto de condiciones ambientales sobre la conducta EP*

En el tercer capítulo, evaluamos si las condiciones adversas antes de la puesta, en la forma de elevación en la temperatura superficial del mar, disminuyen la participación en las conductas EP. Primero, pusimos a prueba si la conducta reproductiva en general se disminuye, y luego si la probabilidad e intensidad de la conducta EP en particular fue reprimida bajo condiciones difíciles. Encontramos que tanto el tiempo que las hembras pasan en la colonia, como su tasa total de cópulas y probabilidad de establecer una puesta (poner al menos un huevo) disminuyeron en cuanto mayor fue la temperatura superficial del mar (peores condiciones). La probabilidad de participar en cortejo y cópulas EP disminuyó, pero entre las hembras que sí participaron, la tasa de conductas EP no cambió con la temperatura. Además, cuando usamos análisis de vías para comprobar si la reducción en la participación en cortejo EP fue debido a la disminución en tiempo que las hembras tuvieron disponible, encontramos que este no fue el caso. En conjunto, nuestros resultados sugieren que las hembras no restringieron su conducta EP solo porque no tuvieron el tiempo o la energía para hacerlo, sino por evitar costos a largo plazo y asociadas a las interacciones EP en sí, como son la reducción del cuidado parental o divorcio del macho social actual o la exposición a patógenos de transmisión sexual. Esto difiere de algunos ejemplos

en aves pequeñas de vida corta en ambientes de frío extremo, donde análisis de paternidad EP sugieren que la reducción en actividades EP es debido a un compromiso inmediato de forrajeo o termorregulación versus interacciones EP (Johnsen y Lifjeld 2003, Hoset et al. 2013).

Cabe mencionar que para este capítulo también intentamos llevar a cabo un experimento en campo durante la temporada del 2013. Intentamos suplementar el alimento de hembras apareadas durante el cortejo, esperando que al disminuir la presión de forrajear, tuvieran mayor probabilidad de participar en conductas EP. Para suplementar el alimento, aventamos sardina (*Sardinops sagax*) fresca al territorio de la hembra. Desafortunadamente, aunque esta técnica se ha utilizado en las Islas Galápagos para suplementar el alimento a hembras del bobo de Nazca antes de la puesta (Clifford y Anderson 2001), aquí las hembras no aceptaron consistentemente el alimento y no hubo forma confiable de evitar que el macho social u otros individuos vecinos se lo comieran, por lo que se abandonó el experimento.

En general, el tema del efecto de condiciones ambientales sobre la conducta EP está poca explorado, con sólo un puñado de estudios empíricos (Westneat 1994, Korpimäki et al. 1996, Hoi-Leitner et al. 1999, Václav et al. 2003, Bouwman y Komdeur 2006, Cameron et al. 2011), y sólo uno que conocemos (el capítulo 3) que se basa principalmente en observaciones directas de conducta EP. Esto deja un campo abierto para mucha exploración, y un siguiente paso intuitivo sería evaluar los posibles mecanismos específicos detrás de la reducción en conducta EP. Por ejemplo, se podría evaluar el efecto de la conducta EP sobre el cuidado parental del macho bajo diferentes condiciones ambientales, evaluar la prevalencia y transmisión de enfermedades de transmisión sexual dependiendo tanto de las condiciones ambientales como de la frecuencia individual y poblacional de conductas EP, o usar implantes hormonales para determinar experimentalmente si las hormonas de estrés (que se aumentan en esta población durante años de temperatura elevada; Wingfield et al. 1999) disminuyen la conducta EP.

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## Anexo 1:

### Información electrónica suplementaria asociada al Capítulo 2

(disponible en <http://www.avianbiology.org/appendix/jav-01061>)

**Table A1: Full results of models testing for correlations between individual standardized heterozygosity and fitness variables.** Brood size and hatch order are categorical variables, such that values refer to the comparison of 2- and 3-chick (in one case, 4-chick) broods to 1-chick broods and of second- and third-hatched chicks to first-hatched chicks, respectively.

Response Variable	<i>n</i>	Fixed Effect	$\beta$	SE	<i>t</i>		
At fledging:							
Fledging probability	806	Standardized Heterozygosity	-5.17	3.61	-1.43		
		Standardized Heterozygosity <sup>2</sup>	-0.887	1.61	-0.550		
		Brood Size:					
		2 chicks	0.479	1.90	0.250		
		3 chicks	1.49	2.63	0.570		
		4 chicks	-8.61	13.99	-0.620		
		Hatch Order:					
		second chick	-1.69	0.936	-1.81		
		third chick	-2.43	1.72	-1.42		
		Proportional Laydate	-6.73	3.48	-1.93		
		Intercept	14.03	2.94	4.77		
		$\sigma^2_{\text{Cohort}} = 171.8$					
Bill length	750	Standardized Heterozygosity	0.101	0.139	0.728		
		Standardized Heterozygosity <sup>2</sup>	0.307	0.404	0.760		
		Brood Size:					
		2 chicks	-0.0288	0.0931	-0.309		
		3 chicks	-0.119	0.123	-0.966		
		Hatch Order:					
		second chick	-0.212	0.0846	-2.50		
		third chick	-0.502	0.202	-2.49		
		Proportional Laydate	0.0988	0.161	0.614		
		Intercept	0.0733	0.112	0.654		
		$\sigma^2_{\text{Cohort}} = 0.0543$					
		Ulna length	748	Standardized Heterozygosity	0.115581	0.115691	0.999
Standardized Heterozygosity <sup>2</sup>	-0.235069			0.335621	-0.700		
Brood Size:							
2 chicks	-0.07278			.077533	-0.939		
3 chicks	-0.039314			0.102643	-0.383		
Hatch Order:							
second chick	0.008182			0.070111	0.117		
third chick	-0.100057			0.167080	-0.599		
Proportional Laydate	-0.088134			0.134639	-0.655		
Intercept	0.217156			0.119819	1.812		
$\sigma^2_{\text{Cohort}} = 0.1311$							

Response Variable	<i>n</i>	Fixed Effect	$\beta$	SE	<i>t</i>
Body mass	698	Standardized Heterozygosity	-0.00552	0.113	-0.049
		Standardized Heterozygosity <sup>2</sup>	-0.104	0.329	-0.316
		Brood Size:			
		2 chicks	-0.0187	0.0755	-0.247
		3 chicks	0.00500	0.0995	0.050
		Hatch Order:			
		second chick	-0.189	0.0682	-2.77
		third chick	-0.635	0.164	-3.86
		Proportional Laydate	-0.576	0.131	-4.40
		Intercept	0.137	0.162	0.847
$\sigma^2_{\text{Cohort}} = 0.277$					
As adults:					
Bill length	642	Standardized Heterozygosity	0.134	0.151	0.889
		Standardized Heterozygosity <sup>2</sup>	-0.114	0.455	-0.250
		Age	$7.30 \times 10^{-5}$	0.0150	0.005
		Intercept	$-2.98 \times 10^{-5}$	0.169	0.000
		$\sigma^2_{\text{Cohort}} = 0.0504$			
Ulna length	642	Standardized Heterozygosity	0.106	0.151	0.703
		Standardized Heterozygosity <sup>2</sup>	-0.111	0.456	-0.244
		Age	0.00426	0.0146	0.291
		Intercept	-0.0153	0.164	-0.093
		$\sigma^2_{\text{Cohort}} = 0.0456$			
Body mass	639	Standardized Heterozygosity	0.240	0.153	1.57
		Standardized Heterozygosity <sup>2</sup>	-0.176	0.463	-0.379
		Age	0.0110	0.0458	0.240
		Age <sup>2</sup>	$-4.20 \times 10^{-4}$	0.00220	-0.190
		Intercept	-0.0278	0.210	-0.132
		$\sigma^2_{\text{Cohort}} = 0.0132$			
Foot color	124	Standardized Heterozygosity	-0.0148	0.288	-0.0510
		Standardized Heterozygosity <sup>2</sup>	-0.757	0.833	-0.909
		Age	-0.0175	0.0740	-0.236
		Age <sup>2</sup>	-0.00135	0.00355	-0.381
		Intercept	0.301	0.346	0.868
		$\sigma^2_{\text{Cohort}} = 0.0031$			
Mean breeding success	749	Standardized Heterozygosity	0.0953	0.0838	1.14
		Standardized Heterozygosity <sup>2</sup>	0.212	0.245	0.868
		Age	-0.00230	0.00947	-0.316
		Age <sup>2</sup>	$-1.33 \times 10^{-5}$	0.00114	-0.012
		Intercept	0.342	0.0358	9.55
$\sigma^2_{\text{Cohort}} = 0.00204$					

**Table A2: Full results of models testing for correlations between individual  $Md^2$  and fitness variables.** Brood size and hatch order are categorical variables, such that values refer to the comparison of 2- and 3-chick (and in one case, 4-chick) broods to 1-chick broods and of second- and third-hatched chicks to first-hatched chicks, respectively.

Response Variable	<i>n</i>	Fixed Effect	$\beta$	SE	<i>t</i>	
At fledging:						
Fledging probability	806	Linear $Md^2$	0.079	0.400	0.200	
		Quadratic $Md^2$	0.206	0.320	0.640	
		Brood Size:				
		2 chicks	0.257	1.80	0.140	
		3 chicks	1.29	2.50	0.520	
		4 chicks	-8.04	13.5	-0.600	
		Hatch Order:				
		second chick	-1.64	0.897	-1.82	
		third chick	-2.15	1.68	-1.28	
		Proportional Laydate	-6.67	3.32	-2.01	
		Intercept	13.1	2.72	4.81	
$\sigma^2_{\text{Cohort}} = 176.6$						
Bill length	750	Linear $Md^2$	0.0364	0.0256	1.42	
		Quadratic $Md^2$	-0.0517	0.0185	-2.79	
		Brood Size:				
		2 chicks	-0.0309	0.0926	-0.334	
		3 chicks	-0.116	0.122	-0.945	
		Hatch Order:				
		second chick	-0.211	0.0841	-2.51	
		third chick	-0.486	0.200	-2.43	
		Proportional Laydate	0.0714	0.160	0.447	
		Intercept	0.198	0.114	1.73	
		$\sigma^2_{\text{Cohort}} = 0.0519$				
Ulna length	748	Linear $Md^2$	0.0319	0.0213	1.50	
		Quadratic $Md^2$	-0.0418	0.0154	-2.72	
		Brood Size:				
		2 chicks	-0.0741	0.0771	-0.962	
		3 chicks	-0.0464	0.102	-0.454	
		Hatch Order:				
		second chick	0.0118	0.0697	0.169	
		third chick	-0.0798	0.166	-0.480	
		Proportional Laydate	-0.107	0.134	-0.802	
		Intercept	0.290	0.123	2.35	
		$\sigma^2_{\text{Cohort}} = 0.136$				

Response Variable	<i>n</i>	Fixed Effect	$\beta$	SE	<i>t</i>
Body mass	698	Linear Md <sup>2</sup>	0.00517	0.0209	0.248
		Quadratic Md <sup>2</sup>	-0.0283	0.0154	-1.84
		Brood Size:			
		2 chicks	-0.0211	0.0753	-0.280
		3 chicks	0.00355	0.0992	0.0360
		Hatch Order:			
		second chick	-0.184	0.0680	-2.71
		third chick	-0.636	0.163	-3.89
		Proportional Laydate	-0.581	0.130	-4.45
		Intercept	0.197	0.165	1.20
$\sigma^2_{\text{Cohort}} = 0.280$					
As adults:					
Bill length	642	Linear Md <sup>2</sup>	-0.0189	0.0273	-0.692
		Quadratic Md <sup>2</sup>	0.00960	0.0199	0.484
		Age	0.000719	0.0150	0.048
		Intercept	-0.0317	0.173	-0.184
		$\sigma^2_{\text{Cohort}} = 0.05105$			
Ulna length	642	Linear Md <sup>2</sup>	-0.00379	0.0274	-0.138
		Quadratic Md <sup>2</sup>	-0.00218	0.0199	-0.109
		Age	0.00459	0.0146	0.314
		Intercept	-0.0226	0.167	-0.135
		$\sigma^2_{\text{Cohort}} = 0.0457$			
Body mass	639	Linear Md <sup>2</sup>	0.0229	0.0279	0.821
		Quadratic Md <sup>2</sup>	-0.00250	0.0202	-0.124
		Age	0.00669	0.0457	0.146
		Age <sup>2</sup>	-0.000201	0.00220	-0.091
		Intercept	-0.0314	0.213	-0.148
		$\sigma^2_{\text{Cohort}} = 0.01294$			
Foot color	124	Linear Md <sup>2</sup>	-0.0453	0.0543	-0.834
		Quadratic Md <sup>2</sup>	-0.0479	0.0383	-1.25
		Age	-0.00808	0.0742	-0.109
		Age <sup>2</sup>	-0.00169	0.00355	-0.474
		Intercept	0.297	0.348	0.852
		$\sigma^2_{\text{Cohort}} = 0.00158$			
Mean breeding success	749	Linear Md <sup>2</sup>	0.00886	0.0155	0.572
		Quadratic Md <sup>2</sup>	0.00353	0.0112	0.314
		Age	-0.00347	0.00948	-0.365
		Age <sup>2</sup>	$6.50 \times 10^{-5}$	0.00114	0.0570
		Intercept	0.343	0.0393	8.71
		$\sigma^2_{\text{Cohort}} = 0.00205$			

**Table A3: Full results of models testing the effect of social male standardized heterozygosity (SH) on probability of extra-pair (EP) behaviors and paternity.**

Response Variable	$n$ (no/yes)	Fixed Effect	$\beta$	SE	Z
EP Courtship Probability:	92 (73/19)	Social male SH	0.716	0.998	0.717
		Social male SH <sup>2</sup>	-1.73	3.10	-0.559
		Intercept	-1.21	0.333	-3.62
		$\sigma^2_{\text{Year}} = 0.00$			
EP Copulation Probability:	19 (11/8)	Social male SH	1.45	2.07	0.701
		Social male SH <sup>2</sup>	-2.02	5.95	-0.339
		Intercept	-0.227	0.572	-0.397
		$\sigma^2_{\text{Year}} = 0.00$			
EP Paternity Probability:	384 (341/43)	Social male SH	-0.0335	0.567	-0.059
		Social male SH <sup>2</sup>	0.839	1.69	0.496
		Intercept	-2.073	0.202	-10.3
		(no random effects)			

**Table A4: Full results of models testing the effect of the interaction of female and social male standardized heterozygosity (SH) on probability of extra-pair (EP) behaviors and paternity.**

Response Variable	$n$ (no/yes)	Fixed Effect	$\beta$	SE	Z
EP Courtship Probability:	54 (42/12)	Female SH	4.51	6.10	0.741
		Social male SH	6.82	6.18	1.10
		Female * Social male SH	-3.83	6.15	-0.623
		Intercept	-8.69	6.29	-1.380
		$\sigma^2_{\text{Year}} = 0.00$			
EP Paternity Probability:	384 (341/43)	Female SH	-0.129	2.60	-0.0500
		Social male SH	0.197	2.49	0.0790
		Female * Social male SH	-0.371	2.53	-0.147
		Intercept	-1.79	2.56	-0.698
		(no random effects)			



**Table A5: Full results of models testing the effect of social male Md<sup>2</sup> on probability of extra-pair (EP) behaviors and paternity.**

Response Variable	<i>n</i> (no/yes)	Fixed Effect	$\beta$	SE	Z
EP Courtship Probability:	92 (73/19)	Linear Social male Md <sup>2</sup>	-0.0984	0.192	-0.511
		Quadratic Social male Md <sup>2</sup>	-0.149	0.143	-1.04
		Intercept	-1.01	0.376	-2.69
		$\sigma^2_{\text{Year}} = 0.00$			
EP Copulation Probability:	19 (11/8)	Linear Social male Md <sup>2</sup>	$-1.93 \times 10^{-3}$	0.363	$-5.00 \times 10^{-3}$
		Quadratic Social male Md <sup>2</sup>	-0.152	0.339	-0.450
		Intercept	-0.0496	0.745	-0.0670
		$\sigma^2_{\text{Year}} = 0.00$			
EP Paternity Probability:	384 (341/43)	Linear Social male Md <sup>2</sup>	0.0456	0.142	0.322
		Quadratic Social male Md <sup>2</sup>	-0.240	0.109	-2.22
		Intercept	-1.63	0.215	-7.55
		(no random effects)			

**Table A6: Full results of models testing the effect of the interaction of female and social male Md<sup>2</sup> on probability of extra-pair (EP) behaviors and paternity.**

Response Variable	<i>n</i> (no/yes)	Fixed Effect	$\beta$	SE	Z
EP Courtship Probability:	54 (42/12)	Female Md <sup>2</sup>	-0.371	0.859	-0.432
		Social male Md <sup>2</sup>	-0.992	1.05	-0.945
		Female * Social male Md <sup>2</sup>	0.163	0.208	0.783
		Intercept	1.39	4.20	0.331
		$\sigma^2_{\text{Year}} = 0.00$			
EP Paternity Probability:	384 (341/43)	Female Md <sup>2</sup>	-0.0741	0.429	-0.173
		Social male Md <sup>2</sup>	-0.196	0.446	-0.439
		Female * Social male Md <sup>2</sup>	0.0387	0.0869	0.445
		Intercept	-1.68	2.19	-0.770
		(no random effects)			

**Table A7: Full results of models testing the effect of the pairwise relatedness of the female and her social male on the probability of extra-pair (EP) behaviors and paternity.**

Response Variable	<i>n</i> (no/yes)	Fixed Effect	$\beta$	SE	Z
EP Courtship Probability:	54 (42/12)	Relatedness	-21.2	13.60	-1.56
		Relatedness <sup>2</sup>	30.4	32.3	0.941
		Intercept	-0.675	0.382	-1.77
$\sigma^2_{\text{Year}} = 2.78 \times 10^{-16}$					
EP Paternity Probability:	384 (341/43)	Relatedness	4.69	3.52	-1.33
		Relatedness <sup>2</sup>	8.95	8.00	1.12
		Intercept	1.88	0.205	-9.20
(no random effects)					

## **Anexo 2:**

### **Información electrónica suplementaria asociada al Capítulo 3**

(disponible en <http://onlinelibrary.wiley.com/doi/10.1111/1365-2656.12537/full#footer-support-info>)

#### **Appendix S1: Details of confirmatory path analysis methods and results**

Generalized multilevel confirmatory path analysis was used to test the hypothesis that the overall negative effect of SSTA on females' participation in EP behaviors was due to higher SSTA increasing the representation of older females or decreasing the time females had available to participate in EP behaviours. If reduced EP courtship is due to increasing SSTA biasing female age distribution upwards, the most supported model should show an increase in female age with increasing SSTA and a decrease in EP courtship participation with increasing age. If reduced EP courtship probability is due to decreased opportunity to participate as SSTA increases, the most supported model should show a negative effect of SSTA on females' time present and/or time alone and a positive effect of either or both of these variables on EP courtship probability.

In path analysis, indirect relationships require that some variables be simultaneously considered both dependent and independent. Since our data are hierarchically structured and include several different variable types (ordinal, binary, count), the "d-sep" method was used to allow the inclusion of random effects as well as the use of the most appropriate modeling technique for each variable class (Shipley 2009; Shipley 2013). In the d-sep method, a set of alternative candidate models is proposed graphically as a series of "Directed Acyclical Graphs" (DAG) and described mathematically by a "basis set" of conditional independence claims (pairs of variables that are not connected in the DAG by arrows and therefore should be independent

when their direct causes are controlled for). Then, the overall congruence between the proposed model and the real relationships in the data is evaluated using the  $P$ -value and  $AIC$  corresponding to Fisher's  $C$ , which follows a  $\chi^2$  distribution with 2 times the number of independence claims degrees of freedom. A significant  $P$ -value ( $P_C$ ) indicates an inappropriate model and higher  $AIC$  indicates a less parsimonious model. Here,  $AIC_C$  was used to correct for small sample size, calculated following Shipley (2013) and Gonzalez-Voyer and von Hardenberg (2014).

Negative binomial GLMMs were used to model effects on females' neighborhood attendance and time alone (with total scans observed and scans present respectively included as offsets to generate proportions) and binomial GLMMs were used to model effects on EP courtship probability. The distributions of female age and latency to lay did not allow them to be modeled using GLMMs, so they were divided into four (2-5, 6-9, 10-13, and 14-21 yrs. old) and three ( $\leq 5$ , 6-30, and  $>30$  days from laying) categories respectively and modeled using mixed ordinal regression (clmm function in the ordinal package for R, Christensen 2015). The proportional odds assumption was verified by assuring overlapping 95% confidence intervals of the slopes of binomial models at each cut-point (see Kim 2003). The clmm function did not allow the inclusion of all three of our random effects, so path analysis was carried out using only the first observation for each individual female ( $n=212$ ) to allow the inclusion of only year and neighborhood random effects. The  $P$ -values for each conditional independence claim were extracted using the drop1 function. The total degrees of freedom (D.F.) for each candidate model (necessary for calculating  $AIC_C$ ), was calculated as the sum of D.F. of the mixed models describing each path contained in the path model, extracted using the logLik function following Brambilla *et. al.* (2014).

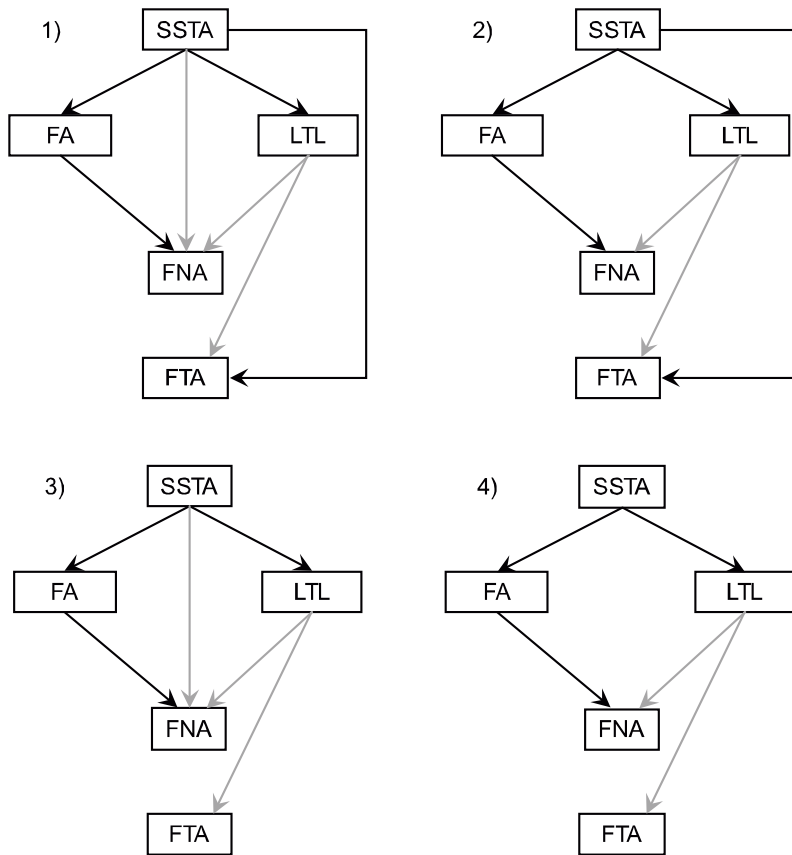
Since EP courtship probability was not proposed as a causal parent in any of our candidate models, we avoided inflating the total number of models to test by first resolving the relationships between SSTA, female age, latency to lay, female neighborhood attendance, and female time alone (hereafter, "pre-models"; see fig. S1),

then leaving these paths unchanged in the four alternative models testing the effect of SSTA, neighborhood attendance, and time alone on EP courtship probability (Gonzalez-Voyer, pers. comm.). A total of 30 candidate pre-models were tested, including the various biologically plausible combinations of relationships. Significant  $P_C$ -values (ranging from  $\ll 0.0001$  to 0.0374) indicated that 26 of these pre-models were inconsistent with the real relationships in the data, and were therefore immediately excluded. The four remaining pre-models (fig. S1) were ranked by  $AIC_C$  (Table S1). Though the first- and second-ranked pre-models were very close in  $AIC_C$ , since this was a preliminary evaluation we decided to use the more complete and slightly higher-ranked pre-model. However, using the simplified second-ranked pre-model yielded practically identical final results (not shown).

The most supported pre-model showed large negative overall effects of SSTA on both females' total neighborhood attendance and their time alone, consistent with the GLMM in the main text and supporting the first prediction. However, these effects were largely indirect, due to substantial effects of SSTA on female age and latency to lay, and of these variables on females' attendance and time alone (coefficients in fig. 3e). Interestingly, the direct effect of SSTA on female time alone was slightly negative, contrary to expected (though this was more than cancelled out by the strong indirect positive effect of latency to lay; see fig. 3e).

Results of the evaluation of candidate models are given in the results section of the main text. Summary statistics for pre-models and candidate models are given in tables S1 and table 3 of the main text, respectively.  $P$ -values for complete basis sets of the candidate models detailed in fig. 3 and table 3 are given in table S2.

**Fig. S1: Candidate pre-models with non-significant  $P_C$ -values.** Gray lines indicate negative and black lines positive effects. Model numbers correspond with table S1. Effect coefficients and significances of the most supported model (1) are given in Fig. 3e. Variables are abbreviated as follows: SSTA=Sea surface temperature anomaly, FA=Female Age LTL= Latency to lay, FNA=Female neighborhood attendance, FTA=Female time alone, EPC=Extra- Pair Courtship Probability.





**Table S1. Summary statistics for candidate pre-models with non-significant  $P_C$ -values.** D.F. refers to the total degrees of freedom in each candidate path model. Fisher's  $C$  and its corresponding  $P$  and  $AIC_C$  values for each model were calculated following (Shiple 2013; Gonzalez-Voyer & von Hardenberg 2014).  $\Delta AIC_C$  is with reference to model 1, the lowest-AIC model.

Model	D.F.	Fisher's $C$	$P_C$	$AIC_C$	$\Delta AIC_C$
1	28	6.608111	0.3586126	71.48243	0
2	27	9.741242	0.2836591	71.95863	0.476
3	28	12.91212	0.1149094	77.78643	6.304
4	27	16.04525	0.09834443	78.26264	6.780

**Table S2. Basis sets describing the five candidate path models proposed in figure 3.**

Using Shipley's (2009) notation,  $(A,B) | \{X,Y\}$  indicates that in a mixed model the effect of  $B$  on  $A$  should be non-significant when their direct causes,  $X$  and  $Y$ , are accounted for by including them as covariates. Gray text indicates independence claims for paths that were previously resolved ("pre-model", see fig. S1, table S1) and are therefore identical in all five candidate models. Significant  $P$ -values (independence claims that are violated) are indicated in bold.

Model	Conditional Independence Claim	$P$ -value
a	(LTL, FA)   {SSTA}	0.715051
	(FTA, FA)   {SSTA, LTL}	0.2873
	(FTA, FNA)   {SSTA, FA,LTL}	0.178811
	(EPC, FA)   {SSTA}	0.31304
	(EPC, FNA)   {FA,SSTA,LTL}	0.18593
	(EPC, FTA)   {SSTA,LTL}	0.6446
	(EPC, LTL)   {SSTA}	0.58046
b	(LTL, FA)   {SSTA}	0.715051
	(FTA, FA)   {SSTA, LTL}	0.2873
	(FTA, FNA)   {SSTA, FA,LTL}	0.178811
	(EPCORT, SSTA)   {FA}	0.09852
	(EPCORT, PTA)   {FA, LtL,SSTA}	0.6885
	(EPCORT, FTPR)   {FA, SSTA, LtL}	0.1958
	(EPCORT, LtL)   {FA, SSTA}	0.48
c	(LTL, FA)   {SSTA}	0.715051
	(FTA, FA)   {SSTA, LTL}	0.2873
	(FTA, FNA)   {SSTA, FA,LTL}	0.178811
	(EPC, FA)   {SSTA, FNA}	0.37409
	(EPC, FTA )   {SSTA, FNA, LTL}	0.7429
	(EPC, LTL)   {SSTA, FNA}	0.82889
	<b>(EPC, SSTA)   {FNA}</b>	<b>0.02118</b>
d	(LTL, FA)   {SSTA}	0.715051
	(FTA, FA)   {SSTA, LTL}	0.2873
	(FTA, FNA)   {SSTA, FA,LTL}	0.178811
	(EPC, FA)   {SSTA, FTA}	0.3113

	<b>(EPC, SSTA)   {FTA}</b>	<b>0.03725</b>
	(EPC, LTL)   {SSTA, FTA}	0.61797
	(EPC, FNA)   {FA, LTL, SSTA, FTA}	0.1261
e	(LTL, FA)   {SSTA}	0.715051
	(FTA, FA)   {SSTA, LTL}	0.2873
	(FTA, FNA)   {SSTA, FA, LTL}	0.178811
	(EPC, FA)   {SSTA, FNA, FTA}	0.36736
	(EPC, LTL)   {SSTA, FNA, FTA}	0.85547
	<b>(EPC, SSTA)   {FNA, FTA}</b>	<b>0.02415</b>
	Latency to lay=LTL, Female Age=FA, SSTA=Sea surface temperature anomaly, FNA=Female neighborhood attendance, FTA=Female time alone, EPC=Extra Pair Courtship Probability	

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