



**UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO**

**DOCTORADO EN CIENCIAS BIOMÉDICAS**

**INSTITUTO DE ECOLOGÍA**

**IMPACTO DE LA EDAD DE LOS ADULTOS REPRODUCTORES Y LA ESTRUCTURA DEL  
HÁBITAT EN LA PATERNIDAD EXTRA-PAREJA DEL BOBO DE PATAS AZULES**

**TESIS**

**QUE PARA OPTAR POR EL GRADO DE:**

**DOCTORA EN CIENCIAS**

**PRESENTA:**

**ALEJANDRA RAMOS GONZÁLEZ**

**TUTOR PRINCIPAL:**

**DR. HUGH DRUMMOND DUREY**

**INSTITUTO DE ECOLOGÍA**

**COMITÉ TUTOR:**

**DRA. ROXANA TORRES AVILÉS**

**INSTITUTO DE ECOLOGÍA**

**DRA. ROBYN ELIZABETH HUDSON**

**INSTITUTO DE INVESTIGACIONES BIOMÉDICAS**

**CIUDAD UNIVERSITARIA, MÉXICO, D. F.**

**Agosto, 2016**



Universidad Nacional  
Autónoma de México

Dirección General de Bibliotecas de la UNAM

**Biblioteca Central**



**UNAM – Dirección General de Bibliotecas**  
**Tesis Digitales**  
**Restricciones de uso**

**DERECHOS RESERVADOS ©**  
**PROHIBIDA SU REPRODUCCIÓN TOTAL O PARCIAL**

Todo el material contenido en esta tesis esta protegido por la Ley Federal del Derecho de Autor (LFDA) de los Estados Unidos Mexicanos (México).

El uso de imágenes, fragmentos de videos, y demás material que sea objeto de protección de los derechos de autor, será exclusivamente para fines educativos e informativos y deberá citar la fuente donde la obtuvo mencionando el autor o autores. Cualquier uso distinto como el lucro, reproducción, edición o modificación, será perseguido y sancionado por el respectivo titular de los Derechos de Autor.

## **Agradecimientos**

*Numerosas personas contribuyeron a la elaboración de esta tesis, sin su asesoría, dedicación, apoyo, infinita paciencia, amor, contribuciones financieras y ánimos, es improbable que hubiera logrado terminarla*

Tutor principal: Hugh Drummond

Comité tutor: Roxana Torres, Robyn Hudson y Constantino Macías

Jurado: Constantino Macías, Alejandro González Voyer, Margarita Martínez, Katherine Renton y Hugh Drummond

Colaboradores: Patricia Adair Gowaty, Brant Faircloth, Stacey Lance, Schyler Nunziata, Cristina Rodríguez, Hugh Drummond, Lynna Kiere y Oscar Sánchez

Ayudantes en campo: David Schneider, Gabriela López, Janeth Rosas y Brenda Flores

Ayudantes en laboratorio: Jonathan Drury, Carolina Álvarez y Santiago Bautista

Asistentes técnicos, Pablo Frank-Bolton, John Stanton-Geddes, Edgar Ávila, Rubén Pérez y Cristina Rodríguez

Compañeros del lab y amigos, Osko, Cris, Nat, Jocelyn, Momo, Cheko, Santi, Ilse, Vero, Fab, Ren, Chelen, Monse, Nadya, Marce, Yoli, Ruth, Yamaha, Laura, Isa, Mariana, Pablo, JP, Eli, Karla y Marina

Mi esposo, David Schneider, je t'aime mon amour

Mis padres, Rebeca González y Ángel Ramos

Mis hermanos, Juan y Moni

Más familia, Ángeles González, Rebeca Díaz, Francisco Ramos, Eddie Arnes, Andrés González, Yoan Schneider, Marlene Schneider, Ellie, Lia, Chrissie, Brent y tod@s mis primates

## INDICE

<b>Resumen.....</b>	<b>1</b>
<b>Introducción general.....</b>	<b>2-20</b>
<b>Capítulo 1.....</b>	<b>21-28</b>
Habitat structure and colony structure constrain extrapair paternity in a colonial bird	
<b>Capítulo 2.....</b>	<b>29-36</b>
Interactive effects of male and female age on extra-pair paternity in a socially monogamous bird	
<b>Capítulo 3.....</b>	<b>37-60</b>
Tick infestation of chicks in a seabird colony varies with local nest density, local breeding synchrony and habitat structure	
<b>Capítulo 4.....</b>	<b>61-74</b>
Parasitism by ticks in blue-footed booby offspring increases when both breeders are either young or old	
<b>Discusión General .....</b>	<b>75-82</b>
<b>Anexo I.....</b>	<b>83-87</b>
Isolation and characterization of microsatellite loci from blue-footed boobies ( <i>Sula nebouxi</i> )	

## RESUMEN

Durante años se pensó que las aves eran estrictamente monógamas, pero con el desarrollo de técnicas moleculares se descubrió que la mayoría de las especies tienen paternidad extra-pareja. Sin embargo, después de cientos de estudios, aún falta explicar por qué la paternidad extra-pareja es tan común, siendo que los beneficios para las hembras no son claros, y por qué su frecuencia varía tanto, incluso entre individuos de la misma especie y población. Se considera que la identificación de factores que influyen en la ocurrencia de paternidad extra-pareja podría ser clave para responder estas preguntas y avanzar nuestro conocimiento sobre sistemas de apareamiento en aves.

Como objetivo principal de esta tesis exploré entonces si, en nidadas de bobos de patas azules (*Sula nebouxi*), la paternidad extra-pareja varía con la estructura local del hábitat y la combinación de edades de la hembra y el macho social, dos factores potencialmente importantes pero poco estudiados. Además, como objetivo secundario, analicé la relación entre la estructura del hábitat, un determinante posible de infestaciones por ectoparásitos, y la abundancia de garrapatas (*Carios denmarki*) en crías, y probé si crías producidas por un padre viejo y otro joven tienen menos garrapatas que crías con dos padres viejos o dos padres jóvenes. Para cumplir estos objetivos, usé combinaciones de diversos métodos, incluyendo desarrollo de marcadores moleculares, análisis de paternidad, uso de base de datos con miles de adultos de edades conocidas y coordenadas espaciales de nidos, estimación de densidades locales y conteos de garrapatas.

Encontré que, para una pareja de bobos, la probabilidad de producir crías extra-pareja aumentó cuando anidaron en hábitats con pocos obstáculos a su locomoción, tal vez por que los obstáculos limitan las incursiones de cópulas extra-pareja, y cuando ambos eran viejos o jóvenes. Es posible que las hembras jóvenes elijan reproducirse con machos viejos por que éstos han demostrado tener buenos genes, y que las hembras viejas los evitan por que aparearse con ellos podría incrementar el riesgo de infertilidad o defectos genéticos en sus crías. Estos resultados sugieren que no todas las hembras, aun perteneciendo a la misma especie y población, obtienen los mismos beneficios de la paternidad extra-pareja ni tienen las mismas oportunidades de copular con machos extra-pareja. La abundancia de garrapatas aumentó con la densidad de rocas, posiblemente porque éstas sirven de resguardo contra depredadores y climas adversos, y las crías producidas por padres de edades distintas tuvieron menos garrapatas, confirmando que podrían tener mejor respuesta inmune.

# **INTRODUCCIÓN GENERAL**

El descubrimiento más importante sobre sistemas de apareamiento en los últimos 30 años es que, a pesar de que la monogamia social es común en varios taxa, especialmente en aves, la monogamia genética es inusual. Originalmente los sistemas de apareamiento se describieron en base a conductas sociales observadas en campo (Wink y Dyreuz 1999) y, dado que en aves las hembras suelen formar lazos de pareja con un solo macho, conocido como “macho social”, se asumió durante mucho tiempo que la mayoría de las aves eran social y genéticamente monógamas. No obstante, con el desarrollo y uso de técnicas moleculares capaces de determinar la paternidad, se han encontrado crías engendradas por uno o más machos distintos al macho social en nidadas del ~90 % de las especies de aves estudiadas (Griffith et al. 2002). La sorprendente frecuencia de estas crías extra-pareja desató una ola de estudios, la mayoría enfocados en explorar los costos y beneficios genéticos de la paternidad extra-pareja, y generó una gran pregunta que continúa siendo de interés actual: *¿Por qué las hembras, de tantas especies de aves, tienen paternidad extra-pareja?*

Uno de los factores más influyentes en la conducta de un individuo son sus genes. Para los machos, obtener paternidad extra-pareja brinda un gran beneficio adaptativo: incrementa su número de descendientes sin inversión en cuidado parental (Trivers 1972, Wakano e Ihara 2005). No obstante, la paternidad extra-pareja es costosa para machos que pierden paternidad en sus propios nidos, y mediante ciertas conductas, como resguardar (Chuang-Dobbs et al. 2001) y copular múltiples veces con su pareja durante sus días fértiles (Fishman et al. 2003), los machos pueden reducir el riesgo de la infidelidad. El éxito reproductivo de las hembras, en cambio, está limitado por el número de huevos que pueden producir y los beneficios adaptativos de su conducta extra-pareja, en la que suelen participar activamente, son menos claros. Se han propuesto numerosas hipótesis adaptativas como la obtención de buenos genes o genes compatibles, la producción de crías genéticamente diversas, la protección contra infertilidad e infanticidio y el acceso a recursos (Forstmeier et al. 2014). Sin embargo, el apoyo de estas hipótesis es pobre, tal vez porque es improbable que en las aves una sola hipótesis pueda ofrecer una explicación general de la ocurrencia de paternidad extra-pareja en hembras (Eliassen y Kokko 2008).

Otro hallazgo que constituye un reto para explicaciones adaptativas es que existe una gran variación en la frecuencia de paternidad extra-pareja entre años, especies, poblaciones de la misma especie e individuos de la misma población (Petrie y Kempenaers 1998, Westneat y Mays 2005). Para que la paternidad extra-pareja ocurra, dos individuos de sexos opuestos, no apareados entre sí, deben encontrarse, interactuar y copular, y estas cópulas deben resultar en fertilización. Este proceso depende de las conductas de al menos tres individuos: una hembra, su pareja social y un macho extra-pareja (Lifjeld y Robertson 1992). Sin embargo, la inaccesibilidad de la conducta extra-pareja a la observación ha limitado los estudios empíricos de las interacciones en las triadas. Los factores potencialmente capaces de generar variación en la conducta extra-pareja incluyen los genes, variables ecológicas y el ambiente social. La identificación de los factores operativos, especialmente a nivel individual (Johnsen y Lifjeld 2003), se considera clave para entender los sistemas de apareamiento y selección sexual en aves (Petrie y Kempenaers 1998).

A nivel poblacional, la densidad de nidos y la sincronía reproductiva son dos de los factores ecológicos más correlacionados con la paternidad extra-pareja (Westneat y Sherman 1997). Otros factores potencialmente importantes, como la estructura local del hábitat donde ocurre la conducta extra-pareja, suelen ser ignorados (Westneat y Stewart 2003). Asimismo, los estudios de paternidad extra-pareja tienden a enfocarse en las hembras o en los machos sociales, y pocas veces consideran a los machos extra-pareja, o más importante aún, a éstos tres actores juntos (Griffith et al. 2002). Para empezar a remediar esta carencia, en esta tesis mis objetivos principales fueron explorar dos factores que potencialmente son de gran importancia, la estructura local del hábitat y la combinación de edades de la hembra y el macho social, en la probabilidad de que una nidada de bobos de patas azules (*Sula nebouxi*) tenga crías extra-pareja.

### ***Estructura del Hábitat***

En 1990, Westneat, Sherman y Morton propusieron a la estructura del hábitat como un determinante importante de paternidad extra-pareja. Se espera



que la eficiencia de monitoreo y resguardo de pareja de los machos sociales varíe con el tipo de hábitat (Sundberg 1994, Tryjanowski et al. 2007). Por ejemplo, se sospecha que las hembras reinita grande (*Icteria virens*) tienen menos crías extra-pareja en hábitats abiertos por que ahí sus parejas pueden observar y resguardarlas fácilmente (Mays y Ritchison 2004), y que las hembras alcaudón real (*Lanius excubitor*) tienen cópulas extra-pareja en las zonas más recónditas de sus territorios para evitar ser descubiertas (Tryjanowski et al. 2007). Además, parece probable que la estructura del hábitat modifique los patrones de movimiento de las hembras, alterando la frecuencia de encuentros que tienen con machos extra-pareja (Westneat y Mays 2005).

No obstante, la estructura del hábitat es compleja y difícil de medir (Taniguchi et al. 2003), y sus efectos pueden confundirse con la disponibilidad de alimento (Charmantier y Blondel 2003, Rubenstein 2007) y el riesgo de depredación. Por ejemplo, hembras en territorios con vegetación densa y alimento abundante podrían tener más crías extra-pareja por que no son observables por sus parejas o sus depredadores o, alternativamente, porque invierten menos tiempo en el forrajeo, dejando más tiempo libre para buscar cópulas extra-pareja (Hoi-Leitner et al. 1999). Hasta donde sabemos, ningún estudio ha documentado la relación entre estructura de hábitat y paternidad extra-pareja en una especie libre de depredadores y con territorios exclusivos para reproducción (es decir, que no se usan para la alimentación).

Las características físicas del hábitat, a pequeñas y grandes escalas, también afectan la abundancia y distribución de parásitos (Sousa y Grosholz 1991), un factor que potencialmente afecta el costo de la infidelidad. Las garrapatas, ectoparásitos hematófagos comunes en colonias de aves marinas, suelen esconderse en las grietas de las rocas para protegerse de depredadores y condiciones climáticas adversas (Samish y Rehacek 1999). En áreas que carecen de refugios adecuados, su mortalidad puede incrementar y su abundancia disminuir (Sousa y Grosholz 1991). Se ha encontrado que gaviotas tridáctilas (*Rissa tridactyla*) que anidan en acantilados naturales, donde hay refugios numerosos para garrapatas, son fuertemente parasitadas, mientras que aquellas que anidan en edificios abandonados, carentes de refugios, permanecen libres de parásitos (Danchin 1992). Las garrapatas de aves marinas pueden causar abandono de nidos, efectos negativos en el desarrollo, y mortalidad de crías y

adultos (Wanless et al. 1997, Gauthier-Clerc et al. 1998). Además, el riesgo de la infestación por garrapatas podría afectar la selección de parejas extra-pareja, ya que las hembras podrían preferir machos en zonas con pocas garrapatas o machos con poca infestación ya que éstos podrían tener genes resistentes a parásitos y presentar un menor riesgo de contagio (Hamilton y Zuk 1982, Borgia y Collis 1989, Moller et al. 1999).

### ***Densidad de co-específicos***

Existen otras variables ecológicas capaces de influir en la paternidad extra-pareja. Según la hipótesis de densidad de co-específicos, la probabilidad de que una hembra produzca una cría extra-pareja debe incrementar con el número de machos sexualmente activos cercanos a su nido (Birkhead 1978). No obstante, los resultados de estudios que han puesto a prueba esta hipótesis son mixtos, y aun cuando muchos han encontrado una relación positiva entre densidad y paternidad extra-pareja a nivel poblacional (e.g., Gibbs et al. 1990, Gelter and Tegelström 1992, Yezerinac et al. 1999) e individual (e.g., Stewart et al. 2010, Mayer and Pasinelli 2013), otros no han encontrado relación (e.g., Barber et al. 1996, Sundberg and Dixon 1996, Lindstedt et al. 2007), o bien, han encontrado una relación negativa (Leisler et al. 2000). La inconsistencia de estos resultados podría deberse en parte a la falta de consideración de otras variables importantes, incluyendo la estructura del hábitat donde ocurre la conducta extra-pareja (Sherman y Morton 1988, Mays y Ritchison 2004) y posibles asociaciones entre densidad de co-específicos y riesgo de infestación por parásitos.

La densidad de huéspedes es considerada un fuerte determinante de la abundancia de parásitos (Lebarbenchon et al. 2006) en numerosos sistemas de huésped-parásito (Rifkin et al. 2012, Patterson y Ruckstuhl 2013). En densidades altas, el número de parásitos que son transmitidos por contacto directo entre huéspedes incrementa por el contacto frecuente y prolongado entre conspecíficos, mientras que el número de parásitos móviles (como mosquitos y crustáceos acuáticos) disminuye por un efecto de dilución (Patterson y Ruckstuhl 2013). Las garrapatas son ectoparásitos móviles y pueden activamente buscar a sus huéspedes (Mugabo et al. 2015), pero su

movilidad es limitada (Godfrey 2010). La transmisión directa podría ser su principal forma de infección, y su abundancia y posible impacto sobre el comportamiento extra-pareja debería entonces incrementar con la densidad de huéspedes.

### ***Edades de la hembra y el macho social***

La relación entre la edad de un macho y la probabilidad de paternidad extra-pareja en su nido ha sido documentada en varias aves (Griffith et al. 2002). En general, comparado con machos jóvenes, los machos viejos pierden menos paternidad en sus propios nidos (Møller y Ninni 1998) y además engendran más crías extra-pareja (Cleasby y Nakagawa 2012), sugiriendo que son los padres biológicos preferidos por las hembras. Los machos viejos podrían ser atractivos como parejas sociales y extra-pareja porque sobrevivir a edades avanzadas indica que tienen buenos genes (Hansen y Price 1995). Sin embargo, la evidencia por esta preferencia es mixta y procrear con machos viejos puede ser riesgoso, ya que con la edad aumentan las mutaciones en su ADN germinal (Beck y Promislow 2007), potencialmente disminuyendo la calidad de las crías producidas (Johnson y Gemmell 2012). La mayoría de los estudios en vida libre desconocen las edades exactas y burdamente catalogan a individuos como jóvenes o viejos según su plumaje, por lo que no es claro si hembras prefieren machos viejos o machos de edades intermedias.

Una falla recurrente en la literatura es que, aun cuando el envejecimiento probablemente afecta la reproducción en ambos sexos, y la combinación de edades de la hembra y el macho social es potencialmente importante, tanto la edad de las hembras como la combinación de las edades tienden a ser ignoradas. En humanos, la edad avanzada de las mujeres puede acentuar los efectos dañinos de la edad avanzada de los hombres en la fertilidad (Kühnert and Nieschlag 2004) y calidad genética de la progenie (Fisch et al. 2003). En aves, la fertilidad del progenitor y la calidad de las crías pueden disminuir con la edad de la hembra (Priest et al. 2002, Saino et al. 2002, Descamps et al. 2008), y en el bobo de patas azules la viabilidad de las crías disminuye con la edad tanto del padre como de la madre (Torres et al. 2011). Además, en otras aves hay evidencia de que la combinación de edades de la pareja puede afectar la probabilidad de paternidad extra-pareja (Rätti et al. 2001, Dietrich et al. 2004,

Bouwman y Komdeur 2005). Finalmente, aun cuando la producción de crías extra-pareja requiere de la participación de tres individuos, hembra, macho social y macho extra-pareja, rara vez se han considerado las edades de éstos tres individuos en el mismo estudio. Es concebible que las hembras senescentes apareadas con machos senescentes podrían rescatar la calidad genética de su progenie consiguiendo padres biológicos más jóvenes que sus parejas sociales.

Cuando investigué esta posibilidad encontré que efectivamente la paternidad extra-pareja para una hembra joven disminuye con la edad de su pareja social mientras que para una hembra vieja incrementa (Capítulo 2). Posteriormente Drummond y Rodríguez (2015) descubrieron que los volantones producidos por un padre joven y otro viejo tienen mayor probabilidad de reclutar que volantones con dos padres jóvenes o dos padres viejos. Mientras que otros rasgos altamente dependientes del cuidado biparental, como tamaño de nidada, éxito de eclosión, número de emplumados, éxito de emplumados y condición corporal de emplumados, no variaron con las distintas combinaciones de edades de los padres, indicando que los efectos observados de las combinaciones de edades de los padres en la viabilidad de las crías podrían ser genéticos (Drummond y Rodríguez 2015).

Considerando que en aves existe un componente hereditario en la resistencia a parásitos (Owen et al. 2010), que la respuesta inmune disminuye con la edad (Miller 1991) y que la edad de los padres posiblemente afecta la inmunidad de las crías, sobretodo su respuesta inflamatoria considerada una de las defensas más importante de las aves en contra de ectoparásitos hematófagos, decidimos explorar la predicción de que crías con un padre joven y un padre viejo tienen menos garrapatas que crías con dos padres viejos o dos padres jóvenes.

### ***Especies de estudio***

Los bobos de patas azules (*Sula nebouxi*) son aves marinas coloniales que se reproducen estacionalmente en islas tropicales del Océano Pacífico (Nelson 1978). En la Isla Isabel (21° 52' N, 105° 54' W), el hábitat predominante es un bosque deciduo compuesto de papelillo (*Euphorbia schlechtendalli*) y roache (*Crataeva tapia*), con parches de pastizal, roca y arena. Los bobos son

fieles a sus territorios de anidación (Osorio-Beristain y Drummond 1993, Kim et al. 2007). Los nidos, en la mayor parte de la colonia, están separados por varios metros unos de otros, pero en zonas de altas densidades pueden estar a sólo 1 m de distancia (Castillo y Chavez-Peón 1983). Las hembras ponen de uno a tres huevos a intervalos de cuatro días y éstos eclosionan secuencialmente después de ~41 días (Nelson 1978, Drummond 1986). Ambos padres brindan cuidado parental durante la incubación y la crianza, tomando turnos para realizar excursiones de cacería de peces. En la colonia de anidación, la única depredación sobre estos bobos es cuando las culebras falso coralillo (*Lampropeltis triangulum*) se alimentan de las crías en su primera semana de vida (Drummond et al. 1986).

Aunque los bobos son socialmente monógamos, es frecuente que las hembras copulen con uno o más machos extra-pareja, usualmente vecinos, en las semanas previas a su primera puesta (Osorio-Beristain y Drummond 1998, Pérez-Staples y Drummond 2005). Estas cópulas son claramente consensuales ya que las hembras son más grandes y en promedio 32% más pesadas que los machos y, para que una cópula sea exitosa, es necesario que la hembra coopere permaneciendo inmóvil (Osorio-Beristain y Drummond 1998, Pérez-Staples y Drummond 2005). Además, en observaciones de conducta extra-pareja, las cópulas son precedidas por cortejo recíproco que puede durar varios días, incluso semanas, y nunca se ha documentado una cópula forzada. Para asegurar la paternidad de las crías en sus nidos, los machos suelen incrementar la frecuencia de cópulas con su pareja y la conducta de resguardo en su período fértil (Osorio-Beristain y Drummond 1998) y destruyen el primer huevo de la puesta cuando su paternidad está en duda (Osorio-Beristain y Drummond 2001).

Los bobos son aves longevas con esperanzas de vida que superan los 20 años y mortalidad anual, de hembras y machos adultos, del ~10% (Oro et al. 2010). Desde 1989, en dos áreas de estudio de Isla Isabel, las crías que logran emplumar se han marcado con anillos que contienen dígitos únicos, por lo que actualmente se conocen las edades e historias de vida del ~90% de los bobos adultos que aquí anidan (detalles en Drummond et al. 2003). A los ~10 años de edad, los bobos alcanzan su mayor éxito reproductivo y, posteriormente, ambos sexos experimentan senescencia reproductiva (Velandó et al. 2006). Con la

edad, decae la calidad espermática de los machos (Velando et al. 2011), la calidad de huevos producidos por las hembras y su cuidado parental, el cuidado parental de las hembras (Beamonte-Barrientos et al. 2010) y la probabilidad de reclutamiento de los volantones (Torres et al. 2011).

Las garrapatas de caparazón blando *Carios denmarki* son ectoparásitos frecuentes y abundantes de bobos de patas azules en Isla Isabel. Es común observarlas adheridas a la piel de crías y adultos, así como en la vegetación y el substrato cercanos a sus nidos. Aunque no se han estudiado los efectos negativos de estas garrapatas en la salud y la adecuación de los bobos, se sabe que las infestaciones por garrapatas en otras especies de aves pueden afectar el crecimiento de las crías (Morbey 1996, Bosch y Figuerola 1999), causar mortalidad en crías y adultos (Duffy 1983), y ocasionar abandono de nidos y, en casos extremos, abandono de colonias enteras (Clayton et al. 2010).

### **Objetivos**

Mi primer objetivo principal fue probar si la paternidad extra-pareja en nidos de bobos de patas azules, una especie con territorios exclusivos para reproducción y sin depredación en adultos, se relaciona con la estructura del hábitat y la densidad local de nidos (Capítulo 1). Además, probé si la estructura del hábitat y la densidad local de nidos alrededor del nido de los machos afecta su probabilidad de adquirir paternidad extra-pareja en otros nidos. Los resultados de estos análisis sugieren que la probabilidad de producir crías extra-pareja, para hembras y machos, se relaciona cuadráticamente con la densidad local de nidos y, que para las hembras, también disminuye con el número de obstáculos alrededor de sus nidos.

Mi segundo objetivo principal fue examinar si las combinaciones de edades de las hembras y machos reproductores influyen en la probabilidad de que una nidada de bobos incluya al menos una cría extra-pareja (Capítulo 2). Adicionalmente, dado que encontré que la paternidad extra-pareja para una hembra joven disminuyó con la edad de su pareja social mientras que para una hembra vieja incrementó, exploré si en parejas de dos individuos jóvenes el macho extra-pareja era de mayor edad que el macho social y si en parejas de dos individuos viejos el macho extra-pareja era de menor edad que el macho social.

Para complementar los resultados que obtuve sobre los efectos del hábitat y densidad local en la paternidad extra-pareja, como objetivo secundario probé la relación de éstas variables ecológicas, consideradas determinantes importantes de infestaciones por ectoparásitos, con la abundancia de garrapatas (*Carios denmarki*) en crías de bobos de patas azules (Capítulo 3). Además, en base a los efectos encontrados de la interacción de las edades de los progenitores en la probabilidad de paternidad extra-pareja (Capítulo 2) y la viabilidad de las crías (Drummond y Rodríguez 2015), probé si crías producidas por un padre viejo y otro joven tienen más garrapatas que crías con dos padres viejos o dos padres jóvenes (Capítulo 4).

Para cumplir con los objetivos de mi tesis, usé combinaciones de diversos métodos, incluyendo muestreos de sangre y extracción de ADN para analizar paternidad (Capítulo 1 y 2), información de la base de datos sobre miles de adultos de edades conocidas (Capítulos 2 y 4) y sobre las coordenadas espaciales de los nidos (Capítulos 1 y 3), medición de la estructura del hábitat (Capítulos 1 y 3), estimación de densidades locales (Capítulo 1 y 3), y cuantificación de garrapatas sobre crías (Capítulos 3 y 4).

Además, para poder excluir y asignar paternidad colaboré con dos grupos de investigadores en Estados Unidos. Primero, realicé una estancia de 6 meses en la Universidad de Los Ángeles California dónde, en colaboración con la Dra. Patricia Gowaty y el Dr. Brant Faircloth, desarrollamos los primeros microsatélites específicos para bobos de patas azules y publicamos las secuencias de sus primers en la revista *Conservation Genetics Resources* en Junio de 2009 (Anexo 1). Segundo, envíe el ADN que extraje de la sangre de 478 familias de bobos a la Dra. Stacey Lance en la Universidad de Georgia quién, con su estudiante Schyler Nunziata, realizó el genotipaje y las pruebas de paternidad usando los microsatélites que reportamos en Faircloth et al. (2009).

## LITERATURA

- Barber, C.A., Robertson, R.J. and Boag, P.T., 1996. The high frequency of extra-pair paternity in tree swallows is not an artifact of nestboxes. *Behavioral Ecology and Sociobiology*, 38(6), pp.425-430.
- Beamonte - Barrientos, R., Velando, A., Drummond, H. and Torres, R., 2010. Senescence of Maternal Effects: Aging Influences Egg Quality and Rearing Capacities of a Long - Lived Bird. *The American Naturalist*, 175(4), pp.469-480.
- Beck, C.W. and Promislow, D.E., 2007. Evolution of female preference for younger males. *PLoS One*, 2(9), pp.e939-e939.
- Birkhead, T.R., 1978. Behavioural adaptations to high density nesting in the common guillemot *Uria aalge*. *Animal Behaviour*, 26, pp.321-331.
- Borgia, G., & Collis, K. (1989). Female choice for parasite-free male satin bowerbirds and the evolution of bright male plumage. *Behavioral Ecology and Sociobiology*, 25(6), 445-453
- Bosch, M. and Figuerola, J., 1999. Detrimental effects of ticks *Ornithodoros maritimus* on the growth of yellow-legged gull *Larus michahellis* chicks. *Ardea*, 87(1), pp.83-89.
- Bouwman, K.M. and Komdeur, J., 2005. Male reed buntings do not adjust parental effort in relation to extrapair paternity. *Behavioral Ecology*, 16(3), pp.499-506.
- Castillo, A., & Chavez-Peón, C. (1983). Ecología reproductiva e influencia del comportamiento en el control del número de crías en el bobo de patas azules *Sula nebouxii* en la Isla Isabel, Nayarit. *Undergraduate thesis, Facultad de Ciencias UNAM*.
- Charmantier, A., & Blondel, J. (2003). A contrast in extra-pair paternity levels on mainland and island populations of Mediterranean blue tits. *Ethology*, 109, 351e363.



- Chuang-Dobbs, H.C., Webster, M.S. and Holmes, R.T., 2001. The effectiveness of mate guarding by male black-throated blue warblers. *Behavioral Ecology*, *12*(5), pp.541-546.
- Clayton, D.H., Koop, J.A., Harbison, C.W., Moyer, B.R. and Bush, S.E., 2010. How birds combat ectoparasites. *Open Ornithology Journal*, *3*, pp.41-71.
- Cleasby, I.R. and Nakagawa, S., 2012. The influence of male age on within - pair and extra - pair paternity in passerines. *Ibis*, *154*(2), pp.318-324.
- Danchin, E., 1992. The incidence of the tick parasite *Ixodes uriae* in Kittiwake *Rissa tridactyla* colonies in relation to the age of the colony, and a mechanism of infecting new colonies. *Ibis*, *134*(2), pp.134-141.
- Descamps, S., Boutin, S., Berteaux, D. and Gaillard, J.M., 2008. Age - specific variation in survival, reproductive success and offspring quality in red squirrels: evidence of senescence. *Oikos*, *117*(9), pp.1406-1416.
- Dietrich, V., Schmoll, T., Winkel, W., Epplen, J.T. and Lubjuhn, T., 2004. Pair identity—an important factor concerning variation in extra-pair paternity in the coal tit (*Parus ater*). *Behaviour*, *141*(7), pp.817-835.
- Drummond, H., González, E. and Osorno, J.L., 1986. Parent-offspring cooperation in the blue-footed booby (*Sula nebouxii*): social roles in infanticidal brood reduction. *Behavioral Ecology and Sociobiology*, *19*(5), pp.365-372.
- Drummond, H., Rodríguez, C., Vallarino, A., Valderrábano, C., Rogel, G. and Tobón, E., 2003. Desperado siblings: uncontrollably aggressive junior chicks. *Behavioral Ecology and Sociobiology*, *53*(5), pp.287-296.
- Drummond, H., & Rodríguez, C. (2015). Viability of Booby Offspring is Maximized by Having One Young Parent and One Old Parent. *PloS one*, *10*(7), e0133213.
- Duffy, D.C., 1983. The ecology of tick parasitism on densely nesting Peruvian seabirds. *Ecology*, pp.110-119.

- Eliassen, S. and Kokko, H., 2008. Current analyses do not resolve whether extra-pair paternity is male or female driven. *Behavioral Ecology and Sociobiology*, 62(11), pp.1795-1804.
- Faircloth, B.C., Ramos, A., Drummond, H. and Gowaty, P.A., 2009. Isolation and characterization of microsatellite loci from blue-footed boobies (*Sula nebouxii*). *Conservation Genetics Resources*, 1(1), pp.159-162.
- Fisch, H., Hyun, G., Golden, R., Hensle, T.W., Olsson, C.A. and Liberson, G.L., 2003. The influence of paternal age on Down syndrome. *The Journal of urology*, 169(6), pp.2275-2278.
- Fishman, M.A., Stone, L. and Lotem, A., 2003. Fertility assurance through extrapair fertilizations and male paternity defense. *Journal of theoretical biology*, 221(1), pp.103-114.
- Forstmeier, W., Nakagawa, S., Griffith, S.C. and Kempenaers, B., 2014. Female extra-pair mating: adaptation or genetic constraint?. *Trends in ecology & evolution*, 29(8), pp.456-464.
- Gauthier-Clerc, M., Clerquin, Y. and Handrich, Y., 1998. Hyperinfestation by ticks *Ixodes uriae*: a possible cause of death in adult king penguins, a long-lived seabird. *Colonial Waterbirds*, pp.229-233.
- Gelter, H.P. and Tegelström, H., 1992. High frequency of extra-pair paternity in Swedish pied flycatchers revealed by allozyme electrophoresis and DNA fingerprinting. *Behavioral Ecology and Sociobiology*, 31(1), pp.1-7.
- Gibbs, H.L., Weatherhead, P.J., Boag, P.T., White, B.N., Tabak, L.M. and Hoysak, D.J., 1990. Realized reproductive success of polygynous red-winged blackbirds revealed by DNA markers. *Science*, 250(4986), pp.1394-1397.
- Godfrey, S.S., Moore, J.A., Nelson, N.J. and Bull, C.M., 2010. Social network structure and parasite infection patterns in a territorial reptile, the tuatara (*Sphenodon punctatus*). *International journal for parasitology*, 40(13), pp.1575-1585.

- Griffith, S., Owens, I. & Thuman, K. (2002) Extra-pair paternity in birds: a review of interspecific variation and adaptive function. *Molecular Ecology*, **11**, 2195–2212.
- Hamilton WD, Zuk M. Heritable true fitness and bright birds: a role for parasites?. *Science*. 1982 Oct 22;218(4570):384-7.
- Hansen, T. F., & Price, D. K. (1995). Good genes and old age: do old mates provide superior genes?. *Journal of Evolutionary Biology*, **8**(6), 759-778.
- Hoi-Leitner, M., Hoi, H., Romero-Pujante, M. and Valera, F., 1999. Female extra-pair behaviour and environmental quality in the serin (*Serinus serinus*): a test of the 'constrained female hypothesis'. *Proceedings of the Royal Society of London B: Biological Sciences*, **266**(1423), pp.1021-1026.
- Johnsen, A., & Lifjeld, J. T. (2003). Ecological constraints on extra-pair paternity in the bluethroat. *Oecologia*, **136**(3), 476-483.
- Johnson, S.L. and Gemmill, N.J., 2012. Are old males still good males and can females tell the difference?. *Bioessays*, **34**(7), pp.609-619.
- Kühnert, B. and Nieschlag, E., 2004. Reproductive functions of the ageing male. *Human reproduction update*, **10**(4), pp.327-339.
- Kim, S. Y., Torres, R., Domínguez, C. A., & Drummond, H. (2007). Lifetime philopatry in the blue-footed booby: a longitudinal study. *Behavioral Ecology*, **18**(6), 1132-1138.
- Lebarbenchon, C., Poulin, R., Gauthier-Clerc, M. and Thomas, F., 2006. Parasitological consequences of overcrowding in protected areas. *EcoHealth*, **3**(4), pp.303-307.
- Leisler, B., Beier, J., Staudter, H. and Wink, M., 2000. Variation in extra - pair paternity in the polygynous Great Reed Warbler (*Acrocephalus arundinaceus*). *Journal für Ornithologie*, **141**(1), pp.77-84.
- Lifjeld, J. T., & Robertson, R. J. (1992). Female control of extra-pair fertilization in tree swallows. *Behavioral Ecology and Sociobiology*, **31**(2), 89-96.

- R Lindstedt E, P Oh K, V Badyaev A. Ecological, social, and genetic contingency of extrapair behavior in a socially monogamous bird. *Journal of Avian Biology*. 2007 Mar 1;38(2):214-23.
- Mayer, C. and Pasinelli, G., 2013. New support for an old hypothesis: density affects extra - pair paternity. *Ecology and evolution*, 3(3), pp.694-705.
- Mays Jr, H.L. and Ritchison, G., 2004. The effect of vegetation density on male mate guarding and extra-territorial forays in the yellow-breasted chat (*Icteria virens*). *Naturwissenschaften*, 91(4), pp.195-198.
- Miller RA. Aging and immune function. *Int Rev Cytol* 1991; 124: 187–215.
- Moller, A. P., Christe, P., & Lux, E. (1999). Parasitism, host immune function, and sexual selection. *Quarterly Review of Biology*, 3-20.
- Møller, A.P. and Ninni, P., 1998. Sperm competition and sexual selection: a meta-analysis of paternity studies of birds. *Behavioral Ecology and Sociobiology*, 43(6), pp.345-358.
- Morbey, Y.E., 1996. The abundance and effects of ticks (*Ixodes uriae*) on nestling Cassin's Auklets (*Ptychoramphus aleuticus*) at Triangle Island, British Columbia. *Canadian journal of zoology*, 74(8), pp.1585-1589.
- Mugabo, M., Perret, S., Decencièrè, B., Meylan, S. and Le Galliard, J.F., 2015. Density-dependent immunity and parasitism risk in experimental populations of lizards naturally infested by ixodid ticks.
- Nelson JB. (1978). *The Sulidae: gannets and boobies*. Oxford, UK: Oxford University Press.
- Oro, D., Torres, R., Rodríguez, C., & Drummond, H. (2010). Climatic influence on demographic parameters of a tropical seabird varies with age and sex. *Ecology*, 91(4), 1205-1214.

- Osorio-Beristain M, Drummond H. Natal dispersal and deferred breeding in the blue-footed booby. *The Auk*. 1993 Apr 1:234-9.
- Osorio-Beristain, M., & Drummond, H. (1998). Non-aggressive mate guarding by the blue-footed booby: a balance of female and male control. *Behavioral Ecology and Sociobiology*, *43*(4-5), 307-315.
- Osorio-Beristain, M., & Drummond, H. (2001). Male boobies expel eggs when paternity is in doubt. *Behavioral Ecology*, *12*(1), 16-21.
- Owen, J.P., Nelson, A.C. and Clayton, D.H., 2010. Ecological immunology of bird-ectoparasite systems. *Trends in parasitology*, *26*(11), pp.530-539.
- Patterson, J.E. and Ruckstuhl, K.E., 2013. Parasite infection and host group size: a meta-analytical review. *Parasitology*, *140*(07), pp.803-813.
- Pérez-Staples, D. and Drummond, H., 2005. Tactics, effectiveness and avoidance of mate guarding in the blue-footed booby (*Sula nebouxii*). *Behavioral Ecology and Sociobiology*, *59*(1), pp.115-123.
- Petrie, M. and Kempenaers, B., 1998. Extra-pair paternity in birds: explaining variation between species and populations. *Trends in Ecology & Evolution*, *13*(2), pp.52-58.
- Priest, N.K., Mackowiak, B. and Promislow, D.E., 2002. The role of parental age effects on the evolution of aging. *Evolution*, *56*(5), pp.927-935.
- Rätti, O., Lundberg, A., Tegelström, H. and Alatalo, R.V., 2001. No evidence for effects of breeding density and male removal on extrapair paternity in the pied flycatcher. *The Auk*, *118*(1), pp.147-155.
- Rifkin, J.L., Nunn, C.L. and Garamszegi, L.Z., 2012. Do animals living in larger groups experience greater parasitism? A meta-analysis. *The American naturalist*, *180*(1), pp.70-82.
- Rubenstein, D. R. (2007). Territory quality drives intraspecific patterns of extrapair paternity. *Behavioral Ecology*, *18*, 1058e1064.

- Saino, N., Ambrosini, R., Martinelli, R., Calza, S., Møller, A.P. and Pilastro, A., 2002. Offspring sexual dimorphism and sex - allocation in relation to parental age and paternal ornamentation in the barn swallow. *Molecular Ecology*, 11(8), pp.1533-1544.
- Samish, M., and J. Rehacek (1999). Pathogens and predators of ticks and their potential in biological control. *Annual Review of Entomology* 44:159-182.
- Sherman, P.W. and Morton, M.L., 1988. Extra-pair fertilizations in mountain white-crowned sparrows. *Behavioral Ecology and Sociobiology*, 22(6), pp.413-420.
- Sousa, W. P., & Grosholz, E. D. (1991). The influence of habitat structure on the transmission of parasites. In *Habitat structure* (pp. 300-324). Springer Netherlands.
- Stewart, S.L., Westneat, D.F. and Ritchison, G., 2010. Extra-pair paternity in eastern bluebirds: effects of manipulated density and natural patterns of breeding synchrony. *Behavioral Ecology and Sociobiology*, 64(3), pp.463-473.
- Sundberg, J., 1994. Paternity guarding in the yellowhammer *Emberiza citrinella*: a detention experiment. *Journal of avian biology*, pp.135-141.
- Sundberg, J. and Dixon, A., 1996. Old, colourful male yellowhammers, *Emberiza citrinella*, benefit from extra-pair copulations. *Animal Behaviour*, 52(1), pp.113-122.
- Taniguchi H, Nakano S, Tokeshi M (2003) Influences of habitat complexity on the diversity and abundance of epiphytic invertebrates on plants. *Freshw Biol* 48:718–728
- Torres, Roxana, Hugh Drummond, and Alberto Velando. "Parental age and lifespan influence offspring recruitment: a long-term study in a seabird." *PloS one* 6.11 (2011): e27245-e27245.
- Trivers, R., 1972. Parental investment and sexual selection. *Sexual Selection & the Descent of Man, Aldine de Gruyter, New York*, pp.136-179.

- Tryjanowski, P., Antczak, M. and Hromada, M., 2007. More secluded places for extra-pair copulations in the great grey shrike *Lanius excubitor*. *Behaviour*, 144(1), pp.23-31.
- Velando, A., Drummond, H. and Torres, R., 2006. Senescent birds redouble reproductive effort when ill: confirmation of the terminal investment hypothesis. *Proceedings of the Royal Society of London B: Biological Sciences*, 273(1593), pp.1443-1448.
- Velando, A., Noguera, J.C., Drummond, H. and Torres, R., 2011. Senescent males carry premutagenic lesions in sperm. *Journal of evolutionary biology*, 24(3), pp.693-697.
- Wakano, J.Y. and Ihara, Y., 2005. Evolution of Male Parental Care and Female Multiple Mating: Game - Theoretical and Two - Locus Diploid Models. *The American Naturalist*, 166(2), pp.E32-E44.
- Wanless, S., Barton, T.R. and Harris, M.P., 1997. Blood hematocrit measurements of 4 species of North Atlantic seabirds in relation to levels of infestation by the tick *Ixodes uriae*. *Colonial Waterbirds*, pp.540-544.
- Westneat, D.F. and Mays, H.L., 2005. Tests of spatial and temporal factors influencing extra - pair paternity in red - winged blackbirds. *Molecular Ecology*, 14(7), pp.2155-2167.
- Westneat, D.F. and Sherman, P.W., 1997. Density and extra-pair fertilizations in birds: a comparative analysis. *Behavioral Ecology and Sociobiology*, 41(4), pp.205-215.
- Westneat, D.F. and Stewart, I.R., 2003. Extra-pair paternity in birds: causes, correlates, and conflict. *Annual Review of Ecology, Evolution, and Systematics*, pp.365-396.
- Wink, M. and Dyrce, A., 1999. *Mating systems in birds: a review of molecular studies*. Muzeum i Instytut Zoologii PAN.

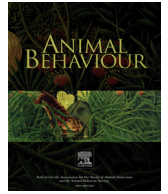
Yezerinac, S.M., Gibbs, H.L., Briskie, J.V., Whittam, R. and Montgomerie, R., 1999.  
Extrapair paternity in a far northern population of yellow warblers *Dendroica  
petechia*. *Journal of Avian Biology*, pp.234-237.



## **CAPÍTULO 1**

### **HABITAT STRUCTURE AND COLONY STRUCTURE CONSTRAIN EXTRAPAIR PATERNITY IN A COLONIAL BIRD**

*Animal Behaviour* 95:121-127



## Habitat structure and colony structure constrain extrapair paternity in a colonial bird



Alejandra G. Ramos<sup>a, \*</sup>, Schyler O. Nunziata<sup>b, c</sup>, Stacey L. Lance<sup>b</sup>, Cristina Rodríguez<sup>a</sup>, Brant C. Faircloth<sup>d</sup>, Patricia Adair Gowaty<sup>d, e, f</sup>, Hugh Drummond<sup>a</sup>

<sup>a</sup> Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México, Mexico

<sup>b</sup> Savannah River Ecology Laboratory, University of Georgia, Aiken, SC, U.S.A.

<sup>c</sup> Department of Biology, University of Kentucky, Lexington, KY, U.S.A.

<sup>d</sup> Department of Ecology and Evolutionary Biology, University of California, Los Angeles, CA, U.S.A.

<sup>e</sup> Institute of the Environment and Sustainability, University of California, Los Angeles, CA, U.S.A.

<sup>f</sup> Smithsonian Tropical Research Institute, Washington, D.C., U.S.A.

### ARTICLE INFO

#### Article history:

Received 30 January 2014

Initial acceptance 2 April 2014

Final acceptance 16 June 2014

Published online

MS. number: A14-00092R

#### Keywords:

blue-footed booby

colonial seabird

extrapair paternity

extrapair sire

habitat structure

microsatellite

nest density

spatial location

*Sula nebouxii*

Individual variation in sexual fidelity and extrapair paternity (EPP) is widely attributed to environmental heterogeneity, but the only variables known to be influential are food abundance and density of conspecific breeders (potential extrapair partners). Habitat structure is thought to impact EPP but is rarely measured and, when considered, is usually confounded with food abundance and predation pressure. To sidestep these confounds, we tested whether EPP is associated with habitat structure variables and with local conspecific density in a species whose nesting habitat is not used for feeding and lacks predators. In a blue-footed booby, *Sula nebouxii*, colony, the probability of EPP in a female's nest was highest in parts of the study plot where there were few obstacles to locomotion, and was quadratically related to local density of sexually active males, even though local males did not sire the EP chicks. The probability of a male breeder siring EP (extrapair) chicks elsewhere was quadratically related to local density of sexually active males around his nest. From these patterns we infer that both sexes may foray for EP interactions, that males and females nesting at intermediate density are most likely to be accessed by forayers, and that obstacles in the vicinity of a female's nest constrain access of foraging males. To our knowledge, this is the first demonstration that individual variation in EPP is associated with habitat structure in the absence of confounding variation in food availability, predation pressure or breeder quality, and the first evidence that EPP opportunities of female and male breeders are reduced by high density of conspecific breeders above a particular threshold.

© 2014 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Infidelity and extrapair paternity (EPP) characterize most avian mating systems and vary considerably among individuals, populations and species (reviews in: Birkhead & Møller, 1992, 1996; Gowaty, 2006). Over several decades researchers have expended considerable effort to explain this variation in terms of population density, nesting synchrony, and the age and secondary sexual characteristics of males (reviewed in Griffith, Owens, & Thuman, 2002), while devoting much less effort to ecological variables such as habitat structure, food availability (Hoi-Leitner, Hoi, Romero-Pujante, & Valera, 1999; Václav, Hoi, & Blomqvist, 2003), weather (Bouwman & Komdeur, 2006) and predation pressure. Habitat structure is widely expected to affect opportunities for

extrapair (EP) interactions (Westneat & Stewart, 2003), for example by constraining the extraterritorial foraging by which males and females of some species gain access to potential EP partners or by obstructing visual monitoring and guarding of partners (Sundberg, 1994). For instance, it is suspected that female great grey shrikes, *Lanius excubitor*, perform their EP copulations in secluded parts of their territories to escape detection (Tryjanowski, Antczak, & Hromada, 2007) and that female yellow-breasted chats, *Icteria virens*, have fewer EP chicks in open habitat because their mates can more easily guard them there (Mays & Ritchison, 2004). However, reported associations between habitat quality and EPP are likely due, not to habitat structure per se, but to associated food availability (e.g. Charmantier & Blondel, 2003; Rubenstein, 2007) or predation threat. Surprisingly, impacts of habitat structure on EPP, independent of effects of food availability and predation, have gone largely unstudied.

\* Correspondence: A. G. Ramos, Departamento de Ecología Evolutiva, Instituto de Ecología, UNAM, A.P. 70-275, México, D.F. 04510, Mexico.

E-mail address: [ramos.alejandra@gmail.com](mailto:ramos.alejandra@gmail.com) (A. G. Ramos).

To address this omission, we studied a colony of birds that nest densely in a complex habitat in which the residents do not forage and in which predators are absent, allowing us to test for associations between EPP and habitat structure in the absence of typical confounds. Blue-footed boobies, *Sula nebouxi*, nest on the forested margins of the study island, on horizontal or gently sloping ground that is heterogeneous for substrate quality and for presence of obstacles to locomotion and visual monitoring of conspecifics. Adults feed entirely at sea and, as is typical for boobies (Nelson, 1978), are not subject to any predation on land. In a part of the colony with a north–south gradient for obstacle density and an east–west gradient for proximity to the shore, we tested whether probability of EPP at each nest was associated with its location on those two axes and with substrate quality, obstacle abundance and conspecific visibility around the nest. In addition, we tested whether these habitat structure variables around a male's nest affect his probability of siring EP chicks elsewhere.

We also tested for an effect of density of sexually active male breeders around each nest on EPP at the nest and on the male's probability of siring chicks elsewhere, because the boobies' fierce territorial defence constrains locomotion of colony neighbours and passers-by and potentially limits access to EP partners or the scope of EP interactions. In avian species, local conspecific abundance can increase the probability of EPP by increasing the rate of encounters with potential EP partners (Griffith et al., 2002; Stewart, Westneat, & Ritchison, 2010), but it is not known whether dense nesting in terrestrial avian colonies can impede extrapair (EP) interactions.

Importantly, female boobies are larger than males, and extensive observation of EP behaviour in the study colony has shown that copulation is always preceded by reciprocal courtship and never by any behaviour resembling coercion (see *Study Colony and Habitat*; Westneat & Stewart, 2003). This enabled us to interpret observed patterns of paternity as an outcome of male willingness and female choice.

## METHODS

### *Study Colony and Habitat*

On the northeast shore of Isla Isabel, Mexico (21°52'N, 105°54'W), blue-footed boobies nest colonially in and at the edges of a forest of garlic pear (*Crataeva tapia*) and papelillo trees (*Euphorbia schlechtendalli*). They feed exclusively by plunge-diving for fish, and adults have no predators on their nesting grounds. The forest floor of the island is studded with embedded boulders and littered with patches of tall grass and remains of dead and sprouting trees, mostly felled by hurricane Rosa in 1994. There, these large birds walk, hop and flutter awkwardly, slipping and snagging on boulders and fallen branches, occasionally even tearing the webs of their feet. Some die snagged on branches while attempting to land through gaps in the tree canopy. In the 8450 m<sup>2</sup> study plot (part of a wider long-term study area in which 90% of breeders are banded), nests can be as close as 1 m to each other but density is heterogeneous. Territories, which are larger at low densities, are defended 24 h per day; males and females threaten and attack neighbours and intruders, particularly during the period of courtship and copulation when boundaries are being expanded and contested (Nelson, 1978). Nevertheless, most boobies nesting in the forest interior walk to and from the shore for take-off and landing for their foraging trips, bypassing and tussling with territory holders along the way.

Direct observation of relatively dense neighbourhoods in two seasons revealed that 53–61% of males and 33–53% of females copulated with habitual EP partners, usually neighbours, on or beside one of their territories before laying eggs. Sexual conflict

over EPP was indicated by a 35-fold increase in the rate of female EP copulations in the absence of their partners, an increase in within-pair courtship by males after observing their partner's EP courtship and destruction of the first-laid egg by males that were prevented from monitoring their female partner (i.e. removed from the territory for 10–12 h) during the female's assumed fertile period (Osorio-Beristain & Drummond, 1998, 2001; Pérez-Staples & Drummond, 2005; Pérez-Staples, Osorio-Beristain, Rodríguez, & Drummond, 2013).

Female boobies can control EP copulation. They are larger, 32% heavier and sensibly stronger (during handling) than males; copulation can only occur if the female stands still to allow the male to perch on her back; all observed EP copulations were preceded by reciprocal courtship, usually spread over days or weeks; and we have never seen EP males showing aggression to females or their partners (Osorio-Beristain & Drummond, 1998; Pérez-Staples & Drummond, 2005).

### *Blood Sampling*

In 2011, we marked and mapped all nests in the 20 800 m<sup>2</sup> long-term study area, and recorded breeder identities and nest contents every 3 or 6 days (details in Drummond, Torres, & Krishnan, 2003). In the study plot for this project, a 8450 m<sup>2</sup> subsection, we took blood samples between February and May from the families of all 478 nests in which any chick survived to at least age 10 days (72.3% of total nests in that section). In 424 sampled families, we obtained blood from both putative parents and all of their nestlings; in 54 families, broods were incompletely sampled because of early death of one or two chicks, but these families were retained in our analyses.

For sampling, we hand-captured adults on dark nights between 2100 and 0500 hours and removed chicks from their nests at ages 10–40 days between 0800 and 1200 hours or between 1400 and 1800 hours. We withdrew 75 µl of blood from the brachial vein and immediately mixed it with 1 ml of storage buffer (1.0 M Tris: 0.5 M EDTA: 5.0 M NaCl: 10% SDS). Manipulation took less than 10 min and bleeding stopped before release at the site of capture. Released adults promptly settled into their original neighbourhoods, and released chicks were readily attended by their putative parents. The Secretaria del Medio Ambiente y Recursos Naturales (SEMARNAT) provided the permit (SGPA/DGVS/08333/10) to collect blood samples.

### *Parentage*

We extracted DNA with illustra blood genomicPrep Mini Spin Kits from GE Healthcare (Buckinghamshire, U.K.). To analyse paternity we used 10 blue-footed booby microsatellite loci that ranged from three to 22 alleles per locus (Faircloth, Ramos, Drummond, & Gowaty, 2009). We performed all PCR amplifications under the conditions specified by Faircloth et al. (2009) and used GeneMapper version 3.7 (Applied Biosystems, Foster City, CA, U.S.A.) to analyse results. To evaluate genotypic linkage disequilibrium and deviations from Hardy–Weinberg equilibrium with Bonferroni corrections, we used GenePop v.4.0.1 (Raymond & Rousset, 1995). We conducted allele frequency estimations and parentage analyses with a likelihood-based approach in CERVUS 2.0 (Marshall, Slate, Kruuk, & Pemberton, 1998).

The sample was reduced from 478 to 453 focal families by excluding 25 families where either the female or male was genotyped in fewer than 6 of the 10 microsatellite loci; 13 chicks were excluded for the same reason. We performed 10 000 tests and assumed that 90% of sampled males and females (the percentage of individuals in the study area that bear our bands) originated from

Isla Isabel's population of about 4000 breeders. Relaxed and strict assignment levels were set at values of 80 and 95%, respectively. To assign paternity of EPP chicks, we first assigned maternity by using the putative mother as the candidate parent. If zero or one mismatch was found between the nestling and its putative mother, we assigned maternity. For nestlings that mismatched their putative mother by more than one locus, maternity was scored as unknown for subsequent paternity analysis. For nestlings for which putative mothers were not sampled ( $N = 36$ ), we examined maternity using all sampled females as candidate mothers. If no candidate mother was assigned maternity with strict confidence, maternity was left unknown for paternity analysis. We then checked paternity using the identified male breeder at the nest as the candidate father. When no mismatches occurred within the trio, paternity and maternity were assigned to the two putative parents. We reran CERVUS when mismatches occurred, using the assigned mother and all males with at least six genotyped loci as candidate fathers. If we could still not assign paternity with confidence, we examined parentage using all males and females as candidates. We found 55 EPP chicks in 48 broods and successfully assigned paternity to 17 chicks.

#### Local Nest Density

We measured local nest density as the number of nests with sexually active males within 10 m of each female's nest. Pairs were included if their male's assumed period of sexual activity (between the 16th day before and the 30th day after their females laid, based on the observed range of EP fertilizations by paired males; see Results) overlapped the female's potential fertile period (her laying period plus 29 days, the average prelaying period: Osorio-Beristain & Drummond, 1998). Local density for analysis of a male breeder's probability of siring chicks at other nests included all nests within 10 m of the male whose males' assumed period of sexual activity overlapped his. We used a radius of 10 m since boobies interact most often with individuals nesting within this distance (Drummond, Torres, Juarez, & Kim, 2010; Montes-Medina, Drummond, & Kim, 2009; Osorio-Beristain & Drummond, 1993).

The local density of sexually active males around nests of females and potential male sires was significantly correlated with densities of local breeders in all reproductive stages (Pearson correlation: females:  $r_{421} = 0.94$ ,  $P < 0.001$ ; males:  $r_{421} = 0.97$ ,  $P < 0.001$ ). However, significance values and changes in deviance in nested generalized linear models indicated that the density of sexually active males was a better predictor for female EP paternity and male EP paternity gain than was density of local breeders in all stages.

#### Habitat Structure

After the breeding season, we measured density of obstacles to locomotion, substrate quality and visibility of standing boobies to each other at 152 focal nest sites: at 100 randomly selected sites and at all sites with EP chicks or EP sires. By error, we omitted eight sites of EP chicks and two sites of EP sires where breeder ages were unknown, leaving samples of 38 and 14 sites, respectively. In a 5 m radius around each site, we counted the objects that could obstruct walking boobies (standing and fallen tree trunks >15 cm in diameter and rocks protruding >30 cm from the substrate), visually estimated the percentage of substrate covered by grass and by rocks, and computed a visibility index by posing a 40 cm high stick (representing a standing booby) 5 m away at eight standard, evenly spaced compass bearings, scoring the proportion of the stick (100%, 50%, 0%) that an observer at the site could see from a height of 35 cm (booby eye-height), and summing the eight scores. A 5 m

radius permitted sensitive discrimination among sites; visibility decreased with distance and, at 10 m, most bearings scored 0% visibility.

#### Analysis

To analyse the probability of a female breeder having an EP chick in her nest and the probability of a male breeder siring a chick at another nest, we used generalized linear models (GLMs) with binomial distribution and logit link function. Full models included local nest density, laying date and spatial coordinates ( $X$  and  $Y$ ) within the sampling area as explanatory variables, plus quadratic terms and interactions of interest (see Results, Table 1). Laying date was expressed as a proportional rank that ranged from zero to one, using all 1360 nests in the study area in 2011. The sample was reduced from 453 to 423 focal families by exclusion of 25 nests located less than 10 m from the edge of the sampling area (whose local nest density could not be fairly calculated), three nests lacking spatial coordinates and two nests with unknown laying dates. At one excluded nest the male breeder sired an EP chick elsewhere, and two of the excluded nests had EP chicks. Excluded nests were included in the analyses as neighbours of focal nests, except when their spatial coordinates or laying date were unknown.

Two additional GLMs tested whether any of the four habitat structure variables (obstacles, grass cover, rock cover and visibility) predicted the probability that females at focal nests would have EP chicks in their broods or that males at focal nests would sire chicks elsewhere. Variance inflation factors (VIFs) for all explanatory variables were less than 2, indicating acceptable levels of multicollinearity (Zuur, Ieno, & Elphick, 2010; Zuur, Ieno, Walker, Saveliev, & Smith, 2009).

We simplified full models with deletion tests, removing nonsignificant interactions, quadratic factors and linear factors sequentially (Crawley, 2007). Each term was individually removed to evaluate its significance and change in deviance, then reintroduced to the model. After evaluating every term, we permanently removed the nonsignificant term that accounted for the least change in deviance and repeated the process until only significant terms remained. We used deletion tests to obtain the  $P$  values associated with each term in the final model. Statistics were performed in R statistical software v.2.12.2 (R Development Core Team, 2008). Means  $\pm$  SD are reported throughout the manuscript.

## RESULTS

#### Females with EP Chicks

Forty-eight (10.6%) of the 453 genotyped families had one or two EP chicks (41 had one EP chick and seven had two EP chicks), and 46 (10.9%) of our reduced sample of 423 focal females had at

**Table 1**

Generalized linear model of the relationship between probability of an extrapair (EP) chick in a nest and its location ( $X$  coordinate) and local nest density ( $N = 423$  nests)

Variables in final model	$B$	SE( $\beta$ )	Deviance <sup>a</sup>	$P^a$
$X$ coordinate	0.016	0.005	10.01	<0.001
Density	0.162	0.103	2.76	0.096
Density <sup>2</sup>	-0.004	0.002	3.74	0.052

Full model: (EPP ~  $X$  coordinate +  $Y$  coordinate + laying date + density + (density)<sup>2</sup> + ( $X$  coordinate)<sup>2</sup> + ( $Y$  coordinate)<sup>2</sup> +  $X$  coordinate \*  $Y$  coordinate + density \* laying date + laying date \*  $Y$  coordinate + laying date \*  $X$  coordinate +  $X$  coordinate \* density +  $Y$  coordinate \* density + ( $X$  coordinate)<sup>2</sup> \* ( $Y$  coordinate)<sup>2</sup>, family = binomial).

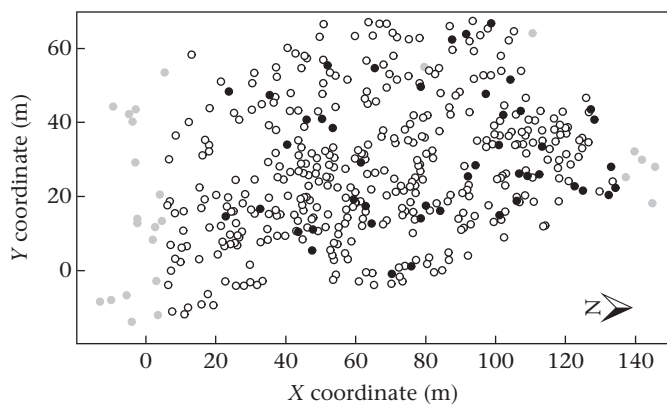
<sup>a</sup> We obtained statistical significance and changes in deviance from comparison tests between nested models.

least one EP chick (39 with one EP chick and seven with two EP chicks). The probability of a brood including an EP chick was related to location in the sampling area, habitat structure and local nest density, but not to laying date (Table 1).

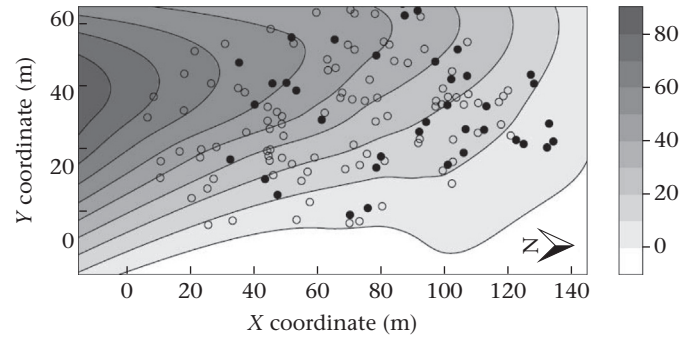
The relationship between EPP and spatial location of nests was significant for the  $X$  coordinate (GLM:  $P < 0.001$ ) but not the  $Y$  coordinate (GLM:  $P = 0.76$ ) or their interaction (GLM:  $P = 0.12$ ): probability of EPP decreased southward in the sampled area regardless of a nest's distance from the shoreline, and there were no EPP chicks in the southernmost 22 m band (Fig. 1).

Analysis of habitat structure revealed only one (marginally) significant association: probability of EPP increased as the density of obstacles to locomotion within 5 m of a female's nest decreased (GLM: deviance = 3.79,  $N = 138$ ,  $P = 0.051$ ). Density of obstacles decreased in the same direction that probability of EPP increased: northward (Fig. 2). Probability of EPP was not related to substrate quality (GLM: grass coverage:  $P = 0.30$ ; rock coverage:  $P = 0.66$ ), visibility (GLM:  $P = 0.58$ ), or any interaction between habitat structure variables.

The observed associations between EPP and both location and obstacle density could potentially be driven by associations between breeder quality and those environmental variables. To test for this, for all boobies of known reproductive history that bred in the study plot in 2011, we sought correlations between the  $X$  coordinate and two indices of their quality: age and previous breeding success. We expressed individuals' previous breeding success as the average of the numbers of fledglings they produced each year (standardized for each year) between birth and 2010. All four Spearman rank correlation coefficients were small and none approached significance (male age:  $r_s = 0.043$ ,  $N = 596$ ,  $P = 0.30$ ; male reproductive success:  $r_s = 0.037$ ,  $N = 324$ ,  $P = 0.51$ ; female age:  $r_s = 0.054$ ,  $N = 587$ ,  $P = 0.19$ ; female reproductive success:  $r_s = -0.017$ ,  $N = 295$ ,  $P = 0.78$ ). In addition, to test whether the probability of EPP was associated with male or female body condition or size, we tested whether those variables were correlated with the  $X$  coordinate, using the 333 males and 326 females in the study plot that were weighed and measured in 2011. Condition was expressed as the residuals of a regression of body mass on body size (ulna length). Again, Spearman rank correlation coefficients were small and none approached significance (male body condition:  $r_s = 0.076$ ,  $P = 0.16$ ; male body size:  $r_s = 0.067$ ,  $P = 0.22$ ; female body condition:  $r_s = 0.017$ ,  $P = 0.75$ ; female body size:  $r_s = 0.016$ ,  $P = 0.77$ ).



**Figure 1.** Distribution of blue-footed booby nests with extrapair (EP) chicks in the sampling area. Focal nests included 46 nests with EP chicks (black dots) and 377 nests without EP chicks (white dots). Grey dots represent 25 nests that were included in the analysis only as neighbours because proximity to the sampling area boundary prevented calculation of local nest density. The shoreline runs roughly north–south, about 20 m beyond the lower (eastern) boundary of the plot.



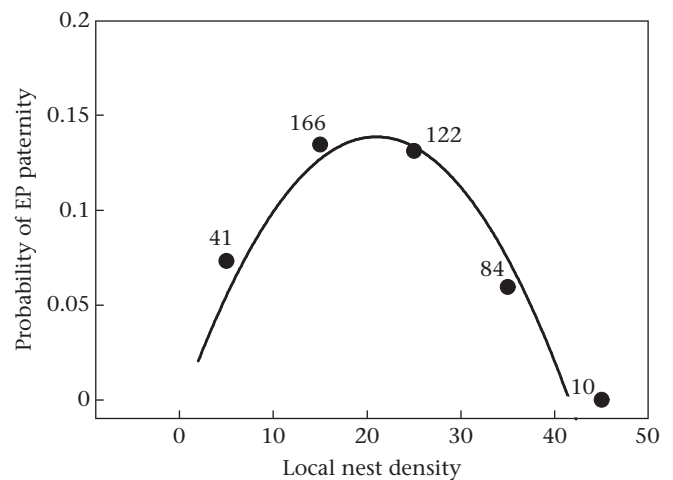
**Figure 2.** Extrapair paternity and obstacle density in blue-footed boobies. Focal nests comprised 99 nests without extrapair (EP) chicks (open dots) and 39 nests with EP chicks (solid dots). Scale shows number of trees and rocks within 5 m of focal nests. Contour plot created with lattice package of R shows estimated obstacle density in each greyscale band (Sarkar, 2008). The shoreline runs roughly north–south, about 20 m beyond the lower (eastern) boundary of the plot.

The probability of EPP was marginally related to quadratic local nest density (GLM:  $P = 0.052$ ; Fig. 3), and piecewise regression (Crawley, 2007) showed that although the initial increase with density in Fig. 3 was not significant (density  $< 26$ : intercept =  $-2.21$ , slope =  $0.012$ ,  $N = 273$ ,  $P = 0.72$ ), the probability of EPP declined significantly above 26 nests/ neighbourhood (density  $\geq 26$ : intercept =  $3.49$ , slope =  $-0.18$ ,  $N = 150$ ,  $P = 0.025$ ). Moreover, visual inspection suggests that, with only one exception, nests with EP chicks did not occur within nest clusters, but rather at their edges and in more isolated nests (Fig. 1).

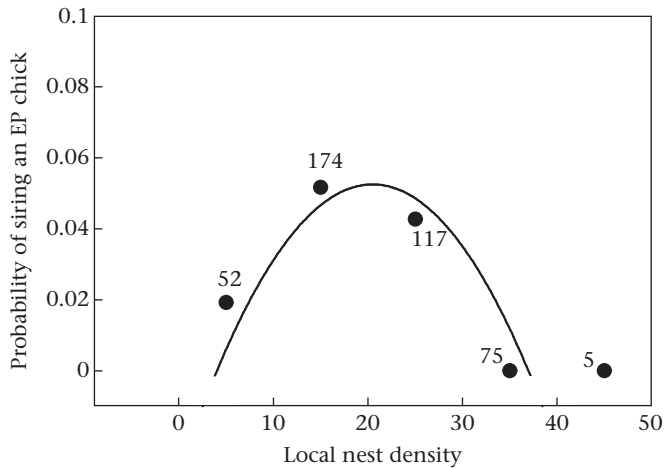
#### Extrapair Sires

We successfully assigned paternity of 17 of the 55 EP chicks genotyped in the sampled area. One of these EP fathers sired two EP chicks in nests that were 33.9 m apart.

The probability of a male breeder in the sampling area siring a chick at another nest in the sampling area was not related to his nest's  $Y$  or  $X$  coordinates (GLM:  $Y$  coordinate,  $P = 0.21$ ;  $X$  coordinate,  $P = 0.43$ ), nor to his social partner's laying date (GLM:  $P = 0.56$ ). It was significantly related to the quadratic local nest density (GLM: deviance = 6.49,  $N = 423$ ,  $P = 0.010$ ; Fig. 4) but not to linear local density (GLM:  $P = 0.37$ ). The probability of siring an EP chick



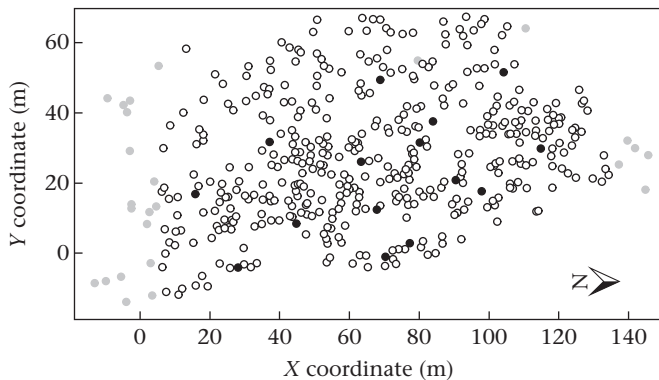
**Figure 3.** Probability of extrapair paternity (EPP) in a female blue-footed booby's nest as a function of local nest density (neighbours within 10 m). Points show mean probabilities at each of five nest density intervals; numbers represent sample sizes at each density interval. We binned the (binomial) data to improve visualization.



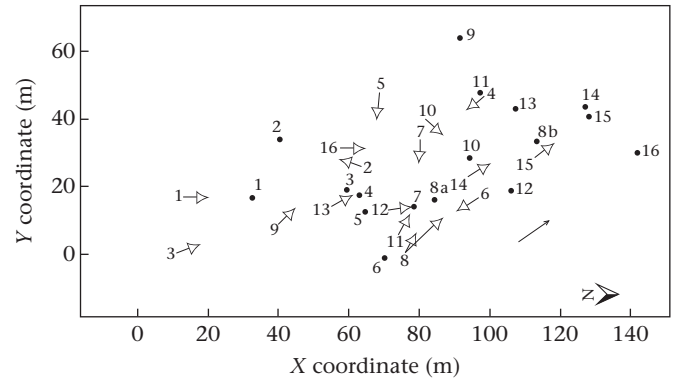
**Figure 4.** Probability of male's gaining extrapair paternity as a function of local nest density (i.e. neighbours within 10 m). Points show mean probabilities at each of five nest density intervals; numbers represent sample sizes at each density interval. We binned the (binomial) data to improve visualization.

declined beyond 19 nests/neighbourhood (density  $\geq 19$ : intercept = 1.02, slope =  $-0.16$ ,  $N = 226$ ,  $P = 0.043$ ), but the initial increase seen in Fig. 4 was not significant (density  $< 19$ : intercept =  $-5.41$ , slope =  $0.16$ ,  $N = 197$ ,  $P = 0.21$ ). Conspicuously, nests of EP sires did not occur within nest clusters, but only at or near their edges and in more isolated sites (Fig. 5). Of the habitat structure variables tested, only density of obstacles came close to showing a significant relationship to the probability of siring chicks elsewhere (GLM:  $N = 113$ : density of obstacles, deviance = 3.29,  $P = 0.069$ ; grass coverage,  $P = 0.81$ ; rock coverage,  $P = 0.34$ ; visibility,  $P = 0.66$ ); hinting that the more obstacles around a male's nest, the less likely he was to sire an EP chick elsewhere.

Surprisingly, EP sires were not neighbours of their EP partners: the 17 EP sires were located an average of 43.7 m from the nests of the EP partners they fertilized, at distances ranging from 14 to 105 m (median = 39 m; Fig. 6). Assuming that EP sires moved directly from their own territories to the territories of their EP partners to inseminate them, we tested randomness of their directions of movement against a circular uniform distribution (Watson, 1967). Directions were not random and followed a mean bearing of  $328.8^\circ$  (test statistic = 0.71,  $N = 17$ ,  $P < 0.001$ ; Fig. 6); this could result from nests with EPP being concentrated in the



**Figure 5.** Distribution of blue-footed booby nests sired by extrapair (EP) males in the sampling area. Focal nests included 15 nests with EP sires (black dots) and 408 nests with no identified EP sires (white dots). Grey dots show 25 nests that were included in the analysis only as neighbours since they were too close to the sampling area's edge. The shoreline runs roughly north–south, about 20 m beyond the lower (eastern) boundary of the plot.



**Figure 6.** Spatial relationship between nests of extrapair (EP) sires and nests of their EP partners. Locations of nests with EP chicks (dots) and nests of the corresponding EP sires (at origin of each arrow). Arrows show the directions of movement of sires, assuming they moved directly from their own territories to the territories of their EP partners to inseminate them. The nest of each EP chick bears the same number as the corresponding EP sire. The large arrow shows the mean movement bearing of  $328.8^\circ$ , assuming that sires moved to the territories of their EP partners. The shoreline runs roughly north–south, about 20 m beyond the lower (eastern) boundary of the plot.

relatively boulder-free north while EP sires were distributed heterogeneously along the north–south axis.

Seven EP chicks were from clutches started  $6.57 \pm 4.66$  days before their sires' social partners started laying (range 2–16 days) and the other 10 were from clutches started  $15.80 \pm 9.60$  days after the sires' social partners started laying (range 2–30 days). Thus, the range of start dates of EP clutches spanned a total 46 days, starting 16 days before the sires' clutches with their social partners.

## DISCUSSION

EPP was more common in nests in areas where obstacles to locomotion were sparse and it increased northward, just as obstacle density around nests declined northward. Obstacles in the vicinity of a booby's nest probably make it more difficult for potential EP mates to approach and for EP interactions to occur, either because boobies circumventing them expose themselves to injury or aggression from guarding males or neighbours, or because obstacles complicate performance of courtship components such as the parading display (Nelson, 1978). It is unlikely that this pattern was driven by an association between nest location and breeder quality (as suspected in oystercatchers, *Haematopus ostralegus*; van de Pol, Bakker, Saaltink, & Verhulst, 2006), because for neither sex was there an association between nest location and any measure of individual quality (previous breeding success, current age, body size or body condition).

Density of sexually active male neighbours (and their partners) within 10 m of a female's nest had a quadratic effect on the probability of EPP. In contrast, nest density is positively related to EPP in many avian species (Griffith et al., 2002), but negative effects of very high density may have been missed because tests of a quadratic relationship are rare. At densities of over  $0.082$  nests/m<sup>2</sup>, the probability of EPP declined as the number of neighbours increased, and females nesting within clusters of nests did not have EP chicks. Very high-density neighbourhoods in a booby colony may be difficult for foraging individuals of either sex to penetrate and sample because of frequent agonistic encounters with territory-defending neighbours of both sexes. In addition, male partners in dense neighbourhoods possibly increase mate guarding effort in response to the high risk of cuckoldry (Komdeur, 2001; Mayer & Pasinelli, 2013). The quadratic effect of density on EPP could imply that neighbourhoods with fewer than  $0.082$  nests/m<sup>2</sup>

are less attractive to foraging males, although the decline in EPP at lower densities was not significant. Foraging males may be most successful in neighbourhoods of medium density because female breeders there are both relatively numerous and relatively accessible.

The average distance between nests of females and their EP sires was 43.7 m, and not a single EP sire was a near neighbour. Fertilization by distant rather than nearby EP males has been detected in other avian species (e.g. Dunn, Robertson, Michaud-Freeman, & Boag, 1994; Hung, Tarof, & Stutchbury, 2009; Stewart, Hanschu, Burke, & Westneat, 2006), although EP fertilization by near neighbours is also common (e.g. Perreault, Lemon, & Kuhnlein, 1997; Webster, Chuang-Dobbs, & Holmes, 2001; Woolfenden, Stutchbury, & Morton, 2005). Mating with distant EP partners may incur increased travel costs for the foraging sex, but if females are more closely related to near neighbours, then mating with distant EP partners could potentially increase offspring heterozygosity (Foerster, Delhey, Johnsen, Lifjeld, & Kempnaers, 2003). Studies of numerous species have reported positive relationships between heterozygosity and fitness traits such as embryo, offspring and adult survival, health and reproductive success (reviewed in Kempnaers, 2007). On average, male and female boobies disperse only 30.5 m and 36.6 m, respectively, from their natal site to their first breeding site, and they breed thereafter within 19.0–28.9 m and 22.5–30.5 m, respectively, of that first site (Kim, Torres, Rodriguez, & Drummond, 2007; Osorio-Beristain & Drummond, 1993), so a heterozygosity benefit to females is plausible.

Foraging by paired boobies has not been observed, and copulations between breeders with territories distant from each other could take place at the territory of the male or the female, or in other areas. It is likely that EP fertilizations mostly occur when foraging males approach the territories of paired females because (1) among avian species with EPP, foraging has been nearly universally documented in males and much less often documented in females (whose foraging may, however, be less detectable; Westneat & Stewart, 2003), (2) six non-neighbouring male boobies of unknown breeding status were observed approaching, courting and copulating with paired females beside those females' territories before they laid in 2011 (Kiere & Drummond, 2013) and (3) location of an individual's own nest in the study plot affected the probability of a female having an EP chick but not the probability of a male siring an EP chick elsewhere. Boobies probably foray by walking through the colony, but they could also access distant parts of the colony by flying.

It is remarkable that male neighbours were almost absent from the sample of identified EP sires despite predominating among EP partners directly observed in other years. Paired and unpaired territorial neighbours represented 60.0% of observed extrapair copulatory partners in the study plot in 2011 (Kiere & Drummond, 2013) and 86.7% in 1991 and 1997 (Osorio-Beristain & Drummond, 1998, 2001; Pérez-Staples & Drummond, 2005; Pérez-Staples et al., 2013). This disjunction could arise partly from movement of unpaired males to new territories after EP copulations, although EP sires' own clutches were mostly laid before, not after, the clutches containing their EP eggs. A more straightforward explanation is that females' EP partners include both local and distant territorial males (paired and unpaired) and female postcopulatory choice among ejaculates may favour distant EP partners. Ejaculate retention and sperm competition in red junglefowl, *Gallus gallus domesticus*, and ruffs, *Philomachus pugnax*, favour males that are more genetically different from the female (Pizzari, Lø, & Cornwallis, 2004; Thuman & Griffith, 2005). Hence, multiple EP mating by female boobies with neighbours and non-neighbours could be followed up by cryptic selection of sperm to optimize genetic compatibility, either by avoiding inbreeding or by achieving

complementarity of major histocompatibility complex haplotypes (Ball & Parker, 2003; Griffith & Immler, 2009; Hasson & Stone, 2011).

Identified sires were breeders (only breeders were blood-sampled) from distant nests that fertilized eggs in clutches laid up to 16 days before and 30 days after their own partners laid, implying that they engaged in EP interactions during their within-pair courtship period and during the time when they were alternating incubation shifts with their social partners (although female seabirds can store sperm for at least 1 month (Hatch, 1983)). A male breeder's probability of siring EP chicks at another nest was not related to the location of his nest within the study plot but increased with local nest density up to  $\geq 0.06$  nests/m<sup>2</sup>, then declined at higher values, similar to the effect of local nest density on a female's probability of producing EP chicks. This could imply that male breeders are visited at their own territories by female breeders and that foraging females are attracted by clusters of sexually active males but are unable to access individuals in the densest neighbourhoods successfully. However, prevalence of male foraging is indicated by the strong relationship between the location of a female's nest in the colony and the probability of EPP there, and the absence of a similar relationship for males.

Different potential of different parts of the nesting habitat for EP interactions could influence foraging patterns and territory selection. Nesting in the southern, boulder-strewn part of the study plot appeared to constrain the EPP opportunities of females more than males. Assuming that foraging was mostly done by males, it appears that males in the south forayed elsewhere, in parts of the study plot where females were less constrained by obstacle density, and that this movement towards abundant nests of unconstrained females (in the north and northwest) imparted an average foraging direction of 328.8° to the sample of males that achieved EPP. In some avian species, males or females may prefer to nest in social environments that either propitiate or render unlikely encounters with potential EP partners (Danchin & Wagner, 1997); likewise, boobies may show similar preferences in relation to features of habitat structure and colony structure that facilitate or constrain EP interactions. For example, males with attractive partners could nest preferentially where abundant obstacles or high breeder density will limit access of other males (without foreclosing their own EP options), and females with poor-quality partners could select locations where obstacles are scarce and they are not enclosed within clusters of conspecifics. It will be a challenge to determine whether individual boobies selectively locate their territories and nests in habitats and social contexts that allow them to pursue their own extrapair interests while limiting those of their partners.

## Acknowledgments

We are grateful to Simon C. Griffith, Lynna M. Kiere, Marcela Osorio-Beristain, Diana Pérez-Staples, Oscar Sánchez-Macouzet and two anonymous referees for helpful comments on the manuscript, and to numerous volunteers and colleagues for help in the field and lab, especially Santiago Bautista-López, Jonathan P. Drury, Gabriela López-Carapia, Janeth Rosas-Morales and David Schneider. We are grateful to Pablo Frank-Bolton, who kindly wrote the script used to estimate neighbourhood nest density. The Armada de México, local fishermen and staff of the Parque Nacional Isla Isabel supplied vital and much appreciated logistical support. This study was financed by UNAM (PAPIIT, IN206610) and CONACyT (104313). This manuscript constitutes a partial fulfilment of the Graduate Program in Biomedical Sciences of the Universidad Nacional Autónoma de México. A.G.R. wishes to thank and acknowledge the scholarship and financial support provided by the Consejo Nacional de Ciencia y Tecnología and the Universidad Nacional Autónoma de

México. This research was partially supported by U.S. Department of Energy under Award Number DE-FC09-07SR22506 to the University of Georgia Research Foundation.

## References

- Ball, M. A., & Parker, G. A. (2003). Sperm competition games: sperm selection by females. *Journal of Theoretical Biology*, 224, 27–42.
- Birkhead, T. R., & Möller, A. P. (1992). *Sperm competition in birds: Evolutionary causes and consequences*. London, U.K.: Academic Press.
- Birkhead, T. R., & Möller, A. P. (1996). Monogamy and sperm competition in birds. In J. M. Black (Ed.), *Partnerships in birds: The study of monogamy* (pp. 323–343). Oxford, U.K.: Oxford University Press.
- Bouwman, K. M., & Komdeur, J. (2006). Weather conditions affect levels of extra-pair paternity in the reed bunting *Emberiza schoeniclus*. *Journal of Avian Biology*, 37, 238–244.
- Charmantier, A., & Blondel, J. (2003). A contrast in extra-pair paternity levels on mainland and island populations of Mediterranean blue tits. *Ethology*, 109, 351–363.
- Crawley, M. J. (2007). *The R book*. Chichester: J. Wiley.
- Danchin, E., & Wagner, R. H. (1997). The evolution of coloniality: the emergence of new perspectives. *Trends in Ecology & Evolution*, 12, 342–347.
- Drummond, H., Torres, R., Juárez, C. R., & Kim, S.-Y. (2010). Is kin cooperation going on undetected in marine bird colonies? *Behavioral Ecology and Sociobiology*, 64, 647–655.
- Drummond, H., Torres, R., & Krishnan, V. V. (2003). Buffered development: resilience after aggressive subordination in infancy. *American Naturalist*, 161, 794–807.
- Dunn, P. O., Robertson, R. J., Michaud-Freeman, D., & Boag, P. T. (1994). Extra-pair paternity in tree swallows: why do females mate with more than one male? *Behavioral Ecology and Sociobiology*, 35, 273–281.
- Faircloth, B. C., Ramos, A., Drummond, H., & Gowaty, P. A. (2009). Isolation and characterization of microsatellite loci from blue-footed boobies (*Sula nebouxi*). *Conservation Genetics Resources*, 1, 159–162.
- Foerster, K., Delhey, K., Johnsen, A., Lifjeld, J. T., & Kempenaers, B. (2003). Females increase offspring heterozygosity and fitness through extra-pair matings. *Nature*, 425, 714–717.
- Gowaty, P. A. (2006). Beyond extra-pair paternity: individual constraints, fitness components, and social mating systems. In J. R. Lucas, & L. W. Simmons (Eds.), *Essays in animal behaviour: Celebrating 50 years of Animal Behaviour* (pp. 221–256). London, U.K.: Academic Press.
- Griffith, S. C., & Immler, S. (2009). Female infidelity and genetic compatibility in birds: the role of the genetically loaded raffle in understanding the function of extrapair paternity. *Journal of Avian Biology*, 40, 97–101.
- Griffith, S. C., Owens, I. P., & Thuman, K. A. (2002). Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Molecular Ecology*, 11, 2195–2212.
- Hasson, O., & Stone, L. (2011). Why do females have so few extra-pair offspring? *Behavioral Ecology and Sociobiology*, 65, 513–523.
- Hatch, S. A. (1983). Mechanism and ecological significance of sperm storage in the northern fulmar with reference to its occurrence in other birds. *Auk*, 593–600.
- Hoi-Leitner, M., Hoi, H., Romero-Pujante, M., & Valera, F. (1999). Female extra-pair behaviour and environmental quality in the serin (*Serinus serinus*): a test of the 'constrained female hypothesis'. *Proceedings of the Royal Society B: Biological Sciences*, 266, 1021–1026.
- Hung, S., Tarof, S. A., & Stutchbury, B. J. M. (2009). Extra-pair mating tactics and vocal behavior of female acadian flycatchers. *Condor*, 111, 653–661.
- Kempenaers, B. (2007). Mate choice and genetic quality: a review of the heterozygosity theory. *Advances in the Study of Behavior*, 37, 189–278.
- Kiere, L. M., & Drummond, H. (2013). [Observations of extra-pair behaviour in blue-footed boobies in the Isla Isabel colony]. Unpublished raw data.
- Kim, S. Y., Torres, R., Rodríguez, C., & Drummond, H. (2007). Effects of breeding success, mate fidelity and senescence on breeding dispersal of male and female blue-footed boobies. *Journal of Animal Ecology*, 76, 471–479.
- Komdeur, J. (2001). Mate guarding in the Seychelles warbler is energetically costly and adjusted to paternity risk. *Proceedings of the Royal Society B: Biological Sciences*, 268, 2103–2111.
- Marshall, T. C., Slate, J., Kruuk, L. E. B., & Pemberton, J. M. (1998). Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, 7, 639–655.
- Mayer, C., & Pasinelli, G. (2013). New support for an old hypothesis: density affects extra-pair paternity. *Ecology and Evolution*, 3, 694–705.
- Mays, H. L., & Ritchison, G. (2004). The effect of vegetation density on male mate guarding and extra-territorial forays in the yellow-breasted chat (*Icteria virens*). *Naturwissenschaften*, 91, 195–198.
- Montes-Medina, A., Drummond, H., & Kim, S.-Y. (2009). Distance from the forest edge matters in habitat selection of the blue-footed booby (*Sula nebouxi*). *Journal of Ornithology*, 150, 845–852.
- Nelson, J. B. (1978). *The Sulidae: Gannets and boobies*. New York, NY: Oxford University Press.
- Osorio-Beristain, M., & Drummond, H. (1993). Natal dispersal and deferred breeding in the blue-footed booby. *Auk*, 110, 234–239.
- Osorio-Beristain, M., & Drummond, H. (1998). Non-aggressive mate guarding by the blue-footed booby: a balance of female and male control. *Behavioral Ecology and Sociobiology*, 43, 307–315.
- Osorio-Beristain, M., & Drummond, H. (2001). Male boobies expel eggs when paternity is in doubt. *Behavioral Ecology*, 12, 16–21.
- Pérez-Staples, D., & Drummond, H. (2005). Tactics, effectiveness and avoidance of mate guarding in the blue-footed booby (*Sula nebouxi*). *Behavioral Ecology and Sociobiology*, 59, 115–123.
- Pérez-Staples, D., Osorio-Beristain, M., Rodríguez, C., & Drummond, H. (2013). Behavioural roles in booby mate switching. *Behaviour*, 150, 337–357.
- Perreault, S., Lemon, R. E., & Kuhnlein, U. (1997). Patterns and correlates of extrapair paternity in American redstarts (*Setophaga ruticilla*). *Behavioral Ecology*, 8, 612–621.
- Pizzari, T., Lø, H., & Cornwallis, C. K. (2004). Sex-specific, counteracting responses to inbreeding in a bird. *Proceedings of the Royal Society B: Biological Sciences*, 271, 2115–2121.
- van de Pol, M., Bakker, T., Saaltink, D. J., & Verhulst, S. (2006). Rearing conditions determine offspring survival, independent of egg quality: a cross-foster experiment with oystercatchers. *Ibis*, 148, 203–210.
- Raymond, M., & Rousset, F. (1995). GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, 86, 248–249.
- Rubenstein, D. R. (2007). Territory quality drives intraspecific patterns of extrapair paternity. *Behavioral Ecology*, 18, 1058–1064.
- Sarkar, D. (2008). *Lattice: Multivariate data visualization with R*. New York, NY: Springer.
- Stewart, I. R. K., Hanschu, R. D., Burke, T., & Westneat, D. F. (2006). Tests of ecological, phenotypic, and genetic correlates of extra-pair paternity in the house sparrow. *Condor*, 108, 399–413.
- Stewart, S. M., Westneat, D., & Ritchison, G. (2010). Extra-pair paternity in eastern bluebirds: effects of manipulated density and natural patterns of breeding synchrony. *Behavioral Ecology and Sociobiology*, 64, 463–473.
- Sundberg, J. (1994). Paternity guarding in the yellowhammer (*Emberiza citrinella*): a detection experiment. *Journal of Avian Biology*, 25, 135–141.
- Thuman, K. A., & Griffith, S. C. (2005). Genetic similarity and the nonrandom distribution of paternity in a genetically highly polyandrous shorebird. *Animal Behaviour*, 69, 765–770.
- Tryjanowski, P., Antczak, M., & Hromada, M. (2007). More secluded places for extra-pair copulations in the great grey shrike (*Lanius excubitor*). *Behaviour*, 144, 23–31.
- Václav, R., Hoi, H., & Blomqvist, D. (2003). Food supplementation affects extrapair paternity in house sparrows (*Passer domesticus*). *Behavioral Ecology*, 14, 730–735.
- Watson, G. S. (1967). Another test for the uniformity of a circular distribution. *Biometrika*, 54, 675–677.
- Webster, M. S., Chuang-Dobbs, H. C., & Holmes, R. T. (2001). Microsatellite identification of extrapair sires in a socially monogamous warbler. *Behavioral Ecology*, 12, 439–446.
- Westneat, D. F., & Stewart, I. R. K. (2003). Extra-pair paternity in birds: causes, correlates, and conflict. *Annual Review of Ecology, Evolution, and Systematics*, 34, 365–396.
- Woolfenden, B. E., Stutchbury, B. J. M., & Morton, E. S. (2005). Male Acadian flycatchers, *Empidonax virescens*, obtain extrapair fertilizations with distant females. *Animal Behaviour*, 69, 921–929.
- Zuur, A. F., Ieno, E. N., & Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution*, 1, 3–14.
- Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009). *Mixed effects models and extensions in ecology with R*. New York, NY: Springer.



## **CAPÍTULO 2**

### **INTERACTIVE EFFECTS OF MALE AND FEMALE AGE ON EXTRA-PAIR PATERNITY IN A SOCIALLY MONOGAMOUS BIRD**

*Behavioral Ecology and Sociobiology 68:1603-1609*

# Interactive effects of male and female age on extra-pair paternity in a socially monogamous seabird

Alejandra G. Ramos · Schyler O. Nunziata · Stacey L. Lance ·  
Cristina Rodríguez · Brant C. Faircloth · Patricia Adair Gowaty ·  
Hugh Drummond

Received: 14 May 2014 / Revised: 26 June 2014 / Accepted: 30 June 2014  
© Springer-Verlag Berlin Heidelberg 2014

**Abstract** Females sometimes obtain older sires for their offspring through extra-pair interactions, but how female age influences paternity is largely unexplored and interactive effects across the age span of both sexes have not been analyzed. To test whether female choice of sire age varies with female age in the blue-footed booby (*Sula nebouxi*), we examined associations between ages of both partners and the probability of extra-pair paternity (EPP) in 350 broods of parents up to 22 years old in a single breeding season. Extra-pair paternity enables a female to select an alternative sire for her offspring and could function to avoid or achieve particular combinations of parental

ages. A male age × female age interaction revealed that in young females ( $\leq 4$  years), EPP decreased with increasing age of the social partner, whereas in old females ( $\geq 8$  years), it increased. Moreover, sires of extra-pair (EP) chicks of young females paired to young males were on average 6.33 years older than the females' social partners. Since female boobies control copulatory access, this pattern could imply that young females choose old sires for their proven genetic quality and that old females avoid very old males because matings with them may risk infertility or genetic defects in offspring. Taking female age into account and observing across the whole age span may be necessary for understanding female age-based mate choice.

Communicated by S. Pruett-Jones

**Electronic supplementary material** The online version of this article (doi:10.1007/s00265-014-1769-9) contains supplementary material, which is available to authorized users.

A. G. Ramos (✉) · C. Rodríguez · H. Drummond  
Departamento de Ecología Evolutiva, Instituto de Ecología,  
Universidad Nacional Autónoma de México, A.P. 70-275,  
04510 Mexico, Mexico  
e-mail: ramos.alejandra@gmail.com

S. O. Nunziata · S. L. Lance  
Savannah River Ecology Laboratory, University of Georgia, Aiken,  
SC, USA

S. O. Nunziata  
Department of Biology, University of Kentucky, Lexington, KY,  
USA

B. C. Faircloth · P. A. Gowaty  
Department of Ecology and Evolutionary Biology, University of  
California Los Angeles, Los Angeles, CA, USA

P. A. Gowaty  
Institute of the Environment and Sustainability, University of  
California Los Angeles, Los Angeles, CA, USA

P. A. Gowaty  
Smithsonian Tropical Research Institute, Washington, DC, USA

**Keywords** Age · Female choice · Extra-pair paternity ·  
Extra-pair sire · Infertility · Good genes

## Introduction

Female age-based mate choice remains an enigma because many studies have found that female animals prefer to mate with old males despite the expectation of senescent increase in mutation load in male germ line DNA (Beck and Promislow 2007) and evidence that old males tend to father poor quality offspring (Johnson and Gemmill 2012). However, there are also theoretical grounds for expecting older males to provide better genes when differential survival of males generates a correlation between age and quality (Manning 1985; Kokko 1998; Brooks and Kemp 2001) as well as counterarguments based on life history considerations holding that older males are likely to be genetically inferior (Hansen and Price 1995). When choosing mates, females should theoretically take into account not only the decreased fertility and genetic quality that may come with germ line senescence but also the change in genetic quality that may be associated with greater male age (Johnson and Gemmill 2012). Currently, our appreciation of

the choices actually made by females is limited because no analysis of a wild population details female choice across the whole age span of both sexes.

In birds, there is growing evidence that senescence prejudices reproductive traits of both sexes, including germ line DNA (Velando et al. 2011), egg quality (Beamonte-Barrientos et al. 2010), parental care (Cтры et al. 2006), and offspring quality (Saino et al. 2002), and in birds, infidelity provides a special window into female choice among male genes because extra-pair (EP) sires generally contribute only their genes and no parental care to offspring. There is some evidence that genes of older males are preferred: old males are more likely to gain extra-pair paternity (EPP) at other nests (Cleasby and Nakagawa 2012; but see Brooks and Kemp 2001), EP sires tend to be older than the males they cuckolded (Akçay and Roughgarden 2007), and older males lose paternity to EP sires less often than younger males (Møller and Ninni 1998). However, the evidence for this preference is mixed (Brooks and Kemp 2001; Cleasby and Nakagawa 2012), and it is possible that inconsistent patterns have arisen through females sometimes preferring middle-aged males and due to fieldworkers almost universally failing to distinguish between middle-aged and old males (Hansen and Price 1995; Jones et al. 2000; Radwan 2003). Although senescent individuals have undoubtedly been included in analyses of female choice in wild avian populations, most have probably contrasted young and middle-aged males and, consequently, could not have revealed effects of senescence.

In theory, female age could affect the choice of social and EP partners, but changes in preference among different aged males across female age span remain to be explored (Johnson and Gemmell 2012). Whether due to maternal effects or defects in their germ line DNA, aging females of many animal species produce poor quality offspring (e.g., Priest et al. 2002; Saino et al. 2002; Descamps et al. 2008), and in humans at least, advanced female age can enhance the deleterious effects of advanced male age on fertility (Kühnert and Nieschlag 2004) and on genetic quality of progeny (Fisch et al. 2003). These interaction effects raise the possibility that old female birds, in particular, might maintain their fertility or offspring quality by avoiding pairing with old males with senescent sperm traits and germ cells. Indeed, there is some evidence that female age affects the incidence of EPP in birds (Stutchbury et al. 1997) and can interact with male age (Rätti et al. 2001; Dietrich et al. 2004; Bouwman and Komdeur 2005). However, interacting effects of male and female age on EPP across the age span of long-lived birds have not been investigated (although see Lubjuhn et al. 2007).

We tested for the influence of male age and female age across the age span on the incidence of EPP in a marked population of a long-lived, socially monogamous species where females frequently engage in consensual relationships with one or more EP partners during the average 30-day

courtship period (Osorio-Beristain and Drummond 1998; Pérez-Staples and Drummond 2005), and 11 % of broods contain EP chicks (Ramos et al. 2014). Importantly, female blue-footed boobies (*Sula nebouxi*) can control within-pair and EP copulation: females are 32 % heavier (Castillo and Chavez-Peón 1983) and physically stronger than males; copulation can only occur if the female stands still while the male perches precariously on her back; EP copulations are preceded by reciprocal courtship, which usually goes on for days or weeks; and we have never seen EP males show aggression to females or their partners. Hence, imposed copulations are highly unlikely and age-related patterns of EPP can be attributed to female choice among candidate mating partners (including the social partner) and cryptic postcopulatory mechanisms. Early life improvement in blue-footed booby breeding traits followed by later senescence has been documented in the field (e.g., Kim et al. 2011). Importantly, there is deterioration in egg quality with female age (Beamonte-Barrientos et al. 2010) and germ line DNA with male age (Velando et al. 2011), and fledgling quality (probability of recruiting) increases steadily up to maternal or paternal age of 5 years and plateaus and then declines abruptly after maternal or paternal age of 12 years (Torres et al. 2011). Using cross-sectional analysis of effects of age combinations on paternity in a single season, we asked how the probability of a female producing EP chicks varies with the age of her partner, how this variation changes over the age span of the female, and how the ages of EP sires differ from the ages of social partners.

## Material and methods

### Study species

In the blue-footed booby colony on Isla Isabel (21° 52' N, 105° 54' W), females lay one to three eggs and raise one to three chicks that are cared for by both parents throughout the incubation and nestling periods (Nelson 1978; Drummond et al. 1986). Life spans of up to 23 years have been observed, but annual mortality of adult males and females is roughly 10 % (Oro et al. 2010) in the 1989 cohort, and 80.3 and 11.7 % of recruits survived to ages 8 and 16 years, respectively.

### Sampling

Annually since 1989, all fledglings in a long-term study area have been individually banded (details in Drummond et al. 2003), so in 2011, roughly 90 % of breeders were birds of known age. In that year, all nests in the study plot (a 8,450-m<sup>2</sup> subsection) were marked, breeders were identified, and nest contents were recorded every 3 days after the start of hatching. Between February and May, we took blood samples from a sample of 478 families that raised any chicks to at least age of

10 days. We extracted DNA with illustra blood genomicPrep Mini Spin kits from GE Healthcare and analyzed paternity using ten blue-footed booby microsatellite loci that had 3 to 22 alleles (Faircloth et al. 2009). All of these loci were checked for null alleles and were in Hardy-Weinberg equilibrium (Raymond and Rousset 1995). We excluded 25 families from all analyses in which either the female or male was genotyped in fewer than six microsatellite loci. We performed parentage analyses with a likelihood-based approach (see Ramos et al. 2014) in Cervus 2.0 (Marshall et al. 1998), setting relaxed and strict assignment levels at values of 80 and 95 %, respectively. In 350 focal pairs, with known breeder ages, male ages spanned 2–22 years (mean±SD, 8.22±4.32 years) and female ages spanned 2–22 years (mean±SD, 7.27±4.25 years). The remaining 103 families were excluded from our focal sample because age data were missing for one or both parents, but the males were included as potential sires in paternity assignments.

### Analysis

We used generalized linear models (GLMs) with binomial error distribution to examine whether the ages of females and their male partners influence the probability of females having at least one EP chick in their broods. The full model contained linear and quadratic ages of males and females, laying dates of females (proportional rank, where 0=the earliest breeder and 1=the latest breeder, using all 1,360 nests in the study area in 2011) and two-way interactions (see Supplementary Material Table S1). Male and female ages were centered before squaring to avoid potential collinearity issues between the linear and quadratic terms (Schielzeth 2010).

We used deletion tests to simplify the full model, first by eliminating nonsignificant interactions, followed by quadratic factors and, lastly, linear factors (Crawley 2007). We temporarily removed each term independently from the model to evaluate its significance, and once all significance values were obtained, we permanently removed the nonsignificant term with the least associated change in deviance. This process was repeated until we attained a final model that contained only significant terms. We used deletion tests to obtain the *p* values associated with each term in the final model. We performed all statistics in R statistical software v.2.12.2 (R Development Core Team 2008). Means±SD are reported throughout the manuscript.

### Results

In the 350 focal broods, 35 contained one EP chick and 6 contained two. In pairs with EP chicks, ages of both females and males ranged from 3 to 18 years. Paternity of 14 of these 47

chicks could be assigned to male breeders from the study plot. In the sample of 453 pairs including, for this analysis only, the 103 excluded families, there were 14 cases in which EPP was assigned and the ages of both males were known; here, the ages of social males (8.28±5.36 years, range=3–17) and EP sires (9.71±4.88 years, range=3–20) did not differ ( $t=0.75$ ,  $p=0.46$ , paired *t* test).

In the focal sample, the probability of having an EP chick was significantly related to the female age×male age interaction (GLM:  $p=0.0046$ ; Table 1), but not to laying date or its interaction with breeder ages. Inspection of the surface in Fig. 1 shows that with an increasing age of their social partner, EPP decreased in females ≤4 years old (hereafter, young females) but increased in females ≥8 years old (hereafter, old females), with no clear effect at intermediate female ages. To confirm that the effect of male age was significant in both cases, we examined young and old females separately, using GLM models that included breeder ages and their interaction as predictor variables. In both models, male age (only) had a significant effect on the probability of EPP, the effect being negative for young females ( $n=139$ , deviance=5.53,  $p=0.018$ ) and positive for old females ( $n=138$ , deviance=5.87,  $p=0.015$ ). Inspection of the two visible peaks in Fig. 1 reveals the approximate magnitude of these effects. The rate of EPP was roughly 2.5 times higher in pairs of two young breeders (both ≤4 years) and in pairs of old female (≥8 years) with very old male (≥13 years) than elsewhere, 20.3 % ( $n=74$ , estimate=-1.37, SE=0.29) and 20.8 % ( $n=24$ , estimate=-1.33, SE=0.50), respectively, versus 8.3 % in the remaining 252 (estimate=-2.40, SE=0.23) pairs.

This pattern could imply that young females paired with young partners seek older sires for their offspring, while old females paired with very old partners seek younger sires for their offspring. Analysis of the seven focal pairs where the age of the EP sire was known supported these hypotheses: the EP chick of the single old female (11 years old) with a very old male partner (17 years old) was sired by a younger male (7 years old), and the EP chicks of six young females (all 3 years old) with young male partners (3.33±0.51 years old, range=3–4 years) were sired by significantly older males (9.66±4.92 years old, range=3–14 years old;  $p=0.021$ , degrees of freedom (*df*)=5,  $t=-3.32$ , paired *t* test). The EP sires of the 6 young females were not older, on average, than the 397 male breeders of known age in the study plot (8.26±4.31 years;  $p=0.51$ ,  $df=5$ ,  $t=-0.69$ , Welch *t* test), but five of those six sires were older than the partners they cuckolded and the sixth was equal in age to the partner it cuckolded.

### Discussion

In the 2011 breeding season, EPP was most probable when young females were paired with young males and when old

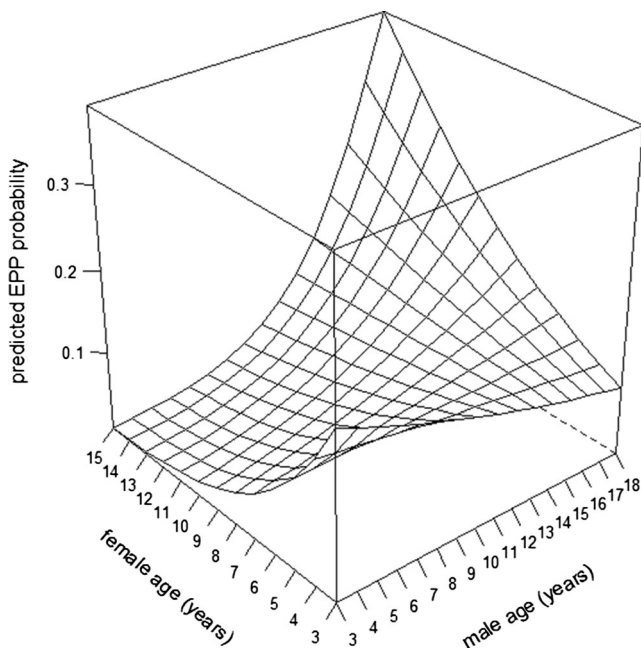
**Table 1** Generalized linear model of the relationship between male and female ages and the probability of a pair having an EP chick in their nest ( $n=350$  pairs)

Factors in final model	<i>B</i>	SE ( $\beta$ )	Change in deviance <sup>a</sup>	<i>p</i> <sup>a</sup>
Female age	-0.45	0.15	5.08	>0.024*
Male age	-0.17	0.08	0.13	0.71
Female age $\times$ male age	0.03	0.01	8.02	0.0046*

<sup>a</sup>We obtained statistical significance and changes in deviance from comparison tests between nested models. Residual deviance = 239.61 on  $df=346$

\*Statistical significance at the 0.05 level

females were paired with very old males. This result was obtained with a cross-sectional sample, so it could be due not to age-related developmental change in females but to differential mortality of females that produce EP chicks when mated to young males or to a cohort effect: females of different cohorts might tend to produce EP chicks when paired with males of different ages. However, neither of these seems likely and nor it is likely that such effects could aggregate over sufficient cohorts to establish patterns over an observed female age span of 20 years. Further, identification of seven EP sires revealed that whereas young males paired to young females were cuckolded by males that were 6.33 years older, an old male with an old female partner was cuckolded by a male that was 10 years younger. When female age was not taken into account, there was no difference in ages of EP sires and the males they cuckolded was evident, highlighting the



**Fig. 1** Probability of EPP as a function of male and female ages in 350 breeding pairs. The surface shows values predicted by the final model (male age  $\times$  female age interaction,  $p=0.0046$ ; Table 1). Although the full sample includes males and females of ages 2–22 and 2–22 years, respectively, we graph only the age ranges of pairs with EP chicks to avoid inflation of peaks at ages with small samples

importance of considering female age when analyzing choice among male ages. Paternity depends on which males seek copulatory access, intermale competition for access and female choice among males. Because females control both copulation and postcopulatory mechanisms of sperm selection (if any), we conclude that the observed pattern of paternity probably reflects female choice among candidate sires. However, age-related variation in male mate guarding motivation or ability, or in male foraging (Kleven et al. 2006), or in mate choices made by EP males cannot be ruled out. We also suggest that mixed support in the literature for the classic good genes predictions that old males should be cuckolded less than young ones and that EP males should be older than the males they cuckolded (Møller and Ninni 1998; Akçay and Roughgarden 2007; Cleasby and Nakagawa 2012) could be partly explained by similar age-dependent female responses to male ages in other species, since female age has seldom been taken into account.

Young females ( $\leq 4$  years old) with young partners appear to select older EP sires, and the younger their partners, the more likely that this substitution will occur. Since sperm traits of male birds tend to degrade with age (Møller et al. 2009), it is unlikely that these young females selectively copulated with older EP males to assure fertilization, unless sperm transfer improves with male age. It is more likely that young females assigned paternity to EP males older than their partners to obtain better genes for their offspring, consistent with predictions from life history theory (Manning 1985; Kokko 1998; Brooks and Kemp 2001). Alternatively, young females' decline in EPP with increasing partner age could be due to diminishing opportunities for this indirect benefit as the availability of relatively older EP sires declines or to improvement in mate guarding with male age (Gowaty and Bridges 1991; Bouwman and Komdeur 2005). Young females' EP sires were not older than the average male breeder in the study plot, but five of the six were older than the males they cuckolded, implying that EP sires were selected not for their absolute age but for their relative age (consistent with good genes theory; Kempnaers and Dhondt 1993). However, females paired with young males could secure sires older on average than their partners even by mating with EP males of random ages, and this could be a beneficial tactic if assessment of male ages is difficult or costly (Hasson and Stone 2011).

Old females ( $\geq 8$  years old), in contrast, were increasingly likely to produce EPP offspring as their partners' ages increased, particularly when their partners were very old ( $\geq 13$  years old). This pattern would generally result in putative offspring of very old males being sired by younger males even if females selected EP mates randomly for age and would do so more reliably if they selected EP males on the basis of absolute or, more likely, relative age. Increased production of EP chicks by old females paired to very old males is not likely to be a result of simple age-related decline in the fertility of

male partners because this would predict frequent EP paternity in young females paired to very old males, which was not observed, nor it is likely to be a result of age-related increase in female ability to avoid mate guarding, because this would predict frequent EP paternity in old females paired to young males, which was not observed either. We propose that when their partners are very old, old females selectively mate with other, probably younger, males in order to reduce the high risk of infertility or offspring defects and diseases that may arise when two old individuals mate. For old females, the genetic benefits offered by very old males (individuals of proven quality) may be outweighed by costs arising from pairing two old individuals.

Although it is unclear whether females paired randomly with EP sires or selected them by their absolute or relative age, it is certainly plausible that they could discriminate males of different ages because evidence suggests that female birds may respond to visual and auditory age cues. In some passerine species, for example, structural plumage coloration, ultraviolet signals, and song performance correlate with male age and possibly influence female mate preferences (Siefferman and Hill 2005; Budden and Dickinson 2009; Kipper and Kiefer 2010), and foot color of blue-footed boobies is a dynamic sexually selected ornament that loses intensity with age (Torres and Velando 2003, 2005, 2010). Olfactory cues could also be used; mice and humans can use smell to discriminate between differently aged conspecifics (Mitro et al. 2012), and some birds discriminate among conspecifics on the basis of their odor (Bonadonna and Nevitt 2004; Strandh et al. 2012).

When two old boobies mate, increased risk of infertility or offspring defects and diseases could arise from an interaction between, on the male side, senescent germ line DNA and, on the female side, either a maternal effect associated with senescence or senescent germ line DNA. This novel proposal is made more plausible by previous findings of senescent decline in male DNA (Velando et al. 2011), female egg quality, and maternal care (Beamonte-Barrientos et al. 2010) in this species; by the observation that after male and female blue-footed boobies reach an age of 12 years, the quality of their fledglings declines (Torres et al. 2011), and by the observation that in humans, old age of females and males interacts to reduce both fertility (Kühnert and Nieschlag 2004) and genetic quality of progeny (Fisch et al. 2003). In animal species, synergistic deleterious effects of male and female age on fertility or on offspring quality have not, to our knowledge, been reported, but they have rarely been sought, since laboratory and field studies typically focus on the age of either fathers or mothers and usually standardize or ignore the age of the other sexes (exceptions in Richard et al. 2005; Liu et al. 2011).

Reported associations between EPP and combinations of male and female ages in birds are few, and all three are based on the analysis of relative ages rather than known ages and may not even include very old individuals. Nonetheless, they

suggest that other avian species may have diverse male/female age interactions. In pied flycatchers (*Ficedula hypoleuca*) and coal tits (*Parus ater*), EPP appears most likely when an old female pairs with a young male (Rätti et al. 2001; Dietrich et al. 2004) and, in reed buntings (*Emberiza schoeniclus*), when old females have more EP chicks than young females and they cuckold young males (but not old males) more often than do young females (Bouwman and Komdeur 2005). The generality and adaptiveness of the booby pattern need to be explored by documenting patterns of paternity across the full natural age spans of other vertebrates, taking ages of all three players into account. More importantly, we need to evaluate the effects of combined breeder ages on fertility and on offspring quality; these questions may not have been addressed for any long-lived animal.

**Acknowledgments** Simon C. Griffith, Lynna M. Kiere, Marcela Osorio-Beristain, Diana Pérez-Staples, Oscar Sánchez-Macouzet, and two anonymous reviewers provided helpful comments on the manuscript. We thank numerous volunteers and colleagues who helped in the field and lab, especially Santiago Bautista-López, Jonathan P. Drury, Gabriela López-Carapia, and Janeth Rosas-Morales. The Armada de México, local fishermen, and staff of the Parque Nacional Isla Isabel supplied vital and much appreciated logistical support. This study was financed by the Universidad Nacional Autónoma de México (PAPIIT, IN206610) and the Consejo Nacional de Ciencia y Tecnología (104313). This manuscript constitutes a partial fulfillment of the Graduate Program in Biomedical Sciences of the Universidad Nacional Autónoma de México. AGR wishes to acknowledge the scholarship and financial support provided by the Consejo Nacional de Ciencia y Tecnología and the Universidad Nacional Autónoma de México. This research was partially supported by US Department of Energy under award number DEF C090 7SR22506 to the University of Georgia Research Foundation.

**Ethical standards** We declare that the present study complies with the current laws and ethical standards of animal research in Mexico. The Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT) provided the permit (SGPA/DGVS/08333/10) to collect blood samples.

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

- Akçay E, Roughgarden J (2007) Extra-pair paternity in birds: review of the genetic benefits. *Evol Ecol Res* 9:855
- Beamonte-Barrientos R, Velando A, Drummond H, Torres R (2010) Senescence of maternal effects: aging influences egg quality and rearing capacities of a long-lived bird. *Am Nat* 175:469–480
- Beck CW, Promislow DE (2007) Evolution of female preference for younger males. *PLoS One* 2:e939
- Bonadonna F, Nevitt GA (2004) Partner-specific odor recognition in an Antarctic seabird. *Science* 306:835–835
- Bouwman KM, Komdeur J (2005) Old female reed buntings (*Emberiza schoeniclus*) increase extra-pair paternity in their broods when mated to young males. *Behaviour* 142:11–12
- Brooks R, Kemp DJ (2001) Can older males deliver the good genes? *Trends Ecol Evol* 16:308–313

- Budden AE, Dickinson JL (2009) Signals of quality and age: the information content of multiple plumage ornaments in male western bluebirds *Sialia mexicana*. *J Avian Evol* 40:18–27
- Castillo A, Chavez-Peón C (1983) Ecología reproductiva e influencia del comportamiento en el control del número de crías en el bobo de patas azules *Sula nebouxii* en la Isla Isabel, Nayarit. Undergraduate thesis, Facultad de Ciencias, UNAM
- Catry P, Phillips RA, Forcada J, Croxall JP (2006) Factors affecting the solution of a parental dilemma in albatrosses: at what age should chicks be left unattended? *Anim Behav* 72:383–391
- Cleasby IR, Nakagawa S (2012) The influence of male age on within-pair and extra-pair paternity in passerines. *Ibis* 154:318–324
- Crawley MJ (2007) *The R book*. Wiley, Chichester
- Descamps S, Boutin S, Berteaux D, Gaillard JM (2008) Age-specific variation in survival, reproductive success and offspring quality in red squirrels: evidence of senescence. *Oikos* 117:1406–1416
- Dietrich V, Schmoll T, Winkel W, Epplen JT, Lubjuhn T (2004) Pair identity—an important factor concerning variation in extra-pair paternity in the coal tit (*Parus ater*). *Behaviour* 141:817–835
- Drummond H, González E, Osorno JL (1986) Parent-offspring cooperation in the blue-footed booby (*Sula nebouxii*): social roles in infanticidal brood reduction. *Behav Ecol Sociobiol* 19:365–372
- Drummond H, Torres R, Krishnan VV (2003) Buffered development: resilience after aggressive subordination in infancy. *Am Nat* 161:794–807
- Faircloth BC, Ramos A, Drummond H, Gowaty PA (2009) Isolation and characterization of microsatellite loci from blue-footed boobies (*Sula nebouxii*). *Conserv Genet Resour* 1:159–162
- Fisch H, Hyun G, Golden R, Hensle TW, Olsson CA, Liberson GL (2003) The influence of paternal age on Down syndrome. *J Urology* 169:2275–2278
- Gowaty PA, Bridges WC (1991) Nestbox availability affects extra-pair fertilizations and conspecific nest parasitism in eastern bluebirds, *Sialia sialis*. *Anim Behav* 41:661–675
- Hansen T, Price D (1995) Good genes and old age: do old mates provide superior genes? *J Evol Biol* 8:759–778
- Hasson O, Stone L (2011) Why do females have so few extra-pair offspring? *Behav Ecol Sociobiol* 65:513–523
- Johnson SL, Gemmill NJ (2012) Are old males still good males and can females tell the difference? Do hidden advantages of mating with old males off-set costs related to fertility, or are we missing something else? *Bioessays* 34:609–619
- Jones T, Balmford A, Quinell R (2000) Adaptive female choice for middle-aged mates in a lekking sandfly. *Proc R Soc Lond B* 267:681–686
- Kempenaers B, Dhondt AA (1993) Why do females engage in extra-pair copulations? A review of hypotheses and their predictions. *Belg J Zool* 123:93–103
- Kim S-Y, Velando A, Torres R, Drummond H (2011) Effects of recruiting age on senescence, lifespan and lifetime reproductive success in a long-lived seabird. *Oecologia* 166:615–626
- Kipper S, Kiefer S (2010) Age-related changes in birds' singing styles: on fresh tunes and fading voices? *Adv Stud Behav* 41:77–118
- Kleven O, Marthinsen G, Lifjeld JT (2006) Male extraterritorial forays, age and paternity in the socially monogamous reed bunting (*Emberiza schoeniclus*). *J Ornithol* 147:468–473
- Kokko H (1998) Good genes, old age and life-history trade-offs. *Evol Ecol* 12:739–750
- Kühnert B, Nieschlag E (2004) Reproductive functions of the ageing male. *Hum Reprod Update* 10:327–339
- Liu XP, Xu J, He HM, Kuang XJ, Xue FS (2011) Male age affects female mate preference and reproductive performance in the cabbage beetle, *Colaphellus bowringi*. *J Insect Behav* 24:83–93
- Lubjuhn T, Gerken T, Brün J, Schmoll T (2007) Yearling male great tits, *Parus major*, suffer more strongly from cuckoldry than older males. *Zoology* 110:387–397
- Manning J (1985) Choosy females and correlates of male age. *J Theor Biol* 116:349–354
- Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Mol Ecol* 7:639–655
- Mitro S, Gordon AR, Olsson MJ, Lundström JN (2012) The smell of age: perception and discrimination of body odors of different ages. *PLoS One* 7:e38110
- Møller AP, Ninni P (1998) Sperm competition and sexual selection: a meta-analysis of paternity studies of birds. *Behav Ecol Sociobiol* 43:345–358
- Møller A, Mousseau T, Rudolfsen G, Balbontin J, Marzal A, Hermosell I, De Lope F (2009) Senescent sperm performance in old male birds. *J Evol Biol* 22:334–344
- Nelson JB (1978) *The Sulidae: gannets and boobies*. Oxford University Press, New York
- Oro D, Torres R, Rodríguez C, Drummond H (2010) Climatic influence on demographic parameters of a tropical seabird varies with age and sex. *Ecology* 91:1205–1214
- Osorio-Beristain M, Drummond H (1998) Non-aggressive mate guarding by the blue-footed booby: a balance of female and male control. *Behav Ecol Sociobiol* 43:307–315
- Pérez-Staples D, Drummond H (2005) Tactics, effectiveness and avoidance of mate guarding in the blue-footed booby (*Sula nebouxii*). *Behav Ecol Sociobiol* 59:115–123
- Priest NK, Mackowiak B, Promislow DE (2002) The role of parental age effects on the evolution of aging. *Evolution* 56:927–935
- Radwan J (2003) Male age, germline mutations and the benefits of polyandry. *Ecol Lett* 6:581–586
- Ramos AG, Nunziata SO, Lance SL, Rodríguez C, Faircloth BC, Gowaty PA, Drummond H (2014) Habitat structure and colony structure constrain extra-pair paternity in a colonial bird. *Anim Behav* (in press)
- Rätti O, Lundberg A, Tegelström H, Alatalo RV (2001) No evidence for effects of breeding density and male removal on extrapair paternity in the pied flycatcher. *Auk* 118:147–155
- Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J Hered* 86:248–249
- Richard M, Lecomte J, de Fraipont M, Clobert J (2005) Age-specific mating strategies and reproductive senescence. *Mol Ecol* 14:3147–3155
- Saino N, Ambrosini R, Martinelli R, Møller AP (2002) Mate fidelity, senescence in breeding performance and reproductive trade-offs in the barn swallow. *J Anim Ecol* 71:309–319
- Schielzeth H (2010) Simple means to improve the interpretability of regression coefficients. *Meth Ecol Evol* 1:103–113
- Siefferman L, Hill GE (2005) Evidence for sexual selection on structural plumage coloration in female eastern bluebirds (*Sialia sialis*). *Evolution* 59:1819–1828
- Strandh M, Westerdahl H, Pontarp M, Canbäck B, Dubois MP, Miquel C, Taberlet P, Bonadonna F (2012) Major histocompatibility complex class II compatibility, but not class I, predicts mate choice in a bird with highly developed olfaction. *Proc R Soc Lond B* 279:4457–4463
- Stutchbury BJM, Piper WH, Neudorf DL, Tarof SA, Rhymer JM, Fuller G, Fleischer RC (1997) Correlates of extra-pair fertilization success in hooded warblers. *Behav Ecol Sociobiol* 40:119–126
- Torres R, Velando A (2003) A dynamic trait affects continuous pair assessment in the blue-footed booby, *Sula nebouxii*. *Behav Ecol Sociobiol* 55:65–72
- Torres R, Velando A (2005) Male preference for female foot colour in the socially monogamous blue-footed booby, *Sula nebouxii*. *Anim Behav* 69:59–65
- Torres R, Velando A (2010) Color in a long-lived tropical seabird: sexual selection in a life-history context. *Adv Stud Behav* 42:155–188

Torres R, Drummond H, Velando A (2011) Parental age and lifespan influence offspring recruitment: a long-term study in a seabird. *PLoS One* 6:e27245

Velando A, Noguera JC, Drummond H, Torres R (2011) Senescent males carry premutagenic lesions in sperm. *J Evol Biol* 24: 693–697



## **CAPÍTULO 3**

### **TICK INFESTATION OF CHICKS IN A SEABIRD COLONY VARIES WITH LOCAL NEST DENSITY, LOCAL BREEDING SYNCHRONY AND HABITAT STRUCTURE**

*Aceptado en Journal of Avian Biology, Agosto 2016*

1 **Tick infestation of chicks in a seabird colony varies with local breeding synchrony, local**  
2 **nest density and habitat structure**

3 Alejandra G. Ramos and Hugh Drummond

4 *Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de*  
5 *México*

6 **Corresponding author:** A. G. Ramos (ramos.alejandra@gmail.com), Departamento de Ecología  
7 Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México, A.P. 70-275,  
8 México D.F. 04510, Mexico. Phone: +52(55)56229007.

9 **Abstract**

10 Parasites are a major risk for group-living animals and seabirds are notoriously susceptible to  
11 ectoparasite infestations because they commonly nest in dense colonies. Ticks parasitize seabirds  
12 across all biogeographical regions and they can be particularly harmful to nestlings, but the  
13 ecological factors that affect their transmission to chicks are little studied and poorly understood.  
14 Here we show that abundance of tick larvae in blue-footed booby (*Sula nebouxii*) broods varies  
15 with local nest synchrony and density, and also with habitat structure: abundance increased with  
16 local breeding synchrony, was linearly and quadratically related to local nest density, and was  
17 highest toward the southern end of the study area which has suitable (boulder-rich) habitat for  
18 ticks. Also, with increasing chick age infestation first increased and then declined. The results of  
19 this study highlight how local physical and social environmental factors influence infestation of  
20 seabird nestlings by ticks.

21

22 **Keywords:** seabird, tick, density, synchrony, habitat structure, age.

## 23 **Introduction**

24 Group-living, which is widespread in the animal kingdom, can offer individuals several benefits,  
25 including protection against predators (Hamilton 1971), enhanced foraging (Brown 1988) and  
26 increased reproductive options (Hoogland and Sherman 1976), but can also entail costs such as  
27 heightened risk of parasite transmission (Alexander 1974). The dynamics of parasite transmission  
28 in wild populations is currently a key issue in ecology, epidemiology and wildlife conservation  
29 (Godfrey 2013).

30 Seabirds are considered attractive hosts for haematophagous ectoparasites. Their colonies  
31 can contain hundreds of thousands of individuals (Coulson 2001), and their long rearing periods  
32 and fidelity to breeding sites make them predictable and reliable hosts (Dietrich et al. 2011).  
33 Ticks are important in seabird ecology but their impacts on seabird reproduction and evolution  
34 are only beginning to be explored. Seabird-ticks parasitize at least 146 seabird species across all  
35 biogeographical regions and climates, and they are vectors of several viral and bacterial  
36 pathogens (reviewed in Dietrich et al. 2011). Heavy tick infestations in seabirds have been linked  
37 to clutch and brood abandonment (Duffy 1983), between-season dispersal away from heavily  
38 infested nesting sites (Boulinier et al. 2001), poor health (Wanless et al. 1997) and development  
39 (Morbey 1996, Bosch and Figuerola 1999) and, in extreme cases, mortality of adults and chicks  
40 (Gauthier-Clerc et al. 1998, Ramos et al. 2001). Chicks are particularly vulnerable as their  
41 immune systems are weak and underdeveloped (Ros et al. 1997, Szép and Møller 1999) and  
42 limited mobility often impedes them from abandoning infested nests (Eggert et al. 2010).

43 Numerous host traits and environmental factors can influence an individual's exposure and  
44 susceptibility to parasites, and parasite distribution across individuals, social groups, populations  
45 and species is often aggregated (Shaw and Dobson 1995, Rifkin et al. 2012). Host density is  
46 considered a major determinant of parasite abundance (Lebarbenchon et al. 2006) in numerous  
47 host-parasite systems (examples in Rifkin et al. 2012, Patterson and Ruckstuhl 2013). At high  
48 densities, frequent and prolonged contact between conspecific hosts increases the number of  
49 passively transmitted parasites, whereas the number of mobile parasites (such as biting flies,  
50 mosquitoes, and aquatic crustaceans), which transmit actively, decreases through a dilution effect  
51 (reviewed in Patterson and Ruckstuhl 2013). Ticks are mobile ectoparasites that can actively seek  
52 out hosts or ambush them (Baulechner et al. 2013, Mugabo et al. 2015), but their mobility is

53 limited (Godfrey et al. 2010). Passive transmission may be their most prevalent method of  
54 infection, in which case their abundance should increase with host density. Indeed, adult king  
55 penguins (*Aptenodytes patagonicus*) were more often infested by *Ixodes uriae* ticks in the high  
56 density part of their colony (Gauthier-Clerc et al. 1999). However, to our knowledge, tick  
57 infestation has not been related to host density in any other seabird.

58 Parasites may maximize their own and their offspring's feeding success by timing their life-  
59 cycles to coincide with the abundance of suitable hosts (Muzaffar and Jones 2004, Cattadori et al.  
60 2005, Singer and Parmesan 2010). For instance, female mosquitoes (*Culex territans*) enhance  
61 their offspring's chances of finding a host by timing their oviposition to coincide with the  
62 appearance of their preferred amphibian host, the green frog (*Rana clamitans*; Bartlett-Healy et  
63 al. 2008). Likewise, adult female ticks (*Ixodes arboricola*), rather than parasitizing adult  
64 songbirds (*Parus major*) delay feeding until their chicks, the favored host, have hatched (Heylen  
65 et al. 2012). There is evidence of ticks synchronizing with their seabird hosts: adult *Amblyomma*  
66 *loculosum* infested only incubating sooty terns (*Sterna fuscata*) and their larvae became  
67 numerous once chicks hatched (Feare and Gill 1997); and questing (host-seeking) by nymphal  
68 *Ixodes uriae* ticks peaked with the temporary abundance of puffin chicks (Muzaffar and Jones  
69 2007).

70 Habitat structure, though rarely studied, can influence parasite distribution and risk of  
71 infestation (Begon et al. 1996), and there is genetic evidence (McCoy et al. 2003) for topographic  
72 characteristics of cliffs limiting the dispersal of tick (*Ixodes uriae*) parasites of kittiwakes (*Rissa*  
73 *tridactyla*). Ticks are predated by arthropods such as spiders, ants, beetles and moths, as well as  
74 many vertebrates, including lizards, rodents and birds (reviewed in Samish and Rehacek 1999),  
75 and in seabird colonies ticks might avoid predation and potentially lethal climatic conditions  
76 (Benoit et al. 2007) by aggregating under rocks during the non-breeding season. In these lithic  
77 havens, multitudes of ticks develop, molt, mate, lay eggs, hatch, and await the seasonal return of  
78 their seabird hosts (Benoit et al. 2007).

79 We tested for effects of local nest density, local breeding synchrony and habitat structure  
80 on infestation of blue-footed booby broods by *C. denmarki*. On the assumption that, for ticks,  
81 passive transmission is more important than active host-seeking, and that interactions at a local  
82 scale are critical for parasite transmission (Vander Wal et al. 2012), we predicted that tick

83 infestation should increase with local abundance of nests and with synchrony of chick presence  
84 due to local nesting cycle synchrony. We also tested whether tick infestation increases with  
85 boulder abundance.

86

## 87 **Methods**

### 88 **Study area and blue-footed boobies**

89 We studied a blue-footed booby breeding colony on Isla Isabel, a tropical island off the Pacific  
90 coast of Mexico (21°52'N, 105°54'W), where *C. denmarki* larvae are seen often on chicks, and  
91 occasionally on surrounding vegetation and soil. Boobies are long-lived and the population of  
92 Isla Isabel is highly philopatric (Osorio-Beristain and Drummond 1993, Kim et al. 2007). More  
93 than 600 booby pairs breed in our 20,800 m<sup>2</sup> study area during the 8-month annual breeding  
94 season in spatially aggregated nests that can be as close as 1 m from each other (Castillo and  
95 Chavez-Peón 1983; Kim et al. 2009). The habitat is mostly deciduous forest of garlic pear  
96 (*Crataeva tapia*) and papelillo trees (*Euphorbia schlehtendalli*), with some beach and grassland;  
97 in the forest there are patches of embedded rocks whose density within 5 m of each nest increases  
98 toward the south (Ramos et al. 2014; see Supplementary material Appendix 1, Fig. A1). At the  
99 eastern limit of the study area the forest gives way to sandy beach. To start their foraging trips,  
100 most boobies that nest in the forest walk to the beach for take-off, often interacting with other  
101 boobies on the way. Females usually lay one or two, and very occasionally three eggs that hatch  
102 sequentially at 4-day intervals after roughly 41 days of incubation (Nelson 1978, Drummond  
103 1986). After hatching naked and blind onto an unlined soil or sand substrate, chicks are largely  
104 covered in down by 21 days and fully feathered by 90-110 days (Nelson 1978).

### 105 **Argasid ticks**

106 Argasid ticks, which have a soft body and leathery cuticle, are common avian parasites in  
107 tropical and subtropical regions (Dietrich et al. 2011) but less studied than hard-bodied ticks.  
108 They can live up to 25 years and, in the absence of hosts, survive famines of several years  
109 (Sonenshine 1993). After a blood-meal, female soft ticks may lay 5-500 eggs that hatch as larvae  
110 and go through 4-7 nymphal instars before molting into adults (Fig. 1). Adult and nymph soft-  
111 bodied ticks take numerous blood meals and feed rapidly, taking only 15 minutes to 60 minutes,  
112 while larvae usually take several days to complete their first meal, before molting into nymphs

113 (Vial 2009, Landulfo et al. 2012). Argasids spend ~90% of their life cycle off-host and they can  
114 exploit a sequence of individual hosts (Sonenshine 1993). Most species live in the nests, burrows  
115 or caves of vertebrates, where they hide in rock crevices, nest material or soil (Sonenshine 1993,  
116 Anderson and Magnarelli 2008). *Carios denmarki* (formerly *Ornithodoros denmarki*), a vector of  
117 Hughes, Quaranfil and Nyamanini viruses (reviewed in Dietrich et al. 2011), parasitizes at least  
118 12 seabird species in the Nearctic, Neotropical and Australian biogeographical zones.

119

## 120 **Data collection**

121 In 2008, locations of all nests in the study area were mapped and laying and hatching dates of all  
122 clutches were either recorded during nest-checks every three days or, for chicks hatched before  
123 the initiation of nest checks, estimated using ulna and culmen growth curves (details in  
124 Drummond et al. 2003). All chicks were marked with unique plastic rings.

125 We selected 50 focal nests, located in forest, grassland and beach, with 15 day-old ( $\pm 2$   
126 days) first-hatched or singleton chicks that hatched between the 6<sup>th</sup> and 16<sup>th</sup> of February and  
127 which were at least 5 m apart. Twenty-one nests had one chick, 19 had two, and ten had three.  
128 When these broods (their first-hatched chicks) were 15, 29 and 43 days old, we counted the ticks  
129 on all 1-3 broodmates. Four of the ten third-hatched chicks died between 15 and 29 days, so we  
130 could only sample them at brood age of 15 days. Though fledging does not start until roughly 90  
131 days, we counted ticks only through age 43 days, to confine our observations to the period of  
132 limited nestling mobility when the local nest environment is most likely to affect tick abundance.  
133 We used the end of a PVC pipe dabbed in non-toxic water paint to mark a circle of 2.54 cm  
134 diameter under each wing under the shoulder joint and counted all ticks inside this 5.06 cm<sup>2</sup> area.  
135 A chick's tick abundance score was the sum of its two underwing counts. The paint disappeared  
136 completely within 1-3 days, leaving no visible signs of skin irritation. Down and feathers are  
137 sparse in this area and ticks are conspicuous (Duffy and Campos de Duffy 1986), enabling fast  
138 and accurate counts (interobserver reliability:  $r_s = 0.94$ ,  $n = 11$ ,  $p < 0.001$ ). Similar underwing  
139 counts on chicks outside the study area were highly correlated with whole body counts ( $r_s = 0.92$ ,  
140  $n = 17$ ,  $p < 0.001$ ).

141 For each focal nest, we identified all breeding pairs within 5 m that were present with a  
142 clutch or brood at any time during the 43 days after hatching of the focal brood. Local nest

143 density was the number of pairs and local breeding synchrony was the standard deviation of their  
144 laying dates. We could not accurately estimate local density and synchrony for two nests located  
145 at the edge of the study area so they were excluded from analysis of the effects of these variables.

## 146 **Analyses**

147 To analyze the effect of ecological factors on tick abundance on broods (summing across all  
148 broodmates), we used generalized linear mixed models (GLMMs) and a model selection  
149 approach (Burnham and Anderson 2002). We generated 38 candidate models that, based on  
150 theory and results from previous studies, made biological sense and reflected alternative  
151 hypotheses of effects of the ecological variables on tick abundance. They comprised unique  
152 combinations of the following covariates: local nest density (linear and quadratic), local breeding  
153 synchrony, nest's spatial location (X and Y coordinates), age of chick at sampling (15, 29 or 43  
154 days), and the interaction between density and the X coordinate, as well as a null model with  
155 intercept only to evaluate relative performance of the selected models. The X coordinate, which  
156 runs roughly north-south and parallel to the shoreline, was a surrogate for boulder abundance,  
157 since we have previously shown that the abundance of boulders within 5 m of a nest increases  
158 from north to south in the segment of the study area where the focal nests were located (Ramos et  
159 al. 2014). The Y coordinate, which runs roughly perpendicularly from the shoreline into the  
160 forest in a westward direction, was a proxy for the transition from beach through grassland to  
161 forest habitats.

162 We included quadratic nest density because theory predicts both positive and negative  
163 relationships between number of hosts and tick abundance (Patterson and Ruckstuhl 2013). Nest  
164 density scores were centered on the mean before squaring to avoid collinearity problems between  
165 the linear and quadratic terms (Schielzeth 2010). Though broods varied from 1 to 3 chicks,  
166 graphical inspection showed no evidence of a relationship between brood size and tick abundance  
167 (Supplementary material Appendix 2, Fig. A2), so we excluded brood size as a covariate to avoid  
168 overparametrization. We fitted models with a negative binomial error distribution to account for  
169 overdispersion, a common issue with parasite abundance data (Qviller 2013), and nest identity as  
170 a random effect.

171 We used Akaike's information criterion, corrected for small sample sizes (AICc), to  
172 identify the best-fitting model (i.e., the model with the lowest AICc value) among the 38

173 candidates (Burnham and Anderson 2002). Then we ranked the remaining models from second-  
174 best to worst based on the difference between their own AICc value and the AICc of the best-  
175 fitting model ( $\Delta_1$  values in Table 1). Following Burnham and Anderson (2002), we estimated  
176 each model's Akaike weight ( $w_i$ ), which indicates the probability of a given model being the best  
177 out of a candidate set. However, we found no support for a "best" model, as the model with the  
178 highest Akaike weight of 0.54 (Table 1), had only a 54% chance of being the best model for our  
179 data. Therefore, to reduce model selection bias, rather than using a single model for inference we  
180 used a multi-model averaging approach (Burnham and Anderson 2002). For this, we selected a  
181 subset of the best-fitting models ( $n = 3$ ) which, together, had a cumulative Akaike weight ( $w_i$ ) of  
182 0.97 (Table 1).

183 We used the glmmADMB package (Skaug et al. 2011) of the R statistical software, version  
184 3.0.2 (R Core Team 2013), which allows for a negative binomial distribution in mixed models.

185

## 186 **Results**

187 There were *C. denmarki* larvae attached to the underwing skin of all 65 chicks that survived to  
188 brood age 43 days, and a single (non-feeding) nymph was found in the breast feathers of a 43  
189 day-old chick. Though we did not find any nymph or adult ticks feeding on chicks, these life  
190 stages are often seen attached to adult boobies in Isla Isabel. The four third-hatched chicks that  
191 died before brood age 29 days had no ticks at brood age 15 days. Other species of ectoparasites  
192 were not observed in the underwing circles and were minimal elsewhere: two chicks bore one and  
193 two fleas (Siphonaptera), respectively, and another bore a flat-fly (Hippoboscidae).

194 We found strong support for an increase in per-brood tick abundance with local breeding  
195 synchrony ( $\beta = -0.027$ , SE = 0.007, 95% CI: -0.039 to -0.014, Fig. 2). Both linear and quadratic  
196 local nest density affected tick abundance (linear:  $\beta = 0.17$ , SE = 0.041, 95% CI: 0.087 to 0.25;  
197 quadratic:  $\beta = -0.031$ , SE = 0.008, 95% CI: -0.047 to -0.014; Fig. 3), and Piecewise regression  
198 (Crawley 2007) revealed a significant increase in larval ticks up to a density score of 8 neighbors  
199 (density  $\leq 8$ : intercept = 2.03,  $\beta = 0.21$ ,  $P < 0.001$ ) followed by a decrease that was not significant  
200 (density  $> 8$ :  $P = 0.89$ ). Ticks were fewest at brood age 15 days, peaked at 29 days, and declined  
201 by 43 days, reflecting an inverted U-shaped relationship (15 vs 29 days,  $\beta = 1.36$ , SE = 0.14, 95%  
202 CI: 1.074 to 1.64; 15 vs 43 days,  $\beta = 0.86$ , SE = 0.15, 95% CI: 0.57 to 1.14; 29 vs 43 days,  $\beta = -$



203 0.49, SE = 0.14, 95% CI: -0.63 to -0.36; Fig. 4). Synchrony, linear and quadratic densities, and  
204 sampled age were all present in the three best-fitting models and scored the same Akaike weight  
205 ( $w_i = 0.96$ ; Table 2).

206 Nest location on the X coordinate, parallel to the shoreline, was also important ( $\beta = -0.007$ ,  
207 SE = 0.004, 95% CI: -0.012 to -0.0028): southward, as embedded boulders increase in abundance  
208 (Ramos et al. 2014), broods were more heavily parasitized by larval ticks (Fig. 5). Although  
209 closeness to the shoreline (the Y coordinate) was present in two of the three best-fitting models,  
210 its relationship with tick abundance was unimportant; its confidence intervals overlapped zero  
211 (CI: -0.0095 to 0.00092) and, compared to the other covariates, it had the lowest Akaike weight  
212 ( $w_i = 0.42$ ; Table 2).

213 To test whether the environmental factors associated with tick abundance were  
214 confounded with breeder quality/condition, we sought correlations between male and female age,  
215 which are associated with measures of blue-footed booby breeding performance (Beamonte-  
216 Barrientos et al. 2010, Torres et al. 2011), and all three significant environmental variables  
217 (synchrony, density, and the X coordinate) using all male ( $n = 439$ ) and female ( $n = 433$ )  
218 breeders of known age in the study year. Most Spearman rank correlation coefficients were very  
219 small and not significant (male age: density,  $r_s = 0.05$ ,  $P = 0.35$ ; synchrony,  $r_s = -0.034$ ,  $P = 0.48$ ;  
220 X coordinate,  $r_s = 0.093$ ,  $P = 0.052$ ; female age: density,  $r_s = -0.02$ ,  $P = 0.64$ ), and those that  
221 were significant were only weakly correlated (female age: synchrony,  $r_s = -0.104$ ,  $P = 0.031$ ; X  
222 coordinate,  $r_s = 0.11$ ,  $P = 0.021$ ).

223

## 224 **Discussion**

225 Abundance of tick larvae increased with local breeding synchrony, implying that *C. denmarki*  
226 ticks time the emergence of their larvae to coincide with the availability of chicks of the preferred  
227 ages (centered at roughly 29 days). To synchronize with their host's life cycle, ticks could  
228 potentially obtain cues from reproductive hormones or melatonin concentrations in the host's  
229 blood, or respond to changes in temperature or photoperiod. The synchrony of *C. denmarki* with  
230 local availability of broods was likely due to reliance on host-related cues rather than seasonal  
231 temperature/photoperiod cues because our sample comprised only chicks hatched within 10 days  
232 of each other and therefore experienced similar seasonal cues.

233 Tick abundance increased with linear density and a significant quadratic effect showed  
234 that it plateaued at the highest densities ( $>0.41$  nests/m<sup>2</sup>). Unlike most other parasites, ticks have  
235 different ways of infesting hosts, and these may alter how host density affects the risk of parasite  
236 infection. For instance, passive transmission which occurs by direct contact between hosts  
237 predicts an increase of parasite abundance with host density, whereas active host-seeking and  
238 ambushing-behavior, in which ticks climb onto vegetation and wait for hosts to pass, predicts the  
239 opposite (Mugabo et al. 2015). A strong, linear increase of tick abundance with density suggests  
240 that, as predicted, the main mode of tick infestation in booby broods was passive transmission.  
241 The plateau in tick abundance at the highest nest densities could imply that individuals breeding  
242 at such densities increased their investment in immunity against parasites (Rifkin et al. 2012).

243 Larval ticks on booby broods increased toward the south, as does boulder abundance  
244 (Ramos et al. 2014), implying that presence of boulders favors tick abundance and parasitism of  
245 boobies. Likewise, kittiwakes (*Rissa tridactyla*) breeding on natural cliffs with plentiful tick  
246 refuges were heavily infested by *Ixodes uriae* ticks, whereas those breeding on old buildings were  
247 tick-free, apparently because the buildings lacked refuges (Danchin 1992). Isla Isabel is home to  
248 many arthropods and three lizard species, one of which (the Mexican whiptail, *Asterias*  
249 *amurensis*) is common in the study area and eats ticks (O. Sánchez-Macouzet, personal  
250 observation). Without shelter, ticks could be at risk not only of predation, but also of desiccation  
251 or, during the rainy and hurricane season, drowning (Ogden et al. 2013).

252 Our longitudinal observations show that tick abundance increased with brood age and then  
253 declined, following an inverted U-shaped pattern first documented in arctic charrs (*Salvelinus*  
254 *alpinus*) infested by cestodes (Pacala and Dobson 1988), and subsequently observed in birds  
255 (kittiwakes, Boulinier and Danchin 1996; barn owls, Roulin 1998; starlings, Liker 2001). The  
256 initial increase in tick abundance with age could be due to declining protection by maternal  
257 antibodies, which in some seabird species lasts for roughly 20 days post-hatch (Garnier et al.  
258 2012), or to longer exposure to ticks (Boulinier and Danchin 1996). Subsequent decline of ticks  
259 could be due to development of defenses such as feather coverage (Boulinier and Danchin 1996,  
260 Wilson et al. 2002) and the immune system (Willadsen 1980). Feathers may limit foraging of  
261 parasites (Roulin 1998), and both ticks (Cordero del Campillo et al. 1999, Millán et al. 2004) and  
262 winged flies (Liker et al. 2001) seem to prefer featherless juvenile birds. Alternatively, assuming

263 a finite local population of ticks, decrease in infestation at 43 days could be due to decreasing  
264 abundance of unfed ticks.

## 265 **Conclusion**

266 Ticks are common and detrimental parasites of seabirds in colonies worldwide and carriers of  
267 numerous pathogens that could or are known to harm humans and livestock. Yet despite their  
268 importance and an increasing risk of tick infestation with shifting climates (Van Hemert et al.  
269 2014), little is known about what determines their abundance and distribution in natural  
270 populations. Here we show that three environmental factors, local nest synchrony and density and  
271 habitat structure, affect tick abundance in blue-footed booby broods. Identifying factors that  
272 influence susceptibility to infection is important for understanding the impact of parasites on their  
273 hosts and may well be important for understanding the evolution of nesting behavior. Also,  
274 knowledge of factors that affect parasite distribution could inform reintroduction and restoration  
275 programs.

276

277 *Acknowledgements* - We thank Alejandro González Voyer, Robyn Hudson, Lynna M. Kiere and  
278 Oscar Sánchez Macouzet for comments on the manuscript, Brenda Flores and Cristina Rodríguez  
279 for their assistance in the field, Elena Gómez-Díaz and Ma. del Carmen Guzmán-Cornejo for  
280 identifying ticks, and Pablo Frank-Bolton for writing the script for calculating local density and  
281 synchrony. The Armada de México, local fishermen and staff of the Parque Nacional Isla Isabel  
282 provided logistical support. This study was financed by CONACYT (1041313). This manuscript  
283 constitutes a partial fulfillment of the Graduate Program in Biomedical Sciences of the  
284 Universidad Nacional Autónoma de México (UNAM). A.G.R. wishes to acknowledge the  
285 scholarship and financial support provided by CONACYT and UNAM. All fieldwork complies  
286 with the current laws and ethical standards of animal research in Mexico and with the  
287 ASAB/ABS Guidelines for the Treatment of Animals in Behavioural Research.

288

## 289 **References**

290 Alexander, R. D. 1974. The evolution of social behavior. – *Annu. Rev. Ecol. Syst.* 5:325-383.

291 Anderson, J. F. and Magnarelli, L. A. 2008. Biology of ticks. – *Infect. Dis. Clin. N. Am.* 22:195-215.

- 292 Bartlett-Healy, K., Crans, W. and Gaugler, R. 2008. Temporal and spatial synchrony of *Culex territans* (Diptera:  
293 Culicidae) with their amphibian hosts. – J. Med. Entomol. 45:1031-1038.
- 294 Baulechner, D., Becker, N. I. and Encarnaçao, J. A. 2013. Host specificity in spinturnicid mites: do parasites share a  
295 long evolutionary history with their host? – J. Zool. Syst. Evol. Res. 51:203-212.
- 296 Beamonte-Barrientos, R., Velando, A., Drummond, H. and Torres, R. 2010. Senescence of Maternal Effects: Aging  
297 Influences Egg Quality and Rearing Capacities of a Long-Lived Bird. Am. Nat. 175:469-80.
- 298 Begon, M., Harper, J. L. and Townsend, C. R. 1996. Ecology – Blackwell Science.
- 299 Benoit, J. B., Yoder, J. A., Lopez-Martinez, G., Elnitsky, M. A., Lee Jr, R. E. and Denlinger, D. L. 2007. Habitat  
300 requirements of the seabird tick, *Ixodes uriae* (Acari: Ixodidae), from the Antarctic Peninsula in relation to  
301 water balance characteristics of eggs, nonfed and engorged stages. – J. Compar. Physiol. B 177:205-215.
- 302 Bosch, M. and Figuerola, J. 1999. Detrimental effects of ticks *Ornithodoros maritimus* on the growth of yellow-  
303 legged gull *Larus michahellis* chicks. – Ardea 87:83-89.
- 304 Boulinier, T. and Danchin, E. 1996. Population trends in kittiwake *Rissa tridactyla* colonies in relation to tick  
305 infestation. – Ibis 138:326-334.
- 306 Boulinier, T., McCoy, K. D. and Sorci, G. 2001. Dispersal and parasitism. – In: J. Clobert, E. Danchin, A. A. Dhondt  
307 and J. D. Nichols (eds), Dispersal. Oxford Univ. Press, pp. 169-179.
- 308 Burnham, K. P. and Anderson, D. R. 2002. Model selection and multimodel inference: a practical information-  
309 theoretic approach, 2nd ed. – Springer.
- 310 Brown, C. R. 1988. Enhanced foraging efficiency through information centers: a benefit of coloniality in cliff  
311 swallows. – Ecology 69:602-613.
- 312 Castillo, A. and Chavez-Peón, C. 1983. Ecología reproductiva e influencia del comportamiento en el control del  
313 número de crías en el bobo de patas azules, *Sula nebouxii*, en la Isla Isabel, Nay. – B. S. Thesis, Universidad  
314 Nacional Autónoma de México, México D. F.
- 315 Cattadori, I. M., Haydon, D. T. and Hudson, P. J. 2005. Parasites and climate synchronize red grouse populations. –  
316 Nature 433:737-741.
- 317 Cordero del Campillo, M., Rojo, F. A., Martínez, A. R., Acedo, S., Hernández, S., Navarrete, I. and Carvalho M.  
318 1999. Veterinary Parasitology. – McGraw-Hill Interamericana de España.

- 319 Coulson, J. C. 2001. Colonial breeding in seabirds. – In: E. A. Schreiber and J. Burger (eds), *Biology of Marine*  
320 *Birds*. CRC Press, pp. 87-114.
- 321 Crawley, M. J. 2007. *The R Book*. – Wiley.
- 322 Danchin, E. 1992. The incidence of the tick parasite *Ixodes uriae* in Kittiwake *Rissa tridactyla* colonies in relation to  
323 the age of the colony, and a mechanism of infecting new colonies. – *Ibis* 134:134-141.
- 324 Dietrich, M., Gómez-Díaz, E. and McCoy, K. D. 2011. Worldwide distribution and diversity of seabird ticks:  
325 implications for the ecology and epidemiology of tick-borne pathogens. – *Vector Borne Zoonotic Dis.*  
326 11:453-470.
- 327 Drummond, H., González, E. and Osorno, J. L. 1986. Parent-offspring cooperation in the blue-footed booby (*Sula*  
328 *nebouxii*): social roles in infanticidal brood reduction. – *Behav. Ecol. Sociobiol.* 19:365-372.
- 329 Drummond, H., Rodríguez, C., Vallarino, A., Valderrábano, C., Rogel, G. and Tobón, E. 2003. Desperado siblings:  
330 uncontrollably aggressive junior chicks. – *Behav. Ecol. Sociobiol.* 53:287-296.
- 331 Duffy, D. C. 1983. The ecology of tick parasitism on densely nesting Peruvian seabirds. – *Ecology* 64:110-119.
- 332 Duffy, D. C. and Campos De Duffy, M. J. 1986. Tick parasitism at nesting colonies of blue-footed boobies in Peru  
333 and Galapagos. – *Condor* 88:242-244.
- 334 Eggert, L. M., Jodice, P. G. and O'Reilly, K. M. 2010. Stress response of brown pelican nestlings to ectoparasite  
335 infestation. – *Gen. Comp. Endocr.* 166:33-38.
- 336 Feare, C. J., and Gill, E. L. 1997. The life cycle of the tick *Amblyomma loculosum* in a Sooty Tern *Sterna fuscata*  
337 colony in the Seychelles. – *J. Zool.* 241:643-648.
- 338 Garnier, R., Ramos, R., Staszewski, V., Militão, T., Lobato, E., González-Solís, J. and Boulinier, T. 2012. Maternal  
339 antibody persistence: a neglected life-history trait with implications from albatross conservation to  
340 comparative immunology. – *Proc. R. Soc. B* 279:2033-2041.
- 341 Gauthier-Clerc, M., Clerquin, Y. and Handrich, Y. 1998. Hyperinfestation by ticks *Ixodes uriae*: a possible cause of  
342 death in adult king penguins, a long-lived seabird. – *Colon. Waterbird.* 21:229-233.
- 343 Gauthier-Clerc, M., Jaulhac, B., Frenot, Y., Bachelard, C., Monteil, H., Le Maho, Y. and Handrich, Y. 1999.  
344 Prevalence of *Borrelia burgdorferi* (the Lyme disease agent) antibodies in king penguin *Aptenodytes*  
345 *patagonicus* in Crozet Archipelago. – *Polar Biol.* 22:141-143.

- 346 Godfrey, S. S. 2013. Networks and the ecology of parasite transmission: a framework for wildlife parasitology. – Int.  
347 J. Parasitol. Parasites Wildl. 2:235-245.
- 348 Godfrey, S. S., Moore, J. A., Nelson, N. J. and Bull, C. M. 2010. Social network structure and parasite infection  
349 patterns in a territorial reptile, the tuatara (*Sphenodon punctatu*). – Int. J. Parasitol. 40:1575-1585.
- 350 Hamilton, W. D. 1971. Geometry for the selfish herd. – J. Theor. Biol. 31:295-311.
- 351 Heylen, D. J. A., White, J., Elst, J., Jacobs, I., Van de Sande, C. and Matthysen, E. 2012. Nestling development and  
352 the timing of tick attachments. – Parasitology 139:766-773.
- 353 Hoogland, J. L. and Sherman, P. W. 1976. Advantages and Disadvantages of Bank Swallow (*Riparia riparia*)  
354 coloniality. – Ecol. Monogr. 46:33-58.
- 355 Kim, S. Y., Torres, R. and Drummond, H. 2009. Simultaneous positive and negative density-dependent dispersal in a  
356 colonial bird species. – Ecology 90:230-239.
- 357 Kim, S. Y., Torres, R., Dominguez, C. A. and Drummond, H. 2007. Lifetime philopatry in the blue-footed booby: a  
358 longitudinal study. – Behav. Ecol. 18:1132-1138.
- 359 Landulfo, G. A., Pevidor, L. V., dos Santos Sampaio, J., Luz, H. R., Onofrio, V. C., Faccini, J. L. H. and Barros-  
360 Battesti, D. M. 2012. Life cycle of *Ornithodoros mimon* (Acari: Argasidae) under laboratory conditions. –  
361 Exp. Appl. Acarol. 58:69-80.
- 362 Lebarbenchon, C., Poulin, R., Gauthier-Clerc, M. and Thomas, F. 2006. Parasitological consequences of  
363 overcrowding in protected areas. – EcoHealth 3:303-307.
- 364 Liker, A., Márkus, M., Vozár, Á., Zemankovics, E. and Rózsa, L. 2001. Distribution of *Carnus hemapterus* in a  
365 starling colony. – Can. J. Zool. 79:574-580.
- 366 McCoy, K. D., Tirard, C. and Michalakis, Y. 2003. Spatial genetic structure of the ectoparasites *Ixodes uriae* within  
367 breeding cliffs of its colonial seabird host. – Heredity. 91:422-429
- 368 Millán, J., Gortazar, C., Martín-Mateo, M. P. and Villafuerte, R. 2004. Comparative survey of the ectoparasite fauna  
369 of wild and farm-reared red-legged partridges (*Alectoris rufa*), with an ecological study in wild populations. –  
370 Parasitol. Res. 93:79-85.
- 371 Morbey, Y. E. 1996. The abundance and effects of ticks (*Ixodes uriae*) on nestling Cassin's Auklets (*Ptychoramphus*  
372 *aleuticus*) at Triangle Island, British Columbia. – Can. J. Zool. 74:1585-1589.

- 373 Mugabo, M., Perret, S., Decencière, B., Meylan, S. and Le Galliard, J. F. 2015. Density-dependent immunity and  
374 parasitism risk in experimental populations of lizards naturally infested by ixodid ticks. – *Ecology* 96:450-  
375 460.
- 376 Muzaffar, S. B. and Jones I. L. 2004. Parasites and diseases of the auks (Alcidae) of the world and their ecology - a  
377 review. – *Mar. Ornithol.* 32:121-146.
- 378 Muzaffar, S. B. and Jones, I. L. 2007. Activity periods and questing behavior of the seabird tick *Ixodes uriae* (Acari:  
379 Ixodidae) on Gull Island, Newfoundland: the role of puffin chicks. – *J. Parasitol.* 93:258-264.
- 380 Nelson, J. B. 1978. *The Sulidae: gannets and boobies.* – Oxford Univ. Press.
- 381 Ogden, N. H., Mechai, S. and Margos, G. 2013. Changing geographic ranges of ticks and tick-born pathogens:  
382 drivers, mechanisms and consequences for pathogen diversity. – *Front. Cell. Inf. Microbiol.* 3:46.
- 383 Osorio-Beristain, M. and Drummond, H. 1993. Natal dispersal and deferred breeding in the blue-footed booby. – *The*  
384 *Auk* 110:234-239.
- 385 Pacala, S. W. and Dobson, A. P. 1988. The relation between the number of parasites/host and host age: population  
386 dynamic causes and maximum likelihood estimation. – *Parasitology* 96:197-210.
- 387 Patterson, J. E. and Ruckstuhl, K. E. 2013. Parasite infection and host group size: a meta-analytical review. –  
388 *Parasitology* 140:803-813.
- 389 Qviller, L., Risnes-Olsen, N., Bærum, K. M., Meisingset, E. L., Loe, L. E., Ytrehus, B., Viljugrein, H. and Mysterud,  
390 A. 2013. Landscape level variation in tick abundance relative to seasonal migration in red deer. – *PloS ONE*  
391 8:e71299.
- 392 R Core Team. 2015. *R: a language and environment for statistical computing.* – R foundation for statistical  
393 computing. ISBN 3-900051-07-0 (<http://www.R-project.org>)
- 394 Ramos, A. G., Nunziata, S. O., Lance, S. L., Rodríguez, C., Faircloth, B. C., Gowaty, P. A. and Drummond, H. 2014.  
395 Habitat structure and colony structure constrain extrapair paternity in a colonial bird. – *Anim. Behav.* 95:121-  
396 127.
- 397 Ramos, J. A., Bowler, J., Davis, L., Venis, S., Quinn, J. and Middleton, C. 2001. Activity patterns and effect of ticks  
398 on growth and survival of tropical Roseate Tern nestlings. – *The Auk* 118:709-716.
- 399 Rifkin, J. L., Nunn, C. L. and Garamszegi, L. Z. 2012. Do animals living in larger groups experience greater  
400 parasitism? A meta-analysis. – *Am. Nat.* 180:70-82.

- 401 Ros, A. F., Groothuis, T. G. and Apanius V. 1997. The relation among gonadal steroids, immunocompetence, body  
402 mass, and behavior in young black-headed gulls (*Larus ridibundus*). – Am. Nat. 150:201-219.
- 403 Roulin, A., Richner, H. and Ducrest, A. L. 1998. Genetic, environmental, and condition-dependent effects on female  
404 and male ornamentation in the barn owl *Tyto alba*. – Evolution 52:1451-1460.
- 405 Samish, M. and Rehacek, J. 1999. Pathogens and predators of ticks and their potential in biological control. – Annu.  
406 Rev. Entomol. 44:159-182.
- 407 Schielzeth, H. 2010. Simple means to improve the interpretability of regression coefficients. – Methods Ecol.  
408 Evol. 1:103-113.
- 409 Shaw, D. J. and Dobson, A. P. 1995. Patterns of macroparasite abundance and aggregation in wildlife populations: a  
410 quantitative review. – Parasitology 111:S111-S133.
- 411 Singer, M. C. and Parmesan, C. 2010. Phenological asynchrony between herbivorous insects and their hosts: signal  
412 of climate change or pre-existing adaptive strategy? – Phil. Trans. R. Soc. B 365:3161-3176.
- 413 Skaug, H., Fournier, D., Nielsen, A., Magnusson, A. and Bolker, B. 2011. glmmADMB: generalized linear mixed  
414 models using AD Model Builder. – R package version 0.6, 5, r143.
- 415 Sonenshine, D. E. 1993. Ecology of nidicolous ticks. – In: D. E. Sonenshine and R. M. Roe (eds), Biology of Ticks.  
416 Oxford Univ. Press, pp. 66-81.
- 417 Szép, T. and Møller, A. P. 1999. Cost of parasitism and host immune defence in the sand martin *Riparia riparia*: a  
418 role for parent-offspring conflict? – Oecologia 119:9-15.
- 419 Torres, R., Drummond, H. and Velando, A. 2011. Parental age and lifespan influence offspring recruitment: a long-  
420 term study in a seabird. PloS ONE 6:e27245.
- 421 Van Hemert, C., Pearce, J. M. and Handel, C. M. 2014. Wildlife health in a rapidly changing North: focus on avian  
422 disease. Front. Ecol. Environ. 12: 548-556.
- 423 Vander Wal, E., Paquet, P. C. and Andres, J. A. 2012. Influence of landscape and social interactions on transmission  
424 of disease in a social cervid. – Mol. Ecol. 21:1271-1282.
- 425 Vial, L. 2009. Biological and ecological characteristics of soft ticks (Ixodida: Argasidae) and their impact for  
426 predicting tick and associated disease distribution. – Parasite 16:191-202.
- 427 Wanless, S., Barton, T. R. and Harris, M. P. 1997. Blood hematocrit measurements of 4 species of North Atlantic  
428 seabirds in relation to levels of infestation by the tick *Ixodes uriae*. – Colon. Waterbird. 20:540-544.



- 429 Willadsen, P. 1980. Immunity to ticks. – *Adv. Parasit.* 18:293-313.
- 430 Wilson, K., Bjørnstad, O. N., Dobson, A. P., Merler, S., Pogliayen, G., Randolph, S. E. and Skorping, A. 2002.  
431 Heterogeneities in macroparasite infections: patterns and processes. – In: P. J. Hudson, A. Rizzoli, B. T.  
432 Grenfell, H. Heesterbeek and A. P. Dobson (eds), *The Ecology of Wildlife Diseases*. Oxford Univ. Press, pp.  
433 6-44.
- 434
- 435 **Supplementary material** (Appendix JXXX at <[www.avianbiology.org/readers/appendix](http://www.avianbiology.org/readers/appendix)>). Appendix 1-2, Fig. A1  
436 and Fig. A2

437 **Table 1.** Summary of model selection results for GLMMs analyzing tick abundance in 48 blue-footed booby broods at ages 15, 29 and  
 438 43 days, in relation to synchrony, density (linear and quadratic), nest location (X and Y coordinates), and sampled age. Table includes  
 439 the three best-fitting models and a null model, ordered from best to worst based on their AICc scores. The evidence ratio shows how  
 440 many times better the 'best' model is than the model of interest, and K represents the number of parameters in a model.

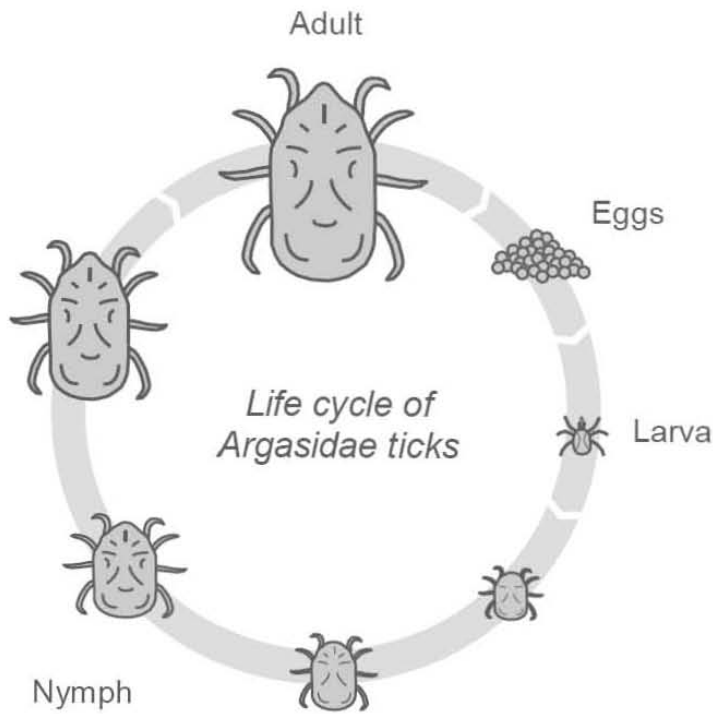
<b>Explanatory variables</b>	<b>AICc</b>	$\Delta_i$	<b>Relative likelihood</b>	<b>K</b>	$w_i$	<b>Evidence ratio</b>
synchrony + density + $X_{\text{coordinate}}$ + age + density <sup>2</sup>	1104.85	0.00	1.00	9	0.54	---
synchrony + density + $X_{\text{coordinate}}$ + $Y_{\text{coordinate}}$ + age + density <sup>2</sup>	1105.57	0.72	0.70	10	0.38	1.43
synchrony + density + $Y_{\text{coordinate}}$ + age + density <sup>2</sup>	1109.91	5.06	0.08	9	0.04	12.53
Null	1183.97	79.12	0.00	3	0.00	13384.91

441 **Table 2.** Model averaged estimates for GLMMs evaluating tick abundance in 48 blue-footed  
 442 booby broods at ages 15, 29 and 43 days.

<b>Variable</b>	<b><math>\beta</math></b>	<b>SE</b>	<b><math>w_i</math></b>	<b>Lower 95% CI</b>	<b>Upper 95% CI</b>
synchrony	-0.027	0.007	0.96	-0.039	-0.014
density	0.17	0.041	0.96	0.087	0.25
density <sup>2</sup>	-0.031	0.008	0.96	-0.047	-0.014
age					
<i>15 vs 29</i>	1.36	0.14	0.96	1.074	1.64
<i>15 vs 43</i>	0.86	0.15	0.96	0.57	1.14
<i>29 vs 43</i>	-0.49	0.14	0.96	-0.63	-0.36
X <sub>coordinate</sub>	-0.007	0.004	0.92	-0.012	-0.0028
Y <sub>coordinate</sub>	-0.004	0.003	0.42	-0.0095	0.00092

443

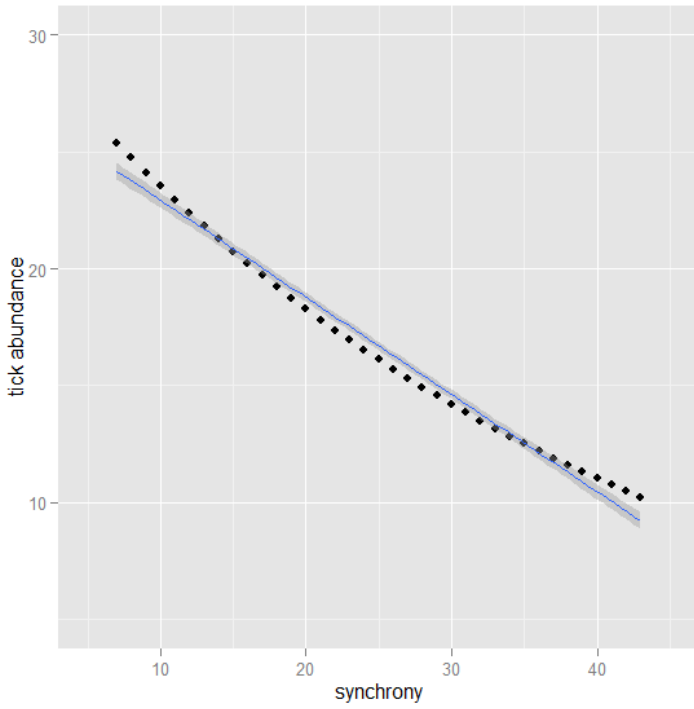
444 **Figure 1.** The life cycle of argasid ticks includes one larval stage, followed by 4-7 nymphal  
445 instars that measure ~3.5-6.5 mm in length, and ends with an adult male or female tick that may  
446 reach up to 7.5 mm or 1.2 cm respectively (Mehlhorn 2015; illustration by Oscar Sánchez-  
447 Macouzet).



448

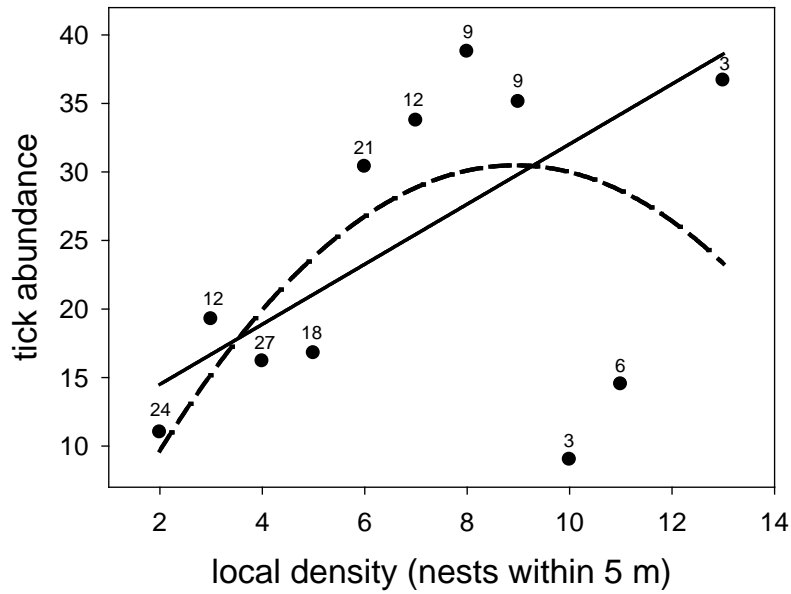
449

450 **Figure 2.** The dotted line shows model-averaged predicted estimates of tick abundance as a  
451 function of synchrony while holding density, density<sup>2</sup>, X coordinate, and Y coordinate constant  
452 at their means. Synchrony is highest at values close to zero.



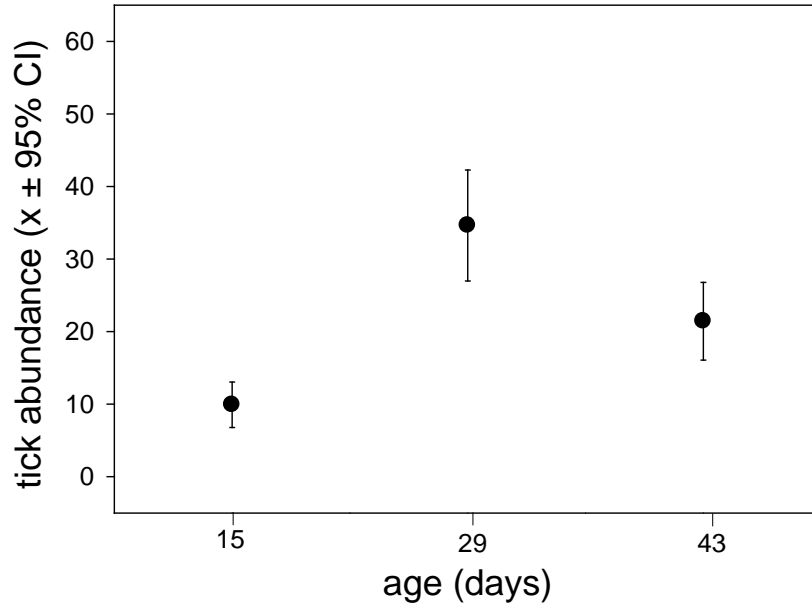
453  
454

455 **Figure 3.** Nest density (linear and quadratic) and tick abundance in 48 blue-footed booby broods.  
456 Points show number of ticks averaged across ages 15, 29 and 43 days at each density value;  
457 numbers represent sample sizes at each density interval.



458  
459

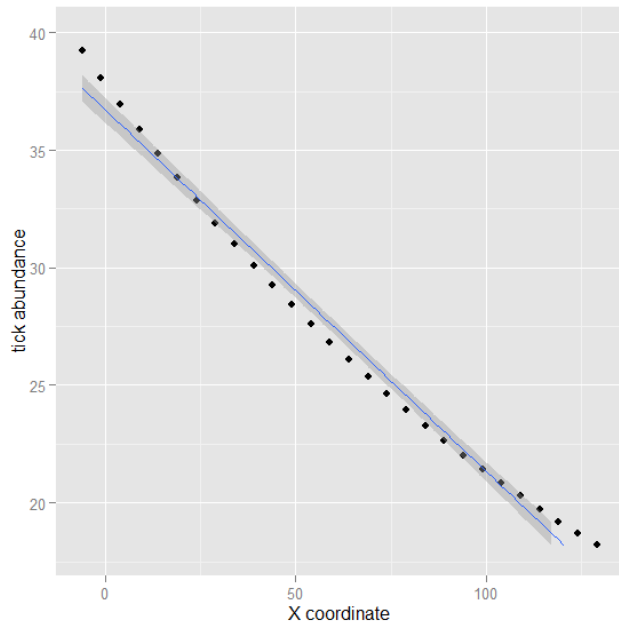
460 **Figure 4.** Tick abundance in 48 blue-footed booby broods when broods were 15, 29 and 43 days  
461 old.



462

463

464 **Figure 5.** The dotted line shows model-averaged predicted estimates of tick abundance as a  
465 function of the X coordinate while holding density, density<sup>2</sup>, synchrony, and Y coordinate  
466 constant at their means.  
467



468



## **CAPÍTULO 4**

**PARASITISM BY TICKS IN BLUE-FOOTED BOOBY OFFSPRING  
INCREASES WHEN BOTH BREEDERS ARE EITHER YOUNG OR OLD**

1 SHORT COMMUNICATION

2 **Booby chicks with different-aged parents have the fewest ticks**  
3 **and best body condition**

4 ALEJANDRA G. RAMOS & HUGH DRUMMOND

5 Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma  
6 de México, A.P. 70-275, México D.F. 04510, Mexico

7 *Running title:* Interactive parental age effects on chick viability

8 *Correspondence:* Alejandra G. Ramos, Departamento de Ecología Evolutiva, Instituto de  
9 Ecología, Universidad Nacional Autónoma de México, A.P. 70-275, México D.F. 04510,  
10 Mexico. Tel.: (+52 55) 562 29007; fax: (+52 55) 561 61976;  
11 e-mail: ramos.alejandra@gmail.com

12

13 **Abstract**

14 There is a widespread expectation that the viability of animal offspring should vary with  
15 parental age, through effects of age on parental care and genetic quality, but very little is  
16 known about variation in wild animals. The probability of blue-footed booby (*Sula nebouxii*)  
17 fledglings recruiting into a breeding population was recently found to be maximal when one  
18 parent was young and the other old, but the mechanism behind this effect is unknown. We  
19 tested the hypothesis that offspring of different-aged parents have superior  
20 immunocompetence and parasite resistance by testing whether they are less infested by the  
21 argasid tick *Carios denmarki* (a common booby parasite) in the nestling phase. In a sample of  
22 breeding pairs including mothers and fathers up to 19 years old, infestation of nestlings at  
23 ages 15-43 days depended on the combination of parental ages: ticks were least abundant  
24 when one parent of either sex was young and the other old and most abundant when both  
25 parents were young or old. Furthermore, nestling body condition was best when one parent  
26 was young and the other old and poorest when both parents were young or old. However, tick  
27 abundance and body condition were not negatively correlated, implying that parental age  
28 combination may affect them independently. Effects of parental age combination on the body  
29 condition or, more likely, the immunocompetence of booby offspring may mediate the  
30 superior viability of offspring with different-aged parents.

31

32 *Keywords:* parental age; offspring viability; ectoparasites; immunity; body condition; genes;  
33 parental care

34

## 35 **Introduction**

36 Ageing of an animal is expected to have complex effects on the viability of the offspring it  
37 produces (Johnson & Gemmell, 2012). In species with parental care, the effect of increasing  
38 age is expected to be positive in early life as individuals gain experience at feeding and  
39 reproducing, but negative in later life as they experience senescent decline in physiology and  
40 behavior (Cichoń, 2003; Limmer & Becker, 2009). Further, although as animals age their  
41 germline DNA is expected to decline in quality (Tarín *et al.*, 2000), in wild populations better  
42 survival of genetically superior individuals is predicted to result in positive correlations  
43 between breeder age and offspring viability (Manning, 1985; Kokko, 1998). How these  
44 factors combine to affect offspring viability and the desirability of different-aged individuals  
45 as mates is largely unknown. Female animals sometimes show a preference for old males and  
46 a few studies, mostly of captive animals of a limited age range, have revealed both positive  
47 and negative effects of breeder age on offspring traits (review in Johnson & Gemmell, 2012).  
48 However, effects of parental age on offspring quality in natural populations have rarely been  
49 examined in nature (but see Torres *et al.*, 2011).

50         A recent study of a long-lived wild vertebrate that, for the first time, sampled across the  
51 lifespan and considered interactions of mother's age and father's age confirmed a surprising  
52 effect that had been predicted from an analysis of offspring viability in the same species:  
53 viability of blue-footed boobies (*Sula nebouxii*, Milne-Edwards) is maximal when one parent  
54 is young and the other old, regardless of whether the young parent is the mother or the father  
55 (Drummond & Rodríguez, 2015). Whereas fledglings with two old parents or two young  
56 parents were the least likely to recruit into the breeding population, fledglings with different-  
57 aged parents were the most likely. The mechanism behind this variation in viability is  
58 unknown; here we report a test of the hypothesis that it is mediated by variation in  
59 immunocompetence. Specifically, we tested the prediction that nestlings with different-aged  
60 parents should show lower levels of tick infestation than nestlings with two old parents or two  
61 young parents. In statistical terms, we expected that parental ages interact to affect tick  
62 infestation of nestlings, just as they interact to affect recruitment of fledglings. We also  
63 measured body condition because nutritional resources govern both growth and immune  
64 function, which are thought to trade off against each other (Owen *et al.*, 2010; Clayton, 2010).

65         In the breeding colony of blue-footed boobies on Isla Isabel (21°52'N, 105°54'W) most  
66 nestlings bear larvae of the argasid tick *Carios denmarki* (Kohls, Sonenshine & Clifford,  
67 1965), whose abundance on them peaks at roughly age 4 weeks (Ramos & Drummond, in

68 review). Ticks are frequent ectoparasites of seabirds, and harm them by injecting toxins,  
69 extracting blood (Lehmann, 1993) and vectoring micropathogens (Chastel, 1988). Nestling  
70 defences against ticks include both innate and acquired components of the immune system,  
71 including antibodies, complement, antigens and T-lymphocytes (Wikel, 1996), as well as  
72 maternal antibodies transferred via the egg (Gasparini *et al.*, 2001). Defence against ticks can  
73 involve high concentrations of leukocytes and antibodies (Dusbábek *et al.*, 1994; Szép &  
74 Møller, 1999). Costs of tick infestation to blue-footed boobies have not been studied, but in  
75 other birds ticks induce poor nestling growth (Morbey, 1996; Bosch & Figuerola, 1999),  
76 increased nestling mortality (Duffy, 1983), and abandonment of nests and even whole  
77 colonies (Clayton *et al.*, 2010).

78 Separate analyses of the two sexes have shown that boobies' reproductive success peaks  
79 at age ~10 years (Velando *et al.*, 2006) although boobies can breed for as many as 23 years.  
80 As they age, females experience decline in egg quality and parental care (Beamonte-  
81 Barrientos *et al.*, 2010) and males in their germ-line DNA (Velando *et al.*, 2011).

82

## 83 **Materials and methods**

84 In the colony on Isla Isabel, blue-footed boobies care for broods of 1-3 chicks on the forest  
85 floor or beach during 70-120 days, dividing all parental duties roughly equally between the  
86 female and male (Drummond *et al.*, 1986).

### 87 **Data collection**

88 In 2008, the ages of most breeders in a 20,800-m<sup>2</sup> study area were known thanks to annual  
89 marking of fledglings since 1989 with numbered metal rings (Drummond *et al.*, 2003). The  
90 hatching dates of all chicks were either recorded during nest-checks every three days or  
91 estimated from growth curves (details in Drummond *et al.*, 2003). In February we identified  
92 35 -15 day-old ( $\pm 2$  days) singleton or eldest chicks in broods where ages of both parents were  
93 known (males,  $9.60 \pm 3.10$  years, range = 4-19; females,  $10.43 \pm 4.03$  years, range = 4-19). At  
94 ages 15, 29 and 43 days, we counted tick larvae in a 5.06-cm<sup>2</sup> circular area under each chick's  
95 wings without damaging or displacing them (Ramos & Drummond in review). Ticks in this  
96 area, which is almost featherless, can be accurately counted and in a sample of chicks from  
97 elsewhere in the colony, underwing counts were strongly correlated with whole body counts  
98 ( $r_s = 0.92$ ,  $N = 17$ ,  $P < 0.001$ ). We also measured ulna length to the nearest 1mm and body  
99 mass to the nearest 5 g at age 15 days and nearest 10 g at 29 and 43 days. We expressed body

100 condition as the residuals of a regression of body mass on body size (ulna length),  
101 independently for each of the three ages.

102 To determine sex, we took 75  $\mu$ l of blood from each chick's brachial vein and stored it  
103 in 1 ml of buffer (1.0 M Tris: 0.5 M EDTA: 5.0 M NaCl: 10% SDS), returning chicks to their  
104 nests within 5 min, after bleeding stopped. We extracted DNA with illustra blood genomic  
105 Prep mini spin kits from GE Healthcare and amplified segments of the CHD-Z and CHD-W  
106 genes to differentiate between heterogametic females (ZW) and homogametic males (ZZ)  
107 (Griffiths *et al.*, 1998).

## 108 **Analysis**

109 We used two sets of generalized linear mixed models (GLMMs) to examine whether  
110 abundance of tick larvae and body condition of focal chicks, respectively, were influenced by  
111 the combination of fathers' and mothers' ages. The fixed term of interest was the interaction  
112 of fathers' and mothers' ages, which were uncorrelated ( $r_s = 0.19$ ,  $P = 0.25$ ,  $N = 35$ ). We  
113 included chick identity as a random effect, with the maximal random structure allowed by our  
114 data (Barr *et al.*, 2013). We controlled for the covariates brood size and chick sex and, in tick  
115 abundance models, we further controlled for chick age. Female blue-footed boobies are larger  
116 than males throughout early development and adult life (Drummond *et al.* 1991). For tick  
117 counts, we used a negative binomial error distribution (Crawley, 2007) to control for  
118 overdispersion, which is common in parasite abundance data (Qviller, 2013); for body  
119 condition, we used a Gaussian distribution.

120 For each response variable, we first built a model containing only the covariates that we  
121 wished to control for, then we included those that were significant in a subsequent model  
122 containing the fixed terms of interest. We used the glmmADMB package (Skaug *et al.*, 2011)  
123 of R statistical software, version 3.02 (R Core Team, 2013), which allows for a negative  
124 binomial error distribution in mixed models.

125

## 126 **Results**

127 Abundance of ticks was significantly related to the mother age\*father age interaction ( $P =$   
128  $0.043$ ; Table 1). The surface in Fig. 1a shows that tick abundance was maximal when a  
129 chick's parents were either both young or both old, minimal when the father was very old and  
130 the mother very young, and low when the mother was very old and the father very young.

131 Body condition was also significantly related to the mother age\*father age interaction  
132 ( $P = 0.02$ : Table 1). The surface in Fig. 1b is approximately the inverse of the surface in Fig.  
133 1a. Body condition was minimal when a chick's parents were both young and low when they  
134 were both old, but maximal when one parent was old and the other young (Fig. 1b).

135 The correlation between body condition and tick abundance was positive and significant  
136 at 29 days ( $r_s = 0.43$ ,  $P = 0.01$ ) and nonsignificant at 15 days and 43 days (respectively:  $r_s =$   
137  $0.24$ ,  $P = 0.17$ ;  $r_s = -0.05$ ,  $P = 0.80$ ).

138

## 139 **Discussion**

140 Minimal tick abundance in nestlings with one young parent (of either sex) and one old parent  
141 suggests that these nestlings are more resistant to parasites than those with two young or old  
142 parents, and supports our hypothesis that chicks with different-aged parents are the most  
143 immunocompetent. Alternative explanations for parental age-based infestation levels are  
144 certainly possible, and experimental confirmation of the role of immunocompetence should  
145 now be a research priority. One of the main avian defences against blood-feeding  
146 ectoparasites is thought to be inflammation, which restricts access of the parasite's  
147 mouthparts to blood (Owen *et al.*, 2010). Chicks with different-aged parents may be more  
148 able to mount an inflammatory response, and consequently more effective at thwarting blood  
149 extraction and repelling ticks than those with similar-aged parents. The explanation for this  
150 peculiar distribution of parasite resistance according to combined parental ages could lie in  
151 maternal/paternal effects or genetic effects, which we consider in turn.

152 Could heavy infestation of chicks with two young parents or two old parents be due to  
153 poor body condition diminishing their immune responses, given that immunocompetence of  
154 nestling birds declines under reduced food availability (Hoi-Leitner *et al.*, 2001) and  
155 underfeeding of nestlings reduces their resistance to ticks (*Ixodes uriae*; McCoy *et al.*, 2002)?  
156 Poor body condition itself could result from poor provisioning of eggs or nestlings by the  
157 young and inexperienced and the old and senescent. For example, young birds sometimes  
158 show poor breeding performance (Balbontin *et al.*, 2007), and old birds sometimes lay low  
159 quality eggs, resulting in nestlings that are undersized and slow-growing (Beamonte-  
160 Barrientos *et al.*, 2010) or less immunocompetent (Saino *et al.*, 2002). However, this  
161 explanation predicts that maximal condition and minimal infestation should occur in chicks  
162 with two middle-aged parents rather than where they were observed: in chicks with one old

163 parent and one young parent. Another possibility is that the interacting effects of parental ages  
164 on body condition and parasite infestation are due to parents strategically adjusting their  
165 investment in the light of their own and their partner's traits, in accordance with Harris &  
166 Uller's (2009) conditional model of differential allocation and reproductive compensation.  
167 For example, male and/or female boobies might respond to old partners by increasing  
168 investment in offspring when they are young and decreasing it when they are old (Drummond  
169 & Rodríguez 2015). These resource-based explanations for the distribution of nestling  
170 immunocompetence in relation to parental ages cannot be dismissed completely, but they are  
171 implausible: both predict a negative correlation between nestling body condition and  
172 infestation but at the three nestling ages tested the correlation was either absent or positive.  
173 However, parental effects could influence other egg components that affect  
174 immunocompetence but are not correlated with body condition, such as antibodies and  
175 carotenoids.

176 A genetically mediated effect of parental ages on offspring immunocompetence and  
177 condition is possible. Birds show heritable polygenic variation in parasite resistance (Owen *et*  
178 *al.*, 2010) and the cell-mediated immune responses and pathogen resistance of house finches  
179 (*Carpodacus mexicanus*) increase with heterozygosity (Hawley *et al.*, 2005). In theory,  
180 genetic contributions of males and females could vary complexly with age, as a result of  
181 individual development or within-cohort natural selection, resulting in higher genetic quality  
182 or increased heterozygosity in offspring of dissimilar-aged parents (Drummond & Rodríguez,  
183 2015). Furthermore, a genetic origin of enhanced immunocompetence in offspring of  
184 dissimilar-aged boobies would be consistent with broods of young pairs and old pairs  
185 showing high levels of extra-pair paternity; females in such pairings may seek alternative  
186 sires in order to endow their progeny with a combination of genes from dissimilar-aged  
187 parents (Ramos *et al.*, 2014), to boost their parasite resistance. If poor gene-based  
188 immunocompetence explains the heavy infestation of nestlings with two young or old parents,  
189 can heavy infestation in turn explain their relatively poor body condition? Although tick  
190 infestation can constrain the growth of seabird nestlings (Morbey, 1996; Bosch & Figuerola,  
191 1999), this is unlikely to be the explanation because infestation and body condition were, if  
192 anything, positively correlated. More likely, the assumed genetic inferiority of nestlings with  
193 two young or old parents had largely independent negative effects on both  
194 immunocompetence and body condition.



195 Finally, the pattern of variation in infestation might be due to pairs of young or old  
196 parents exposing their nestlings to greater risk of transmission because they themselves carry  
197 a heavy tick load. The uropygial gland appears to be smaller and provide less defence in  
198 young adults (González, 2014), and immunosenescence has been documented in several avian  
199 species (*e.g.*, Cichoñ *et al.*, 2003; Haussmann *et al.*, 2005). Young and old parents could be  
200 relatively deficient in self-grooming, chemical defences and parental hygienic behaviour  
201 (review in Clayton *et al.*, 2010) or occupy more heavily infested nest sites. Again though,  
202 age-related variation in parental parasite load or hygienic behaviour might be able to explain  
203 high infestation of chicks with two young parents or two old parents, but it cannot account for  
204 minimal infestation of chicks with different-aged parents.

205 Our study shows that parental age combinations affect the parasite load and body  
206 condition of blue-footed booby nestlings in a pattern that mirrors their effects on fledglings'  
207 prospects for recruiting into the breeding population (Drummond & Rodríguez 2015). The  
208 effect of age combination on recruitment prospects is unlikely to be mediated by nestling  
209 body condition because by the time chicks fledge at age 70 d, their body condition is  
210 unrelated to parental age combination (Drummond & Rodríguez 2015). The effect on  
211 recruitment is more likely to be mediated by damage inflicted on nestlings by parasites or  
212 longer-term costs of diminished immunocompetence that persists into juvenile and adult life.

213

## 214 **Acknowledgments**

215 We are grateful to Brenda Flores and Cristina Rodríguez for their substantial aid in the field,  
216 and to the Armada de México, local fisherman and the staff of Parque Nacional Isla Isabel for  
217 providing vital logistical support. This study was financed by the Consejo Nacional de  
218 Ciencia y Tecnología (CONACYT; 1041313). This manuscript constitutes a partial fulfilment  
219 of the Graduate Program in Biomedical Sciences of the Universidad Nacional Autónoma de  
220 México (UNAM). AGR acknowledges the scholarship and financial support provided by the  
221 CONACYT and the UNAM.

222

## 223 **References**

- 224 Balbontin, J., Hermosell, I.G., Marzal, A., Reviriego, M., de Lope, F. & Møller, A.P. 2007. Age-  
225 related change in breeding performance in early life is associated with an increase in  
226 competence in the migratory barn swallow *Hirundo rustica*. *J. Anim. Ecol.* **76**: 915–925.
- 227 Barr, D. J., Levy, R., Scheepers, C. & Tily, H. J. 2013. Random effects structure for confirmatory  
228 hypothesis testing: keep it maximal. *J. Mem. Lang.* **68**: 255–278.
- 229 Beamonte-Barrientos, R., Velando, A., Drummond, H. & Torres, R. 2010. Senescence of maternal  
230 effects: aging influences egg quality and rearing capacities of a long-lived bird. *Am. Nat.* **175**:  
231 469–480.
- 232 Bosch, M., & Figuerola, J. 1999. Detrimental effects of ticks *Ornithodoros maritimus* on the growth of  
233 yellow-legged gull *Larus michahellis* chicks. *Ardea* **87**: 83–89.
- 234 Chastel, C. 1988. Tick-borne virus infections of marine birds. *Adv. Dis. Vec. Res.* **5**: 25–60.
- 235 Cichoń, M. 2003. Does prior breeding experience improve reproductive success in collared flycatcher  
236 females? *Oecologia* **134**: 78–81.
- 237 Clayton, D.H., Koop, J.A., Harbison, C.W., Moyer, B.R. & Bush, S.E. 2010. How birds combat  
238 ectoparasites. *Open Ornithol. J.* **3**: 41–71.
- 239 Crawley, M. J. 2007. *The R book*. Wiley, Chichester.
- 240 Drummond, H., J.L. Osorno, C. García, R. Torres & H. Merchant. 1991. Sexual dimorphism and  
241 sibling competition: implications for avian sex ratios. *American Naturalist*, 138, 623-641.
- 242 Drummond, H. y C. Rodríguez. 2015. Viability of booby offspring is maximized by having one young  
243 parent and one old parent. *PLoS ONE*, 10(7): e0133213
- 244 Drummond, H., Rodríguez, C., Vallarino, A., Valderrábano, C., Rogel, G. & Tobón, E. 2003.  
245 Desperado siblings: uncontrollably aggressive junior chicks. *Behav. Ecol. Sociobiol.* **53**: 287–  
246 296.
- 247 Drummond, H., González, E. & Osorno, J.L. 1986. Parent-offspring cooperation in the blue-footed  
248 booby (*Sula nebouxii*): social roles in infanticidal brood reduction. *Behav. Ecol. Sociobiol.* **19**:  
249 365–372.
- 250 Duffy, D.C. 1983. The ecology of tick parasitism on densely nesting Peruvian seabirds. *Ecology* **64**:  
251 110–119.
- 252 Dusbábek, F., Uhlíř, J. & Borský, I. 1994. Age-dependent immune response of chickens to feeding by  
253 *Argas persicus* larvae. *Vet. Parasitol.* **51**: 307–319.

- 254 Gasparini, J., McCoy, J.D., Haussy, C., Tveraa, T. & Boulinier, T. 2001. Induced maternal response to  
255 the Lyme disease spirochaete *Borrelia burgdorferi sensu lato* in a colonial seabird, the kittiwake  
256 *Rissa tridactyla*. *Proc. R. Soc. B* **268**: 647–650.
- 257 González, C.A. 2014. *Changes in mass of the preen gland in rock ptarmigans (Lagopus muta) in*  
258 *relation to sex, age and parasite burden 2007-2012*. M.Sc. Thesis, Faculty of Life and  
259 Environmental Sciences, University of Iceland.
- 260 Griffiths, R., Double, M.C., Orr, K. & Dawson, R.J. 1998. A DNA test to sex most birds. *Mol. Ecol.* **7**:  
261 1071–1075.
- 262 Harris, W.E. & Uller, T. 2009. Reproductive investment when mate quality varies: differential  
263 allocation versus reproductive compensation. *Philos. T. R. Soc. B* **364**: 1039–1048.
- 264 Haussmann, M.F., Winkler, D.W., Huntington, C.E., Vleck, D., Sanneman, C.E., Hanley, D. *et al.*  
265 2005. Cell-mediated immunosenescence in birds. *Oecologia* **145**: 269–274.
- 266 Hawley, D.M., Sydenstricker, K.V., Kollias, G.V. & Dhondt, A.A. 2005. Genetic diversity predicts  
267 pathogen resistance and cell-mediated immunocompetence in house finches. *Biol. Lett.* **1**: 326–  
268 329.
- 269 Hoi-Leitner, M., Romero-Pujante, M., Hoi, H. & Pavlova, A. 2001. Food availability and immune  
270 capacity in serin (*Serinus serinus*) nestlings. *Behav. Ecol. Sociobiol.* **49**: 333–339.
- 271 Johnson, S.L. & Gemmell, N.J. 2012. Are old males still good males and can females tell the  
272 difference? *Bioessays* **34**: 609–619.
- 273 Kokko, H. 1998. Good genes, old age and life-history trade-offs. *Evol. Ecol.* **12**: 739–750.
- 274 Lehmann, T. 1993. Ectoparasites: direct impact on host fitness. *Parasitol. Today* **9**: 8–13.
- 275 Limmer, B. & Becker, P.H. 2009. Improvement in chick provisioning with parental experience in a  
276 seabird. *Anim. Behav.* **77**: 1095–1101.
- 277 Manning, J.T. 1985. Choosy females and correlates of male age. *J. Theor. Biol.* **116**: 349–354.
- 278 McCoy, K.D., Boulinier, T., Schjørring, S. & Michalakis, Y. 2002. Local adaptation of the  
279 ectoparasite *Ixodes uriae* to its seabird host. *Evol. Ecol. Res.* **4**: 441–456.
- 280 Morbey, Y.E. 1996. The abundance and effects of ticks (*Ixodes uriae*) on nestling Cassin's Auklets  
281 (*Ptychoramphus aleuticus*) at Triangle Island, British Columbia. *Can. J. Zool.* **74**: 1585–  
282 1589.
- 283 Owen, J.P., Nelson, A.C. & Clayton, D.H. 2010. Ecological immunology of bird-ectoparasite  
284 systems. *Trends Parasitol.* **26**: 530–539.

285 Qviller, L., Risnes-Olsen, N., Bærum, K.M., Meisingset, E.L., Loe, L.E., Ytrehus, B. *et al.* 2013.  
286 Landscape level variation in tick abundance relative to seasonal migration in red deer. *PloS*  
287 *ONE* **8**: e71299.

288 R Core Team. 2013. *R: A Language and Environment for Statistical Computing*. R Foundation for  
289 Statistical Computing, Vienna, Austria. URL:

290 Ramos, A.G., Nunziata, S.O., Lance, S.L., Rodríguez, C., Faircloth, B.C., Gowaty, P.A. *et al.* 2014.  
291 Interactive effects of male and female age on extra-pair paternity in a socially monogamous  
292 seabird. *Behav. Ecol. Sociobiol.* **68**: 1603–1609.

293 Saino, N., Ambrosini, R., Martinelli, R. & Møller, A. P. 2002. Mate fidelity, senescence in breeding  
294 performance and reproductive trade-offs in the barn swallow. *J. Anim. Ecol.* **71**: 309–319.

295 Skaug, H., Fournier, D., Nielsen, A., Magnusson, A. & Bolker, B. 2011. *Generalized linear mixed*  
296 *models using AD Model Builder*. R package version 0.6 5, r143.

297 Szép, T., & Møller, A.P. 1999. Cost of parasitism and host immune defence in the sand martin *Riparia*  
298 *riparia*: a role for parent-offspring conflict? *Oecologia* **119**: 9–15.

299 Tarín, J.J., Pérez-Albalá, S. & Cano, A. 2000. Consequences on offspring of abnormal function in  
300 ageing gametes. *Hum. Reprod. Update* **6**: 532–549.

301 Torres, R., Drummond, H. & Velando, A. 2011. Parental age and lifespan influence offspring  
302 recruitment: a long-term study in a seabird. *PloS one* 6.11: e27245.

303 Velando, A., Noguera, J.C., Drummond, H. & Torres, R. 2011. Senescent males carry premutagenic  
304 lesions in sperm. *J. Evol. Biol.* **24**: 693–697.

305 Velando, A., Drummond, H. & Torres, R. 2006. Senescent birds redouble reproductive effort when ill:  
306 confirmation of the terminal investment hypothesis. *Proc. R. Soc. B* **273**: 1443–1448.

307 Wikel, S.K. 1996. Host immunity to ticks. *Ann. Rev. Entomol.* **41**: 1–22.

308

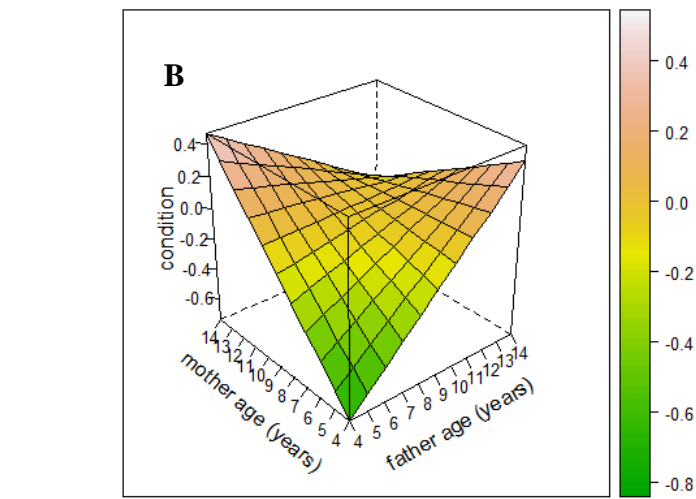
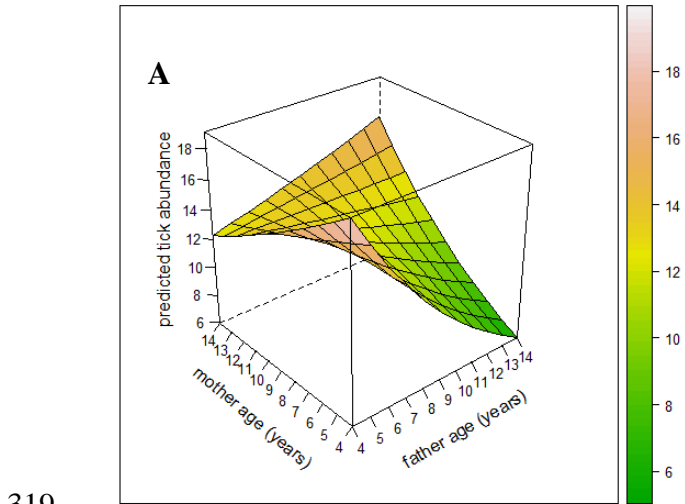
309 **Table 1.** Generalized linear mixed models of the relationships between parents' ages and tick  
 310 abundance and body condition of booby chicks at 15, 29 and 43 days ( $N = 105$ ).

	<b>Estimate <math>\pm</math> SE</b>	<b><i>P</i></b>
<b>Ticks</b>		
Age 15 vs. 29	1.12 $\pm$ 0.15	< 0.0001
Age 29 vs. 43	-0.63 $\pm$ 0.14	< 0.0001
Brood 1 vs. 2	0.40 $\pm$ 0.25	0.11
Brood 2 vs. 3	-0.91 $\pm$ 0.31	0.003
Father age	-0.25 $\pm$ 0.10	---
Mother age	-0.19 $\pm$ 0.10	---
Father age*Mother age	0.02 $\pm$ 0.01	0.043
<b>Body condition</b>		
Chick sex	1.14 $\pm$ 0.16	< 0.0001
Father age	0.21 $\pm$ 0.07	---
Mother age	0.16 $\pm$ 0.07	---
Father age*Mother age	-0.02 $\pm$ 0.01	0.023

311

312

313 **Figure 1.** Abundance of tick larvae (a) and body condition (b) of 35 booby chicks, at ages 15,  
314 29 and 43 days, as a function of their fathers' and mothers' ages. The surfaces show values  
315 predicted by the father age\*mother age interactions (tick abundance,  $P = 0.043$ ; body  
316 condition,  $P = 0.023$ ) in GLMMs (Table 1). Although the full sample includes males and  
317 females of up to 19 years, we graph only the ranges of observed age combinations of booby  
318 pairs.



## **DISCUSIÓN GENERAL**

En esta tesis exploré si la probabilidad de paternidad extra-pareja en nidadas de bobos de patas azules en la Isla Isabel varía con la estructura del hábitat y las combinaciones de edades de los padres, dos variables potencialmente importantes pero usualmente ignoradas. Encontré que los bobos, al igual que la mayoría de las especies de aves consideradas socialmente monógamas, son genéticamente promiscuos. El ~ 11% de las nidadas tuvieron al menos una cría extra-pareja, siendo éste el primer y único registro de paternidad extra-pareja en ésta especie hasta ahora. Para una pareja de bobos, la probabilidad de producir nidadas con una o más crías extra-pareja aumentó cuando anidaron en hábitats con pocos obstáculos a su locomoción (Capítulo 1), y cuando ambos eran o viejos o jóvenes (Capítulo 2).

El hábitat de la colonia en Isla Isabel es heterogéneo. Los bobos anidan en playa arenosa, bosques de papelillo y roache, sitios de transición con pastizal abundante y sitios en el bosque con altas densidades de rocas y árboles. La paternidad extra-pareja fue más común en el norte de la zona de estudio, donde la densidad de rocas y árboles disminuye (Capítulo 1). La baja incidencia de extra-paternidad en áreas con alta densidad de obstáculos podría deberse a la compleja conducta de cortejo de los bobos: individuos de ambos sexos hacen despliegues tales como caminar con pasos exagerados exhibiendo el color de sus patas, levantar sus picos hacía el cielo mientras revierten y extienden sus alas hacia arriba, y colocar material de anidación cerca de las patas del individuo al que cortejan (Nelson 1978, 2005). Es posible que una alta densidad de obstáculos dificulta la expresión de los despliegues, afectando el establecimiento del lazo de pareja, la frecuencia o eficiencia de las cópulas, y el acceso de los individuos en búsqueda de interacciones extra-pareja.

Alternativamente, zonas rocosas o los individuos que aniden en ellas podrían ser menos atractivos por la alta abundancia de garrapatas y la mayor severidad de infestaciones de polluelos. Un posible e importante costo de copular con individuos infestados con garrapatas es el alto riesgo de contagio, ya que se transmiten por contacto directo y los parásitos pueden afectar la selección sexual en aves (Borgia y Collis 1989). Aunque no contamos garrapatas en adultos, la abundancia de garrapatas en crías aumentó, al igual que la densidad de rocas, hacía el sur de la zona de estudio (Capítulo 3). Las garrapatas en su fase libre, cuando están fuera del huésped, sufren de depredación y



mortalidad por condiciones climáticas adversas. En colonias de aves marinas, es común encontrar garrapatas refugiadas entre las grietas de las rocas, y es factible que en zonas con densidades altas de rocas incremente la sobrevivencia de las garrapatas y su abundancia. Sin embargo, los resultados que presento son meras correlaciones y hace falta usar una aproximación experimental para comprobar la importancia de los parásitos en la conducta extra-pareja.

Encontré una relación cuadrática, nunca antes reportada, entre la probabilidad de paternidad extra-pareja en nidadas de bobos y la densidad local de nidos, número de nidos vecinos ubicados a distancias de 10 m o menos (Capítulo 1). En nidadas con más de 26 nidos vecinos, la probabilidad de paternidad extra-pareja disminuyó conforme aumentó el número de vecinos. La distancia promedio entre los nidos de hembras y los padres biológicos fue de 43.7 m, y nunca fueron vecinos entre sí. En vecindades de más de 26 nidos, las incursiones en búsqueda de cópulas extra-pareja, por individuos de ambos sexos, podrían ser obstruidas por interacciones agonísticas frecuentes con coespecíficos. Además, es posible que los machos que anidan en densidades altas incrementen su conducta de resguardo para responder al alto riesgo de infidelidad de sus parejas (Komdeur 2001, Mayer y Pasinelli 2013). Tal vez los machos que buscaron cópulas extra-pareja fueron más exitosos en vecindades de densidades medias porque las hembras son relativamente numerosas y accesibles.

La densidad local de nidos tuvo un efecto lineal y un efecto cuadrático, menos fuerte, en la abundancia de garrapatas (Capítulo 3). A diferencia de muchos otros parásitos, las garrapatas pueden infectar a sus huéspedes de diferentes formas, y éstas pueden modificar la relación entre la densidad local de coespecíficos y el riesgo de infección. Cuando un parásito se transmite por contacto directo se espera que su abundancia incremente con la densidad de huéspedes y, en contraste, cuando un parásito es móvil y busca activamente a sus huéspedes se espera lo contrario (Mugabo et al. 2015). La magnitud del efecto lineal sugiere que la principal forma de contagio de *Carios denmarki* fue por contacto directo. Si esto es cierto, es posible que los bobos que anidaron en densidades altas hayan invertido más en su inmunidad contra parásitos (Rifkin et al. 2012), y esto podría explicar el efecto cuadrático que indica que el incremento de garrapatas con el número de vecinos ocurre hasta alcanzar

densidades de 0.41 nidos/m<sup>2</sup>.

La combinación de las edades de los padres afectó tanto la probabilidad de paternidad extra-pareja como la abundancia de garrapatas en las crías (Capítulos 3 y 4, respectivamente). Hembras jóvenes apareadas con un macho joven y hembras viejas apareadas con un macho viejo tuvieron mayor probabilidad de tener al menos una cría extra-pareja en sus nidadas. Es posible que estos patrones reflejen las preferencias entre machos progenitores de diferentes edades, ejercidas por hembras de distintas edades, ya que ellas controlan las cópulas y posiblemente usan mecanismos de selección espermática postcópula. De ser así, las hembras jóvenes podrían estar dando preferencia a los machos viejos y las hembras viejas a los machos jóvenes. Ciertamente, cuando las hembras jóvenes apareadas con machos jóvenes produjeron crías extra-pareja, los padres biológicos fueron más grandes en edad que sus parejas sociales, y una hembra vieja apareada con un macho viejo produjo crías extra-pareja con un macho extra-pareja que era 10 años más joven que su pareja.

La explicación más plausible para la preferencia de las hembras jóvenes por machos extra-pareja mayores a sus parejas sociales, es que, al lograr sobrevivir a edades avanzadas los machos viejos demuestran tener buenos genes, y éstos son transmisibles a sus crías (Manning 1985, Kokko 1998, Brooks y Kemp 2001). Para las hembras viejas, los beneficios genéticos ofrecidos por machos muy viejos podrían ser atenuados por los costos resultantes de la unión de dos individuos viejos. Con la edad, las capacidades reproductivas tanto de hembras como de machos deterioran, incluyendo el ADN germinal (Velandó et al. 2011) y la calidad de los huevos (Beamonte-Barrientos et al. 2010) y crías (Saino et al. 2002). Es concebible, entonces, que las hembras viejas apareadas con machos viejos reducen los riesgos de infertilidad y de defectos genéticos, acentuados por la unión de dos individuos viejos, copulando con machos extra-pareja relativamente más jóvenes.

La compleja relación observada entre combinación de edades de los progenitores y la probabilidad de paternidad extra-pareja indica que las combinaciones de edades más exitosas son las de un macho o hembra joven y otro individuo viejo. Sin embargo, cuando publiqué el Capítulo 3 de esta tesis no existían estudios en animales sobre los efectos de las combinaciones de edades

de los progenitores en la fertilidad y sobre la calidad de las crías. Un estudio provocado por estos resultados confirmó la expectativa de que la probabilidad del reclutamiento disminuye cuando los volantones tienen dos padres jóvenes o dos padres viejos (Drummond y Rodríguez 2015). Este resultado tiende a confirmar la hipótesis adaptativa de que las hembras en parejas con combinaciones de edades desfavorables aumentan la viabilidad de sus crías buscando un padre biológico cuya edad permite una combinación mas favorable. La posibilidad de que la alta viabilidad en volantones producidos por un padre viejo y otro joven surge de su calidad genética, tal vez mediada por alta heterocigosidad, está actualmente investigándose aplicando Genotipado por Secuenciación (GBS) al ADN de las muestras que tomé para los Capítulos 1 y 2.

Finalmente encontré que las crías producidas por un bobo joven y otro viejo tuvieron menos garrapatas y mejor condición corporal (Capítulo 4). La respuesta inmune y los anticuerpos maternos son las dos defensas principales que tienen las crías de bobos en contra de parásitos, y la eficiencia de ambas podría ser afectada por las edades de sus padres. En carpodacos domésticos (*Carpodacus mexicanus*), la respuesta inmune incrementa con la heterocigosidad genética (Hawley et al. 2005) y, en teoría, las crías producidas por padres de edades distintas podrían tener mayor heterocigosidad. La intensidad de infestaciones por garrapatas en colonias de aves marinas fluctúa (Oorebeek y Kleindorfer 2008, Dietrich et al. 2012). Si en consecuencia de la variación ecológica interanual los bobos de diferentes cohortes enfrentan distintas presiones selectivas durante sus vidas, las crías producidas por padres de edades distintas podrían ser más heterocigóticas y estar mejor equipadas para defenderse contra garrapatas (Drummond y Rodríguez 2015). Mi estudio muestra que la combinación de edades de los padres afecta la carga parasitaria de las crías con un patrón que se asemeja a los patrones observados de paternidad extra-pareja y la probabilidad de reclutamiento de crías emplumadas, reafirmando que las parejas formadas por un progenitor joven y otro viejo podrían ser óptimas para producir crías de alta calidad.

### ***Conclusiones y perspectivas***

Los 4 capítulos de la tesis revelan patrones novedosos en los contextos ecológicos, sociales y posiblemente genéticos en los que las hembras del bobo de

patas azules, una especie socialmente monógama, recurren a la paternidad extra-pareja. Estos patrones demuestran la importancia de la variación entre individuos en el comportamiento extra-pareja. No todas las hembras de una misma especie y población obtienen los mismos beneficios al tener crías extra-pareja, ya que los beneficios dependen de la combinación de edades de la pareja, ni tampoco tienen las mismas oportunidades de copular con machos extra-pareja, ya que éstas pueden fluctuar con el hábitat local y la densidad local de conspecíficos.

Las correlaciones que encontré resaltan la importancia de continuar explorando e identificando factores asociados con la probabilidad de extra-paternidad en distintas especies ya que, a pesar de cientos de estudios, sus causas siguen siendo controversiales e inciertas. También, podría probarse si las correlaciones descritas en mi tesis ocurren en otros años, ya que mis análisis abarcan una sola temporada reproductiva, y en otras especies, y la validez de sus interpretaciones podría ponerse a prueba con estudios experimentales. La variación en probabilidades de paternidad extra-pareja y en preferencias por machos extra-pareja de distintas edades en función de las edades de la hembra y su macho social, indican la importancia de considerar las edades de estos tres participantes en estudios de conducta y paternidad extra-pareja, y no solo al macho social como suele hacerse. A su vez, los estudios de extra-paternidad, viabilidad de crías y abundancia de garrapatas en crías según las distintas combinaciones de edades de los progenitores, demuestran la importancia del monitoreo a largo plazo de individuos de organismos de vida larga y la elaboración de bases de datos.

Quizá la pregunta más importante que surge de esta tesis es si otras especies de vertebrados obtienen ventajas reproductivas cuando un individuo joven se reproduce con uno viejo. Si este patron es comun, las implicaciones podrían ser valiosas en diversas áreas, incluso podrían ser aplicables. Por ejemplo, en programas de reproducción y reintroducción de especies amenazadas es común seleccionar individuos para formar parejas reproductoras según su compatibilidad genética, pero podría ser importante además aprovechar las bondades de las mejores combinaciones de edades de progenitores.

## LITERATURA

- Beamonte - Barrientos, R., Velando, A., Drummond, H. and Torres, R., 2010. Senescence of Maternal Effects: Aging Influences Egg Quality and Rearing Capacities of a Long - Lived Bird. *The American Naturalist*, 175(4), pp.469-480.
- Borgia, G., & Collis, K. (1989). Female choice for parasite-free male satin bowerbirds and the evolution of bright male plumage. *Behavioral Ecology and Sociobiology*, 25(6), 445-453
- Brooks, R. and Kemp, D.J., 2001. Can older males deliver the good genes?. *Trends in Ecology & Evolution*, 16(6), pp.308-313.
- Dietrich, M., Kempf, F., Gómez - Díaz, E., Kitaysky, A.S., Hipfner, J.M., Boulinier, T. and McCoy, K.D., 2012. Inter - oceanic variation in patterns of host - associated divergence in a seabird ectoparasite. *Journal of biogeography*, 39(3), pp.545-555.
- Drummond, H., & Rodríguez, C. (2015). Viability of Booby Offspring is Maximized by Having One Young Parent and One Old Parent. *PloS one*, 10(7), e0133213.
- Hawley, D.M., Sydenstricker, K.V., Kollias, G.V. and Dhondt, A.A., 2005. Genetic diversity predicts pathogen resistance and cell-mediated immunocompetence in house finches. *Biology Letters*, 1(3), pp.326-329.
- Kokko, H., 1998. Good genes, old age and life-history trade-offs. *Evolutionary Ecology*, 12(6), pp.739-750.
- Komdeur, J., 2001. Mate guarding in the Seychelles warbler is energetically costly and adjusted to paternity risk. *Proceedings of the Royal Society of London B: Biological Sciences*, 268(1481), pp.2103-2111.
- Manning, J.T., 1985. Choosy females and correlates of male age. *Journal of Theoretical Biology*, 116(3), pp.349-354.
- Mayer, C. and Pasinelli, G., 2013. New support for an old hypothesis: density affects extra - pair paternity. *Ecology and evolution*, 3(3), pp.694-705.
- Mugabo, M., Perret, S., Decenièrè, B., Meylan, S. and Le Galliard, J.F., 2015. Density-dependent immunity and parasitism risk in experimental populations of lizards naturally infested by ixodid ticks.

Nelson JB. (1978). *The Sulidae: gannets and boobies*. Oxford, UK: Oxford University Press.

Nelson, J.B., 2005. *Pelicans, cormorants, and their relatives. The Pelecaniformes*.

Oorebeek, M. and Kleindorfer, S., 2008. Climate or host availability: what determines the seasonal abundance of ticks?. *Parasitology research*, 103(4), pp.871-875.

Rifkin, J.L., Nunn, C.L. and Garamszegi, L.Z., 2012. Do animals living in larger groups experience greater parasitism? A meta-analysis. *The American naturalist*, 180(1), pp.70-82.

Saino, N., Ambrosini, R., Martinelli, R., Calza, S., Møller, A.P. and Pilastro, A., 2002. Offspring sexual dimorphism and sex - allocation in relation to parental age and paternal ornamentation in the barn swallow. *Molecular Ecology*, 11(8), pp.1533-1544.

Velando, A., Noguera, J.C., Drummond, H. and Torres, R., 2011. Senescent males carry premutagenic lesions in sperm. *Journal of evolutionary biology*, 24(3), pp.693-697.

## **ANEXO I**

### **ISOLATION AND CHARACTERIZATION OF MICROSATELLITE LOCI FROM BLUE-FOOTED BOOBIES (SULA NEBOUXII)**

*Conservation Genetics Resources 1:159-162*

## Isolation and characterization of microsatellite loci from blue-footed boobies (*Sula nebouxii*)

Brant C. Faircloth · Alejandra Ramos ·  
Hugh Drummond · Patricia Adair Gowaty

Received: 29 May 2009 / Accepted: 2 June 2009 / Published online: 23 June 2009  
© The Author(s) 2009. This article is published with open access at Springerlink.com

**Abstract** Blue-footed boobies (*Sula nebouxii*) are socially monogamous, colonial seabirds exhibiting intra-specific nest parasitism and extra-pair copulations. Prior DNA fingerprinting assays failed to detect extra-pair offspring in the nests of congeners, and the rate of intra-specific nest parasitism has not been estimated using molecular techniques. We describe the development and characterization of 11 microsatellite DNA loci, tested using 31 individuals collected on Isla Isabel, Nayarit, México. The number of alleles per locus ranged from three to 22, averaging seven; total exclusionary power of the microsatellite panel was 0.99; no loci deviated from Hardy-Weinberg equilibrium; and we did not detect linkage disequilibrium following Bonferroni correction. This microsatellite panel will facilitate future studies of nest parasitism and extra-pair paternity in blue-footed boobies.

**Keywords** Microsatellites · SSRs · Blue-footed booby · *Sula nebouxii* · Sulidae

---

B. C. Faircloth (✉) · P. A. Gowaty  
Department of Ecology and Evolutionary Biology,  
University of California, 621 Charles E. Young Drive South,  
Los Angeles, CA 90095, USA  
e-mail: brant@ucla.edu

A. Ramos · H. Drummond  
Departamento de Ecología Evolutiva, Instituto de Ecología,  
Universidad Nacional Autónoma de México, AP 70-275, México  
DF 04510, México

P. A. Gowaty  
Institute of the Environment, University of California,  
Los Angeles, CA 90095, USA

P. A. Gowaty  
Smithsonian Tropical Research Institute, Unit 0948,  
AP AA 34002-0948 Panama City, Republic of Panama

Blue-footed boobies (*Sula nebouxii*) are colonial seabirds that nest annually on small Pacific Ocean islands (Nelson 1978). They reproduce several times during their lifetime of up to 20 years, forming pair bonds lasting 1–5 years (Torres and Drummond, unpublished data). Female boobies lay clutches of 1–3 eggs and provide parental care jointly with males until chicks fledge at approximately 90 days. Though socially monogamous, both sexes perform consensual, extrapair copulations that peak in frequency during the female's presumed fertile period (Osorio-Beristain and Drummond 1998; Perez-Staples and Drummond 2005) and some females lay eggs in the nests of neighbors [intraspecific nest parasitism (INP)]. Females also lay eggs in the nests of their male extra-pair partners [quasi nest parasitism (QNP)]. Observation revealed consistently hostile responses by incubating (host) females to INP and flexible responses by males to QNP—ranging from egg destruction to acceptance (Osorio-Beristain et al. 2005).

While the occurrence of extrapair and unrelated chicks in *S. nebouxii* nests is plausible, it remains unproven. Doubts arise because fingerprinting analyses failed to detect extrapair chicks in all congeners tested: nazca (*Sula granti*, Anderson and Boag 2006), masked (*Sula dactylatra*) and brown (*Sula leucogaster*) boobies (Baumgarten et al. 2001).

Understanding the evolution of extra-pair mating, mate-guarding and nest parasitism in blue-footed boobies, as well as violent conflict between broodmates (Drummond et al. 1986; Gonzalez-Voyer et al. 2007), requires assignment of genetic parentage to and estimation of relatedness between brood mates. Mendelian inheritance, combined with high levels of polymorphism, makes microsatellite loci ideal for these purposes and for analyses of small and isolated populations, like blue-footed boobies (Hedrick 1999; Selkoe



and Toonen 2006). In this manuscript, we describe the isolation and characterization of 11 microsatellite loci, which we tested using 31 *S. neboxii*. These are the first microsatellites characterized in members of the genus *Sula*.

We developed a double-enriched microsatellite library following Glenn and Schable (2005) using DNA purified (5-Prime ArchivePure Blood Kit) from blood collected from a female blue-footed booby on Isla Isabel, Nayarit, México. We modified the enrichment protocol of Glenn and Schable (2005) using MyOne C1 streptavidin beads (Invitrogen) with 2X (10  $\mu$ M Tris HCl, pH 7.5; 1  $\mu$ M EDTA; 2 M NaCl; 0.2% Tween 20) and 1X B&W Buffer (Invitrogen) during the enrichment steps. From this library, we selected 285 positive (white) colonies using the  $\beta$ -galactosidase gene and bi-directionally sequenced colony PCR products of 500–1,200 base pairs using 1/16th BigDye [v3.1, Applied Biosystems (ABI)] sequencing reactions and an ABI PRISM 3730xl sequencer. We aligned and edited sequences and assembled 179 contigs using Sequencher 4.2 (Gene Codes Corp.). Prior to primer design, we tested for sequence homology using BLAST (Altschul et al. 1990).

Using MSATCOMMANDER (Faircloth 2008), we located microsatellite repeat arrays within 68 contigs, designed 45 primers, and applied 5'-tags (CAG or M13R) to primer pairs for subsequent polymorphism testing (Boutin-Ganache et al. 2001; Glenn and Schable 2005). We reviewed contigs containing repeats for which the automated software indicated primer design errors, and we manually designed primers for these regions ( $N = 15$ ) using Oligo 6.0 (Molecular Biology Insights) and the 5'-tagging approach. We added GTTT "pigtailed" to the 5' end of each primer lacking either CAG or M13R tag to facilitate the addition of adenosine by *Taq* polymerase (Brownstein et al. 1996).

We tested 60 primer pairs for amplification using phenol-chloroform purified DNA collected from three blue-footed boobies on Isla Isabel during 1994. We performed PCR amplifications in 10  $\mu$ L volumes using ABI 9700 thermal cyclers. Concentrations for reactions were 10 mM Tris pH 8.4, 50 mM KCl, 0.5  $\mu$ M "pigtailed" primer, 0.05  $\mu$ M CAG or M13-reverse (M13R) tagged primer (CAG or M13R + primer), 0.45  $\mu$ M dye labeled tag (Dye + CAG or M13R), 1.5 mM MgCl<sub>2</sub>, 0.5 mM dNTPs, 0.5 U AmpliTaq Gold DNA Polymerase (ABI), and 20 ng DNA. We labeled M13R and CAG universal primers with VIC, NED, FAM, or PET fluorescent dyes (ABI). We performed reactions using a touchdown thermal cycling program (Don et al. 1991), and we evaluated each primer at a starting annealing temperature of 60°C. We used the following cycling parameters: 1 cycle of 95°C for 5 m; followed by 20 cycles of 95°C for 20 s, 60°C for 30 s minus 0.5°C per annealing cycle, and 72°C for 90 s;

followed by 20 cycles of 95°C for 20 s, 50°C for 30 s, 72°C for 90 s; and a final extension period of 10 m at 72°C. We scored each amplicon using an ABI Prism 3730xl DNA Sequencer in combination with LIZ600 fluorescent size standard (ABI), GeneMapper 4.0 Software (ABI) and the Local Southern size calling method.

Based on the performance of primers during the initial test, we selected 18 primer pairs for subsequent optimization and polymorphism testing. The primers we did not select for additional testing were monomorphic ( $N = 25$ , 78%) or failed to amplify ( $N = 7$ , 22%) during the initial test. We optimized and screened primers using phenol-chloroform purified DNA collected from 31 individuals sampled on Isla Isabel and amplification conditions identical to those presented above. When visual assessment of resulting peak morphologies indicated that increased annealing temperatures might improve amplification quality, we also genotyped and scored loci using a starting annealing temperature of 65°C (–0.5°C step). We selected the starting annealing temperature resulting in the most precise peaks without inducing allelic dropout, and we removed any loci from the candidate set yielding ambiguous peaks or inconsistent results.

We calculated observed ( $H_O$ ) and expected ( $H_E$ ) heterozygosity and polymorphic information content (PIC) using Cervus 3.0 (Marshall et al. 1998; Kalinowski et al. 2007), and we tested for deviations from Hardy-Weinberg equilibrium (HWE) and evaluated genotypic linkage disequilibrium (LD) using Genepop (Raymond and Rousset 1995). We conducted *a posteriori* Bonferroni correction (Rice 1989) for each analysis consisting of multiple, concurrent statistical tests (HWE and LD).

Table 1 presents the characteristics of 11 primer pairs amplifying microsatellite loci in blue-footed boobies. **The number of alleles ranged from three to 22, averaging seven.** No loci deviated from HWE, and we did not detect LD following Bonferroni correction. Total exclusionary power with both parents unknown was 0.99.

Incorporation of the MyOne C1 Streptavidin beads to the process did not appear to increase the efficiency of enrichment for fragments containing microsatellite repeats (38%) when compared to the older M-280 beads ( $\approx$ 50–60%, Crawford et al. 2009, BC Faircloth *unpublished data*). This observation may be species-specific or a general indication that the M-280 beads are more efficient for the purpose of microsatellite enrichment. Others (S Lance and T Glenn, University of Georgia, *personal communication*) have observed reduced enrichment efficiencies using MyOne C1 beads and B&W hybridization buffer.

The microsatellite loci identified here will serve as a useful resource for future studies of blue-footed boobies requiring a panel of polymorphic DNA markers.

**Table 1** Characteristics of 11 primer pairs amplifying microsatellite DNA loci in blue-footed boobies (*Sula nebouxi*) sampled from Isla Isabel, Nayarit, México

Locus	Sequence	Accession	Anneal	N	A	Range	Repeat	HO	HE	PIC
BOOB-RM2-F07 U	GTTTGCTAATCCCTGGTGCAAGTG	FJ587350	60	30	3	389–397	(CT) <sup>8</sup>	0.60	0.59	0.50
BOOB-RM2-F07 L	<u>GGAAACAGCTATGACCATACATCCGGTGCTCAGTTGTG</u>									
BOOB-RM3-D07 U	GTTTGGCTACTGCCATCCAGGG	FJ587394	60	31	15	325–399	(AAAG) <sup>35</sup> ... (AAAG) <sup>5</sup>	0.87	0.90	0.87
BOOB-RM3-D07 L	<u>CAGTCGGGGGTCATCAGTTGCTCATAAACAGGCTGGTATTC</u>									
BOOB-RM3-F11 U	GTTTAGAGGGTGTAGGCTGAAATC	FJ587411	60	31	22	281–420	(CTTTT) <sup>41</sup>	0.97	0.95	0.94
BOOB-RM3-F11 L	<u>CAGTCGGGGGTCATCATCTCTAGGCAAGGATCAATACC</u>									
BOOB-RM4-A08 U	GTTTCTGAAAAGTCTTTTATTGTGGAGGG	FJ587430	60	31	3	398–406	(AG) <sup>6</sup> ... (ACAG) <sup>6</sup> ... (ACAG) <sup>6</sup>	0.58	0.54	0.42
BOOB-RM4-A08 L	<u>CAGTCGGGGGTCATCAGCCTAGCACAGGGGTG</u>									
BOOB-RM4-B03 U	<u>CAGTCGGGGGTCATCATGGACCAGCAGCTAAGGC</u>	FJ587372	65	31	3	385–389	(AT) <sup>6</sup> ... (ATGT) <sup>5</sup>	0.29	0.37	0.32
BOOB-RM4-B03 L	GTTTAGGTGAGTGGCTGGGTGG									
BOOB-RM4-C03 U	<u>CAGTCGGGGGTCATCATGGTCAAAACAGTAACATGACAAAGGAC</u>	FJ587442	60	31	11	336–432	(AAAG) <sup>29</sup>	0.84	0.80	0.77
BOOB-RM4-C03 L	GTTTCCAGTAACAACCACTCAGTTCAGG									
BOOB-RM4-D07 U	<u>CAGTCGGGGGTCATCAGCCACCCTCAAGCCATTCC</u>	FJ587311	65	31	3	213–239	(GGAT) <sup>13</sup>	0.52	0.45	0.36
BOOB-RM4-D07 L	GTTTCCAAACAGTTCTGTGCTCAC									
BOOB-RM4-E03 U	GTTTGAAGCTGTGTTCCAAAAGTTGCTC	FJ587457	65	31	3	313–317	(ATCT) <sup>7</sup> ... (CT) <sup>10</sup> ... (ATCC) <sup>4</sup>	0.39	0.36	0.33
BOOB-RM4-E03 L	<u>CAGTCGGGGGTCATCATTTCTGCTCCTGGTAAAATTCCC</u>									
BOOB-RM4-E10 U	<u>CAGTCGGGGGTCATCAAGTCCGACGGGAAAACAG</u>	FJ587461	65	31	5	251–267	(ATCT) <sup>15</sup> ... (ATCT) <sup>4</sup>	0.65	0.62	0.53
BOOB-RM4-E10 L	GTTTGGCTGGCAGGGCTTTGTG									
BOOB-RM4-F11 U	GTTTGTGCTCAGGACATGCATCC	FJ587469	60	31	5	287–307	(AATAG) <sup>12</sup>	0.61	0.61	0.54
BOOB-RM4-F11 L	<u>CAGTCGGGGGTCATCACAAAAGTCCAAATTGACAGCCAG</u>									
BOOB-RM4-G03 U	<u>CAGTCGGGGGTCATCAGGCAGCACTCAAGCTGAAGG</u>	FJ587472	65	30	5	167–197	(ATCCT) <sup>11</sup>	0.67	0.64	0.56
BOOB-RM4-G03 L	GTTTCTCAAAGGTAGGGCAGGGTG									

Sequences used to introduce sites for the universal, fluorescent tags are in bold italics. Underlined bases indicate sharing of nucleotides between the CAG tag (5' - CAGTCGGGGGTCATCA - 3'), the M13R tag (5' - GGAAACAGCTATGACCAT - 3'), or the locus-specific primer binding site and the GTTT "pigtail". U upper primer; L lower primer; N number of individuals successfully genotyped at each locus; A number of alleles; HO observed heterozygosity; HE expected heterozygosity; PIC polymorphic information content

**Acknowledgements** Financial support was provided by: Universidad Nacional Autónoma de México; a UC-Mexus Small Grant to PAG and HD; and setup funds for PAG from the University of California, Los Angeles. We conducted blue-footed booby blood collection on Isla Isabel, Nayarit, Mexico under permits granted to HD by SEDESOL and SEMARNAT as part of his long-term research program on the island. The authors thank Tania del Rio Albrechtsen, Alejandra Nuñez and Sergio Ancona for collecting blood, and the Mexican Navy, San Blas fishermen and Isla Isabel staff for providing transportation and logistical support. BCF thanks Jonathan Drury, Kevin Tan and Alexandra Title for their assistance during the microsatellite enrichment process. PAG and HD acquired funding for this research and provided the physical and intellectual resources for its pursuit, BCF and AR conducted the data collection, BCF conducted the data analysis, and BCF and AR wrote the manuscript.

**Open Access** This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

## References

- Altschul SF, Gish W, Miller W et al (1990) Basic local alignment search tool. *J Mol Biol* 215:403–410
- Anderson DJ, Boag PT (2006) No extra-pair fertilization observed in nazca booby (*Sula granti*) broods. *Wilson J Ornithol* 118: 244–247
- Baumgarten MM, Kohlrausch AB, Miyaki CY et al (2001) DNA fingerprinting and parentage in masked (*Sula dactylatra*) and brown (*S. leucogaster*) boobies. *Ornithol Neotrop* 12:319–326
- Boutin-Ganache I, Raposo M, Raymond M et al (2001) M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. *BioTechniques* 31:24–28
- Brownstein MJ, Carpenter JD, Smith JR (1996) Modulation of non-templated nucleotide addition by *Taq* polymerase: primer modifications that facilitate genotyping. *BioTechniques* 20: 1004–1010
- Crawford NG, Peters MB, Hagen C et al (2009) Polymorphic microsatellite loci from Sprague's pipit (*Anthus spragueii*), a grassland endemic passerine bird. *Mol Ecol Res* 9:315–317
- Don RH, Cox PT, Wainwright BJ et al (1991) Touchdown PCR to circumvent spurious priming during gene amplification. *Nucleic Acids Res* 19:4008
- Drummond H, González E, Osorno JL (1986) Parent-offspring cooperation in the blue-footed booby (*Sula nebouxii*): social roles in infanticidal brood reduction. *Behav Ecol Sociobiol* 19:365–372
- Faircloth BC (2008) MSATCOMMANDER: detection of microsatellite repeat arrays and automated, locus-specific primer design. *Mol Ecol Res* 8:92–94
- Glenn TC, Schable NA (2005) Isolating microsatellite DNA loci. In: Zimmer EA, Roalson EH (eds) *Methods in enzymology* 395, molecular evolution: producing the biochemical data, Part B. Academic Press, San Diego, CA
- Gonzalez-Voyer A, Székely T, Drummond H (2007) Why do some siblings attack each other? Comparative analysis of aggression in avian broods. *Evol Int J org Evol* 61:1946–1955
- Hedrick PW (1999) Perspective: highly variable loci and their interpretation in evolution and conservation. *Evol Int J org Evol* 53:313–318
- Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol Ecol* 16:1006–1099
- Marshall TC, Slate J, Kruuk LEB et al (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Mol Ecol* 7:639–655
- Nelson B (1978) *The Sulidae: gannets and boobies*. Oxford University Press, Oxford
- Osorio-Beristain M, Drummond H (1998) Non-aggressive mate guarding by the blue-footed booby: a balance of female and male control. *Behav Ecol Sociobiol* 43:307–315
- Osorio-Beristain M, Perez-Staples D, Drummond H (2005) Does booby egg dumping amount to quasi-parasitism? *Ethology* 112:625–630
- Perez-Staples D, Drummond H (2005) Tactics, effectiveness and avoidance of mate guarding in the blue-footed booby (*Sula nebouxii*). *Behav Ecol Sociobiol* 59:115–123
- Raymond M, Rousset F (1995) GENEPOP (version 1.2) population genetics software for exact tests and ecumenicism. *J Hered* 86:248–249
- Rice WR (1989) Analyzing tables of statistical tests. *Evol Int J org Evol* 43:223–225
- Selkoe KA, Toonen RJ (2006) Microsatellites for ecologists: a practical guide using and evaluating microsatellite markers. *Ecol Lett* 9:615–629