UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
POSGRADO EN CIENCIAS DEL MAR Y LIMNOLOGÍA

DINÁMICA DE UNA POBLACIÓN DEL CHORLO NEVADO (*Charadrius nivosus*) E IMPLICACIONES DEL COMPLEJO MAYOR DE HISTOCOMPATIBILIDAD EN SU ADECUACIÓN BIOLÓGICA

TESIS
(POR ARTÍCULOS CIENTÍFICOS)

QUE PARA OPTAR POR EL GRADO ACADÉMICO DE:
DOCTOR EN CIENCIAS
(BIOLOGÍA MARINA)

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(Graduación con trabajo escrito)

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Dinámica de una población de Chorlo nevado (*Charadrius nivosus*) e implicaciones del complejo mayor de histocompatibilidad en su adecuación biológica.

Que presenté para obtener el grado de **Doctorado** es original, de mi autoría y lo realicé con el rigor metodológico exigido por mi programa de posgrado, citando las fuentes de ideas, textos, imágenes, gráficos u otro tipo de obras empleadas para su desarrollo.

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Resumen

La información demográfica de una población, es relevante para estimar las tasas vitales, evaluar la tendencia poblacional o comprender el impacto de la estocasticidad demográfica y ambiental. Además, procesos demográficos influyen en la dinámica de genes de las poblaciones, lo que bajo ciertas circunstancias podrían reducir sus tasas de crecimiento poblacional. En este estudio, se evaluó la viabilidad de una población de Chorlo nevado que ha presentado cambios en su tamaño poblacional. Además, se analizó la contribución proporcional de las tasas vitales en las fluctuaciones del tamaño poblacional. El análisis de viabilidad indicó que la población presenta una probabilidad alta de desaparecer en un mediano plazo, siendo la supervivencia de adultos y crías los parámetros demográficos limitantes del crecimiento poblacional. Para poder evaluar si la diversidad genética estaba relacionada a componentes de adecuación biológica, se caracterizó los genes del Complejo Mayor de Histocompatibilidad (MHC por sus siglas en inglés) en ocho poblaciones a través de su distribución. Se observó una mayor diversidad alélica en el exón 3 del MHC clase I, con hasta cuatro loci presentes por individuo, en comparación con un solo gen (locus) para el MHC clase II. Estas diferencias, indican una fuerte presión selectiva por parte de patógenos intracelulares en esta especie. Finalmente, se investigó si la diversidad genética del MHC estaba asociada con la supervivencia y reclutamiento en crías del Chorlo nevado. No se encontró evidencia de que la diversidad del MHC confiera una ventaja a componentes de adecuación biológica en el Chorlo nevado en etapas tempranas de vida. Incluso, otros factores como la intensidad de cuidado biparental son más relevantes para la supervivencia de crías. Los descubrimientos en esta disertación nos ayudan a comprender que tasas vitales se deberían considerar en planes de manejo, para incrementar el tamaño poblacional en el Chorlo nevado. Adicionalmente, se contribuye al escaso conocimiento de la arquitectura y evolución de estos genes inmunes, dentro de los Charadriiformes. Finalmente, otros factores no genéticos, parecen ser más relevantes en la supervivencia de esta especie, al menos en etapas tempranas de vida. Este estudio ofrece nuevas perspectivas, de cómo el uso de genes inmunes para evaluar componentes de adecuación debe ser considerado con precaución, sobre todo cuando otros factores pueden tener un efecto más inmediato en la supervivencia.

Abstract

Demographic information on a population is relevant for estimating vital rates, assessing population trends, or understanding the impact of demographic and environmental stochasticity. In addition, demographic processes influence the gene dynamics of populations, which under certain circumstances could reduce population growth rates. In this study, we evaluated the viability of a Snowy Plover population undergoing changes in population size. In addition, the proportional contribution of vital rates to population size fluctuations was estimated. The viability analysis indicated that the population has a high probability of disappearing in the medium term, with adult and offspring survival being the demographic parameters limiting population growth. To evaluate whether genetic diversity was related to fitness components, the genes of the Major Histocompatibility Complex (MHC) in eight populations were characterized through their distribution. Greater allelic diversity was observed in exon 3 of MHC class I, with up to four loci present per individual, compared to a single gene (locus) for MHC class II. These differences indicate a strong selective pressure from intracellular pathogens in this species. Finally, we investigated whether MHC genetic diversity was associated with survival and recruitment in Snowy Plover offspring. We found no evidence that MHC diversity confers an advantage to the fitness in Snowy Plovers at early life stages. Moreover, other factors such as biparental care intensity are more relevant to offspring survival. This work contributes to understanding what vital rates should be considered in management plans to increase population size in Snowy Plovers. Additionally, it contributes to the scarce knowledge of the architecture and evolution of these immune genes within the Charadriiformes. Finally, other non-genetic factors seem to be more relevant in the survival of this species, at least in early life stages. This study offers new insights into how the use of immune genes to assess fitness components should be considered with caution, especially when other factors may have a more immediate effect on survival.

Capítulo 1. Introducción general

1.1. Pérdida de biodiversidad

La biodiversidad es el resultado de billones de años de evolución y se refiere a todas las formas de vida en nuestro planeta (Royal Society, 2021). Al mismo tiempo, hace referencia de cómo éstas formas de vida o especies, interactúan unas con otras en una determinada área o ecosistema (Royal Society, 2021). La biodiversidad es fundamental para los procesos que soportan la vida en nuestro planeta, incluyendo a los humanos. Sin una amplia variedad de animales, plantas y microorganismos, no es posible mantener los ecosistemas sanos de los cuales dependemos y que nos proporcionan alimentos, agua, refugio, medicinas, combustibles, etc. A su vez, la biodiversidad ayuda a limpiar la contaminación ambiental, influye en el clima y el ciclo de nutrientes que son indispensable para la vida (Levin, 2020).

La biodiversidad o la diversidad de vida desde el nivel de gen hasta el nivel de ecosistemas, se encuentra disminuyendo rápidamente alrededor del planeta (Díaz *et al.* 2019). En las últimas décadas, la biodiversidad del planeta ha disminuido como consecuencia directa e indirecta de las actividades humanas, lo que se refleja en un número indeterminado de especies que se han extinguido y otras tantas en las que sus poblaciones se encuentran disminuyendo o son más susceptibles a efectos estocásticos ambientales, demográficos y genéticos (Frankham *et al.* 2002; Frankham, 2003). Los principales factores que contribuyen a estas disminuciones son la sobreexplotación y la pérdida o alteración del hábitat por factores como cambios en el uso del suelo, contaminación, introducción de especies exóticas, cambio climático y perturbación (Frankham, 2003).

La extinción de una especie es el resultado más conspicuo de la pérdida de biodiversidad. Actualmente, el nivel y la tasa de pérdida de especies es tan grande que se le ha comparado con los ocurridos en otros periodos de extinciones en masa, como los ocurridos 65 millones de años atrás durante el Cretácico tardío; incluso se le ha denominado como “la sexta extinción masiva” (Lande 1988; Frankham *et al.*

2002; Ceballos *et al.* 2015). Sin embargo, antes de que una especie llegue a extinguirse, la diversidad genética entre poblaciones puede ser afectada de manera substancial en respuesta a factores demográficos. Se ha estimado que las poblaciones pueden llegar a extinguirse tres veces más rápido que una especie por completo (Hughes 1997; Manel y Holderegger, 2013), pues la fragmentación y declive en tamaño poblacional eventualmente conduce a la pérdida de diversidad genética y podría afectar su adecuación (Pertoldi *et al.* 2007).

La pérdida de biodiversidad genética influye sobre procesos evolutivos. Afectando en la capacidad de adaptación de los organismos, ante nuevas circunstancias en un planeta en rápida transformación (Mace y Purvis, 2008). Dado que, la diversidad genética es la materia prima de cambios evolutivos, de esta dependerá el potencial evolutivo de una especie y de sus poblaciones silvestres ante cambios ambientales, nuevas enfermedades, patógenos, competidores o depredadores y contaminación (Frankham, 1996). Por tal motivo, conservar la diversidad genética es de relevancia en biología evolutiva y de la conservación. Así, la Unión Internacional para la Conservación de la Naturaleza (IUCN por sus siglas en inglés) recomienda que la diversidad genética debe ser uno de los niveles de diversidad que es necesario conservar (Reed y Frankham, 2003).

1.2. Genética de la conservación

Durante las últimas décadas el papel de la genética en la conservación y la ecología ha sido fundamental para ayudar a comprender los efectos de la fragmentación del hábitat y sus consecuencias en la erosión genética y el riesgo de extinción. Estos aspectos, en conjunto con la dinámica de adaptación de una especie a nuevas condiciones ambientales, se ha integrado actualmente en una disciplina científica conocida como "genética de la conservación" (Pertoldi *et al.* 2007).

La genética de conservación integra los principios de la biología molecular, ecología, genética de poblaciones, modelación matemática y sistemática evolutiva (Wan *et al.* 2004). Esta disciplina refiere principalmente a los efectos que trae consigo la pérdida de diversidad genética y los cambios en la estructura de las poblaciones sobre la supervivencia a largo plazo de especies amenazadas o en peligro de extinción (Martínez-Cruz 2011). Su enfoque principal es el manejo adecuado de poblaciones pequeñas, la definición o delimitación de unidades de conservación o manejo, y comprender integralmente la biología de una especie con la finalidad de disminuir el riesgo de extinción de especies amenazadas (Frankham *et al.* 2002; Wan *et al.* 2004).

1.3. Diversidad genética

La diversidad genética se manifiesta en diferencias de los caracteres, donde podemos incluir la morfología (tamaño, forma, color, etc.), las respuestas fisiológicas (enzimas y otras proteínas) y el genotipo (variaciones en la secuencia de ADN) de los organismos (Frankham *et al.* 2002). Los genes son secuencias de nucleótidos en una región en particular de la molécula de ADN y la diversidad genética está dada, en su mayor parte, por ligeras variaciones en las secuencias de ADN de cada gen. Estas variaciones dentro el ADN pueden deberse a diversos factores. Se ha demostrado que la variación en la expresión de genes es un recurso importante de variabilidad biológica dentro y entre poblaciones (Skelly *et al.* 2009; Keren *et al.* 2016). La mayoría de los estudios se han enfocado a comprender como los cambios o diferencias en la secuencia del ADN resultan en la expresión de diferentes proteínas (Townsend *et al.* 2003). Estas variaciones en la cantidad y el tipo de proteínas pueden resultar en disimilitudes bioquímicas, morfológicas y conductuales, las cuales traduzcan en tasas diferenciales de reproducción y supervivencia de los individuos (Frankham *et al.* 2002).

La diversidad genética es crucial para mantener la adecuación de poblaciones al proveer de resistencia ante cambios ambientales a corto plazo y evolucionar en respuesta a estos cambios a largo plazo (Frankham *et al.* 2002). Generalmente, las poblaciones grandes presentan una elevada diversidad genética. Pero, en poblaciones amenazadas, con un número reducido de individuos, la diversidad genética es baja (Frankham *et al.* 2002). Estas poblaciones pequeñas y aisladas son susceptibles a la pérdida de su diversidad genética, como resultado de la deriva genética o bien como resultado de la endogamia. Bajo deriva genética, el número de las diferentes variantes de genes (alelos), aumentan o disminuyen por azar a través del tiempo. Con ciertos alelos que se pierden o bien son fijados en una población (Martínez-Cruz, 2011). Por su parte, la endogamia puede conducir a la depresión endogámica al incrementar la frecuencia de individuos homocigotos con alelos recesivos deletéreos, lo que se evidencia en una pérdida en la adecuación o éxito reproductivo (Keller y Waller, 2002; Whitehorn *et al.* 2011) y podría incrementar el riesgo de extinción de poblaciones locales (Frankham, 2005).

Desde la llegada de las investigaciones basadas en el ADN a la genética de la conservación, la diversidad genética se estimaba normalmente con marcadores presumiblemente neutros, como los marcadores mitocondriales, los microsatélites y los AFLP, debido a su alta variabilidad y disponibilidad (Leroy *et al.* 2018; Willi *et al.* 2021). La teoría neutral de evolución propone que la mayoría de cambios evolutivos a nivel molecular están determinados por el efecto de la deriva genética y la mutación, influenciadas principalmente por el tamaño de las poblaciones, pero también por la intensidad y direccionalidad del flujo genético entre ellas (Kimura, 1983) Estos marcadores son útiles para investigar procesos evolutivos a nivel individual y poblacional como la estructura y el tamaño efectivo poblacional, estimar las fuerzas e importancia de la mutación o recombinación, deriva genética, flujo de genes, diversidad dentro y entre poblaciones, y los efectos de la endogamia (Höglund, 2009). Históricamente, los estudios sobre diversidad genética y adecuación biológica han utilizado marcadores neutrales, principalmente debido al desafío que presenta encontrar genes sometidos a selección (adaptativos). Aunque en un principio se asumió que la variación neutral y la adaptativa se encontraban correlacionadas, esta correlación suele ser débil y en algunos casos, inexistente (Hedrick, 2001; Leroy *et al.* 2018). En contraste con los marcadores neutrales, la existencia de variación individual en marcadores funcionales es relevante para mantener la viabilidad de las poblaciones al brindarles capacidad de adaptarse a los cambios ambientales (Sommer, 2005; Höglund, 2009; Allendorf, 2017). En poblaciones que han sufrido cuellos de botellas, se ha observado que la pérdida de variación genética puede ser incluso más severa en genes adaptativos que en regiones neutrales del genoma (Holderegger *et al.* 2006; Agudo *et al.* 2012).

El mantenimiento de la variación genética en partes neutras del genoma, bajo fuerzas evolutivas no selectivas, suele depender principalmente de procesos de mutación, deriva genética, tamaño efectivo poblacional (N_e), tasas de recombinación y patrones de dispersión (flujo genético) (Ellegren y Galtier, 2016). En contraste, la capacidad de las poblaciones silvestres para mantener la variación en genes adaptativos depende de las presiones de selección implicadas (Sommer, 2005). Cambios genéticos de rasgos sometidos a selección, sugieren que la evolución adaptativa ocurre de manera común en poblaciones silvestres (Hendry *et al.* 2018). En la actualidad los científicos se han interesado en evaluar la diversidad de genes adaptativos por el potencial para la identificación de genes que causan enfermedades o que podrían mejorar el desempeño y, por ende, el rendimiento agropecuario (Kirk y Freeland, 2011).

Investigar cambios en genes adaptativos es importante, principalmente en dos contextos: Los cambios ambientales que actualmente experimentamos y la degradación genética. Ambos procesos actúan de manera constante provocando una mala adaptación y disminución de la adecuación biológica de los organismos (Hendry *et al.* 2018). La supervivencia de las especies podría ser determinada por una rápida adaptación, que solo podría ser posible si se ha mantenido un nivel adecuado de diversidad genética (Jump y Penuelas, 2005; Kirk y Freeland, 2011), debido a que solo la diversidad o variabilidad de genes adaptativos podría permitir adaptarse a cambios en las condiciones ambientales. Se esperaría que, la diversidad genética neutral y adaptativa tendrá diferentes impactos en la supervivencia y permanencia de una determinada población (Gebremedhin *et al.* 2009; Kirk y Freeland, 2011).

1.4. El Complejo Mayor de Histocompatibilidad

El análisis de loci adaptativos es más apropiado cuando se investiga la adecuación biológica en especies silvestres y los genes del Complejo Mayor de Histocompatibilidad (MHC por sus siglas en inglés) son excelentes candidatos para este tipo de estudios. El MHC es un complejo de genes de los más variables en el genoma de vertebrados (Piertney y Oliver, 2006) y tienen un papel fundamental en la lucha y eliminación de patógenos. La diversidad de genes del MHC parece estar relacionada con la adecuación biológica de los individuos en una población (Agudo *et al.* 2012), así como con procesos evolutivos, dentro y entre poblaciones (Sommer, 2005, Radwan *et al.* 2020); influye en características biológicas importantes para la viabilidad de poblaciones, tal como, éxito reproductivo, supervivencia y resistencia a patógenos (Bernatchez y Landry, 2003; Sommer, 2005; Schewensow *et al.* 2007; Wutzler *et al.* 2012).

Se ha planteado que la diversidad tan alta en genes del MHC, con respecto a otros genes o marcadores neutrales, puede ser producto de selección balanceadora mediados por patógenos a través de la combinación de tres mecanismos: ventaja de heterocigotos, ventajas de alelos raros y selección fluctuante (Aguilar *et al.* 2004; Sommer, 2005; Radwan *et al.* 2010).

Primero, la hipótesis de ventaja de heterocigotos sugiere que individuos heterocigotos tendrán una mayor adecuación que aquellos organismos homocigotos, especialmente si se enfrenta a múltiples patógenos (Pierini y Lenz, 2018). Esta suposición está basada en el antecedente teórico que individuos heterocigotos pueden ser capaces de detectar y presentar un mayor espectro de antígenos impulsado por la actividad de los patógenos, esto debido al mayor número de moléculas diferentes en el MHC (Bernatchez y Landry, 2003; Piertney y Oliver, 2006; Spurgin y Richardson, 2010).

Segundo, la hipótesis de ventajas de alelos raros propone que existe una fuerte selección del patógeno por superar los alelos más comunes del MHC que les confiere una mayor resistencia al hospedero (Lenz, 2018). Esto podría disminuir la adecuación de los hospederos con estos genotipos más comunes (Bernatchez y Landry, 2003). Por lo tanto, los alelos nuevos que surgen dentro de la población podrían ofrecer una mayor protección contra patógenos que los alelos más comunes (Bernatchez y Landry, 2003; Spurgin y Richardson, 2010). Un alelo puede disminuir en frecuencia dentro de una población debido a la evolución de la resistencia del patógeno, pero una vez que se convierte en alelo raro, la adaptación del patógeno puede disminuir o desaparecer, causando la ventaja selectiva del alelo y aumentar de nuevo (Lenz, 2018). Esto resulta en una carrera armamentista cíclica, donde patógenos y alelos del MHC fluctúan en frecuencia, manteniendo la diversidad vía este proceso dinámico (Spurgin y Richardson, 2010).

Tercero, la hipótesis de selección fluctuante menciona que la heterogeneidad espacial y temporal en el tipo y abundancia de patógenos a los cuales son expuestos los individuos podría mantener la alta diversidad del MHC (Migalska *et al.* 2022) Si el tipo de patógeno que enfrenta un organismo presenta fluctuaciones espacio-temporales, la intensidad de selección direccional en genes del MHC también fluctuaría. Esto dará lugar a que diferentes subconjuntos de alelos del MHC se seleccionen en un diferente punto en espacio y/o tiempo, y esto mantendría la diversidad genética a través de subpoblaciones (Spurgin y Richardson, 2010).

Una fuerte estructura teórica se ha establecido soportando la idea que cualquiera de estos tres mecanismos, o una combinación de estos, puede estar conduciendo a la alta diversidad del MHC (Spurgin y Richardson, 2010; Lighten *et al.* 2017; Migalska *et al.* 2022). Al mismo tiempo, otros mecanismos asociados a selección sexual como la selección de pareja basados en genotipo diferentes del MHC podrían mantener la alta diversidad del MHC (Piertney, 2006; Radwan *et al.* 2020). Tratar de entender cómo y por qué existe esta extraordinaria diversidad ha hecho del MHC un paradigma de la investigación en genética evolutiva y de la conservación (Radwan *et al.* 2010; Ujvari y Belov, 2011).

1.5. Estructura y función del MHC

La estructura y función del MHC ha sido bien estudiada durante los últimos 50 años. Los primeros estudios sobre el MHC se realizaron en mamíferos, sobre todo aquellos enfocados al antígeno leucocitario (HLA por sus siglas en inglés) en el proyecto del genoma humano. El HLA es la versión del MHC en humanos, está conformado por aproximadamente 4 millones de pares de bases) y representa la región más diversa dentro del genoma humano, con alrededor de 14,000 variantes alélicas conocidas alrededor del mundo (Robinson *et al.* 2016). Éste se encuentra casi en su totalidad localizado en el cromosoma 6 y se estima que cerca del 40% de los genes expresados tienen una función inmune (Hess y Edwards, 2002). A pesar de que la estructura general y función del MHC se ha conservado entre vertebrados, existen grandes diferencias en la organización genómica del MHC entre los diferentes linajes de vertebrados, especialmente entre mamíferos y aves (Kelley *et al.* 2005). En la mayoría de los linajes, mamíferos incluidos, los genes del MHC están duplicados y evolucionan de manera independiente en cada especie. En contraste, en las aves, los genes del MHC suelen ser similares en grupos de especies, lo que indica que las duplicaciones son recientes o un frecuente intercambio genético entre genes duplicados por recombinación o conversión de genes (Hess y Edwards, 2002).

Dentro de la región genómica del MHC existen dos familias principales de multigenes que codifican moléculas de unión-antígeno, las cuales están encargadas de presentar péptidos extraños a los receptores de superficie CD8+ y CD4+ de células T (Rock *et al.* 2016; Radwan *et al.* 2020). Estas familias son conocidas como genes del MCH clase I y clase II, y son esenciales para activar la inmunidad mediada por células, por lo que, se considera que este mecanismo se originó desde que evolucionó el sistema inmune adaptivo en vertebrados mandibulados alrededor de 500 millones de años atrás (Flajnik y Kasahara, 2010; Rock *et al.* 2016). Las moléculas del MHC están conformadas por dos dominios-Ig (inmunoglobulinas) rematados por dos hélices alfa paralelas que descansan sobre una plataforma de láminas plegadas beta. Esta conformación estructural genera un región o ranura de unión a péptidos (sitios PBR (por sus siglas en inglés) encargados de reconocer los antígenos derivados de patógenos) entre las hélices alfa (Piertney y Oliver, 2006; Rock *et al.* 2016).

El papel del MHC es reconocer proteínas extrañas derivadas de patógenos, estos genes codifican glicoproteínas que unen péptidos, tanto propios como extraños, en el interior de la célula y los llevan a la superficie para que sean inspeccionados por las células T y las células asesinas naturales (Natural Killer; NK), para iniciar una respuesta inmune especifica (Sommer, 2005; Radwan *et al.* 2020). Los genes del MHC clase I codifican para proteínas que son expresadas en la superficie de todas las células somáticas, excepto para ciertas neuronas y son encargan de presentar péptidos a los CD8 de células T citotóxicas, las cuales son asociadas con la defensa contra patógenos intracelulares como virus (Hughes y Yeager, 1998; Piertney y Oliver, 2006). El MHC clase I es heterodímero y consiste en una cadena pesada transmembrana α, y posee tres dominios extracelulares (α1, α2 y α3) y una molécula β_2 microglobulina (figura 1). La región de unión de péptidos consta de dos hélices α que bordean una hoja β plegada formada a partir de los dominios α1 y α2 de la cadena pesada (Hughes y Yeager, 1998).

Genes del MHC clase II se expresan principalmente en células presentadoras de antígenos del sistema inmune. Estas presentan antígenos a los CD4 de células T colaboradoras y son asociadas con infecciones inmunes derivados de patógenos extracelulares (Piertney y Oliver, 2006). Las células T liberan citoquinas que disparan una respuesta inmune adecuada, la cual puede incluir producción de anticuerpos (Hughes y Yeager, 1998). MHC clase II está organizado estructuralmente por una cadena α y una cadena β ambas como proteína de transmembrana. Cuenta con cuatro dominios extracelulares (similar al MHC clase I), pero dos de los dominios del MHC clase II son formados por cadenas α (α_1 y α_2) y dos dominios son cadenas β (β_1 y β_2) (figura 1). La región de unión de péptido es formada por una cadena α y una cadena β (Hughes y Yeager, 1998).

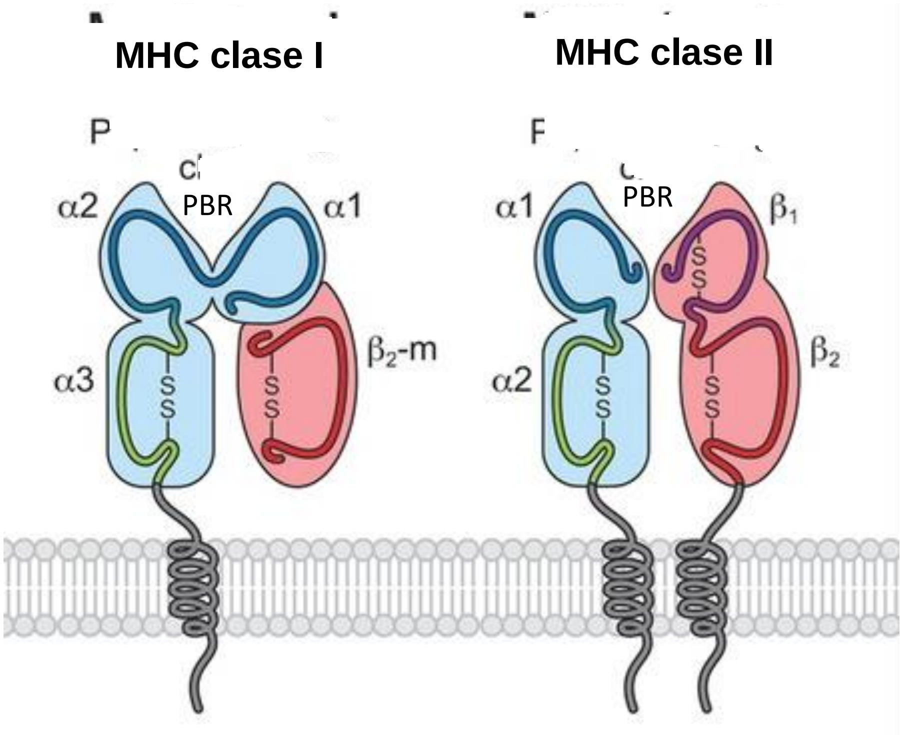

Figura 1. Representación esquemática de la estructura del MHC clase I (izquierda) y MHC clase II (derecha), PRB se refiere a la región de unión de péptidos. Figura tomada y modificada de Schumacher *et al.* 2017.

1.6. MHC en aves

En aves, la especie donde se ha estudiado el MHC de manera detallada es el complejo *B* y *Y* de la gallina (*Gallus gallus*) (Kaufman *et al.* 1999; Hess y Edwards, 2002). Comparado con los mamíferos el MHC de la gallina posee una región genómica densa de genes, cuenta con apenas 92 kb de longitud y contiene 19 genes, ubicada en el micro-cromosoma 16 con pocas copias del MHC clase I y II, es decir, 20 veces más pequeño que el MHC de humanos. Debido a estas diferencias entre el MHC de humanos y de la gallina es que se le ha nombrado al MHC de la gallina como el “mínimo esencial” (Kaufman *et al.* 1999; Westerdahl, 2007; Ekblom *et al.* 2011). Aves como el faisán de cuello anillado (*Phasianus colchicus*) y el urogallo negro (*Tetrao tetrix*) presentan similitud en la estructura y función del MHC de la gallina. Sin embargo, otras especies de aves suelen presentar un mayor número de genes del MHC clase I y II, y estas diferencias en estructura y función suelen ser más pronunciadas dentro del orden de los paseriformes (Westerdahl, 2007). Por ejemplo, la evidencia sugiere una fuerte selección del MHC clase I en paseriformes, mientras que, en aves no-paseriformes la selección actúa con mayor presión en el MHC clase II (Minias *et al.* 2018a). Indicando, que la dinámica para ambos MHC es controlada mayormente por las presiones de patógenos intra y extracelulares, que afectan de diferente manera a aves paseriformes y no-paseriformes, asociadas a las historias de vida, tamaño corporal o diferencias en tasas de evolución entre estos dos grupos de aves (Alcaide *et al.* 2013, Minias *et al.* 2018b).

En el orden de los paseriformes encontramos un alto número de genes y de mayor tamaño comparado con el de la gallina (Westerdahl *et al.* 2004b). Por ejemplo, se han reportado hasta 3,500 alelos del MHC clase I en el Carricero Común (*Acrocephalus schoenobaenus*) con un promedio de hasta 36.3 alelos por individuo (Biedrzycka *et al.* 2017), mientras que, en la Mascarita Común (*Geothlypis trichas*) se encontraron alrededor de 1,000 alelos del MCH clase II y un promedio de 27.7 alelos por individuo (Bollmer *et al.* 2012). Por otra parte, se ha reportado pseudogenes (genes no funcionales) para el MHC clase I y II en paseriformes, mientras que, en el MHC de la gallina no se han reportado pseudogenes (Westerdahl 2007). En aves rapaces se reportó que la arquitectura y complejidad de genes del MHC I se encuentran más estrechamente relacionados a la de la gallina, siendo el complejo clase I en rapaces simple y compacto, con un bajo número de loci clase I (de uno a dos) en comparación a paseriformes donde se ha reportado hasta 33 loci en un mismo individuo (Biedrzycka *et al.* 2017). De manera general, el MHC en paseriformes es más complejo en términos de duplicación de genes y diversidad que en aves no paseriformes, con un mayor número de genes, intrones grandes y la presencia de pseudogenes (Westerdahl, 2007, Alcaide *et al.* 2014; Minias *et al.* 2018b).

En el orden de los Charadriiformes (aves playeras, gaviotas y asociados) existen pocos trabajos sobre el MHC. Un estudio sobre los patrones de polimorfismos del

MHC en la Becacina Real (*Gallinago media*), encontraron que el número de genes, la longitud de las secuencias en los intrones y exones son intermedios entre aquellos de la gallina y los paseriformes (Ekblom *et al.* 2003). Cloutier (2012) provee la primera información acerca de la organización a nivel de genoma del MHC de los Charadriiformes y sustenta la compleja historia evolutiva de la organización del MHC en aves. En la caracterización de los genes del MHC clase I en *Calidris canutus*, se reportó 36 alelos en tan solo ocho individuos y evidencia de seis genes funcionales del MHC clase I en un solo individuo, esta alta diversidad del MHC en esta especie es sorprendente (posiblemente explicada por la variabilidad genética de las seis subespecies reconocidas y sus migraciones de largas distancias), sobre todo cuando se tiene en cuenta el bajo tamaño efectivo poblacional con el que cuenta esta especie (Buehler *et al.* 2013, Conklin *et al.* 2022). Por su parte, Pardal y colaboradores (2017) caracterizaron el MHC clase I en la Aguja Colinegra (*Limosa limosa*) mostrando que la diversidad y divergencias del MHC clase I en esta especie, es similar a lo antes reportado en otras aves del orden de los Charadriiformes, con hasta cuatro loci (2 a 7 alelos) por individuo y un máximo de 47 alelos provenientes de 84 individuos (Pardal *et al.* 2017).

1.7. El MHC en genética de la conservación

El nivel de impacto causado por el ser humano trae consigo consecuencias ecológicas y genéticas en las poblaciones de vertebrados y sus patógenos. La degradación de hábitat y la variación de las condiciones climáticas son factores determinantes en la distribución, trasmisión y desarrollo de patógenos (Sommer, 2005). Contar con niveles de variabilidad altos en el MHC es esencial por el potencial para responder ante nuevos desafíos de patógenos y asegurar la supervivencia a largo plazo de una especie (Bernatchez y Landry, 2003; Sommer, 2005; Ujvari y Belov, 2011; Hoelzel *et al.* 2019); se ha observado que los genes del MHC están asociados a la variación en la carga de parásitos, adaptación local, interacciones madre-feto y éxito reproductivo. Además, también se ha demostrado que el MHC influye en la elección de pareja, en función de lo que puede proveer a las crías de un repertorio adecuado del MHC (Ujvari y Belov, 2011).

Estudios en el Demonio de Tasmania (*Sarcophilus harrisii*) ha revelado la importancia de comprender mecanismos inmunes asociados al MHC para la conservación de esta especie. Durante las últimas dos décadas, esta especie ha desarrollado un tumor facial y sus poblaciones han enfrentado importantes disminuciones (Siddle *et al.* 2007). Un grupo de investigadores han encontrado que las células del tumor facial no expresan moléculas de superficie celular del MHC, debido a una baja regulación de genes que son primordiales para las vías y transportadores asociados con el procesamiento de antígenos (Siddle *et al.* 2013, Caldwell y Siddle, 2017). Conocer la estructura del MHC en esta especia, ha ayudado en desarrollar terapias inmunes y vacunas para ayudar a recuperar y conservar a este icónico marsupial de Australia. En otro estudio realizado en Pingüinos de las Galápagos (*Spheniscus mendiculus*) en el que se caracterizó y comparó la diversidad del MHC con otros sietes especies de pingüinos, se encontró que esta presenta los niveles más bajos como consecuencia de una historia demográfica con reducciones severas en tamaño poblacional (cuellos de botella) asociadas a eventos El Niño a través de cientos de años (Bollmer *et al.* 2007).

Se sabe que entre algunas especies con tamaño poblacional reducido o niveles bajos de diversidad genética neutral el MHC exhibe niveles altos de polimorfismo (Winternitz y Wares, 2013; Schwason *et al.* 2019). Aguilar y colaboradores (2004) realizaron un estudio en una población de zorros de la Isla de San Nicolás (*Urocyon littoralis dickeyi*), en esta especie se ha reportado que no existe variación genética en marcadores neutrales como microsatélites, y se piensa que esta nula variación en genes neutrales es debido a la reciente disminución de sus poblaciones —en la Isla de Santa Catalina la población disminuyó de 1,000 a 10 individuos en una sola generación a causa de una enfermedad, caso similar con la población de San Nicolás—. Sin embargo, en este estudio encontraron altos valores de heterocigocidad en cuatro de cinco loci muestreados (0.36, 0.000, 0.62, 0.57, 0.33) del MHC, estos valores fueron similares a los encontrados en las poblaciones más grandes, en otras islas, de esta especie (Aguilar *et al.* 2004). En el Chita (*Acinonyx* *jubatus*), se encontró que a pesar de que la diversidad alélica para ambas clases de MHC es baja, la especie es capaz de mantener un nivel de diversidad alélica lo suficientemente divergente para brindar protección contra patógenos (Schwason *et al.* 2019). Estos estudios, permiten sugerir que la selección es capaz de mantener la diversidad del MHC a pesar de fuerte deriva genética.

Como la selección actúa a escala espacial, brinda información acerca de procesos y el grado de adaptación local de poblaciones silvestres (Gonzalez-Quevedo, *et al.* 2016). Genes del MHC han sido utilizados como marcadores moleculares para estudiar la variación genética a escala geográfica (Bernatchez y Landry, 2003). En el Guppy (*Poecilia reticulata*) un estudio realizado en dos islas de origen geológico similar, separadas entre 10,000 a 15,000 años. Se encontró que en la isla de mayor tamaño presenta una mayor diversidad alélica respecto a la isla más pequeña (probablemente dado por una menor disponibilidad de ríos y bajas densidades observadas en los ríos de la isla de menor tamaño). A pesar de compartir algunos alelos entre las poblaciones en ambas islas. También, se encontró que existen alelos locales del MHC en ambas islas, esto se podría ser explicado por una alta tasa de "nacimiento" de alelos o a una alta probabilidad de retención (Herdegen-Radwan *et al.* 2021). En un trabajo sobre la variación geográfica del MHC en la Becacina Real (*Gallinago media*) se encontró una estructura geográfica a gran escala en el MHC de clase II entre regiones ecológicamente diferentes, la cual se atribuyó a la adaptación local hacia ciertos patógenos (Ekblom *et al.* 2007). Por otro lado, el análisis de marcadores genéticos MHC ha permitido inferir el origen y la conectividad migratoria entre poblaciones de aves, demostrando el uso de estos genes como una herramienta poderosa para la trazabilidad de organismos silvestres (Rodríguez *et al.* 2011).

Las reducciones en el tamaño de las poblaciones suelen ser causadas por procesos demográficos estocásticos antes que por causas genéticas (Lande, 1988; Allendorf y Luikart, 2007). Sin embargo, estos cambios en el tamaño poblacional afectan la composición genética, ya sea mediante procesos demográficos o como consecuencia de una respuesta selectiva (Allendorf y Luikart, 2007; (Pertoldi *et al.* 2007). Pese a la importancia de la variación genética en términos del potencial adaptativo, la interacción entre la diversidad genética, la demografía y los factores ambientales no se ha comprendido del todo en poblaciones silvestres (Wright, 2014).

Conocer los niveles de variabilidad genética nos permite evaluar si especies amenazadas presentan baja diversidad como resultado de disminuciones en poblaciones o cuellos de botellas, lo cual, podría incrementar la susceptibilidad a la extinción (Marsden, 2010). Pero, además, el uso de marcadores genéticos del MHC proporciona un mejor conocimiento acerca de la variación adaptativa, lo que permite evaluar el efecto de la selección, conocer sobre la adaptación local, la adecuación biológica y entender su relación con el potencial evolutivo en poblaciones silvestres (Marsden, 2010; Radwan *et al.* 2020).

1.8. Chorlo nevado (Charadrius nivosus) como modelo de estudio

El Chorlo nevado (*Charadrius nivosus*), es una especie de ave playera del orden Charadriiformes, de talla pequeña (15-17 cm largo, 30-48 gr) y nativa del continente americano (Küpper *et al.* 2009), algunas poblaciones reproductoras del interior de Norte América suelen migrar en su totalidad hacia zonas costeras como California y Baja California, mientras algunos individuos de poblaciones reproductoras costeras suelen presentar individuos que migran e individuos que permanecen todo el año (Page *et al.* 1995).

Su distribución está bien documentada para Norte América y comprende desde la costa del Pacífico en el Sur de Canadá hasta México, incluyendo el Golfo de California, así como la costa del Golfo de México y las planicies y zonas altas del centro de Estados Unidos y México (Thomas *et al.* 2012). Sin embargo, se sabe que existen también poblaciones reproductoras en la costa del Pacífico en Centro y Sudamérica hasta Perú (figura 2). Su distribución es amplia pero discontinua, debido principalmente a la dependencia del uso de costas y cuerpos de agua salinos, que en muchas zonas han sido afectados negativamente por las actividades humanas (Thomas *et al.* 2012). Para Norte América se estima una población de 23,500 individuos, mientras que específicamente para México son aproximadamente 2,400 individuos reproductores y, actualmente, sus poblaciones se encuentran en declive debido a la degradación y pérdida de hábitat por el desarrollo humano y especies invasoras (Page *et al.* 2009; Thomas *et al.* 2012). Debido a lo anterior, el Chorlo nevado se encuentra sujeto a protección por las autoridades de Estados Unidos (Page *et al.* 2009) y las autoridades mexicanas (NOM 059 ECOL 2010).

El Chorlo nevado presenta un sistema de apareamiento polígamo, en el cual las hembras de muchas de las poblaciones, y con menor frecuencia los machos, desertan de sus primeras nidadas pocos días después de la eclosión de las crías con la finalidad de buscar una nueva pareja (Warriner *et al.* 1896; Page *et al.* 2009), Dejando el cuidado parental a cargo de uno de los padres (frecuentemente los machos). Generalmente, el tamaño de nidada es de tres huevos y con menor frecuencia dos huevos (Page *et al.* 2009). Las crías son precociales y dejan sus nidos a las primeras pocas horas de haber eclosionado, por lo que enfrentan ambientes hostiles casi inmediatamente después de su eclosión. Los padres guían a sus crías a territorios donde se alimentan por si solas, estos territorios de alimentación suelen ser abiertos o con vegetación esparcida, y las familias y sus crías pueden ser seguidas fácilmente (Küpper *et al.* 2010).

Algunas poblaciones de Chorlo nevado han sido monitoreadas durante las últimas décadas. Ambos sexos tienen alta fidelidad a los sitios de reproducción y un alto porcentaje de las crías regresan como reproductores a sus sitios natales en años subsecuentes (Stenzel *et al.* 2011; Küpper *et al.* 2012). Un estudio realizado por Funk y colaboradores, logró identificar tres subespecies. Pero, no encontraron evidencia de estructura genética para las poblaciones de Norteamérica (*nivosus*), indicando un elevado flujo entre poblaciones de Norteamérica. No obstante, se evidenciaron diferencias genéticas entre las poblaciones del Caribe (*tenuirostris*) y Sudamérica (*occidentalis*) con respecto a las poblaciones de Norteamérica (Funk *et* *al.* 2007). Recientemente, un estudio donde se utilizaron cuatro tipos de marcadores genéticos reafirmó las tres subespecies propuestas por Funk y colaboradores. Sin embargo, este estudio aporta evidencia que los individuos de Florida están más relacionados con poblaciones de la subespecie *nivosus* (Oeste de Norteamérica) y no con *tenuirostris* (Caribe), como antes se les había asignado a las poblaciones de Florida (D'Urban Jackson *et al.* 2020). Como resultado, tenemos representados cuatro demes en este estudio, con las poblaciones de Florida considerándose como parte de la subespecie *nivosus* del Este en Norteamérica (D'Urban Jackson *et al.* 2020).

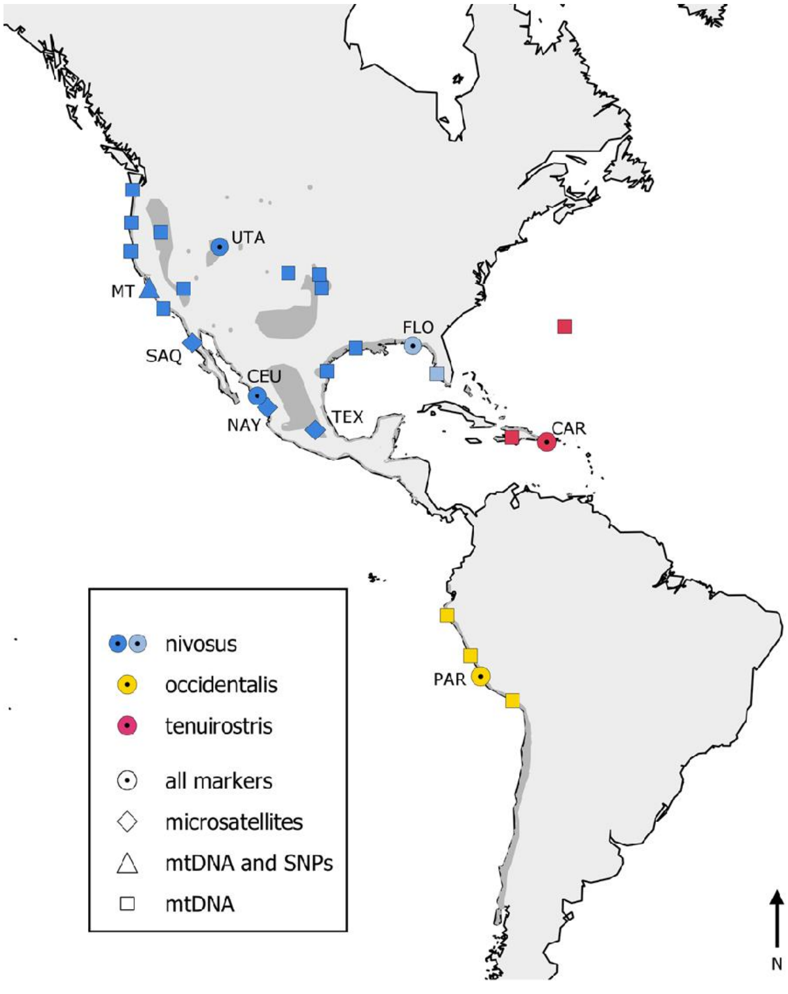

Figura 2. Rango de distribución del Chorlo nevado (gris obscuro) y los cuatro demes identificados por el estudio de D'urban Jackson y colaboradores. La identidad de los

demes es dada por los colores (azul: *nivosus* del Oeste, azul claro: *nivosus* del Este, rojo: *tenuirostris* y amarillo: *occidentalis*). Tomado de D'urban Jackson *et al.* 2020.

Desde el 2006 se ha llevado a cabo esfuerzos de monitoreo e investigación en una población reproductora del Chorlo nevado en el noroeste mexicano (Cruz-López *et al.* 2017). Gracias al esfuerzo de captura de adultos reproductores y crías se ha logrado marcar cerca del 95% de los individuos que se reproducen en el área. Cada individuo ha sido marcado con una combinación única de anillos de color y un anillo metálico con código único, lo que ha permitido dar seguimiento a lo largo de la temporada reproductiva anual y entre temporadas. Este seguimiento a largo plazo, ha permitido registrar diferentes parámetros demográficos y obtener datos sobre la historia de vida de ~ 95% de los individuos reproductores. Adicionalmente, en esta población se han obtenido muestras de sangre para cada individuo capturado (adultos y crías) desde el 2006.

Durante los últimos años se ha logrado establecer una red de trabajo del Chorlo nevado en Norte América, contando con la participación de nueve grupos de trabajo en México y uno más en Estados Unidos. Esto ha permitido cubrir en su mayor parte la distribución del Chorlo nevado en Norte América y contar con muestras genéticas para las poblaciones bajo el escrutinio de esta red de monitoreo. Además, la colaboración con otros grupos de trabajo en Estados Unidos, el Caribe y Sudamérica permitió la obtención de muestras de ADN prácticamente a lo largo y ancho de su distribución.

Se ha documentado que muchas de las poblaciones del Chorlo nevado presentan disminuciones a lo largo de su distribución en Norte América (Page *et al.* 2009), pero se desconocen los efectos de estas disminuciones en los niveles de variabilidad genética de la especie. Conocer la variabilidad genética dentro y entre poblaciones es importante para evaluar su estado de conservación y diseñar o adecuar los planes de manejo de especies en alguna categoría de riesgo, particularmente cuando se usan marcadores genéticos que podrían estar relacionados directamente con la adecuación biológica de los individuos de una población.

La mayoría de los trabajos de investigación sobre el Chorlo nevado provienen de poblaciones de Estados Unidos y se sabe muy poco de esta especie en otras regiones. Por ello es necesario obtener información sobre tendencia poblacional y la variabilidad genética de genes adaptativos como los del MHC, con lo que se podría saber si estos genes están relacionados con la adecuación biológica individual en una especie que se encuentra amenazada, prácticamente en todo su rango de distribución.

Objetivos de la tesis

Objetivo general

Investigar como factores demográficos y genéticos podrían estar asociados con la historia de la dinámica poblacional del Chorlo nevado, una especie de ave playera que presenta disminuciones en gran parte de su rango de distribución.

Objetivos particulares

1. Evaluar como las diferentes tasas vitales y estrategias reproductivas afectan los cambios en el tamaño poblacional y la viabilidad de una población de Chorlo nevado en la región neotropical de Norteamérica.

2. Caracterizar el Complejo Mayor de Histocompatibilidad clase I y II, para indagar en los procesos evolutivos que pudieran estar moldeando la variabilidad y arquitectura de los genes inmunes (adaptativos) en el Chorlo nevado.

3. Investigar como diversas medidas de variabilidad genéticas o alelos específicos del Complejo Mayor de Histocompatibilidad clase I y II, se relacionan con la supervivencia y reclutamiento en crías de Chorlo nevado.

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Capítulo II. La difícil situación de un Chorlo: Viabilidad de una importante población de Chorlo nevado con cuidado flexible de sus crías en México.

Chapter II. The plight of a plover: Viability of an important snowy plover population with flexible brood care in Mexico.

Medardo Cruz-López, Luke J. Eberhart-Phillips, Guillermo Fernández, René Beamonte-Barrientos, Tamás Székely, Martín A. Serrano-Meneses, Clemens Küpper.

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Resumen

Las poblaciones de aves playeras suelen depender de hábitats de humedales, por lo cual son consideradas importantes indicadores de la salud de los ecosistemas. Las poblaciones que residen en latitudes bajas continúan siendo muy poco estudiadas en comparación con poblaciones de latitudes altas. En este trabajo utilizamos censos y observaciones detalladas de comportamiento, en combinación con modelos específicos para cada etapa de vida, para predecir la trayectoria poblacional de una población de Chorlo nevado (*Charadrius nivosus*) en Bahía de Ceuta, Sinaloa, México. En Norteamérica esta ave playera se encuentra amenazada y con evidencia de que muchas de sus poblaciones están disminuyendo. El modelo matricial estocástico empleado para la población de Ceuta exhibió un ajuste muy bueno al historial de observaciones en campo y sugiere que la población se encuentra en un sumidero, con una probabilidad del 99,8% de extinguirse en 25 años. La supervivencia aparentemente baja de los adultos y que disminuyó a lo largo del periodo de estudio, probablemente como consecuencia de éxito reproductivo reducido y/o a la emigración permanente asociada a la degradación del hábitat, fue el factor de mayor impacto en la trayectoria de la población. Recomendamos acciones urgentes de manejo del hábitat para hacer

frente a la volatilidad de los niveles de agua y aumentar el éxito reproductivo de los individuos en este sitio tan importante para la especie. Reconocer los efectos relativos del cuidado flexible de las crías sobre la adecuación biológica individual y la dinámica de la población plantea un intrigante dilema para la conservación. Observamos que el sistema de cuidado parental flexible del Chorlo nevado afecta a la supervivencia de las crías: las nidadas abandonadas por hembras poliándricas poco después de la eclosión presentan una supervivencia significativamente menor que las nidadas no abandonadas o las abandonadas tardíamente; en general, las hembras que desertaron criaron menos volantones que las hembras que cuidaron la nidada. En conjunto, nuestro estudio revela una variación insostenible en la dinámica de las tasas vitales locales. Para comprender cómo contribuye esta población a la dinámica fuente-sumidero regional, futuras investigaciones deberían evaluar la importancia de la inmigración y la emigración entre poblaciones vecinas.

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The plight of a plover: Viability of an important snowy plover population with flexible brood care in Mexico

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ABSTRACT

Shorebird populations often rely on wetland habitats, for which they are considered important indicators of ecosystem health. Populations residing at low latitudes remain vastly understudied in comparison with populations from high latitudes. Here we use detailed behavioural and demographic observations during all life stages in combination with stage specific modelling to predict the population trajectory of a snowy plover (*Charadrius nivosus*) population at Bahía de Ceuta, Sinaloa, Mexico. In North America this shorebird is threatened, with many monitored populations declining. Our stochastic matrix model for the Ceuta population, which closely matched our field observations, suggests that the population is a sink with a 99.8% probability of going extinct within 25 years. Low apparent adult survival, which declined over time presumably because of poor reproductive success and/or permanent emigration in response to habitat degradation, had the largest impact on the population trajectory. We recommend urgent habitat management actions to address volatile water levels and hence increase reproductive success of this species at this important breeding site. Acknowledging the relative effects of flexible brood care on individual fitness and population dynamics presents an intriguing dilemma for conservation. We found that the flexible parental care system of snowy plovers affected chick survival: broods deserted by polyandrous females early after hatching had significantly lower survival than broods not deserted or those deserted late. Overall, deserting females raised fewer fledglings in this population than females that cared. Taken together, our study reveals unsustainable variation in local vital rate dynamics. To understand how this population contributes to regional source-sink dynamics, future research should evaluate the importance of immigration and emigration among neighbouring populations.

1. Introduction

Wetlands cover approximately 6% of Earth's surface and harbour high levels of biodiversity (Junk et al., 2013). Recent estimates suggest that >50% of wetlands worldwide have been lost, which will detrimentally affect the diversity and persistence of wetland biota (Zedler and Kercher, 2005). For example, 40% of North American species that show population declines depend on coastal habitats such as wetlands (NABCI, 2016). Most shorebirds (order Charadriiformes) rely heavily on wetland habitats for food, reproduction and survival meaning that their populations are important indicators of wetland health worldwide (Thomas et al., 2006). Habitat loss and degradation, predation, and invasive species competition are identified as key threats to shorebird populations (Cruz et al., 2013) with low chick and adult survival negatively impacting breeding populations (Koivula et al., 2008; Larson et al., 2002; Rickenbach et al., 2011).

Most information on shorebird population dynamics comes from breeding populations in temperate and arctic zones of the northern hemisphere (Bart et al., 2007; Laaksonen and Lehikoinen, 2013;

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Thomas et al., 2006; Lindström et al., 2015). By contrast, there are only a few studies from tropical and subtropical breeding populations, which are typically located in developing countries where monitoring is less well established. Wetlands in these regions often harbour the highest level of biodiversity (Sekercioglu et al., 2012). Moreover, human populations are projected to increase particularly in the coastal zones of developing countries (Neumann et al., 2015), which will further increase pressures on these wetlands and shorebird populations.

The snowy plover (*Charadrius nivosus*) is a near threatened shorebird native to the Americas (Küpper et al., 2009; BirdLife International-IUCN, 2016). It is one of the least abundant shorebirds in North America (estimated population size: 25,869) with many populations in decline and requiring intensive management (Thomas et al., 2012). Coastal snowy plover populations are especially at risk due to the threat of rising sea levels caused by climate change (Aiello-Lammens et al., 2011). Mexico harbours 9% of the entire North American snowy plover population (Thomas et al., 2012) and they occur in a variety of habitats from the Pacific coast to the Gulf of Mexico (DeSucre-Medrano et al., 2011; Galindo-Espinosa and Palacios, 2015; Mellink et al., 2009). The snowy plover has been listed as federally 'threatened' in Mexico since 2010 (SEMARNAT, 2010).

Snowy plovers exhibit a flexible mating system, dominated by female polyandry and uniparental male care (Warriner et al., 1986). Typically, females desert broods remate with a different male; sometimes even dispersing to other sites within the breeding season to search for new mates (Stenzel et al., 1994). Mating system and sex-biases can influence population growth dynamics via reproductive constraints on the limiting sex (Bessa-Gomes et al., 2004). A recent study suggests that this polyandrous mating system is driven in part by an adult sex ratio bias caused by male-biased survival (Eberhart-Phillips et al., 2017).

Since parental care improves offspring survival and reproductive success (Clutton-Brock, 1991), variation in parental care may have severe implications on population dynamics. For example, variation in parental care may affect offspring survival (Székely and Cuthill, 1999), a major vital rate that is important for population growth. Yet this variation is largely ignored when assessing population viability. Quantifying the costs that desertion has on chick survival provides an important first step to understand how individual behaviour modulates population dynamics.

A comprehensive survey conducted in 2007 concluded that one of most important snowy plover breeding populations in Mexico is located at Bahía de Ceuta (hereafter "Ceuta"), Sinaloa, a coastal wetland protected by the RAMSAR convention (Thomas et al., 2012). The snowy plovers at Ceuta are polyandrous (60% of females mate with more than one male per season, including first year breeders), and the population has a male-biased adult sex ratio estimated from surveys and population matrix modelling (mean ASR: 0.60–0.63, Carmona-Isunza et al., 2017; Eberhart-Phillips et al., 2017). Mean local tenure of snowy plovers is 2.7 years and the oldest individual recorded at Ceuta is at least 10 years old. Since annual monitoring efforts commenced in 2006, the population has shown dramatic fluctuations in population size probably caused by annual variation in environmental conditions and habitat changes. The main habitat change documented was the 150 m expansion of mangrove vegetation. This expansion increased sedimentation of the substrate, which in turn reduced the water storage capabilities of the salt flats and evaporation ponds where the plovers breed.

In this study, we aimed to quantify the viability of this population. Using seven years of detailed breeding surveys we (1) investigated temporal variation in the breeding population size and nesting activity, (2) identified factors explaining variation in survival during critical life stages, (3) evaluated the effect of parental desertion on offspring survival, (4) forecasted the threat of extinction, and (5) conducted sensitivity analyses that highlighted the most important vital rates contributing to past and future population changes.

2. Material and methods

2.1. Study area

We studied snowy plovers breeding at Ceuta, a subtropical lagoon (annual average temperature: 23.3 °C, annual average precipitation: 473 mm) surrounded by mangrove forests. Ceuta is located on the coastal plain of Sinaloa in northwest Mexico (23°54′N, 106°57′W, Fig. 1a). The plovers concentrate breeding mainly on salt flats that contain a number of abandoned evaporation ponds. This habitat (hereafter "flats") is characterised by open substrates with sparse vegetation. The breeding season starts when flood water recedes at the beginning of April and concludes usually by mid-July when rains and high tides flood the flats again. In drought years or at the end of the breeding season, plovers have been observed nesting and tending broods in a section of the lagoon northwest of the main study area where water bodies are accessible throughout the breeding season to snowy plover families (CK, MC-L, unpublished data). This area is separated from the flats by a stretch of mangrove forest (Fig. 1b).

2.2. Data collection

We monitored plovers daily between April and July from 2006 until 2012 using methodology described by Székely et al. (2008). We used a car and mobile hides to search for nests, identifying incubating plovers with binoculars or scopes. The coordinates of each nest (±3 m) were recorded with a hand-held GPS device (Garmin, USA). For each nest, we estimated laying date and calculated hatching date by floating the eggs in lukewarm water assuming an incubation period of 25 days (Piersma and Wiersma, 1996). Clutches were revisited every 3–5 days and we documented causes of nest failure (i.e. depredated, flooded, abandoned, or unknown). After 20 days, we visited clutches daily to mark chicks before they left the nest.

Adults were captured using a funnel trap during the incubation period and marked with a metal ring and a unique colour combination of 3–4 darvic rings. Adult sex was established using plumage, molecular and/or behavioural characters (Vincze et al., 2017). We regularly resighted banded plovers to document individual movements and survival within and between years. Most chicks (90%) were captured in the immediate vicinity of the nest at hatching day, and 4% of chicks were captured and marked at opportunistic encounters with their parents. All captured chicks were marked with one metal and one colour ring, which allowed us to individually identify them based on their colour ring and the unique ring combination of their tending parents (Székely et al., 2008). We resighted broods approximately every other day to monitor daily survival over the course of the breeding season. Active broods were followed for a period of 25 days since hatch, after which we assumed fledging (earliest fledging occurred at 22 days, CK unpublished data). For each brood resighting we recorded the identity of the tending parent and the chicks present (Székely and Cuthill, 1999). We classified broods as deserted if one of the parents was not present in two consecutive encounters that were at least one day apart. We calculated the desertion date as the mean date between the last date that both parents were seen attending the brood and the first date that only one parent was tending the chicks. We searched the study area extensively to confirm that missing broods had not moved elsewhere and concluded that the last unfledged chicks had died if the tending parent(s) was seen alone and did not display any alarming behaviour when we approached it.

2.3. Statistical analyses

2.3.1. Population trends

We evaluated temporal trends using generalized linear models (GLMs). The annual number of nests, number of male and female

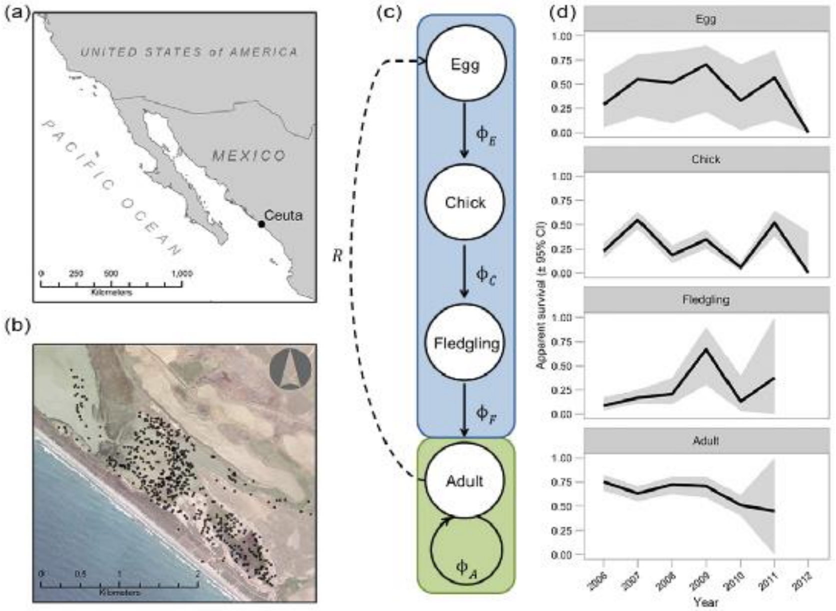

Fig. 1. (a) Location of Bahía de Ceuta, Mexico. (b) Snowy plover nests distribution at Ceuta from 2006 until 2012. (c) Life cycle used in the projection matrix model. Transition probabilities between life stages are egg survival (ϕ_E), chick survival (ϕ_C), fledgling survival (ϕ_F), adult survival (ϕ_A) and fecundity (*R*). Each coloured polygon represents one year, the projection interval of the model. (d) Annual variation in the apparent survival of eggs, chicks, fledglings, and adults between 2006 and 2012. Note that fledgling and adult survival is expressed as the survival from year *t* to year $t + 1$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

breeders captured were used as response variables using a quasi-Poisson distribution and log link function to account for overdispersion.

To assess within and between year trends in reproductive success, we used a generalized linear mixed model (GLMM) to describe annual variation in the number of fledglings per breeding male and female. The models were fitted with a Poisson distribution and a log link function. Only chicks with known fates (i.e. fledged or died) were used for this analysis. Standardised hatch date of brood per male and year were fitted as fixed effects. Since some males had more than one nest within a year, we added male ID as a random effect to account for the non-independence of siblings. The GLMM was constructed using the R package "lme4" (Bates et al., 2015).

We tested whether causes of nest failure changed over the time in the study site by employing log-likelihood ratio tests (G-test) with a Bonferroni correction (Blackmer et al., 2004). In a separate analysis we tested whether the cause of nest failures differed between early (2006 and 2007) and later years (2011 and 2012) of the study.

2.3.2. Vital rate estimation

We estimated five stage-specific vital rates describing important transitions in life history (Fig. 1c). These included egg survival (ϕ_E, probability that an egg will hatch), chick survival (ϕ_C, probability of a chick fledging), fledgling survival (ϕ_F, probability that a fledgling raised in year *t* will recruit into the adult population in year $t + 1$), adult survival (ϕ_A, annual survival and retention of adults in the population), and fecundity (*R*, annual average number of eggs attended by an adult male).

To quantify vital rates, we employed mark-recapture models, which account for temporal and stage-specific variation in encounter and survival probabilities, imperfect detection of marked individuals during surveys, and the inclusion of individuals with unknown fates. We conducted egg and chick survival analyses separately from their respective survival analyses of fledgling and adults because of differences in the temporal duration of encounter intervals (i.e. 25 days vs. one year, respectively; Fig. 1c). Chicks that were known to have died before fledging were not included in the analyses of fledgling and adult survival. All survival models were constructed with the R package "RMark" (Laake and Rexstad, 2008), and subsequently analysed using maximum likelihood estimation in program MARK (White and Burnham, 1999).

Daily nest survival (DNS) was estimated based on variation in DNS as a function of year, nest initiation date, and nest age including all relevant two-way additive and interactive combinations (Table S1). The DNS parameter estimate of the top model was raised to the power of 25, the incubation time, to transform DNS to apparent hatching success.

We estimated daily chick survival (DCS) using Cormack-Jolly-Seber models with one-day encounter intervals modelled as a function of year, hatch date, and age. We first assessed the most appropriate structure of encounter probability (*p*) by fitting the three aforementioned variables and evaluating their ΔAIC_c support while holding DCS constant (Colwell et al., 2013). We then estimated variation in DCS as a function of all relevant two-way additive and interactive combinations of the three variables considered (Table S2). The DCS estimate of the top model was raised to 25, the brooding period, to transform DCS to apparent fledgling success. We estimated annual apparent survival of fledglings and adults using a similar approach but used one-year encounter intervals. We modelled apparent survival as a function of age (i.e., fledgling or adult) and year, with additive and interactive combinations included (Table S3).

2.3.3. Influence of parental care on chick survival

We used survival records of chicks to determine the influence of temporal and behavioural covariates on survival by employing Cox proportional hazards mixed regression models (CPHMs) implemented in the R package "coxme" (Therneau, 2009). By fitting brood ID as a random effect we accounted for non-independence of chick survival of the same family. Chick death was the terminal event. Chicks that reached the fledging age or families with unknown chick fates were censored (Küpper et al., 2010). Our covariates of the initial model included desertion age (i.e. age of the oldest chick at which one of parents abandoned the brood), standardised hatching date, and body mass of chicks at hatching. We removed nonsignificant terms during model simplification. In CPHMs, the statistic of interest is the hazards ratio exp (β), which provides a direct measure for the difference in death hazard between groups; when exp $(\beta) < 1$, the hazard decreases, whereas it increases when exp $(\beta) > 1$ (Rivers et al., 2012). For graphical presentation only, we split covariates of interest into two groups that describe the age of desertion (≤7 and >7 days, respectively). We chose this cut-off based on observations that the first week is critical for chick survival since (i) 47% of chicks with known fate died during the first seven days and (ii) 77% of brood desertions occurred within one week after hatch (MC-L & CK, unpublished data).

2.3.4. Population viability

Population viability and extinction risk were assessed using projection matrix models (Caswell, 2001). Our population projection matrix model is described by the equation:

$$\boldsymbol{n}(t+1) - \mathbf{A}n(t)$$

where t is an annual time step, $\boldsymbol{n}$ is a vector of the population distributed across life stages and the 2×2 matrix $\mathbf{A}$ is expressed as:

$$\begin{pmatrix} 0 & R \\ \phi_E \times \phi_C \times \phi_F & \phi_A \end{pmatrix}$$

where transition probabilities (ϕ) between life stages are survival of eggs (ϕ_E), chicks (ϕ_C), fledglings (ϕ_F), and adults (ϕ_A). Fecundity (R) is annual average per capita number of eggs attended adult males. We implemented both deterministic and stochastic versions of the matrix model. The deterministic model used a matrix built from the seven-year averages of each vital rate whereas the stochastic model incorporated annual variation in vital rates by using year-specific matrices to maintain the covariation structure among vital rates.

To assess the validity of our model structure, we ran the stochastic simulation with a starting population structured the same as what was observed in 2006 (i.e., $N_E = 454$, $N_A = 204$) and projected 10,000 iterations over the 7-year period. We then compared the distribution of final population sizes from the simulation to the actual population size recorded in 2012 (Fig. S1a). We also visually inspected the distributions of the coefficient of annual variation (CV) and the final population sizes of all iterations in the simulation by comparing them with the observed coefficient of annual variation and final population size observed in the population over the 2006 to 2012 study period (Fig. S1b).

We evaluated population viability using three common metrics: 1) deterministic population growth, 2) stochastic extinction probability, and 3) a sensitivity analysis of population growth (λ) to changes in vital rates. We calculated deterministic population growth as the dominant eigen-vector of the deterministic matrix $(\hat{A}_{2007-2012})$. Stochastic population extinction risk was estimated by projecting the current population into the future with a randomly drawn matrix for each annual time step. We ran 10,000 iterations of the stochastic simulation, with each annual matrix (i.e. $\hat{A}_{2007}, \hat{A}_{2008}, \ldots, \hat{A}_{2012}$) being randomly drawn with an equal probability at each time step. We estimated extinction probability by determining the proportion of iterations that hit population size of two at 10, 25, 50, and 100 years after the last year of study. We calculated the deterministic sensitivities and elasticities of lambda to lower-level vital rates using partial derivatives. All statistical analyses and population viability models were conducted using the R version "Supposedly Educational" (R Core Team, 2016). All means are expressed with ± one standard error (SE) or standard deviation (SD). We provide all computer code and documentation as a PDF file written in Rmarkdown (Supplementary Material Appendix A) together with all raw datasets needed to reproduce the analysis (Supplementary Material Appendix B).

3. Results

3.1. Population trends

A total of 231 females and 215 males were individually marked and 659 nests were monitored. The number of breeding females (GLM: $\beta = -0.178 \pm 0.046$ SE, $t_6 = -3.862$, $P = 0.011$), breeding males ($\beta = -0.165 \pm 0.042$ SE, $t_6 = -3.871$, $P = 0.011$) and nests ($\beta = -0.196 \pm 0.045$ SE, $t_6 = -4.341$, $P = 0.007$) declined over the study period reducing the population size from 204 in 2006 to 58 breeding adults in 2012. A decline was also detected by the deterministic matrix model ($\lambda = 0.859$). The stochastic matrix model predicted an average final population size of 52.12 ± 35.69 SD (Fig. S1a). The average coefficient of variation of all 10,000 iterations was 0.61 ± 0.31 SD, compared to an coefficient of variation of 0.42 observed between 2006 and 2012 (Fig. S1b).

3.2. Temporal patterns of reproductive success

The average number of fledglings per male (0.980 ± 0.073 SE) did not vary significantly over the study period (GLMM: $\beta = -0.017 \pm 0.041$ SE, $Z = -0.423$, $P = 0.673$). Nor did this vary annually for females ($\beta = 0.014 \pm 0.043$ SE, $Z = 0.327$, $P = 0.744$). Males breeding early in the season had significantly more fledglings than those breeding later in the season (1.153 ± 0.085 SE vs 0.471 ± 0.119 SE, respectively; $\beta = -0.457 \pm 0.105$ SE, $Z = -4.346$, $P < 0.001$). Likewise, females with early broods produced more fledglings ($\beta = -0.582 \pm 0.110$ SE, $Z = -5.288$, $P < 0.001$).

3.3. Nests fate and survival

Variation in DNS was best described by an interaction between temporal variation between and within years ($w_i = 0.92$, Table S1). DNS^{25} varied over the study period with a maximum of 0.706 in 2009, a minimum of 0.00001 in 2012, and an average of 0.453 ± 0.08 SE (Fig. 1d). The fate of nests (i.e. successful vs. failure) changed over the study period (G-test: $G = 13.82$, $df = 6$, $P = 0.030$), with more successful nests in early years compared to latter years. The causes of nest failure also changed over time (G-test: $G = 68.31$, $df = 3$, $P < 0.001$) with predation being the most common cause of failure in early years and flooding being the most common cause of failure in late years.

3.4. Chick survival and brood desertion

Over the seven year study period, only five chicks known to have survived the first week evaded our capture efforts. In total we marked 895 chicks, with 310 (34.6%) chicks dying before fledging, 229 (25.6%) fledged, and the fate of 356 (39.8%) chicks were unknown. DCS was best described by an interaction between temporal variation between and within years (Table S2), this model received maximum support ($w_i = 1$). Average DCS^{25} was 0.27 ± 0.08 SE (Fig. 1d), with desertion, hatch date, and year being strongly associated with chick survival. Desertion increased chick death hazard by 8% per day (Fig. 2, CPHM: $\beta = -0.076 \pm 0.014$ SE, $Z = -5.49$, $P < 0.001$). Females that deserted the first brood before the chicks reached an age of at least one week produced significantly fewer fledglings than females that stayed to care for this period (0.900 ± 0.075 SE vs 1.666 ± 0.172 SE, respectively; GLMM: $\beta = -0.728 \pm 0.169$ SE, $Z = -4.285$, $P < 0.001$). Chick survival also decreased over the breeding season ($\beta = 2.016 \pm 0.144$ SE, $Z = 13.99$, $P < 0.001$) and decreased 17% per year over the study period ($\beta = -0.175 \pm 0.040$ SE, $Z = -4.31$, $P < 0.001$). Body mass at hatching did not affect the survival of the chicks ($\beta = -0.211 \pm 0.138$ SE, $Z = -1.53$, $P = 0.130$).

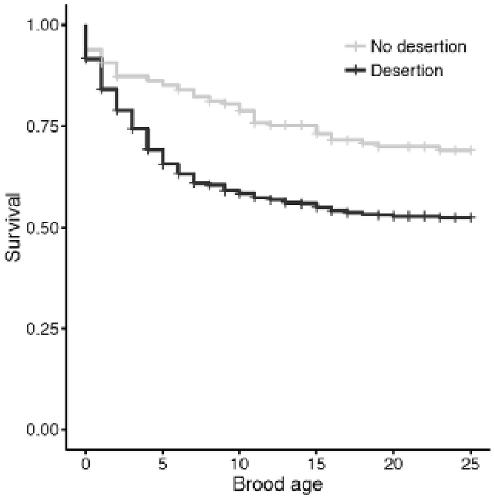

Fig. 2. Survival of snowy plover chicks from hatching to fledging in relation to desertion by one of the parents at Ceuta. Black line illustrate chick survival of broods where one parent deserted within seven days of hatching, whereas the grey line represents chick survival of broods where both parents cared for at least seven days.

3.5. Fledgling and adult survival

Variation in annual survival was best described by life-stage (i.e. fledgling or adult) and year ($w_i = 0.99$; Table S3). The maximum fledgling survival was 0.52 between 2009 and 2010, the minimum was 0.09 between 2010 and 2011, and the average was 0.213 ± 0.066 SE (Fig. 1d). The maximum adult survival estimated was 0.753 between 2006 and 2007, the minimum was 0.275 between 2011 and 2012, and the average was 0.603 ± 0.073 SE (Fig. 1d).

3.6. Population viability

Our stochastic matrix projection model forecasts a rapid decline in the Ceuta breeding population in line with the observed dynamics in the population (Fig. 3a). According to our model, the population has a 47.5% probability of extinction within 10 years after 2012, 99.8% within 25 years, and a 100% chance of extinction in 50 years (Figs. 3b and S2). The sensitivity analysis revealed that population growth rate was most sensitive to perturbations in adult survival (Fig. 3c), whereas egg, chick, fledgling, and fecundity all had equal elasticities (Fig. 3d and e).

4. Discussion

Our study reveals that subtropical resident shorebird populations may face similar problems as those at higher latitudes. Long-term monitoring of this population showed a strong decline by >70% between 2006 and 2012 and it is likely that the decline already started before our study. Based on the observed vital rates, this population is predicted to go extinct within 25 years if no conservation measures are undertaken. Similar declines of other snowy plover populations have been observed in the United States (Colwell et al., 2010; Page et al., 1991; Saalfeld et al., 2013). In Mexico, a study carried out in Baja California reported a decline of 42% between 1991 and 2008 (Galindo-Espinosa and Palacios, 2015). The growth rate was below replacement in our population but slightly higher than the other well-studied populations (Humboldt, California: $\lambda = 0.77$, Mullin et al., 2010). The Humboldt population has been maintained by strong immigration (Eberhart-Phillips and Colwell, 2014) and similarly, immigration at Ceuta slowed down the decline with annual immigration rates of 15–77% for males, and 28–77% for females (CK & MC-L, unpublished data).

Apparent nesting success at Ceuta was higher than most other snowy plover populations without predator control for which comparable data are reported (0.20 to 0.32; Ellis et al., 2015; Galindo-Espinosa, 2015; Hardy and Colwell, 2012; Saalfeld et al., 2011) and comparable to those where lethal predator removal has been enforced (0.24–0.60; Lauten et al., 2014; Powell et al., 2002; Stern et al., 1990). A likely explanation for the observed high nest survival is the absence of avian nest predators at Ceuta (Stoddard et al., 2016), which are the major cause of nest failure in other populations (Colwell et al., 2011; Ellis et al., 2015; Powell et al., 2002). Causes of nest failure varied over the seven-year period, with predation contributing most failure in early years, and flooding being most significant in later years. Flooding and severe weather events have also been responsible for nest failures in other snowy plover populations (Ellis et al., 2015; Saalfeld et al., 2011; Sexson and Farley, 2012). At Ceuta and the surrounding region, range shifts in the natural vegetation are tightly linked to agri- and aqua-cultural development, which has affected water flow, tidal drainage, and spatio-temporal regimes of flooding and sedimentation (Alonso-Pérez et al., 2003) that have also affected the nesting behaviour of plovers. We observed that plovers moved to areas of the breeding site where water levels and therefore foraging opportunities are higher. However, in these areas the risk of nest flooding is also higher. For example, in 2012 when most plovers nested in this area, 35% of all nests were flooded during a single spring tide in early June. Thus, this newly colonised area may serve as an ecological trap for nesting plovers.

In contrast to the high nest survival, chick survival at Ceuta was low in comparison with most other plover populations (Catlin et al., 2013; Colwell et al., 2007; Stenzel et al., 2007). In plovers, chick mortality is often influenced by habitat type and quality, predation, and parental care (Colwell et al., 2007; Székely and Cuthill, 1999). Similar low chick survival is found for beach nesting plovers (Catlin et al., 2013; Colwell et al., 2007) whereas the main habitat at Ceuta is a mudflat. Chick mortality also frequently varies temporally (Székely et al., 2004; Verhulst and Nilsson, 2008). At Ceuta the number of fledglings per male did not change annually but decreased with time over the breeding season as reported from other bird populations (Verhulst and Nilsson, 2008). The seasonal decline has been explained with deterioration of environmental conditions and food availability over the breeding season. At Ceuta, deterioration is caused by the rapid evaporation of water, which reduces the availability of accessible wet foraging habitat for chicks. This effect may have been compounded by habitat changes induced by sedimentation (both natural and anthropogenic) and changes in the hydrological regime at Ceuta (Alonso-Pérez et al., 2003).

Chick survival increases with age, with the lowest probabilities of survival occurring during the first week after hatch (Colwell et al., 2007; Loegering and Fraser, 1995; Székely et al., 2004). This suggests that young chicks depend on parental care for thermoregulation, predator avoidance, and defence from aggressive conspecifics (Colwell et al., 2007; Kosztolányi et al., 2006). We found that desertion of one parent is related to chick survival, with biparental broods surviving better than uniparental broods when controlling for seasonal effects. However, brood desertion may also increase the overall productivity of the population since deserting females have the opportunity to produce more offspring than females restrained to care for their first brood. Snowy plovers rarely have clutches containing more than three eggs. Therefore, by deserting broods and pairing with a second or third male, females can more readily produce more offspring. Notably, females that deserted broods within the first seven days after hatch did not produce more fledglings locally than females that cared. It is possible that deserting females that left Ceuta and bred elsewhere were more successful. However, at least locally, desertion did not benefit individual reproductive success nor did it increase fledgling production at the population level and currently appears to be maladaptive. We suspect that seasonal variation in habitat quality could contribute to this result,

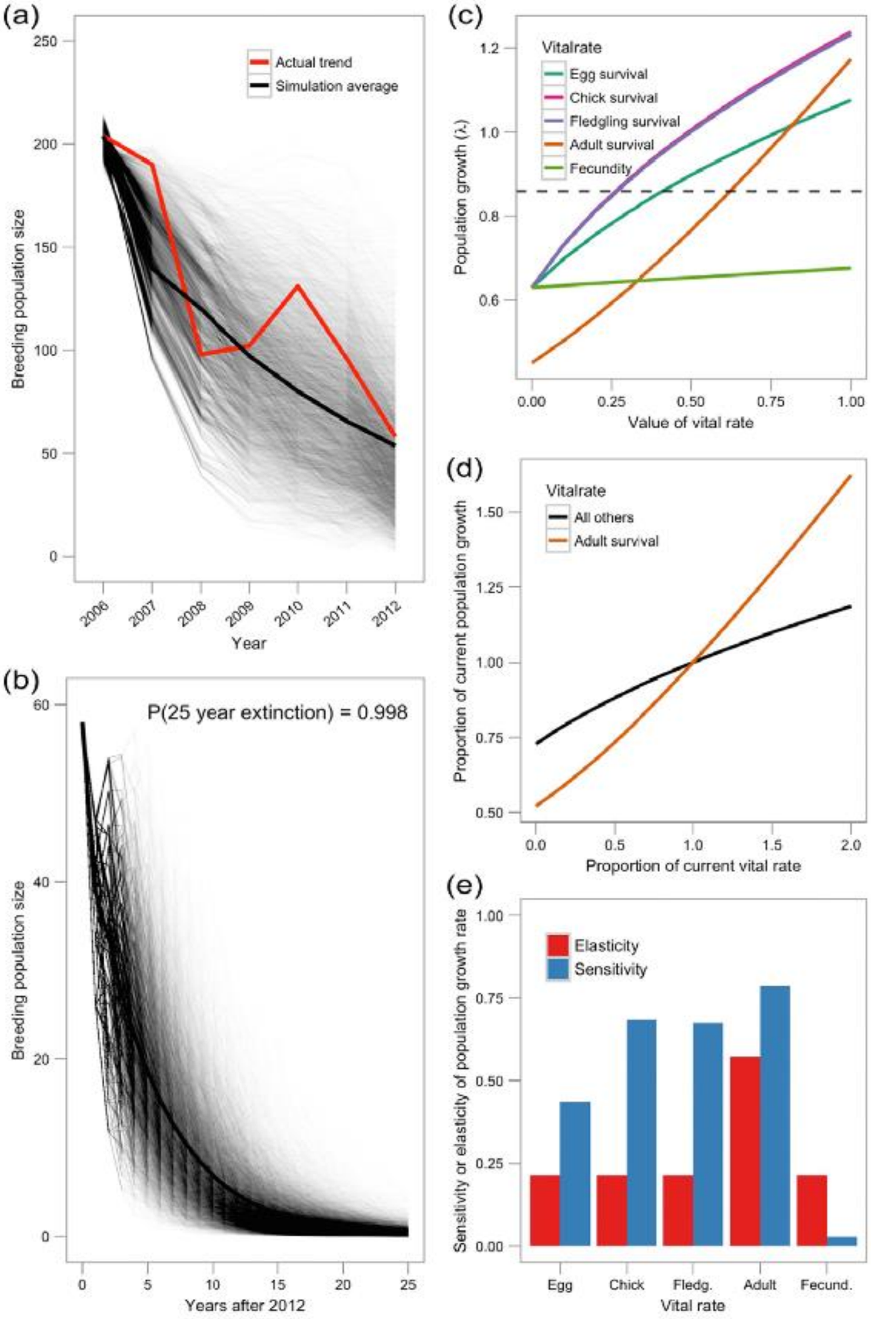

Fig. 3. (a) Validation model of the population trend of Ceuta snowy plovers between 2006 and 2012. Red line represents the real trend across the years and thick black line represents the simulation average. (b) Forecasted population size and extinction risk for 25 years after 2012. Black thin lines represent the 10,000 simulated trajectories and thick line represents the simulation average. (c) The effects of perturbing vital rates on population growth rate (λ). Horizontal dashed line indicates the deterministic population growth rate between 2006 and 2012 (λ = 0.859). (d) Influence of elasticities on vital rates. Fecundity and egg, chick, and fledgling survival all had the same trend line and thus were grouped into the "All others" category. (e) Comparison of stage-specific sensitivities and elasticities. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

because biparental care is shown to increase brood survival especially when the environmental quality is low and/or competition for resources is high (Kosztolányi et al., 2006; Székely and Cuthill, 1999). It is unclear whether and how the flexible brood care system of snowy plovers contributes to population productivity and the species' conservation risk. Acknowledging the relative effects of flexible brood care on individual fitness and population dynamics presents an intriguing dilemma for the conservation and management of such species and requires further work.

Juvenile survival at Ceuta was lower than that reported from any other plover population in North America so far. Estimates across snowy plover populations of the Pacific coast ranged from 0.40 to 0.46 (Mullin et al., 2010; Stenzel et al., 2007; USFWS, 2007). Estimating juvenile survival is challenging given that fledged juveniles frequently disperse. For example, juveniles may move to other sites nearby, which are poorly monitored. Notably there have been no reported juvenile immigrants from Ceuta at a monitored population ~200 km South of Ceuta (Carlos Villar, personal communication). As reported in other shorebirds (Sandercock, 2003), we found that adult survival was higher than juvenile survival although it was decreasing over the years. Average apparent adult survival at Ceuta was lower than most rates reported from other snowy plover populations (Paton, 1994; Saalfeld et al., 2013) or other plover (Foppen et al., 2006; Sandercock et al., 2005,) with the exception of the Humboldt population, which had similar low apparent adult survival rates (Mullin et al., 2010).

The population viability analysis suggested that apparent adult survival is the limiting demographic parameter for population growth. Adult survival showed the highest elasticity, which implies that it has the greatest effect on population change (Larson et al., 2002; Sandercock, 2003). However, it is important to note that our estimate of apparent survival includes both true survival and permanent emigration. We did not observe any dead individuals nor were there any reports of environmental or epidemiological disturbances that could affect adult mortality. Hence the steady decline in apparent survival is likely the result of permanent emigration, perhaps in response to consistent low reproductive success or habitat degradation. Water availability during the spring arrival time to breeding area has been shown to influence regional fidelity rates in snowy plovers, with a decrease in site fidelity following low water levels (Saalfeld et al., 2013). In addition, plovers are long-lived site faithful animals but poor breeding success may provoke adults to abandon the breeding site and thus reduce their site fidelity between years (Haas, 1998; Porneluzi, 2003).

We have knowledge of neighbouring populations or suitable habitat for breeding plovers within a 200 km radius or Ceuta, however, only one of these locations has been regularly monitored. At this site, only a few marked emigrants from Ceuta have been resighted, and only once have we documented a marked immigrant originating elsewhere (MC-L & CK unpublished data). To fully grasp adult movement among these sites, a region-wide monitoring effort is needed. Snowy plovers have a flexible breeding biology that is characterised by long-distance breeding dispersal (Stenzel et al., 1994). This is particularly the case for polyandrous plover females, which can breed at multiple distant sites (Küpper et al., 2012). Moreover, every year we newly banded a large number of unmarked adults despite having marked nearly all hatchlings in the previous season. Taken together, this suggests that substantial adult movements occur regionally. Therefore, it is plausible that an improvement of the vital rates for eggs, chicks and especially juveniles at Ceuta will improve the retention of adults in the population and hence increase population growth.

Retaining adults in a population is a challenging task in avian conservation, since these individuals are highly mobile. One feasible approach could be to increase the per capita reproductive success of the Ceuta population by managing water levels at an optimal level for nest and chick survival. Local retention would also serve as social information to attract prospective immigrants to increase the size of the breeding population and improve overall future reproductive success and hence population viability (Anthony and Blumstein, 2000; Blumstein, 2010).

In conclusion, our findings reveal that one of the most important breeding populations of snowy plovers in Mexico has low vital rates and is in severe decline. We suggest that habitat degradation is the principal reason for the decline. Urgent conservation management actions should first aim to improve chick and juvenile survival by addressing the fluctuating water levels at Ceuta. Abandoned evaporation ponds are ideal habitat for breeding plovers but require active management, which occasionally provides new conservation dilemmas. For example, the water channels feeding the ponds have been blocked by encroaching mangrove vegetation, a plant that is protected under Mexican law and illegal to remove. A possible solution would be to pump up ground water using a wind mill and flood the ponds during the breeding season—a restoration strategy that would not interfere with conservation and local agriculture. In time this could increase the retention of breeding adults, a life-stage identified in our models as being most important for population growth. Improving the situation for breeding snowy plovers will benefit other coastal wetland specialists and therefore generally increase ecosystem health.

Decisive actions are also needed at other levels since predictive models of human development forecast an ever-increasing pressure on coastal ecosystems (Neumann et al., 2015) and the pattern of declining breeding populations of snowy plovers suggest that suitable habitats in Mexico are becoming less and less, meaning that emigrating plovers face similar threats elsewhere in the region. Around Ceuta, we have witnessed large scale deforestation of federally protected mangrove forests combined with illegal beach development. We expect that these disturbances will put further pressure on the local snowy plover population, which are not yet captured by our current models. Taken together, we anticipate a genuine risk that the protected RAMSAR site of Sistema Lagunar Ceuta will quickly lose this iconic bird species. Given the vulnerability of snowy plovers to the consequences of climate change (Aiello-Lammens et al., 2011) the local extinction of the Ceuta population would severely hamper the intensive international efforts to recover the species.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.biocon.2017.03.009.

The following material can be downloaded from the GitHub repository found here: https://github.com/leberhartphillips/Ceuta_snowy_plover_PVA.

Appendix A. RMarkdown PDF file containing all computer code and documentation to reproduce all analyses presented in the paper.

Appendix B. Four datasets containing the raw data used in all analyses.

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Capítulo III. Diversidad alélica y patrones de selección en los loci clase I y II del Complejo Mayor de Histocompatibilidad en una especie de ave playera amenazada, el Chorlo nevado (*Charadrius nivosus*).

Chapter III. Allelic diversity and patterns of selection at the major histocompatibility complex class I and II loci in a threatened shorebird, the Snowy Plover (*Charadrius nivosus*).

Medardo Cruz-López, Guillermo Fernández, Helen Hipperson, Eduardo Palacios, John Cavitt, Daniel Galindo-Espinosa, Salvador Gómez del Angel, Raya Pruner, Oscar Gonzalez, Terry Burke and Clemens Küpper.

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Resumen

Comprender la estructura y variabilidad de loci adaptativos como los genes del complejo mayor de histocompatibilidad (MHC) es uno de los objetivos principales de la investigación en genética evolutiva y de la conservación. Típicamente, los genes clásicos del MHC muestran un elevado polimorfismo y están sometidos a una fuerte selección balanceadora, ya que sus productos desencadenan la respuesta inmunitaria adaptativa en vertebrados. Aquí evaluamos la diversidad alélica y los patrones de selección para los loci del MHC de clase I y clase II en una especie de ave playera amenazada y con un comportamiento de apareamiento y de cuidado parental muy flexible, el Chorlo nevado (*Charadrius nivosus*), a lo largo de su distribución geográfica tan amplia. Determinamos la diversidad nucleotídica y alélica para los genes del MHC clase I y clase II utilizando muestras de 250 individuos de ocho poblaciones reproductoras de Chorlo nevado. Encontramos 40 alelos para el MHC clase I, con individuos portando de dos a siete alelos (media de 3,70), mientras que para el MHC clase II se observaron seis alelos, con hasta dos alelos por individuo (media de 1,45). La diversidad fue mayor en la región de unión a péptidos,

lo que sugiere una selección balanceadora. El locus del MHC clase I mostró señales más fuertes de selección positiva y negativa que el locus del MHC de clase II. La mayoría de los alelos estaban presentes en más de una población. Los alelos privados ocurrieron en frecuencias muy bajas, excepto para los alelos del MHC clase I en una población insular (Puerto Rico, linaje *tenuirostris*). El Chorlo nevado presenta un nivel intermedio de diversidad en el MHC, similar a lo observado en otros Charadriiformes. Las diferencias observadas en los patrones de selección entre los loci de clase I y II concuerdan con la hipótesis de que distintos mecanismos conforman la evolución de las secuencias de los genes del MHC de clase I y clase II. La rareza de los alelos privados entre poblaciones es congruente con la ausencia de estructura genética evidenciada previamente en estudios con marcadores genéticos neutros en la especie, consecuencia de una elevada dispersión natal y reproductora (dispersión a poblaciones vecinas para tratar de incrementar el acceso a nuevas parejas).

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BMC Evolutionary Biology

RESEARCH ARTICLE **Open Access**

Allelic diversity and patterns of selection at the major histocompatibility complex class I and II loci in a threatened shorebird, the Snowy Plover (*Charadrius nivosus*)

Medardo Cruz-López[1*], Guillermo Fernández[2], Helen Hipperson[3], Eduardo Palacios[4], John Cavitt[5], Daniel Galindo-Espinosa[6], Salvador Gómez del Angel[1], Raya Pruner[7], Oscar Gonzalez[8,9], Terry Burke[3] and Clemens Küpper[10*]

Abstract

Background: Understanding the structure and variability of adaptive loci such as the major histocompatibility complex (MHC) genes is a primary research goal for evolutionary and conservation genetics. Typically, classical MHC genes show high polymorphism and are under strong balancing selection, as their products trigger the adaptive immune response in vertebrates. Here, we assess the allelic diversity and patterns of selection for MHC class I and class II loci in a threatened shorebird with highly flexible mating and parental care behaviour, the Snowy Plover (*Charadrius nivosus*) across its broad geographic range.

Results: We determined the allelic and nucleotide diversity for MHC class I and class II genes using samples of 250 individuals from eight breeding population of Snowy Plovers. We found 40 alleles at MHC class I and six alleles at MHC class II, with individuals carrying two to seven different alleles (mean 3.70) at MHC class I and up to two alleles (mean 1.45) at MHC class II. Diversity was higher in the peptide-binding region, which suggests balancing selection. The MHC class I locus showed stronger signatures of both positive and negative selection than the MHC class II locus. Most alleles were present in more than one population. If present, private alleles generally occurred at very low frequencies in each population, except for the private alleles of MHC class I in one island population (Puerto Rico, lineage *tenuirostris*).

Conclusion: Snowy Plovers exhibited an intermediate level of diversity at the MHC, similar to that reported in other Charadriiformes. The differences found in the patterns of selection between the class I and II loci are consistent with the hypothesis that different mechanisms shape the sequence evolution of MHC class I and class II genes. The rarity of private alleles across populations is consistent with high natal and breeding dispersal and the low genetic structure previously observed at neutral genetic markers in this species.

Keywords: Major histocompatibility complex (MHC), Balancing selection, Peptide-binding region (PBR), MHC class I, MHC class II, Private alleles, *Charadrius*

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Background

The genes of the major histocompatibility complex (MHC) are crucial for the immune response in vertebrates [1, 2]. Their encoded proteins are involved in presenting antigen derived from pathogens to immune cells, which then trigger a cascade of immune responses [3, 4]. Because of their functional importance and the direct link between MHC diversity, fitness and individual behaviour [3, 5], the MHC has been the subject of ecological and evolutionary studies ranging from assessing individual survival and mate choice to the processes of speciation and practical conservation management [6–10]. Adaptive genes, which are directly associated with individual fitness, are important for population viability and hence conservation [2, 11]. The loss of adaptive genetic diversity has been associated with an increase in the risk of extinction, especially in species with low population sizes [11]. The maintenance of MHC diversity is crucial for pathogen resistance, which represents one of the principal selective forces impacting fitness and long-term survival of endangered species [2, 12].

MHC genes display the highest degree of polymorphism within vertebrate genomes [5, 13]. Pathogen-mediated selection results in positive selection and the substitution of amino acids in the codons of the peptide-binding region (PBR), as well as balancing selection including heterozygote advantage, frequency-dependent selection and fluctuating selection [2, 4, 14]. Recently, a number of studies showed that the evolutionary dynamics of the MHC genes is driven by high rates of recombination, duplication and conversion [15–17]. Through these processes populations can respond to a great number of antigens [10, 17]. The MHC genes are divided into two principal classes: class I, which is responsible for immune defence against intracellular pathogens such as viruses, and class II, which is responsible for dealing with extracellular pathogens such as bacteria and nematodes [2].

The number of MHC genes varies between and within species [10]. In mammals, MHC genes are organized into a dense genomic region and are characterized by complex organization and many pseudogenes, leading to extraordinary genetic diversity. For example, in humans approximately 9000 class I alleles and 3000 class II alleles have been described [18]. In birds, the structure and organization of the MHC region varies not only between, but also within the same family [9]. Some groups, such as chicken *Gallus gallus* and some birds of prey, have an extraordinarily compact MHC region (coined as the "minimal essential" MHC, [19, 20]). However, other galliform species have duplications, leading to many MHC alleles [21, 22]. In contrast, in Passeriformes, the MHC shows a complex architecture, and is frequently composed of multiple expressed loci and pseudogenes [1, 23, 24]. Other groups of birds, such as the Charadriiformes, appear to have a diversity and complexity intermediate between chicken and Passeriformes [25, 26]. The differences in the number and organization of the MHC genes in vertebrates might be best explained by different evolutionary dynamics in the birth and death of genes [27]. Here, new genes are generated by duplication, with some daughter copies conserving their function while others are inactivated or eliminated from the population [10, 27].

Within the order Charadriiformes, study of the MHC have so far been restricted to three families: Alcidae, Laridae and Scolopacidae. These studies revealed considerable differences in the diversity and organization of the Charadriiform MHC [16, 25, 26, 28, 29]. To investigate which mechanisms generate and promote MHC evolution and diversity [9], studies in more phylogenetically distinct families are required.

We investigated the diversity and organization of the MHC in the Snowy Plover *Charadrius nivosus*, a member of the Charadriidae [30]. Until recently, Snowy Plovers were lumped with Kentish Plovers *Charadrius alexandrinus* and considered to be a cosmopolitan species, but the analysis of genetic and phenotypic traits has shown that the two species are separate and that Snowy Plovers are characterized by low genetic diversity at neutral genetic markers [31–33]. Three Snowy Plover lineages are commonly recognized and distinguishable with genetic markers: Western Snowy Plovers (*C. n. nivosus*) in North America, Cuban Snowy Plovers (*C. n. tenuirostris*) in the Caribbean, and the Peruvian Snowy Plover (*C. n. occidentalis*) in South America [31]. Coastal populations in particular have declined throughout the range and now require active conservation management [34–37]. Here, we develop markers for MHC class I and class II genes to examine adaptive genetic diversity in Snowy Plovers. Our study has four aims: First, we characterize functionally essential regions of the MHC class I and class II loci to provide novel genetic markers for studying adaptive diversity in this species. Second, we investigate the genetic diversity (number of segregating sites and nucleotide diversity), evolutionary distance and type of selection acting on MHC class I and class II alleles. Third, we compare the diversity of MHC classes I and II with the respective diversity observed in other Charadriiformes. Finally, we describe the pattern of diversity across the geographic range and test for the presence of private alleles within Snowy Plover populations.

Results

MHC class I: exon 3

We discarded 32 samples because they did not pass the quality filters or because they did not have the minimum number of reads per amplicon. Among the 218 remaining samples from eight populations, we found a total of 40 alleles. Five alleles ('long alleles'; Chni-UA*12 to 16) showed a 3-bp insertion at position 178–180 (amino acid position 60 in the alignment) in the α2 domain, retaining the correct reading frame. Meanwhile, the remaining 35 alleles ('short alleles'; Chni-UA*01 to UA*11 and Chni-UA*17 to UA*40) did not show this insertion. The alignment of exon 3 displayed five of the eight highly conserved amino acid (peptide main-chain) sites in birds (amino acids: TKWYY, Fig. 1). Alleles Chni-UA*01 to UA*10 had an amino acid substitution at the second of these conserved sites (N for K), whereas in alleles Chni-UA*17 to UA*21 the fourth site was substituted (C for Y). Four of the 18 highly conserved intra- and interdomain contacts described in vertebrates [38] were present, and none of these showed a polymorphism.

Allelic diversity

We found two to seven alleles per individual ($\overline{x} \pm SD$: 3.70 ± 0.92), which suggested that we obtained alleles from up to four loci, assuming heterozygosity. We detected up to three non-classical alleles in 216 of 218 samples. The number of alleles did not differ between individuals across populations (Table S1). However, we found differences in the number of alleles per population, with populations Nayarit and Puerto Rico showing fewer alleles than the other populations (Table S2). Long alleles were less common among the 218 individuals genotyped, with only 23 individuals displaying one or two long alleles, whereas the short alleles were present in all individuals. Chni-UA*21 was the most common allele and detected in 173 individuals (83.5%), followed by Chni-UA*20 in 102 individuals (49.2%) and Chni-UA*30 in 69 individuals (33.3%). All individuals genotyped had at least two alleles. Most individuals (48.3%) had four alleles,

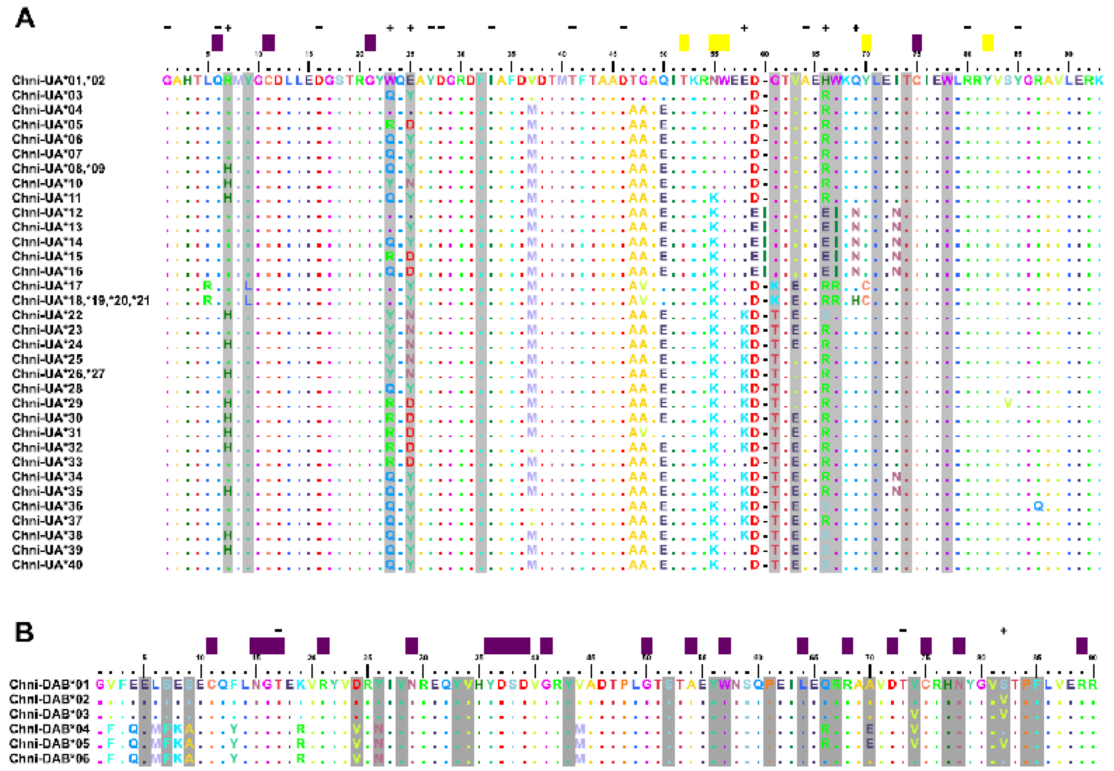

Fig. 1 Alignments of MHC class I alleles **(a)** and MHC class II alleles **(b)** detected among sampled Snowy Plovers *Charadrius nivosus*. Alleles with an identical translation of amino acids are shown in a single sequence. Dots indicate identity to the consensus sequence and dashes indicate gaps. Gray boxes indicate the peptide binding regions (PBR) inferred for MHC class I and MHC class II. Intra- and inter-domain contacts are in purple, and main chain binding sites to peptides are in yellow. Sites under positive (+) and negative (–) selection were identified using FUBAR (Murrel et al. 2013; http://www.datamonkey.org/)

24.6% had three alleles, 13% had five alleles, 11.6% had two alleles, 2.4% had six alleles and 0.9% had seven alleles.

Diversity and inference of selection

The average nucleotide diversity (π) for the complete sequence was similar among the three lineages, ranging from 0.05 to 0.07. Populations at Nayarit and Puerto Rico showed the highest levels of nucleotide diversity, the nucleotides distance and average nucleotide diversity at PBR sites, whereas populations at Utah, San Quintin and Ceuta had the lowest levels of nucleotide diversity (both complete sequence and PBR sites) and nucleotide distance (Table 1). Within all populations, PBR sites showed higher diversity than non-PBR sites, suggesting balancing selection at these sites (Table 1). Bayesian analysis of selection (FUBAR: Fast Unconstrained Bayesian AppRoximation) identified six sites (sites 7, 23, 25, 58, 66 and 69) under positive selection and 11 sites (sites 1, 6, 16, 27, 28, 33, 41, 46, 64, 80 and 85) that displayed diversity patterns consistent with negative, purifying selection (Fig. 1a). Sites with purifying selection were exclusively located in the non-PBR region. Also, differences in nonsynonymous substitution rate and synonymous substitution rate (*dN*/*dS*) suggested stronger positive selection at PBR sites in comparison with non-PBR sites (Table 1). Among genetic lineages, *C. n. tenuirostris* (Puerto Rican population) showed the lowest level of positive selection. Using GARD (Genetic Algorithm for Recombination Detection) we found no evidence for recombination among the 40 alleles.

Table 1 Diversity at MHC class I exon 3 in the Snowy Plover (*Charadrius nivosus*). Segregating sites of amino acids (S_{aa}), average nucleotide diversity (π), evolutionary distance of nucleotide sequences (d_{nt}) and amino acid sequences (d_{aa}). The measures of diversity, and the synonymous and non-synonymous substitution rates, were calculated for the complete sequence (All), and separately for the PBR and non-PBR sites within each population. The number of genotyped individuals per population (*N*) and the number of samples that passed the quality filters (*n*) are shown. To have comparable measures of diversity we randomly selected 15 allele sequences for each population, with the exception of Nayarit and Puerto Rico, where we found only 13 and 9 alleles, respectively

Population	Lat	Long	*N* (*n*)		*dN* ± S.E	*dS* ± S.E	*dN* /*dS*	S_{aa}	π	d_{nt} ± S.E	d_{aa} ± S.E
C. n. nivosus (UTA)	40.9	−112.13	40 (40)	All	0.05 ± 0.01	0.08 ± 0.02	0.62	21	0.06	0.07 ± 0.01	0.09 ± 0.02
				PBR	0.33 ± 0.13	0.12 ± 0.07	2.75	8	0.21	0.35 ± 0.15	0.34 ± 0.09
				Non-PBR	0.02 ± 0.01	0.08 ± 0.02	0.25	13	0.04	0.04 ± 0.01	0.05 ± 0.01
C. n. nivosus (SAQ)	30.4	− 115.97	47 (35)	All	0.05 ± 0.01	0.08 ± 0.02	0.62	20	0.05	0.06 ± 0.01	0.08 ± 0.02
				PBR	0.34 ± 0.12	0.11 ± 0.07	3.10	8	0.21	0.36 ± 0.21	0.34 ± 0.08
				Non-PBR	0.02 ± 0.01	0.07 ± 0.02	0.28	12	0.03	0.03 ± 0.01	0.04 ± 0.01
C. n. nivosus (CEU)	23.91	− 106.95	49 (49)	All	0.05 ± 0.01	0.07 ± 0.02	0.71	20	0.05	0.06 ± 0.01	0.08 ± 0.02
				PBR	0.33 ± 0.12	0.11 ± 0.07	3.00	8	0.21	0.35 ± 0.16	0.35 ± 0.08
				Non-PBR	0.02 ± 0.01	0.07 ± 0.02	0.28	12	0.03	0.03 ± 0.01	0.04 ± 0.01
C. n. nivosus (NAY)	22.41	−105.62	13 (10)	All	0.06 ± 0.01	0.13 ± 0.03	0.46	19	0.07	0.07 ± 0.01	0.09 ± 0.02
				PBR	0.38 ± 0.14	0.18 ± 0.11	2.11	8	0.25	0.46 ± 0.29	0.37 ± 0.08
				Non-PBR	0.02 ± 0.01	0.12 ± 0.03	0.17	11	0.05	0.05 ± 0.01	0.05 ± 0.02
C. n. nivosus (TEX)	19.46	−98.97	23 (14)	All	0.06 ± 0.01	0.09 ± 0.02	0.67	19	0.06	0.07 ± 0.01	0.09 ± 0.02
				PBR	0.39 ± 0.14	0.14 ± 0.09	2.80	8	0.23	0.42 ± 0.21	0.37 ± 0.08
				Non-PBR	0.02 ± 0.01	0.09 ± 0.02	0.22	11	0.03	0.04 ± 0.01	0.05 ± 0.01
C. n. nivosus (FLO)	30.02	−85.57	40 (36)	All	0.05 ± 0.01	0.10 ± 0.02	0.50	19	0.06	0.07 ± 0.01	0.08 ± 0.02
				PBR	0.35 ± 0.12	0.13 ± 0.08	2.70	8	0.22	0.38 ± 0.19	0.36 ± 0.08
				Non-PBR	0.02 ± 0.01	0.09 ± 0.02	0.22	11	0.04	0.04 ± 0.01	0.05 ± 0.01
C. n. occidentalis (PER)	−13.84	−76.24	21 (21)	All	0.06 ± 0.01	0.09 ± 0.02	0.70	20	0.06	0.07 ± 0.01	0.09 ± 0.02
				PBR	0.36 ± 0.13	0.14 ± 0.08	2.60	8	0.23	0.41 ± 0.19	0.37 ± 0.09
				Non-PBR	0.02 ± 0.01	0.09 ± 0.02	0.22	12	0.03	0.04 ± 0.01	0.05 ± 0.01
C. n. tenuirostris (PUR)	17.93	−67.18	17 (13)	All	0.06 ± 0.01	0.15 ± 0.04	0.40	18	0.07	0.07 ± 0.01	0.09 ± 0.02
				PBR	0.36 ± 0.15	0.22 ± 0.13	1.64	8	0.24	0.49 ± 0.36	0.34 ± 0.07
				Non-PBR	0.03 ± 0.01	0.14 ± 0.04	0.21	10	0.05	0.05 ± 0.01	0.06 ± 0.02

Comparison and phylogenetic relationships with other Charadriiformes

Average nucleotide diversity and number of segregation sites were lower than those reported from Red Knot, Icelandic Black-tailed Godwit and Red-billed Gull (Table 2; Figure S1). Nevertheless, the *dN/dS* proportion at PBR sites was higher for the Snowy Plover, indicating stronger positive selection at these sites in comparison with the other three charadriiform species.

As in Red Knots and Red-billed Gulls, we found alleles with putatively non-classical functions among the Snowy Plover alleles. These non-classical alleles (Chni-UA*17 to UA*21) formed a well-defined cluster in the phylogenetic network (Fig. 2a) and showed a low level of polymorphism (Figs. 1a and 2a). Non-classical alleles were present in 216 of 218 samples. These alleles clustered together with the Red-billed Gull non-classical allele Lasc-UDA*03 in the neighbour-joining tree (Fig. 3a).

MHC class II: exon 2

We discarded 36 samples, as they did not pass the quality filters. In total, we found six alleles across 214 individuals of eight populations. We found no more than two alleles per individual ($\bar{x}$ ± SD: 1.45 ± 0.50), which suggests that we genotyped only one locus. The most common allele was Chni-DAB*01, which we detected in 171 individuals (79.9%), whereas the least common allele was Chni-DAB*03, present in four individuals (1.9%). Most individuals (55.6%) were homozygous.

Table 2 Comparison of genetic diversity at MHC class I exon 3 in four different charadriiform species. Segregating sites of amino acids (S_{aa}), nucleotides (S_{nt}), and average nucleotide diversity (π), from 21 sequences randomly chosen by each of these species. Diversity indices and the synonymous (*dS*) and non-synonymous (*dN*) substitution rates were calculated for the complete sequences (All), the PBR and the non-PBR sites

	dN ± S.E	*dS* ± S.E	*dN/dS*	S_{nt}	S_{aa}	π
	Snowy Plover (*Charadrius nivosus nivosus*)					
All	0.05 ± 0.01	0.07 ± 0.02	0.71	47	19	0.05
PBR	0.29 ± 0.10	0.09 ± 0.05	3.22	19	8	0.19
Non-PBR	0.02 ± 0.01	0.06 ± 0.02	0.33	28	11	0.03
	Red-billed Gull (*Chroicocephalus scopulinus*)					
All	0.08 ± 0.01	0.09 ± 0.02	0.90	64	34	0.08
PBR	0.36 ± 0.13	0.20 ± 0.13	1.80	20	9	0.24
Non-PBR	0.05 ± 0.01	0.08 ± 0.02	0.62	44	25	0.05
	Red Knot (*Calidris canutus*)					
All	0.13 ± 0.02	0.15 ± .02	0.87	110	53	0.11
PBR	0.46 ± 0.17	0.24 ± 0.11	1.92	25	9	0.28
Non-PBR	0.09 ± 0.01	0.14 ± 0.03	0.64	85	44	0.09
	Black-tailed Godwit (*Limosa limosa*)					
All	0.09 ± 0.02	0.10 ± 0.02	0.90	70	35	0.09
PBR	0.36 ± 0.14	0.32 ± 0.18	1.12	23	9	0.26
Non-PBR	0.05 ± 0.01	0.08 ± 0.02	0.62	47	26	0.05

Diversity and inference of selection

The nucleotide diversity (π) for subspecies *nivosus* and *occidentalis* were similar, with the PBR sites showing higher diversity in comparison to the non-PBR sites, suggesting that balancing selection could be acting at PBR sites (Table 3). The Bayesian site-by-site test in FUBAR suggested positive selection for one site (site 82), and purifying selection for two sites (sites 17 and 73, Fig. 1b). Sites with purifying selection were exclusively located in the non-PBR region. The analysis of rates of changes *dN/dS* indicated positive selection for the PBR sites in comparison to non-PBR sites for the *nivosus* and *occidentalis* subspecies (Table 3). All *tenuirostris* samples were homozygous. GARD identified one recombination breakpoint, which was located at position 222. When we re-ran a codon-specific model for the non-recombinant fragment of the sequences, our results remained unchanged.

Diversity and phylogenetic relationships within the Charadriiformes

The average nucleotide diversity of Snowy Plovers (π = 0.06) was well within the range of diversity observed in other Charadriiformes (range 0.02 to 0.15, Table 4; Figure S1). This was also true for the nucleotide diversity at PBR sites (Table 4). The phylogenetic network and the neighbor joining tree showed two distant allele groups for MHC Class II (Figs. 2b and 3b). The neighbour-joining tree showed that MHC class II alleles of Snowy Plovers (Chni-DAB) were located on a different branch than most other charadriiform MHC II alleles identified so far (Fig. 3b).

Geographic pattern of MHC diversity

We found that 25% of alleles were private alleles for MHC class I and 16.7% private alleles for MHC class II. For MHC class I, Snowy Plover populations from Utah, Ceuta and Puerto Rico showed private alleles (Fig. 4a). For Utah, four (Chni-UA*03, 04, 31 and 37) of 30 alleles were private, for Ceuta, four (Chni-UA*25, 28, 32 and 33) of 34 alleles were private and for Puerto Rico, two (Chni-UA*05 and 17) of nine alleles were private. Other populations lacked private alleles (notably the Peruvian population of the subspecies *occidentalis*) but showed similar numbers of alleles (San Quintin: 23 alleles, Nayarit: 13 alleles, Texcoco: 19 alleles, Florida: 17 alleles and

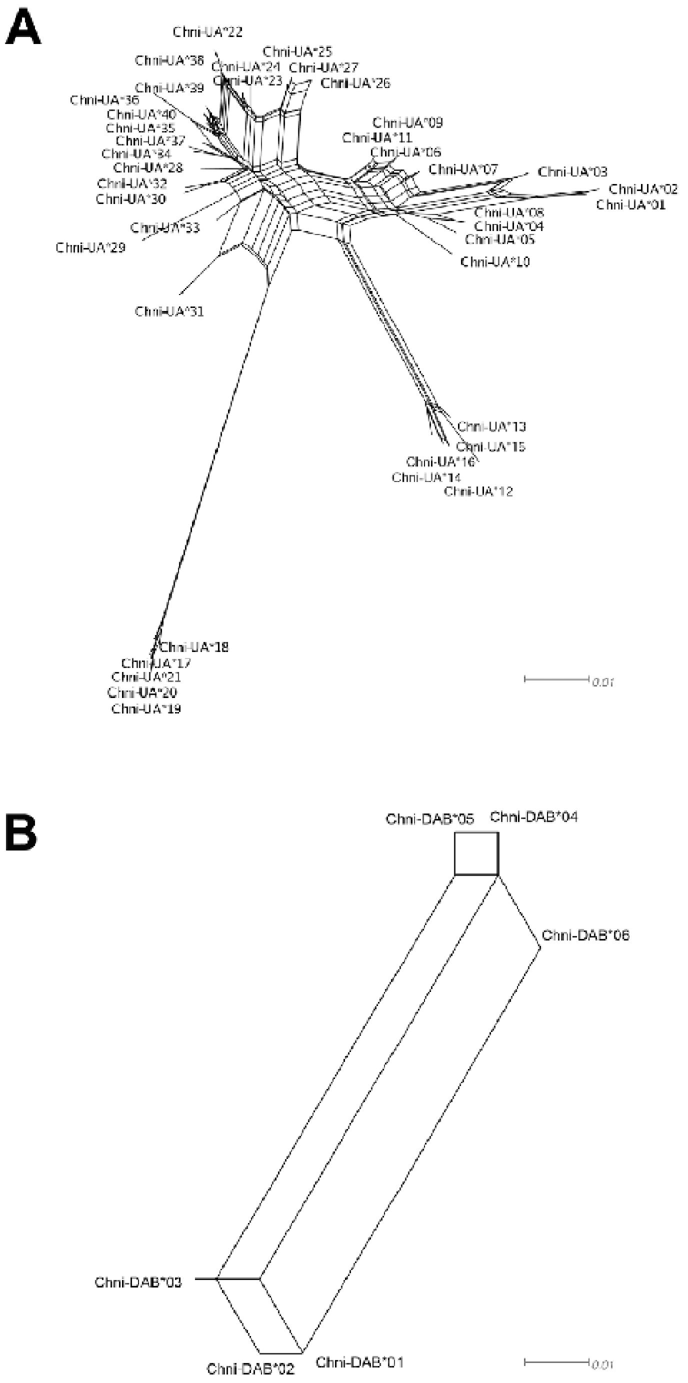

Fig. 2 Phylogenetic network showing relationships among Snowy Plover MHC class I alleles (**a**), and MHC class II alleles (**b**) characterized from eight populations. Long alleles at MHC class I are represented with a white square, whereas non-classical alleles are represented by black squares

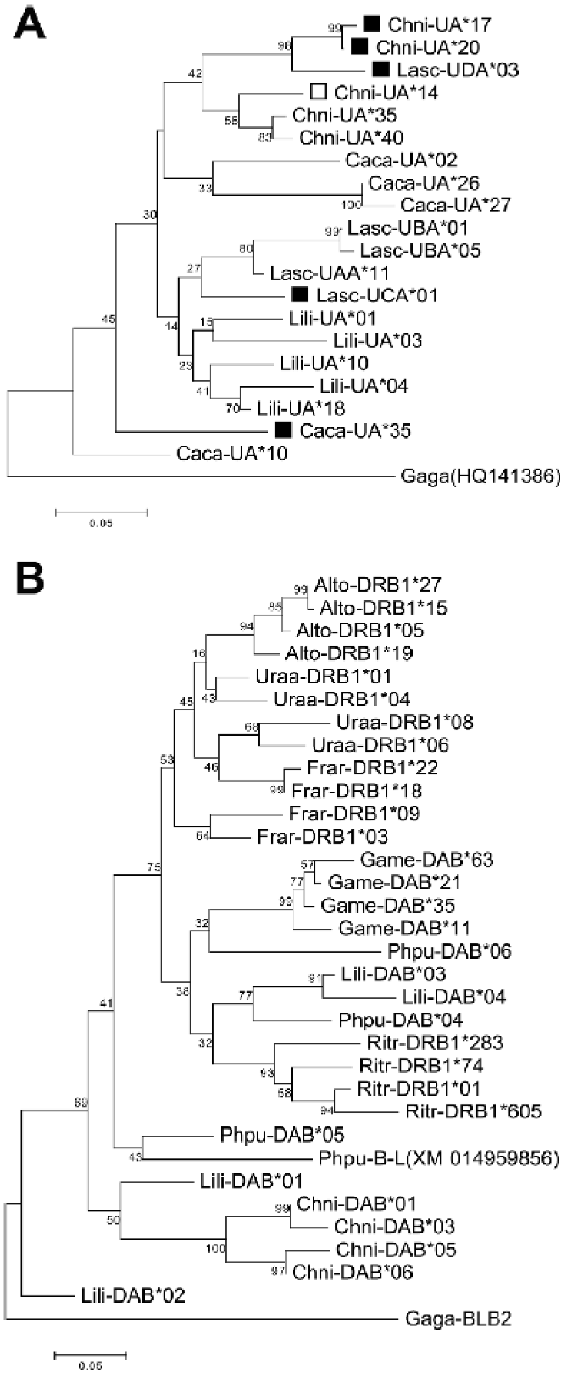

Fig. 3 (See legend on next page.)

(See figure on previous page.)
Fig. 3 Neighbour-joining tree of amino acid sequences for MHC class I and II from different Charadriiformes. For MHC class I, four species are represented (Snowy Plover (Chni), Black-tailed Godwit (Lili), Red Knot (Caca) and Red-billed Gull (Lasc)) and (**b**) MHC class II represented by eight species (Snowy Plover (Chni), Black-tailed Godwit (Lili), Ruff (Phpu), Great Snipe (Game), Black-legged Kittiwake (Ritr), Razorbill (Alto), Common Murre (Uraa) and Atlantic Puffin (Frar)). Long alleles are indicated by a white square, non-classical alleles with a black square. The homologous MHC sequences of the chicken *Gallus gallus* (Gaga-HQ141386 and Gaga-BLB2) served as an outgroup

Peru: 18 alleles). With the exception of the island population of Puerto Rico (lineage *tenuirostris*), private alleles generally occurred in low frequencies (Fig. 4). At MHC class II, there were no private alleles within lineages *tenuirostris* or *occidentalis*. Only the Ceuta population had a private allele (Chni-DAB*03), and we found all six alleles in this population. The other populations had three to five alleles, except for Puerto Rico, where we detected only one allele (Fig. 4b).

Discussion

In this study, we developed new MHC markers that amplified with high success exon 3 of MHC class I and exon 2 of MHC class II in the Snowy Plover, a shorebird species of high conservation concern. This allowed us to examine adaptive genetic diversity at this important locus in the family Charadriidae and provides novel and useful markers for future studies in other *Charadrius* species. Surveying both MHC classes in more than 200 individuals from eight populations, we report differences in allelic diversity across both MHC classes, with nearly seven times as many alleles at MHC class I than at MHC class II. The genotypic variation in individuals suggests that the markers amplified four highly similar loci for MHC class I, as we registered up to seven alleles at MHC class I, whereas a maximum of two alleles per individual is consistent with amplification of a single locus at MHC class II.

Our network analysis suggests the presence of classical and non-classical genes among the amplified MHC class I loci. Non-classical loci have evolved from classic MHC genes to perform specific tasks in the immune recognition [39]. Five alleles (Chni-UA*17 to 21), formed a well-defined cluster in the phylogenetic network, which is characteristic for non-classical alleles. Non-classical alleles have also previously been described in other shorebirds (Red-billed Gull: [29]; Red Knot: [25]). Consistent with expectations derived from other non-classical alleles, these five alleles showed a low level of polymorphism [40, 41]. Four alleles were only differentiated by synonymous substitutions, whereas the fifth allele, found only in the Puerto Rico population, deviated in a single amino acid substitution. We observed that two of three non-classical alleles Lasc-UCA*01 and Caca-UA*35 clustered with the classical alleles of their respective species Red-billed Gull and Red Knot. This suggests that they may have evolved through recent duplications that have occurred after the divergence of the species in this tree. By contrast, Lasc-UDA*03 and the Snowy Plover non-classical alleles may have been created through a more ancient duplication event.

We observed a second well-defined cluster in our phylogenetic network at MHC class I; this cluster corresponds to five alleles (Chni-UA *12 to 16) that shared a 3-bp insertion. The observed insertion kept the reading frame intact, presumably preserving their function. Contrary to the non-classical alleles, these five alleles with a 3-bp insertion show a higher nucleotide diversity, including several non-synonymous changes between these five alleles, suggesting these alleles have classical functions. In birds, insertions are less frequent than deletions in MHC genes, and it has been suggested that insertions have a reduced adaptive advantage [42]. However, some insertions may contribute to adaptive MHC variation due to possible changes in the PBR [42, 43]. Unlike the MHC class I, the six alleles at MHC class II formed an undifferentiated cluster, with both synonymous and non-synonymous substitutions present. Together, with the observation of no more than two alleles per individual, we concluded that our markers amplified a single classical MHC II locus. When we compared the nucleotide diversity at the PBR sites, we found that the MHC class I showed moderately higher values of diversity (0.16 ± 0.03) than at MHC class II (0.12 ± 0.03). This result may be a consequence of having amplified four loci at MHC I and only one locus at MHC II.

For both MHC classes, we observed that the number of sites under negative (purifying) selection was higher than sites under positive (diversifying) selection when we considered the full sequence. As PBR sites are interacting directly with the antigens derived from pathogens, it is expected that these sites are subject to stronger positive diversifying selection than non-PBR sites. Consistent with this, we found that sites with signatures of purifying selection were predominantly non-PBR sites, whereas five of the six sites under diversifying selection in the MHC class I and in one site at MHC class II corresponded to PBR sites. Similar patterns were observed in another shorebird, the Icelandic Black-tailed Godwit [26], and shown in a recent comparative study of selection at the avian MHC [42].

Our finding that more sites showed signatures of positive selection at MHC class I than at MHC class II

Table 3 Diversity at the MHC class II (segregating sites of amino acids (S_{aa}) and average diversity of nucleotides (π)) and evolutionary distance (nucleotide sequences (d_{nt}) and amino acids (d_{aa})) of the alleles of the exon 2 in the Snowy Plover (*Charadrius nivosus*). The measures of diversity and the synonymous and non-synonymous substitution rates were calculated for the complete sequences (All), the PBR and the non-PBR sites for each population. The number of genotyped individuals per population (*N*) and number of samples that passed the quality filter (*n*) are shown

Population	Lat	Lon	*N* (*n*)		dN ± S.E	dS ± S.E	dN /dS	S_{aa}	π	d_{nt} ± S.E	d_{aa} ± S.E
C. n. nivosus (UTA)	40.9	−112.13	40 (40)	All	0.06 ± 0.01	0.10 ± 0.03	0.60	15	0.06	0.07 ± 0.01	0.10 ± 0.02
				PBR	0.15 ± 0.05	0.08 ± 0.05	1.87	8	0.11	0.15 ± 0.05	0.20 ± 0.06
				Non-PBR	0.03 ± 0.01	0.11 ± 0.04	0.27	7	0.05	0.05 ± 0.01	0.07 ± 0.02
C. n. nivosus (SAQ)	30.4	−115.97	47 (36)	All	0.06 ± 0.01	0.10 ± 0.03	0.60	15	0.06	0.07 ± 0.01	0.10 ± 0.02
				PBR	0.15 ± 0.05	0.08 ± 0.05	1.87	8	0.11	0.15 ± 0.05	0.20 ± 0.06
				Non-PBR	0.03 ± 0.01	0.11 ± 0.04	0.27	7	0.05	0.05 ± 0.01	0.07 ± 0.02
C. n. nivosus (CEU)	23.91	−106.95	49 (34)	All	0.06 ± 0.01	0.11 ± 0.03	0.54	15	0.06	0.08 ± 0.01	0.10 ± 0.02
				PBR	0.14 ± 0.05	0.10 ± 0.05	1.40	8	0.12	0.15 ± 0.05	0.19 ± 0.05
				Non-PBR	0.03 ± 0.01	0.11 ± 0.04	0.27	7	0.05	0.05 ± 0.01	0.07 ± 0.02
C. n. nivosus (NAY)	22.41	−105.62	13 (10)	All	0.07 ± 0.02	0.12 ± 0.04	0.58	15	0.07	0.08 ± 0.01	0.11 ± 0.02
				PBR	0.17 ± 0.06	0.09 ± 0.05	1.88	8	0.13	0.17 ± 0.05	0.22 ± 0.06
				Non-PBR	0.04 ± 0.01	0.13 ± 0.05	0.30	7	0.05	0.06 ± 0.01	0.07 ± 0.02
C. n. nivosus (TEX)	19.46	−98.97	23 (17)	All	0.05 ± 0.01	0.09 ± 0.03	0.55	15	0.06	0.07 ± 0.01	0.09 ± 0.02
				PBR	0.14 ± 0.05	0.07 ± 0.05	2.00	8	0.11	0.13 ± 0.04	0.19 ± 0.05
				Non-PBR	0.03 ± 0.01	0.10 ± 0.04	0.30	7	0.04	0.04 ± 0.01	0.05 ± 0.02
C. n. nivosus (FLO)	30.02	−85.57	40 (39)	All	0.07 ± 0.02	0.12 ± 0.04	0.58	15	0.07	0.08 ± 0.01	0.11 ± 0.02
				PBR	0.17 ± 0.06	0.09 ± 0.06	1.88	8	0.13	0.17 ± 0.05	0.22 ± 0.06
				Non-PBR	0.04 ± 0.01	0.13 ± 0.05	0.30	7	0.05	0.06 ± 0.01	0.07 ± 0.02
C. n. occidentalis (PER)	−13.84	−76.24	21 (21)	All	0.07 ± 0.02	0.12 ± 0.04	0.58	15	0.07	0.08 ± 0.01	0.11 ± 0.02
				PBR	0.17 ± 0.06	0.09 ± 0.06	1.88	8	0.13	0.17 ± 0.05	0.22 ± 0.06
				Non-PBR	0.04 ± 0.01	0.13 ± 0.05	0.30	7	0.05	0.06 ± 0.01	0.07 ± 0.02
C. n. tenuirostris (PUR)	17.93	−67.18	17 (17)	All	–	–	–	–	–	–	–
				PBR	–	–	–	–	–	–	–
				Non-PBR	–	–	–	–	–	–	–

differs from a recent global analysis of selection at the avian MHC [42]. Here, the authors suggested that the pressure of extracellular pathogens is higher in non-passerines, resulting in a stronger signature of selection for the MHC class II in non-passerines than in passerines. The order Charadriiformes does not seem to fit to this proposed rule. Consistent with Minias et al. [42], gulls and most of the alcids show a strong signature of selection at MHC class II and weaker selection at MHC class I, but the paraphyletic group of shorebirds (plovers and sandpipers) instead shows a pattern more similar to passerines [16, 25, 26, 28, 29, 44]. Several morphological or ecological variables may explain this discrepancy. First, body size may be related to parasite abundance, as larger hosts may provide a greater variety of niches and, in turn, support a higher number of parasites than smaller birds [45]. Snowy Plovers are relatively small birds with a mean body mass of 38–50 g [32, 46]. Second, the selective pressures imposed by parasites may be habitat dependent. Although aquatic birds (mainly non-passerines) show a more diverse parasite community than their terrestrial counterparts [45], there is a difference between freshwater and saltwater habitats. Snowy Plovers inhabit the shores of alkaline water bodies, such as salt lakes, salt evaporation ponds and sandy beaches [46]. These saline habitats are typically considered to have a lower abundance of extracellular parasites [47–49], which would be consistent with the observed low diversity at MHC class II. In general, shorebirds show a low prevalence in intracellular pathogens, although viral infections (West Nile virus; [50], Newcastle disease virus [50], avian influenza; [51]), avian haemosporidians (*Plasmodium* and *Haemoproteus* spp.; [52]) and bacterial infections (*Mycobacterium*; [53]) have all been reported. Furthermore, Snowy Plovers inhabit low latitudes, where the diversity of intracellular

Table 4 Comparison of genetic diversity at MHC class II (segregating sites of amino acids (S_{aa}) and nucleotides (S_{nt}) and average nucleotide diversity (π)) in eight species of Charadriiformes, from six alleles randomly selected for each species (except for Black-tailed Godwit and Ruff where only four alleles were available). The measures of diversity and the synonymous and non-synonymous substitution rates were calculated for the complete sequences (All), the PBR and the non-PBR sites

	dN ± S.E	*dS* ± S.E	*dN/dS*	S_{nt}	S_{aa}	π
Snowy Plover (*Charadrius nivosus nivosus*)						
All	0.06 ± 0.01	0.10 ± 0.03	0.60	31	15	0.06
PBR	0.14 ± 0.05	0.10 ± 0.05	1.40	15	8	0.12
Non-PBR	0.03 ± 0.01	0.11 ± 0.04	0.27	16	7	0.05
Common Murre (*Uria aalge*)						
All	0.09 ± 0.02	0.05 ± 0.02	1.80	42	26	0.07
PBR	0.19 ± 0.05	0.11 ± 0.05	1.72	22	12	0.15
Non-PBR	0.06 ± 0.02	0.04 ± 0.02	1.50	20	14	0.05
Razorbill (*Alca torda*)						
All	0.03 ± 0.01	0.02 ± 0.01	1.50	17	10	0.02
PBR	0.07 ± 0.03	0.02 ± 0.02	3.50	10	6	0.05
Non-PBR	0.02 ± 0.01	0.02 ± 0.01	1.00	7	4	0.01
Atlantic Puffin (*Fratercula arctica*)						
All	0.10 ± 0.02	0.05 ± 0.02	2.00	44	26	0.08
PBR	0.26 ± 0.07	0.04 ± 0.03	6.50	24	14	0.16
Non-PBR	0.05 ± 0.01	0.06 ± 0.02	0.83	20	12	0.05
Black-legged Kittiwake (*Rissa tridactyla*)						
All	0.12 ± 0.02	0.06 ± 0.02	2.00	56	31	0.09
PBR	0.29 ± 0.06	0.05 ± 0.03	5.80	29	15	0.18
Non-PBR	0.07 ± 0.02	0.07 ± 0.02	1.00	27	16	0.06
Great Snipe (*Gallinago media*)						
All	0.03 ± 0.01	0.04 ± 0.01	0.75	20	10	0.03
PBR	0.08 ± 0.03	0.04 ± 0.03	2.00	10	6	0.06
Non-PBR	0.02 ± 0.01	0.04 ± 0.02	0.50	10	4	0.02
Ruff (*Philomachus pugnax*)						
All	0.17 ± 0.03	0.15 ± 0.04	1.13	67	36	0.14
PBR	0.33 ± 0.09	0.18 ± 0.01	1.83	29	15	0.23
Non-PBR	0.12 ± 0.02	0.14 ± 0.03	0.85	38	21	0.11
Black-tailed Godwit (*Limosa limosa*)						
All	0.18 ± 0.03	0.14 ± 0.03	1.28	68	40	0.15
PBR	0.31 ± 0.08	0.21 ± 0.08	1.47	27	16	0.23
Non-PBR	0.14 ± 0.03	0.12 ± 0.04	1.16	41	24	0.12

pathogens, as well as of their vectors, is expected to be high [48, 52]. In addition to the copy number variation for the fragment amplified at MHC I, the high abundance of intracellular pathogens in the tropics may contribute to the high allelic diversity at MHC class I. Further research is needed to determine whether Charadriiformes themselves show unusual group-specific variation in intra- and extracellular pathogens, or whether other life-history, ecological and evolutionary differences explain the observed differences in signatures of selection.

Across continental populations (subspecies *nivosus* and *occidentalis*), we found a similar number of alleles and nucleotide diversity. This result is consistent with the high gene flow observed among Snowy Plover populations [31, 33, 54]. At MHC I only, we found that the Nayarit population had a lower number of alleles (13 alleles), although this may reflect the sample size, as we only genotyped ten birds from this population. Among continental populations, private alleles were generally rare, and when present, occurred at low frequencies, suggesting that these represent rare alleles rather than geographically distinct alleles. By contrast, the island population of Puerto Rico (lineage *tenuirostris*) showed two privates alleles at MHC class I at moderate frequencies (Fig. 4): these alleles may represent true geographic variants. Interestingly, the *tenuirostris* population was monomorphic at MHC class II. Together with the lower positive selection at PBR sites at MHC class I, this might suggest that pathogen pressure is weaker in this population. The low genetic diversity may be a common feature of island populations that are thought to be exposed to fewer pathogens than continental pathogens [55]. For example, among shorebirds, Icelandic Black-tailed Godwits did not show positive selection at MHC class I PBR sites [26]. Other biogeographic features could contribute to the observed differences in genetic diversity. In contrast to most other populations, *tenuirostris* inhabits the Atlantic Ocean and the diversity of pathogens is thought to be lower in the Atlantic Ocean than the Pacific Ocean [56]. However, the other Atlantic population located in Florida did not show lower diversity than Pacific populations. Demography may also have played a role in shaping MHC diversity. A recent study showed that all Snowy Plover lineages went through a bottleneck within the last 1000 years, with particularly strong effects observed in *C. n. tenuirostris* [33]. A similar pattern of loss of adaptive diversity has been observed in other bird populations subject to recent bottlenecks (see [57–59]).

Comparing allelic diversity across species, we found that diversity at MHC class I in Snowy Plovers (40 alleles across four loci amplified) was similar to other Charadriiformes species (Red-billed Gull [29]; (38 alleles), Red Knot [25]; (36 alleles) and Black-tailed Godwit [26]; (47

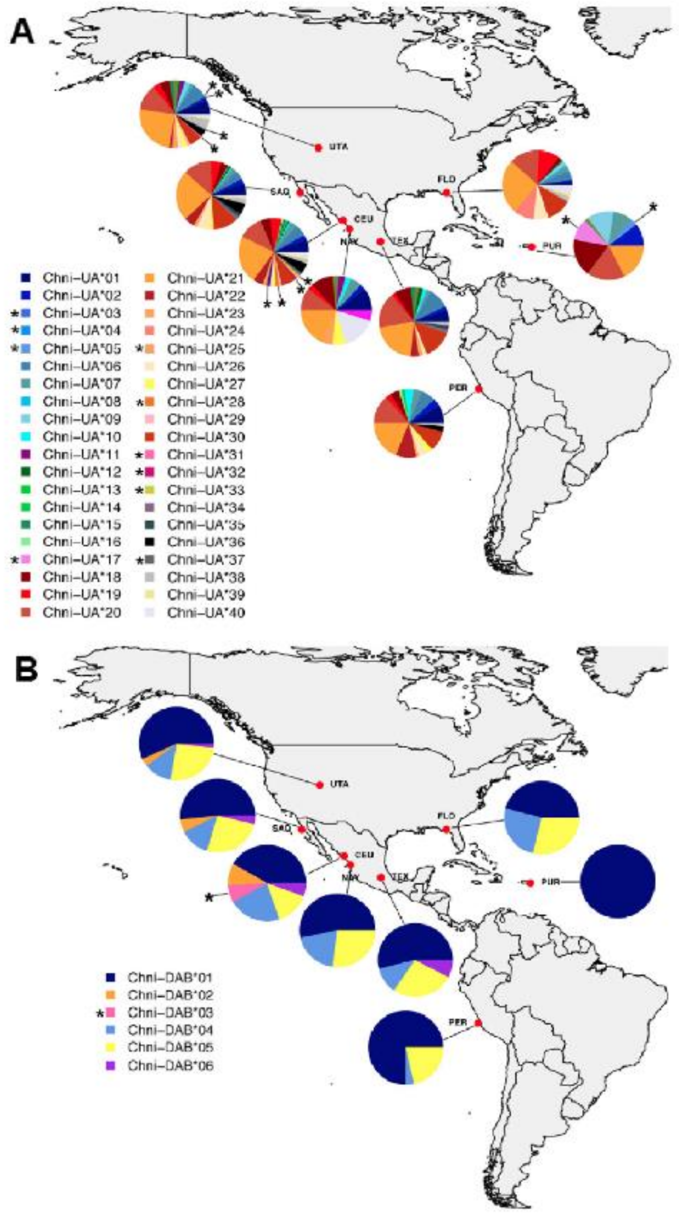

Fig. 4 Geographical distribution of MHC class I (a) and II (b) alleles among eight sampled Snowy Plover populations. Populations are abbreviated (Utah = UTA San Quintin = SAQ, Ceuta = CEU, Nayarit = NAY, Texcoco = TEX, Peru = PER, Florida = FLO and Puerto Rico = PUR). Long alleles of the MHC class I are in green colors and the asterisk shows alleles exclusive to single populations. The figure was created using software RStudio (Version 1.0.153; https://rstudio.com/)

alleles)). By contrast, MHC class II (six alleles) showed a lower allelic diversity than other Charadriiformes (Great Snipe – 50 alleles, [28]; Marbled Murrelet *Brachyramphus marmoratus* – 27 alleles, [60]). Despite the copy number variation, the nucleotide diversity at MHC class I in Snowy Plovers was lower than in other Charadriiformes (Table 2). However, the observed values were within the range of other non-passerines (such as the

Humboldt Penguin *Spheniscus humboldti* – 0.03 to 0.04 – and Magellanic Penguin *Spheniscus magellanicus* – 0.05 to 0.06, [61]; or grouse species – on average 0.05, [58]). For MHC class II, we found intermediate nucleotide diversity in comparison to other Charadriiformes (Marbled Murrelet – 0.08; [60]) and other non-passerines (Magellanic Penguin – 0.02, [61]; Eurasian Coot *Fulica atra* – 0.11, [62]; and Black Grouse *Tetrao tetrix* – 0.11, [63]).

Conclusions

We developed novel MHC markers to amplify the PBR exon 3 of MHC class I and PBR exon two of MHC class II for the threatened Snowy Plover. These are the first markers for MHC in the family Charadriidae and we anticipate that they will be of high utility for studying MHC in other plover species. Overall, genetic diversity at MHC in Snowy Plovers was low to moderate and likely to be shaped by past demographic processes such as bottlenecks and island colonization. In line with population genetic studies, we find that there is limited genetic differentiation attributable to geographic variation, consistent with the high gene flow observed in this species. Contrasting differences in the allelic diversity between MHC class I and class II indicate stronger positive selection at MHC class I than at MHC class II. These differences may reflect variation in exposure to intracellular and extracellular pathogens [42], but further studies are needed to confirm this.

Methods

Population sampling

We collected blood samples of 250 adult and juvenile Snowy Plovers from eight populations across North, Central and South America between 2006 and 2016 (Table 1). We captured adults in funnel traps placed on nests during incubation or used mist nets at other times using the methods described in Székely et al. [64]. After capturing and banding the individuals, we took 20 to 75 μl blood with heparinized capillaries from the brachial vein. We captured chicks at or near to the nest a few hours after hatching and took ~ 20 to 50 μl blood from the tarsal vein. We stored the blood in 1 ml of Queen's lysis buffer [65] at 4 °C or pure ethanol at room temperature until further processing.

DNA extraction

We isolated genomic DNA using the ammonium acetate precipitation method [66]. We checked the integrity of the DNA using a 0.8% agarose gel stained with SYBRsafe (Invitrogen). We measured DNA concentration using either a fluorometer (FLUOstar OPTIMA) or Nanodrop ND800 (Thermo Fisher Scientific).

Primer design for the MHC loci

We designed primers to capture the most polymorphic PBR sites in exonic regions for both MHC class I and MHC class II genes in Snowy Plovers. For MHC class I, we initially used the primers MHCI-int2F [20] and MHCI-ex3R [67] to isolate exon 3 of non-passerine birds. We undertook polymerase chain reactions (PCRs) in a total volume of 20 μl containing 12 μl Multiplex PCR Master Mix (MM, Qiagen), 4 μl Q-Solution (Qiagen), and 1 μl of each primer (1 μM) and 2 μl DNA (~ 15 ng/μl). The PCR program started with an initial denaturation step at 95 °C for 15 min, followed by 30 cycles at 94 °C for 30 s, 56 °C for 90 s and 72 °C for 90 s, and a final elongation step at 72 °C for 10 min. For MHC class II, we first used primers MHC05 [68] and 305 [69], and the primers 306 [69] and RapEx3CR [70] to capture introns 1 and 2, and parts of exons 1 and 3, respectively. We ran PCRs in a total volume of 20 μl, containing 16 μl MM, 1 μl of each primer (1 μM) and 2 μl of DNA (~ 15 ng/μl). The PCR program consisted of one cycle at 95 °C for 3 min, followed by 30 cycles at 94 °C for 30 s, 60 °C for 90 s and 72 °C for 90 s, followed by a final step at 72 °C for 10 min. All PCRs were run on a thermocycler PTC-225 DNA Tetrad Engine. We visualized PCR products using an agarose gel at 1.5% stained with ethidium bromide. For MHC class II we obtained multiple bands and subsequently cut out the visible bands of the expected size and extracted the amplified fragment using the QIAquick Gel Extraction Kit (QIAGEN). For MHC class I we only observed a single band, and the product did not require gel excision. We cleaned up MHC class I and II amplicons with ExoSap and sequenced the products using the BigDye terminator v.3.1 chemistry (Applied Biosystems) on an ABI 3730 automated sequencer (Applied Biosystems). For each MHC locus we aligned the sequences of six individuals using CodonCode Aligner 5.0.2 (CodonCode Corporation). We confirmed the identity of the sequences through blast hits in GenBank (NCBI). We then designed new primers Chni-Ex2F 5′-GAACTGCCTCCCTGCACAAA-3′ and ChCR-Ex2R 5′-TTCCCCGGGGAAATGTTCT-3′ to amplify the complete exon 2 for the MHC class II; and ChCR_MHC I_Ex2aF 5′-GGGTCTGTGCCCCACT-3′ for use with primer MHCI-ex3R 5′- CTCACCTTTCCTCTCCAG-3′ [41] to amplify the complete exon 3 of MHC class I.

Amplification and sequencing

We adopted a two-step PCR protocol to amplify the PBRs of both MHC classes and enable multiplexing. For PCR1 we created new oligonucleotides adding the Illumina overhang sequencing adapters F 5′-TCTACACGTTCAGAGTTCTACAGTCCGACGATC-3′ and R 5′-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-3′ to the MHC primer sequences (following Campbell et al. [71]). We performed PCRs in a total volume of 10 μl that contained; for MHC class I, 3.5 μl MM, 1.25 μl Q-solution, 1 μl of each primer (10 μM), 1 μl DNA (25 ng/μl) and 2.25 μl water, and for MHC class II, 4 μl MM, 1 μl of each primer (10 μM), 1 μl DNA (25 ng/μl) and 3 μl water. We used the same PCR programs as before but reduced the number of cycles to 28 to minimize the impact of chimera formation [72]. We then checked 1 μl of the products on an agarose gel and cleaned up the remainder of the solution using 8 μl (concentration of 0.8 X) of AMPure XP magnetic beads (Beckman Coulter, Indianapolis, USA) according to the manufacturer's protocol. We suspended the clean product in 20 μl TE and transferred 4 μl of MHC class I and II amplicons to a new 96-well plate, combining the amplicons of both classes for the same individual for the PCR2. We then added 0.5 μl of 0.5 M forward and reverse Illumina indexes (dual-plexed Fi5 [12 indexes] and Ri7 [16 indexes]; index primers in the format 5′-[Illumina i5 or i7 capture sequence][6-bp i5 or i7 barcode] [overhang sequence]-3′, 10 μl MM and 1 μl water, and ran PCR2 using the following program: 95 °C for 15 min, followed by 9 cycles at 98 °C for 10 s, 66 °C for 30 s and 72 °C for 30 s, with a final step at 72 °C for 5 min.

We determined the concentration of the PCR2 product using a fluorometer (FLUOstar OPTIMA) and 2 μl of the product. We pooled samples from eight individuals by taking 20 ng per sample and cleaning up the multiplexed PCR products with AMPure XP beads, as described above, with volumes adjusted to a 50 μl solution (concentration of 0.5 X). We used a TapeStation 4200 (Agilent Genomics) to confirm that there were no primer dimers present in the purified samples. We then quantified the PCR products using a qPCR with the KAPA library quantification kit (KAPA Biosystem) using 10 μl reaction volume (8 μl of SYBR Master Mix and 2 μl of template/standard or control), with the program: 95 °C for 5 min, followed by 35 cycles of 94 °C for 30 s and 60 °C for 45 s. We then pooled equimolar amounts per library, preparing six libraries in total, quantified the concentration of the pool with a Qubit (ThermoFisher Scientific, Waltham, USA) and submitted 4 nM per library for sequencing using 250 bp paired-end (500 cycles) Illumina sequencing on the MiSeq (Illumina Inc., San Diego, CA, USA) in six separate runs at the Sheffield Diagnostic Genetics Service.

Processing of data and MHC alleles validation

For the raw MiSeq data processing, we used the Amplicon Sequencing Analysis Tool (AmpliSAT) web server [73]. This tool is divided into different modules that allow the merging, cleaning and assignment of genotypes. First, we used AmpliMERGE with the FLASH algorithm to merge the pair-end reads. Then, we used AmpliCLEAN to filter out low-quality reads (<Q30 score and < 270 bp). After running AmpliCHECK with the default parameters for Illumina sequences, we retained all the remaining reads with lengths of 350 (325) ± 5 bp for MHC class I (II). Finally, we used AmpliSAS to demultiplex, cluster and filter the retained reads using default parameters for Illumina data for clustering, a minimum read depth per amplicon of 2000 and merging minimally different sequences to the dominant sequences when they differed by less than 3 bp and had ≤25% of the read depth in comparison with the dominant sequences [73]. Sequences that differed by 1–3 bp from the dominant sequences, but had more than 25% of the read depth, were classified as 'subdominants' and formed a new cluster representing a putative allele [26, 73]. We discarded all sequences that had a frequency of less than 5%, and those identified as chimera sequences. The minimum amplicon depth was set to 150 reads and the maximum amplicon depth set to 5000 reads due to computational limitations [73].

Allele validation

We blasted all putative alleles from Illumina sequencing to the GenBank (NCBI) nucleotide database to examine their similarity to known MHC alleles from other species. The alleles were named Chni-UA*01 to UA*40 (MHC class I) and Chni-DAB*01 to DAB*06 (MHC class II), following the nomenclature suggested by Klein et al. [74].

Diversity analysis and tests for selection

We used MEGA 7.0.18 [75] for initial diversity and selection analysis. First, we aligned the putative alleles using the MUSCLE algorithm [76] implemented in MEGA. We manually checked indel sites and curated alignments in order to preserve triplets within exons. We then estimated the number of segregating amino acid sites (S_{aa}), nucleotide diversity (π), evolutionary distance for nucleotide sequences (d_{nt}) and evolutionary distance for amino acid (d_{aa}), for each of the eight populations included in the study. For the evolutionary distance analyses, we inferred the appropriate substitution models based on the best-fit model (using AIC_C) using JModeltest 2.1.10 [77]. For MHC class I, we employed the Kimura two-parameter model [78] with a gamma distribution, setting the transition rate α to 0.9 for nucleotide sequences, and using the *p*-distance model with uniform rates to assess amino acid sequences distances. For MHC class II, we implemented the Jukes-Cantor + G model with a gamma distribution, setting the substitution rate α to 0.8 for nucleotide sequences, and we calculated amino acid sequence distances using a *p*-distance model with uniform rates.

We calculated standard errors of the mean evolutionary distances from 1000 bootstrap replicates. Positive selection, as a response to the selection imposed by pathogens, will lead to an excess of non-synonymous over synonymous substitutions in the PBR ($\omega = dN/dS > 1$). To assess the impact of positive selection on nucleotide diversity, we compared ω at PBR sites and non-PBR sites. We inferred PBR sites based on previously documented transcripts (MHC class I: [79], MHC class II: [80]). We calculated synonymous and non-synonymous substitution rates through the modified Nei–Gojorobi method [81] with Jukes–Cantor correction. Also, we tested site-by-site selection applying Fast Unconstrained Bayesian AppRoximation (FUBAR; http://www.datamonkey.org/fubar, [82]. As recombination is frequent in MHC genes and recombination may lead to overestimation of the number of positively selected sites [62], we tested for evidence of recombination in our MHC alignments using Genetic Algorithm for Recombination Detection (GARD; http://www.datamonkey.org/gard, [83]).

Phylogenetic diversity and relationships

We compared nucleotide diversity at MHC loci across the Charadriiformes using data available at GenBank. For MHC class I, we obtained sequences from three other charadriiform species: Red Knot *Calidris canutus* [25], Icelandic Black-tailed Godwit *Limosa limosa islandica* [26] and the Red-billed Gull *Larus scopulinus* [29]. As only sequences from 21 alleles were available for Red-billed Gull, we randomly drew sequences of 21 alleles from each species in this comparison to obtain a comparable sample size for the diversity estimate (Table S3). For MHC class II, we obtained data from seven further species: Common Murre *Uria alge* [44], Razorbill *Alca torda* [44], Atlantic Puffin *Fratercula artica* [44], Black-legged Kittiwake *Rissa tridactyla* [44], Great Snipe *Gallinago media* [28], Ruff *Philomachus pugnax* [16] and Black-tailed Godwit *Limosa limosa* [16]. As we had only six putative alleles in Snowy Plover, we capped the number of sequences to six per species that we randomly drew for this comparison (Table S3). We evaluated the amino acid (S_{aa}) and nucleotide (S_{nt}) segregation sites, nucleotide diversity (π), as well the synonymous (dS) and non-synonymous (dN) substitution rates, using the same parameters described above. We visualized phylogenetic relationships between the MHC class I and class II alleles in Snowy Plovers through the Neighbor-net algorithm implemented in SplitsTree 4.14.6 [84]. This method allows deduction of alternative phylogenetic histories and model incompatibilities in the dataset that may lead to conflicting phylogenetic signals because of duplication, recombination and gene conversion, which are all common in MHC genes [20, 25]. Finally, we inferred the phylogenetic relationships among charadriiform MHC alleles using a Neighbour-Joining Tree with Maximum Likelihood implemented in MEGA 7.0.18. We calculated branch support through 1000 bootstrap replications.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12862-020-01676-7.

Additional file 1: Figure S1. Graphical representation of nucleotide diversity and dN/dS ratios for MHC class I and MHC class II. Silhouettes represent Charadriiform species, i.e. MHC class I (from top to bottom: Red Knot, Black-tailed Godwit, Red-billed Gull and Snowy Plover) and MHC class II (from top to bottom: Black-tailed Godwit, Ruff, Black-legged Kittiwake, Atlantic Puffin, Common Murre, Snowy Plover, Great Snipe and Razorbill). Arrows represent strength of selection in both MHC classes for the Snowy Plover in comparison to the other species. **Table S1.** Results of generalized linear models testing the differences in the number of alleles per individual between populations for the MHC class I and class II in the Snowy Plover. **Table S2.** Results of generalized linear models testing the differences in the number of alleles between populations for the MHC class I and class II in the Snowy Plover. **Table S3.** Sequences ID list randomly drawn for the MHC class I and MHC class II species comparison.

Abbreviations

AICc: Corrected Akaike information criterion; AmpliSAT: Amplicon Sequencing Analysis Tool; dN: Nonsynonymous substitution rate per nonsynonymous site; dS: Synonymous substitution rate per synonymous site; FUBAR: Fast Unconstrained Bayesian AppRoximation; GARD: Genetic Algorithm for Recombination Detection; MHC: Major histocompatibility complex; NCBI: National Center for Biotechnology Information; PBR: Peptide-binding region; PCR: Polymerase chain reaction; S_{aa}: Amino acid segregation sites; S_{nt}: Nucleotide segregation sites

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Authors' contributions

MCL, GF and CK designed the study. MCL, CK, FP, JC, DGF, SGA, RP and OG collected field samples. MCL and CK conducted the laboratory work, with assistance from HH. MCL, CK and HH performed the analyses. MCL and CK wrote and revised the manuscript with assistance from GF, HH and TB. All authors have read and approved the final manuscript.

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Availability of data and materials

All MHC allele sequences are available at GenBank, the sequence data base of the National Institutes of Health (NIH), USA. Accession numbers for MHC class I alleles are MT888135 MT888174 and for MHC class II alleles MT888175 MT888180.

Ethics approval and consent to participate

Fieldwork and blood sampling was conducted under permit by relevant authorities, i.e. Institutional animal care and use committee at the University of Florida, project no. E877 and the Florida Fish and Wildlife Conservation Commission (permit #21980); US Fish and Wildlife Service (permit #23021); Dirección General de Vida Silvestre (SGPA/DGVS/01717/10; SGPA/DGVS/01367/11; SGPA/DGVS/02078/12; SGPA/DGVS/02898/12; SGPA/DGVS/06485/13; SGPA/DGVS/00098/14) for Mexico, permit 042 C/C-2009-INRENA-IANP for Peru and, US Fish and Wildlife Service, Cabo Rojo and Departamento de Recursos Naturales y Ambientales, San Juan, Puerto Rico. The study was conducted in accordance with the current and local laws of each country and in accordance with the approved ethical guidelines outlined by local governments.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Publisher's Note

Capítulo IV: Los genes del Complejo Mayor de Histocompatibilidad y su relación con componentes de adecuación biológica en crías del Chorlo nevado (*Charadrius nivosus*) en una población que presenta disminuciones en su tamaño poblacional.

Chapter IV: Major Histocompatibility Complex genes and their relationship with fitness components in Snowy Plover (*Charadrius nivosus*) offspring in a population showing declines in population size.

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Manuscrito

Abstract

The Major Histocompatibility Complex (MHC) is one of the most highly polymorphic regions within the vertebrate genome. MHC product proteins are critical for initiating an adaptive immune response and their diversity could give individuals in a population an advantage during critical life stages. We investigated whether MHC class I and class II diversity, measured as the number of alleles or supertypes, was associated with survival and recruitment rates in Snowy Plover (*Charadrius nivosus*) offspring. We tested the above using detailed data of survival and MHC I and II genotypes of 197 Snowy Plover offspring from the Ceuta Bay population. We controlled our analyses for other non-genetic predictors of survival that have been identified in previous studies. We found no evidence of a correlation between survival or recruitment rates and MHC class I or II diversity in Snowy Plover offspring. Additionally, we assessed the occurrence of temporal changes in the frequency of MHC alleles or supertypes in the population, but no clear pattern was observed over 11 years of study; except for one MHC class I supertype that appears to have increased in frequency over the years, a large variation in supertype or allele frequencies was observed for both MHC classes. Based on our

results, we found no evidence that MHC diversity is a determinant of Snowy Plover survival or offspring recruitment, suggesting other life history factors, such as predation and habitat availability/stability, have a greater influence on early life stage survival in this species.

Los genes del Complejo Mayor de Histocompatibilidad y su relación con componentes de adecuación biológica en crías del Chorlo nevado (*Charadrius nivosus*) en una población que presenta disminuciones en su tamaño poblacional.

Resumen

El Complejo Mayor de Histocompatibilidad (MHC) es una de las regiones de mayor polimorfismo dentro del genoma de los vertebrados. Las proteínas producto del MHC son fundamentales para iniciar una respuesta inmune adaptativa y su diversidad podría dar ventajas a los individuos de una población durante etapas críticas de la vida. Investigamos si la diversidad del MHC clase I y clase II, medida como el número de alelos o supertipos, estaba asociada con la supervivencia y tasas de reclutamiento en crías de Chorlo nevado (*Charadrius nivosus*). Pusimos a prueba lo anterior usando datos detallados de supervivencias y genotipos del MHC I y II de 197 crías de Chorlo nevado provenientes de la población de Bahía Ceuta. Controlamos nuestros análisis por otros predictores no genéticos de supervivencia que han sido identificados en estudio previos. No encontramos evidencia de que existiera una correlación entre la supervivencia o tasas de reclutamiento y la diversidad del MHC clase I o II en crías del Chorlo nevado. Adicionalmente, evaluamos la ocurrencia de cambios temporales en la frecuencia de alelos o supertipos del MHC en la población, pero no se observó ningún patrón claro a lo largo de 11 años de estudio; con excepción de un supertipo del MHC clase I que parece haber aumentado su frecuencia a través de los años. Se observó una gran variación en las frecuencias de supertipos o alelos para ambas clases de MHC. Con base en nuestros resultados, no encontramos evidencia de que la diversidad del MHC sea determinante para la supervivencia o reclutamiento de crías del Chorlo nevado, lo que sugiere otros factores de su historia de vida, como la depredación y la disponibilidad/estabilidad del hábitat, tienen una influencia mayor en la supervivencia en etapas tempranas de vida en esta especie.

Introducción

La evidencia acumulada durante las últimas cinco décadas, ha mostrado que la diversidad genética tiene un papel fundamental en la conservación de las poblaciones naturales (Allendorf 2017). Debido a que la pérdida de diversidad genética se relaciona con la endogamia, y la endogamia reduce la aptitud reproductiva al incrementar el número de individuos homocigotos, reduciendo la adecuación biológica (fitness) como resultado de la expresión de alelos deletéreos parcialmente recesivos, se espera que la heterocigocidad esté relacionada con la adecuación biológica en los individuos de una población (Hansson y Westerberg, 2002; Reed y Frankham, 2003). En el Buitre Egipcio (*Neophron percnopterus*) individuos con bajos niveles de endogamia reclutaron más temprano a la población reproductora, dándoles una ventaja para iniciar su vida reproductiva antes que los reclutas tardíos (Agudo *et al.* 2012). Por otro lado, en un experimento con el crustáceo *Americamysis bahia* encontraron que aquellas poblaciones con niveles altos de diversidad genética individual sobrevivieron durante un experimento de estrés, mientras que las que exhibían diversidad genética baja presentaron mortalidades promedio del 20% de la población, pero en algunos casos hasta un 73% de los individuos de poblaciones con diversidad genética baja murieron antes de finalizar el estudio (Markert *et al.* 2010). Además de aumentar la homocigosidad individual, la pérdida de diversidad genética de una población también reduce su potencial adaptativo. Asimismo, niveles de diversidad genética bajos se han asociado con una menor esperanza de vida y salud de los individuos, lo que repercute en la tasa intrínseca de crecimiento de la población (Spielman *et al.* 2004). Por lo tanto, la prevalencia a largo plazo de las poblaciones depende en cierta medida de que cuente con una cantidad adecuada de diversidad genética, más aún en los tiempos actuales donde se presentan nuevas presiones selectivas y cambios en el ambiente.

Uno de los principales efectos de la variación ambiental es la fluctuación en el tamaño de poblaciones naturales (Ceballos *et al.* 2017), la cual tienen como consecuencia alteraciones en parámetros demográficos como la reproducción y la supervivencia (Reid *et al.* 2004). Diversos trabajos han demostrado que la supervivencia de los adultos es una de las tasas vitales que guía la demografía o dinámica en poblaciones de vertebrados (Crone 2001; Sæther y Bakke 2000; Manlik *et al.* 2016). Las aves playeras no son la excepción, puesto que la supervivencia de adultos suele ser uno de los factores que más afecta las tasas de crecimiento poblacionales (Sandercock 2003, Cruz-López *et al.* 2017). Estimar la supervivencia de adultos en poblaciones animales abiertas se complica por el hecho de que los individuos pueden integrarse o abandonar la población libremente, particularmente en especies con gran capacidad de locomoción y dispersión como las aves. La desaparición de individuos en poblaciones monitoreadas puede deberse tanto a la muerte como a la emigración, razón por la que se trabaja con "estimaciones de supervivencia aparente" en las que se incluyen ambos fenómenos.

Recientemente se demostró que la supervivencia en etapas tempranas de vida, es el segundo parámetro más importante que afecta las tendencias de sus poblaciones (Weiser *et al.* 2020), particularmente en especies de aves con crías precociales (Rickenbach *et al.* 2011). Las tasas de supervivencia y mortalidad en etapas tempranas de vida son difíciles de evaluar en aves playeras, ya que las crías son móviles y es difícil seguirlas (por ejemplo, las crías suelen ocultarse en la vegetación y presentar un alto grado de camuflaje) Además, estos parámetros podrían tener una mayor relevancia para la dinámica de sus poblaciones (Rickenbach *et al.* 2011), sobre todo si consideramos lo mencionado anteriormente sobre la supervivencia aparente en adultos. Por lo tanto, conocer las causas de mortalidad en etapas tempranas de vida, es fundamental para conocer la dinámica de poblaciones e implementar estrategias de conservación, sobre todo en especies amenazadas y con tendencias poblacionales negativas.

La mortalidad de crías asociada a la depredación en etapas tempranas de vida es uno de principales factores que influyen en la demografía de las aves (Ibáñez-Álamo *et al.* 2015). De hecho, la depredación de nidos (huevos) es la causa principal del bajo éxito reproductivo en varias especies de aves (Martin 1993). De igual forma, la depredación durante los primeros días después de la eclosión es una de las causas de mortalidad más importantes en crías precociales, en las que se ha estimado entre 60 y 87% (Rickenbach *et al.* 2011). Por su parte, otro de los componentes importantes para la supervivencia de crías es la disponibilidad de alimento; en especies precociales, asociadas a ambientes húmedos, se ha encontrado que la disminución en la humedad (suelos secos) disminuye las probabilidades de supervivencia en crías al reducir la cantidad de invertebrados (Eglington *et al.* 2010; Rickenbach *et al.* 2011).

Más recientemente, se ha demostrado también que las enfermedades infecciosas causadas por patógenos (bacterias, hongos, virus y parásitos) impactan de manera importante la supervivencia de las aves (Robinson *et al.* 2010; Staley y Bonnaeud 2015). Sin embargo, la mortalidad asociada a patógenos en las crías ha sido menos estudiada que la de adultos debido a la dificultad de dar seguimiento o encontrar a las crías (por ejemplo, en aves con crías precociales). En un estudio con Cormorán Orejón (*Phalacrocorax auritus*), especie de ave altricial, se demostró que hasta un 50% de las crías de una colonia reproductiva en Canadá murió a causa de una enfermedad viral (Leighton *et al.* 2021). Además, los individuos infectados por algún patógeno no son solo susceptibles a morir por la infección, sino que, al destinar recursos energéticos para activar una respuesta inmune, disminuye también su desempeño en general y aumentan sus probabilidades de morir.

La supervivencia de juveniles en mamíferos y aves está influenciada principalmente por las condiciones ambientales y la calidad del cuidado parental (Clutton-Brock, 1988; Karlsson *et al.* 2015). Sin embargo, algunos trabajos han demostrado que el genotipo de los individuos se encuentra relacionado a la supervivencia. En el Carricero Tordal (*Acrocephalus arundinaceus*), crías con niveles altos de heterocigocidad tuvieron tasas de reclutamiento superiores. En esta especie, el reclutamiento local está fuertemente relacionado con la adecuación biológica, ya que la mayoría de las crías mueren antes de llegar a la adultez (Hansson *et al.* 2001). De manera similar, un estudio en el Águila Real (*Aquila chrysaetos*) en América del Norte, encontró que los juveniles de esta especie exhiben en promedio menor heterocigosidad y una menor supervivencia en sus etapas tempranas de vida, lo que se refleja en niveles promedio de heterocigosidad más altos en los adultos sobrevivientes (Doyle *et al.* 2016). En el caso del Chorlo Patinegro (*Charadrius alexandrinus*), en un estudio donde se evaluó la relación de la heterocigosidad con la supervivencia se encontraron resultados ambiguos; la variabilidad en uno de los marcadores moleculares mostró una correlación positiva con la supervivencia, mientras que para un segundo marcador la correlación fue negativa (Küpper *et al.* 2010).

Debido a que la mayoría de estos estudios han utilizado marcadores selectivamente neutrales o casi-neutrales, los cuales no están expuestos directamente a procesos selectivos, la pérdida de variación genética puede ocurrir más rápido que para genes implicados en la adecuación biológica (Reed y Frankham, 2003). Por otro lado, la pérdida de variación genética neutral, no implica necesariamente una pérdida de adecuación biológica o de potencial adaptativo (Reed y Frankham, 2003). Por tal motivo, comprender los mecanismos detrás de las correlaciones encontradas entre componentes de diversidad genética neutral y componentes de adecuación biológica, como la supervivencia, es complicado. Por ejemplo, en el Zorro de la Isla de San Nicolas (*Urocyon littoralis dickeyi*) no se encontró variación genética en loci neutrales, pero si niveles altos de variación genética en cuatro de cinco loci adaptativos asociados al Complejo Mayor de Histocompatibilidad (Aguilar *et al.* 2004).

En las últimas décadas, los genes del Complejo Mayor de Histocompatibilidad (MHC por sus siglas en inglés) se han utilizado para evaluar cómo es que la diversidad genética adaptativa influye en la supervivencia, elección de pareja y el estado de conservación en poblaciones silvestres (Sepil *et al.* 2012). Los genes del MHC son los encargados de detectar a patógenos o moléculas exógenas, y presentarlas a las células T para iniciar una respuesta inmune adaptativa (Bernatchez y Landry 2003). El interés sobre los genes del MHC se debe a que es una de las regiones más polimórficas del genoma de los vertebrados (Garrigan y Hedrick 2003). Este gran polimorfismo es mantenido, en parte, por la selección balanceadora mediada por la exposición a patógenos (Radwan *et al.* 2010). Existen tres hipótesis principales que explican este alto grado de polimorfismo en el MHC: 1) La hipótesis de *ventaja de heterocigotos*, que plantea que aquellos individuos con loci heterocigotos son capaces de enfrentar a un mayor número de patógenos en comparación con los homocigotos, brindando una mayor probabilidad de supervivencia (Spurgin y Richardson 2010); 2) La hipótesis de *ventaja de los alelos raros (o dependiente de la frecuencia alélica)*, que sugiere que los patógenos pueden evolucionar para ser más resistentes a los alelos más comunes del MHC, pero cuando un nuevo alelo aparece en una población, este podría brindar mayor protección al presentar una respuesta novedosa ante algunos patógenos (Spurgin y Richardson 2010); y 3) La hipótesis de la *selección fluctuante*, la cual hace referencia a que los individuos pueden estar expuestos a patógenos que fluctúan en el tiempo y espacio, lo que hace que diferentes alelos del MHC sean seleccionados de manera espacio-temporal, fluctuando y manteniendo la diversidad genética (Spurgin y Richardson 2010).

Los genes del MHC juegan un papel fundamental para la adecuación biológica de los individuos, brindándoles tolerancia o resistencia contra ciertos patógenos e influyendo directamente en la probabilidad de supervivencia de los individuos (Sepil *et al.* 2013). Un estudio realizado en Ovejas de Soay (*Ovis aries*) mostró que la supervivencia de juveniles de la especie y la resistencia a parásitos intestinales estaba asociada con la presencia de alelos específicos y no tanto con la diversidad del MHC (Paterson *et al.* 1998). En otro caso de estudio, la supervivencia de juveniles del Carricero de Seychelles (*Acrocephalus sechellensis*) estuvo relacionada tanto a la diversidad como a alelos específicos del MHC (Brouwer *et al.*

2010). El análisis de una población de Carbonero Común (*Parus major*) en el Reino Unido reveló que ciertos individuos que poseían supertipos particulares del MHC (conjunto de alelos que presentan características funcionales similares) tenían una mayor tasa de supervivencia o tasas de reproducción mayores, aumentando con ello sus probabilidades de retorno en años posteriores (Sepil *et al.* 2013).

A pesar de la evidencia que existe de que la diversidad genética está relacionada con la supervivencia de individuos en las poblaciones silvestres, otros factores como los ambientales y aquellos asociados a la calidad del cuidado parental también son importantes, sobre todo en etapas tempranas de vida (Clutton-Brock 1991; Klug y Bonsall 2014). En especies con crías precociales el cuidado parental se limita en guiar a sus crías para obtener alimento, así como a protegerlas del frío, depredadores y conespecíficos (Naef-Daenzer y Grüebler 2016). A pesar de que el cuidado parental en especies precociales es menor que en especies altriciales y podría parecer poco importante para la supervivencia de las crías (Alonso-Alvarez y Velando 2012; Naef-Daenzer y Grübler 2016), algunos estudios han demostrado que no es así, sobre todo en ambientes que presentan cambios temporales en la calidad del hábitat. Por lo anterior, es relevante evaluar la medida en que el cuidado parental impacta en la supervivencia de las crías y, por ende, en la dinámica de las poblaciones naturales.

El estudio de las aves playeras es de interés, en un contexto ecológico y evolutivo, en relación con las conductas poco comunes, a diferencia, de otros grupos de aves. Por ejemplo, dentro del grupo se presentan diversos sistemas de apareamiento (monogamia, poliandria, poliginia) y el cuidado parental puede ser proveído tanto por ambos como por solo uno los padres (Székely *et al.* 2006). El Chorlo nevado (*Charadrius nivosus*) es una especie de ave playera que presenta este tipo de conductas “inusuales”; la poliandria y el cuidado uniparental proveído por los machos son frecuentes en esta especie. Las crías son precociales y son capaces de salir del nido pocas horas después de haber eclosionado, pero también sus poblaciones presentan una proporción sexual de adultos sesgada hacia los machos, por lo que las hembras tienen mayores posibilidades de aparearse más de una vez por temporada y son los machos quienes cuidan en nido (Eberhart-Phillips *et al.* 2017, Kupán *et al.* 2021).

Las poblaciones de Chorlo nevado de Estados Unidos de Norteamérica y México están protegidas por las autoridades federales debido a que en muchas de sus poblaciones se ha evidenciado una reducción reciente importante (Cruz-López *et al.* 2017; Galindo-Espinosa y Palacios, 2015). Aunado a estas disminuciones contemporáneas, la especie también ha estado sujeta a procesos de cuello de botella en los últimos 1,000 años (D'Urban-Jackson *et al.* 2020), lo que se refleja en nivel de diversidad genética bajos en las tres subespecies reconocidas. (D'Urban-Jackson *et al.* 2017, 2020). En el Chorlo nevado, la diversidad genética es baja incluso en genes del MHC que típicamente exhiben mucha variabilidad en vertebrados (Cruz-López *et al.* 2020).

En este estudio se puso a prueba cómo los genes del MHC clase I y II, están asociados con la supervivencia y tasas de reclutamiento de crías en una población subtropical del Chorlo nevado monitoreada a lo largo de una década. De acuerdo con nuestras predicciones, la supervivencia de crías estaría asociada con niveles más altos de heterocigosidad o la presencia de alelos específicos del MHC clase I y II (número total de alelos por individuo). Esto se verá reflejado en altas tasas de reclutamiento de individuos con mayor diversidad de genes o con alelos particulares en ambos loci. La supervivencia fue investigada en crías de hasta 25 días post-eclosión, edad a la cual se consideran independientes, mientras que para estimar la tasa de retorno se tomaron en cuenta aquellas crías que se incorporaron como reproductores en años subsecuentes. Por otro lado, investigamos la variación temporal de los alelos del MHC clase I y II, bajo la premisa de que la frecuencia de alelos específicos asociados a componentes de adecuación biológica ha cambiado a lo largo del periodo de estudio.

Métodos

Descripción del área de estudio

Estudiamos una población del Chorlo nevado (*Charadrius nivosus*) durante las temporadas reproductivas (abril a julio) de 2006 a 2016 en la Bahía de Ceuta, Sinaloa, México (23°54′N, 106°57′W). En esta localidad, entre 50 y 100 parejas de la especie utilizan una serie de estanques abandonados de una salinera, para anidar y criar sus polluelos. El hábitat de la zona se caracteriza por tener un substrato con poca vegetación y espacios abiertos rodeados por bosques de mangle. La temporada reproductiva inicia típicamente a mediados de abril, cuando el agua retrocede y deja expuestos los estanques en los que ocurre la puesta y la crianza, concluyendo a mediados de julio, cuando las lluvias y las mareas altas de verano inundan los estanques (Cruz-López *et al.* 2017). Avanzada la temporada reproductiva, los chorlitos suelen hacer uso de una zona ubicada aproximadamente tres kilómetros al norte del sitio principal de anidación, la cual se encuentra separada de los estanques de la salina por una franja de mangle y parte del cuerpo de agua de la bahía.

Captura y obtención de datos de la población de Chorlo nevado en Ceuta

Un total de 604 crías, las cuales representan once temporadas reproductivas, fueron genotipificadas para este estudio. La búsqueda de nidos (familias o individuos incubando) en la zona de los estanques se llevó a cabo con ayuda de binoculares y telescopios, desde un escondite móvil o un vehículo. Cada nido fue georreferenciado con un GPS de mano, registrando las fechas estimadas de puesta y eclosión que se obtuvieron mediante la técnica de flotación de huevos y considerando un período de incubación de 25 días (Székely *et al.* 2008). Los adultos fueron capturados en sus nidos mediante una trampa de embudo, asegurándonos que la nidada (total de huevos en el nido) estuviera completa (al menos 5 días de puesta) para disminuir probabilidad de deserción del nido. Los adultos capturados fueron marcados con una combinación única de tres anillos de color y un anillo metálico con un código numérico (Székely *et al.* 2008). Por otro lado, las crías que fueron capturadas en un mismo nido o en sus cercanías, se marcaron colocándoles un anillo metálico y un anillo de color distinto para poder dar seguimiento individual a cada hermano, registrando además la fecha estimada de eclosión de cada uno (Székely *et al.* 2008). Para cada individuo capturado se tomaron datos morfométricos (longitud de tarso y peso en todos y longitud de ala solo en los adultos) y una muestra de sangre (25-50 microlitros) que se almacenó en 1 mililitro de amortiguador de lisis de Queen o alcohol al 96% para su posterior procesamiento y análisis genético.

Para obtener los datos de supervivencia de las crías se realizaron observaciones de las familias de manera frecuente (al menos cada tres días) a lo largo de las temporadas reproductivas de 2006 a 2012. Para las temporadas del 2013 al 2016 no se realizó el mismo esfuerzo de seguimiento de las familias, por lo que fue necesario restringir los análisis de supervivencia de las crías a un conjunto de datos reducido (2006 al 2012). A las familias activas se les dio seguimiento los 25 días posteriores a la fecha estimada de eclosión, periodo después del cual se asumió que alcanzaron la etapa de independencia (Székely *et al.* 2008). Durante cada avistamiento se observó por al menos 15 minutos a cada familia, o bien hasta que todos los miembros de la familia eran observados, registrando al miembro de la pareja parental que se encontraba atendiendo las crías y las crías que estaban presentes. Para aquellos casos en los que no se observaron una o más crías en dos avistamientos consecutivos, se asumió que estas habían muerto (Székely *et al.* 2008), menos del 5% de las crías a las que se consideró como muertas son observadas de nuevo o reclutadas en años posteriores. Lo que nos da certeza que las crías murieron. Determinamos la deserción por alguno de los padres, si en dos eventos de avistamiento consecutivos, alguno de los padres no estaba presente. Calculamos la fecha de deserción como la fecha promedio entre el día que ambos padres fueron observados atendiendo a la familia por última vez y la primera fecha cuando solo uno de los padres fue observado atendiendo a las crías (Székely *et al.* 2008). De manera regular se revisaron los alrededores de la Bahía en busca de familias marcadas que pudieran haberse movido a otras áreas cercanas a los estanques de la salinera. El reclutamiento de crías como de reproductores, fue confirmado una vez que localizamos el nido de individuos previamente marcados solo con anillo metálico y uno de color, los cuales fueron recapturados para colocarles una combinación completa de anillos de color. Debido al esfuerzo intensivo de búsqueda de nidos y captura de adultos, tenemos la confianza que la mayoría de los reclutas fueron capturados (>85% adultos capturados) en todas las temporadas reproductivas que fueron incluidas en este estudio (2006 al 2016). Todos los datos utilizados en este estudio están disponibles en la base de datos "Ceuta Open" (Eberhart-Phillips *et al.* 2020).

Extracción de DNA y sexado molecular

La extracción de DNA genómico se realizó usando el método de precipitación de acetato de amonio (Nicholls *et al.* 2003). La calidad y la concentración del DNA fueron evaluadas usando un gel de agarosa al 0.8% teñido con SYBRsafe y un fluorómetro o Nanodrop ND800, respectivamente. En breve, la identificación molecular del sexo se realizó con base a la amplificación por PCR del marcador Z-002B (Dawson, 2007) y verificado con el marcador Calex-31 que corresponde a un microsatélite W-específico (Küpper *et al.* 2007). Las condiciones de la reacción de la cadena de la polimerasa (PCR) para el sexado se pueden consultar en dos Remedios *et al.* (2015). Todos los datos del sexado de crías usados en nuestro estudio son parte de la base de datos "Ceuta Open" (Eberhart-Phillips *et al.* 2020).

Genotipificación del MCH en el Chorlo nevado

Para secuenciar las regiones que presentan mayor polimorfismo (sitios PBRs) en el MHC, amplificamos el exón 3 en su totalidad para el MHC clase I y el exón 2 del MHC clase II en el Chorlo nevado. Para esto utilizamos "primers" previamente diseñados para amplificar las regiones de nuestro interés (Cruz-López et al 2020). Para amplificar el exón 3 del MCH clase I, utilizamos los primers ChCR_MHCI_Ex2aF 5'-GGGTCTGTGCCCCACT-3' para ser utilizados con el primer MHCI-ex3R 5'- CTCACCTTTCCTCTCCAG-3' y los primers Chni-Ex2F 5'-GAACTGCCTCCCTGCACAAA-3' y ChCR-Ex2R 5'-TTCCCCGGGGAAATGTTCT-3' para amplificar el exón 2 en el MHC clase II.

Amplificación y secuenciación

Seguimos en protocolo de PCR de dos pasos para amplificar los sitios PBR para ambas clases de MHC. Para la PCR1 colocamos los adaptadores de secuenciación de Illumina F 5'-TCTACACGTTCAGAGTTCTACAGTCCGACGATC-3' y R 5'-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-3' a los primers del MHC para crear nuevos oligonucleótidos (Campbell *et al.* 2015). El volumen total de las PCRs fue de 10 µl y estos contenían, para el MHC clase I, 3.5 µl de Master Mix (MM), 1.25 µl de Q-soultion, 1 µl de cada primer (10 µM) 1 µl de DNA (25ng/ µl) y 3 µl de agua. El programa de PCR se inició con la desnaturalización a 95 °C por 15 minutos, seguido de 28 ciclos, para disminuir la formación de quimeras (Lenz y Becker, 2008) a 94 °C por 30 segundos, 56 °C por 90 segundos, 72 °C por 90 segundos y una elongación final a 72 °C por 10 minutos (Cruz-López *et al.* 2020). Para el MHC clase II, la PCR se realizó con 4 µl de MM, 1 µl de cada primer (10 µM), 1 µl de DNA (25ng/ µl) y 3 µl de agua. El programa de PCR consistió de un ciclo a 95 °C por 3 minutos, seguido de 28 ciclos a 94 °C por 30 segundos, 60 °C por 90 segundos, 72 °C por 90 segundos y un paso final de 72 °C por 10 minutos (Cruz-López *et al.* 2020). Los productos obtenidos fueron verificados en un gel de agarosa para proceder con la limpieza del resto del producto, usando una solución de 8 µl de perlas magnéticas AMPure XP (concentración al 0.8). Para la PCR2, suspendimos el producto limpio en 20 µl de low TE y transferimos 4 µl del amplicón (un conjunto de lecturas derivadas de un PCR de una muestra y puede comprender productos de varios loci coamplificantes) del MHC clase I y II a una placa de 96-pocillos, combinando los amplicones de ambas clases de MHC para un mismo individuo (Cruz-López *et al.* 2020). Entonces para esta PCR agregamos: 0.5 µl al 0.5M de índices "adelante" y "atrás" de Illumina (dual-plexed Fi5 [12 índices] y Ri7 [16 índices]; primers índices en formato 5'-[secuencia de captura Illumina i5 o i7] [códigos de barra de 6-pb i5 o i7][secuencia overhang]-3'); 10 µl de MM y 1 µl de agua. Seguimos el programa de PCR: 95 °C por 15 minutos, seguido de 9 ciclos a 98 °C por 10 segundos, 66 °C por 30 segundos y 72 °C por 30 segundos, con un paso final a 72 °C por 5 minutos (Cruz-López *et al.* 2020).

Determinamos la concentración del producto de la PCR2 usando 2 µl del producto con un fluorómetro. Agrupamos las muestras de ocho individuos, tomando 20 ng por muestra y se procedió a limpiar el producto de la PCR multiplexada con perlas AMPure XP. Para la limpieza usamos un volumen ajustado de una solución de 50 µl (concentración 0.5X). Utilizamos la TapeStation 4200 para verificar que no existían dímeros de los primers en las muestras purificadas. Entonces, cuantificamos el producto usando una qPCR con un kit de cuantificación (KAPA Biosystem) usando 10 µl de volumen de reacción (8 µl de SYBR Master Mix y 2 µl del producto/estándar o control) siguiendo el programa: 95 °C por 5 minutos, seguido de 35 ciclos de 94 °C por 30 segundos y 60 °C por 45 segundos. Finalmente, agrupamos las librerías en cantidades equimolares, preparando un total de seis librerías, para cuantificar la concentración de los agrupamientos con un Qubit (Thermo Fisher Scientfic) y las librerías a 4 nM fueron enviadas para su secuenciación usando un secuenciador Illumina MiSeq (Illummina, Inc., CA, USA) de 250 pb pair-end (500 ciclos) en seis corridas separadas (Cruz-López *et al.* 2020).

Procesamiento de datos del MHC

El procesamiento de las secuencias obtenidas del IIlumina MiSeq se realizó utilizando las herramientas disponibles en el servidor “Amplicon Sequencing Analysis Tool” (AmpliSAT) desarrollado por Sebastian *et al.* (2016). Primero, las lecturas extremas fueron fusionadas con la herramienta AmpliMERGE, que está basada en el algoritmo FLASH, para optimizar el parámetro de traslape. Para después utilizar AmpliCLEAN para filtrar lecturas de baja calidad (<Q30 y < 270 pb). Seguido de AmpliCHECK con los parámetros por defecto para secuencias provenientes de Illumina (mínimo de frecuencia por amplicón del 1%), decidimos conservar las lecturas con una longitud de 350 pb para el MHC clase I y 325 pb para el MHC clase II. Finalmente se utilizó AmpliSAS para demultiplexar, agrupar y filtrar las lecturas restantes usando los parámetros por defecto para el agrupamiento de secuencias procedentes de Illumina (1% de errores de substitución, 0.001% de errores de inserción o deleción), con un mínimo de profundidad de lectura de amplicón de 2,000 y una diferencia mínima de fusión de secuencias respecto a una secuencia dominante cuando difiere por menos de 3 pb y con ≤25% de la profundidad de lectura en comparación con la secuencia dominante (Sebastian *et al.* 2016). Aquellas secuencias que diferían por 1 a 3 pb de una secuencia dominante, pero tuvieron más del 25% de profundidad de lectura, fueron catalogadas como alelos verdaderos. En el proceso de filtrado, descartamos aquellas secuencias que tuvieron frecuencias menores al 5% y aquellas identificadas como quimeras. La profundidad mínima de una variante (alelos) se estableció en 150, mientras que, el número máximo de profundidad de lecturas para un amplicón se estableció en 5,000, según lo aconsejado por razones de rendimiento del servidor AmpliSAS (Sebastian *et al.* 2016).

Validación de alelos

Los alelos identificados, fueron comparados con los obtenidos previamente en un trabajo donde se caracterizó el MHC en el Chorlo nevado (Cruz-López *et al.* 2020). Además, usando la base de datos de nucleótidos de GenBank (NCBI) verificamos la similitud con alelos del MHC de esta misma especie y otras previamente reportados. Una vez revisados y que comprobamos que los alelos encontrados coincidían con los antes reportados o bien no habían sido reportados previamente, procedimos a nombrar los alelos siguiendo la nomenclatura sugerida por Klein *et al.* Para el MHC clase I, utilizamos la nomenclatura Chni-UA*01 al UA*41 y para el MHC clase II Chni-DAB*01 al DAB*06.

Diversidad del MHC y asignación de supertipos

La diversidad del MHC fue evaluada como: 1) Número total de alelos por individuo (MHC clase I), 2) El número total de supertipos funcionales (MHC clase I), 3) Alelos y supertipos raros y/o comunes (MHC clase I y II) y 4) sí presentaban heterocigocidad (MHC clase II). Los supertipos están basados en la idea que moléculas que codifican para diferentes alelos del MHC presentan diferentes especificidades en la unión a péptidos de antígenos, pero algunas moléculas del MHC reconocen los mismos sitios de unión a péptidos y, por ende, los alelos del MHC pueden ser agrupados por su diversidad funcional con alelos que presentan sitios de unión a péptidos potencialmente similares. Los supertipos para el MHC clase I, fueron caracterizados con base a cinco propiedades físico-químicas (z1[hidrofobicidad], z2 [masa estérica], z3 [polaridad], z4 y z5 [efectos electrónicos]) presentes en las regiones de unión a péptidos (Doytchinova y Flower, 2005; Sepil *et al.* 2012). Para esto, realizamos una matriz de datos con los alelos representados en las filas y los valores de las cinco propiedades físico-químicas (z1-z5) en las columnas (Sandberg at al. 1998). En el caso de los supertipos para el MHC clase I, se les nombró como S1 al S7, donde "S" representa a un supertipo seguido del número para cada uno de los siete (S1 a S7) grupos que se formaron al caracterizar el total de alelos para asignar los supertipos. Posteriormente, realizamos un análisis discriminante de componentes principales para agrupar los alelos en supertipos funcionales. Para el MHC clase II, el análisis discriminante de componentes principales falló en agrupar los alelos en supertipos. Por lo cual, no fue posible utilizar este estimado de diversidad y en su lugar se utilizó el número de alelos. Los alelos o supertipos se consideraron raros cuando estuvieron presentes con frecuencias menores al 10% en la población (Bonnaeud *et al.* 2006).

Análisis estadísticos

Utilizamos los diferentes índices de diversidad genética para evaluar si: a) la supervivencia de crías hasta la edad de volantón, y b) el reclutamiento como reproductores, estaba relacionada con la diversidad genética del MHC clase I y II, en el Chorlo nevado. Consideramos como volantones aquellas crías que alcanzaron una edad de 25 días. Las crías nacidas en el sitio de estudio que fueron observadas en años posteriores, como reproductores, se les consideró como reclutas. Decidimos utilizar para nuestros análisis de supervivencia y reclutamiento la diversidad de alelos (número total de alelos por individuo) y de supertipos (número total de supertipos por individuo) para el MHC clase I. El supertipo-5 (S5) fue removido de los análisis debido a que, al presentarse en todos los individuos, no resultaba informativo en los análisis de supervivencia y tasas de reclutamiento diferenciales. Por otro lado, con base en estudios previos en esta población en los que se demostró que la condición corporal y la longitud del tarso no tienen un efecto en la supervivencia de crías del Chorlo nevado, estas variables también se excluyeron de los análisis (Kupán *et al.* 2021).

Modelos: análisis de supervivencia

Pusimos a prueba la relación de la diversidad genética del MHC con la supervivencia en el Chorlo nevado. Para esto, realizamos un modelo de efectos mixtos de riesgos proporcionales de Cox, utilizando el paquete “coxme” en R (Therneau 2012). Estos modelos permiten incluir crías de familias con destino incierto, como en el caso de las que se observaron días antes de finalizar el seguimiento en campo y para las que no se sabe si llegaron a la edad de independencia (muchas de estas crías habían pasado el umbral de una semana, tiempo crítico donde ocurren la mayoría de las muertes). Esto nos permitió incrementar el tamaño de muestra y, por lo tanto, la resolución y el poder estadístico del análisis. La muerte de las crías se consideró como el evento terminal, mientras que las crías que alcanzaron la edad de volantón, o en las que se desconocía su destino, se consideraron eventos censurados. La identidad de la familia, así como, la identidad del macho, la hembra y la temporada reproductiva (año) se integraron al modelo como efectos aleatorios con la idea de incluir la no independencia para el caso de crías pertenecientes a una misma familia, con los mismos padres entre familias dentro y entre temporadas reproductivas.

En otros estudios de esta población se ha demostrado que las crías que eclosionan temprano durante la temporada reproductiva y en familias donde ambos padres permanecen por mayor tiempo al cuidado del nido, la supervivencia es mayor (Cruz-López *et al.* 2017; Kupán *et al.* 2021). Con base en lo anterior, decidimos incluir como covariables a la fecha de eclosión estandarizada y la intensidad del cuidado biparental (número de días en que ambos padres cuidaron de sus crías) en nuestros modelos iniciales. De igual forma, considerando que en esta población de Chorlo nevado la supervivencia en etapas tempranas de vida (juveniles) es diferencial entre sexos (Eberhart-Phillips *et al.* 2017), se incluyó el sexo de las crías en los análisis. Dentro de las variables de diversidad genética incluimos, para el MHC clase I:

número total de supertipos por individuo, supertipos comunes y raros; y para el MHC clase II: número total de alelos por individuo, heterocigosidad, alelos comunes y raros. Los modelos fueron comparados entre sí y seleccionados mediante el método de Criterio de Información de Akaike (AIC_C por sus siglas en inglés) con la función MuMIn de R (Barton 2016). Aunque los modelos con valores de AIC_C más bajos son considerados más parsimoniosos (modelo más simple con mejor poder explicativo), aquellos con valores de AIC_C <2 se consideran igualmente parsimoniosos y bien soportados. Cuando este fue el caso, se seleccionó el modelo con la menor cantidad de parámetros como el más parsimonioso (Burnham y Anderson 2002).

Resultados

Diversidad del MHC en crías del Chorlo nevado

Después de aplicar los filtros de densidad de lectura de los amplicones por individuo (2,000) mediante el sub-programa AmpliCHECK, nueve individuos fueron descartados. Un total de 501 individuos (2006 al 2012) cumplieron con los criterios de control de calidad de los filtros y fueron considerados como exitosamente genotipificados para ambos MHC clase I (profundidad de lecturas promedio de 4,170 con rangos entre 2,204 a 5,000) y II (profundidad de lectura promedio 4,950 y rangos entre 2,695 a 4,998). En el caso del MHC clase I, encontramos un total de 35 alelos (previamente se había reportado 34 alelos para a población de Ceuta (Cruz-López *et al.* 2020), uno de estos alelos no había sido reportado antes para la especie y se detectó en tres crías de diferentes familias. El número de alelos por individuo varía de uno a siete, pero con un promedio de 3.4 alelos por individuo (± 0.9 SD), lo que sugiere al menos cuatro loci para el MHC clase I. Para el MHC clase II, reportamos un total de seis alelos (similar a lo reportado en un estudio previo (Cruz-López *et al.* 2020)), con individuos que presentaron de uno a dos alelos y un promedio de 1.5 alelos por individuo (± 0.5 SD), lo que nos indica que fue genotipado un solo locus para el MHC clase II.

Para el MHC clase I, tuvimos un total de siete supertipos funcionales. Dos supertipos ocurren en baja frecuencia (menor al 10%) y fueron considerados como supertipos raros. Hasta 69 crías presentaron supertipos raros para el MCH clase I. Por su parte, como no fue posible asignar supertipos para el MHC clase II, utilizamos alelos raros y comunes. Tres alelos ocurren en frecuencias menores al 10% en la población y fueron considerados alelos raros. Un total de 52 crías presentaron alelos raros para el MCH clase II. El número total de reclutas fue de 60 individuos, lo que representa un 12% del total de las crías que fueron genotipificadas.

Una vez filtrados los datos, obtuvimos el conjunto de “datos rigurosos” y el número de crías incluidas en los análisis de supervivencia y reclutamiento se redujo a 197 (39% de las crías genotipificadas para el periodo 2006-2012). De éstas, 110 fueron sexadas como hembras y 87 como machos. Para este conjunto de datos, el número de alelos para el MHC clase I varía entre dos a cinco, con un promedio de 3.3 alelos por individuo (± 0.8 SD). Mientras para el MHC clase II, se mantuvo el número de uno a dos alelos por individuo y un promedio de 1.5 alelos (± 0.5 SD). Un total de 27 crías presentaron uno de los dos supertipos raros, para el MCH clase I y 19 crías presentaban alguno de tres alelos raros observados en el MHC clase II. Finalmente, para este conjunto de datos registramos 25 reclutas (5% del total de crías genotipificadas y un 13% del subconjunto genotipificado con “datos rigurosos”).

Influencia del MHC en la supervivencia de crías del Chorlo nevado

La supervivencia de crías del Chorlo nevado no estuvo relacionada de manera significativa con los diferentes índices de diversidad genética del MHC clase I y II. En nuestro análisis, controlamos por la fecha de eclosión, sexo y el cuidado biparental proveído a las crías. Encontramos que la intensidad de cuidado biparental tiene un efecto positivo en la supervivencia de crías del Chorlo nevado (Tabla 2). En el modelo donde se puso a prueba la supervivencia con relación a los supertipos individuales del MHC clase I, encontramos una tendencia a que las crías que presentan el supertipo 1 (S1), su supervivencia tiende a ser hasta 5 veces menor respecto a crías con otros supertipos, (Tabla 2). Sin embargo, es probable que las tendencias observadas estén influenciadas por la cantidad de modelos y parámetros analizados, lo que aumenta la probabilidad de que un resultado sea significativo por un efecto estocástico.

Influencia del MHC en el reclutamiento del Chorlo nevado

No encontramos evidencia que el reclutamiento estuviera asociado a la diversidad genética del MHC clase I (Tabla 6) y II (Tabla 8) en el Chorlo nevado. De igual manera, el sexo de las crías, la fecha de eclosión y la cantidad de cuidado biparental, no está relacionada con las tasas de reclutamiento dentro de esta población.

Variación temporal del MHC

Para MHC clase I, la frecuencia de los supertipos entre cohortes presentó una gran variación (Figura 1). Con la excepción de una caída drástica en la frecuencia de algunos supertipos comunes para el año 2012, la mayoría de los supertipos comunes no presentaron cambios drásticos en sus frecuencias a través de los años. El supertipo 7 (S7), mostró una gran variación en sus frecuencias a través de los años, con una caída bastante pronunciada en sus frecuencias hacia el año 2012 y un incremento considerable para el año 2013. Por su parte, el S1 (supertipo 1) considerado como raro incrementó en su frecuencia hacia los últimos años de este estudio.

Por su parte la frecuencia de alelos para el MHC clase II mostró una amplia variación entre el período es estudio del 2006 al 2016. Alelos que fueron categorizados como raros (alelos 2, 3 y 6), presentaron pocos cambios en sus frecuencias a través de los años. En el caso de los alelos más comunes (alelos 1, 4 y 5), éstas mostraron cambios drásticos en sus frecuencias hacia la mitad del periodo de estudio (Figura 2), con dos de ellos disminuyendo y uno incrementando su frecuencia hacia el año 2012, para posteriormente recuperar su comportamiento en cambio de frecuencia a través de los últimos años de este estudio.

Discusión

Los genes del MHC, al estar directamente asociados a características ligadas a la adecuación biológica (Piertney y Oliver 2006), son candidatos idóneos para poner a prueba si la diversidad o alelos/supertipos específicos está asociada con la supervivencia. En este trabajo se investigó como la diversidad genética del MCH clase I y II se encontraba relacionado con la supervivencia y reclutamiento en una población de Chorlo nevado, controlando por factores que podrían causar confusión como lo son fecha de eclosión, cuidado bi-parental y el sexo de las crías. Encontramos que la cantidad de cuidado biparental da una ventaja en la supervivencia de las crías de Chorlo nevado, más que la diversidad de alelos o supertipos del MHC. Nuestro estudio nos indica, que la supervivencia o reclutamiento de crías del Chorlo nevado no se encuentra relacionada con supertipos o alelos específicos del MHC clase I y II. Esto a pesar de que se observa una tendencia de uno de los supertipos que afecta la supervivencia de las crías. Además, exploramos los cambios temporales en las frecuencias de alelos para el MHC clase I y II a través del tiempo. encontrando que existe una amplia variación en cambio de frecuencias de alelos a través de los años.

Estudios donde se evaluó la importancia de la diversidad de alelos o supertipos del MHC han mostrado resultados mixtos. Brouwer y colaboradores (2010), encontraron que juveniles del Carricero de Seychelles, que presentaban una mayor diversidad de alelos del MHC clase I, sobreviven mejor que juveniles con baja diversidad (Brouwer et al 2010). Sin embargo, esta asociación positiva entre una alta diversidad del MHC y supervivencia, no fue evidente en adultos de esta especie (Brouwer *et al.* 2010). Por su parte, en el Carbonero Común no se encontró una ventaja en cuanto a la supervivencia de adultos que presentaban una alta diversidad del MHC (Sepil et al 2013). En el presente estudio, no se encontró una relación de la diversidad de alelos o supertipos del MHC clase I y II, con la supervivencia o reclutamiento en crías de Chorlo nevado. En aves, el desarrollo de una respuesta inmune adaptiva (como la iniciada por los genes del MHC) adecuada, se espera sea desarrollada por completo entre la cuarta a la sexta semana después de la eclosión

(Killpack y Karasov 2012). Por lo que una relación entre la supervivencia y la diversidad de alelos del MHC podría no ser esperada en etapas muy tempranas de vida. En nuestra población de estudio, sabemos que la mayoría de crías mueren entre la primera y segunda semana (Cruz-López et al 2017). Por lo que otros factores parecen ser más importantes para la supervivencia en etapas tempranas de vida del Chorlo nevado.

En nuestros modelos incluimos covariables de historia de vida como la fecha de eclosión y cuidado bi-parental. La fecha de eclosión fue excluida de subsecuentes análisis al no ser significativa. Por el contrario, conservamos la covariable de cuidado biparental en análisis sucesivos, al mostrar una relación positiva y significativa con la supervivencia de crías del Chorlo nevado, para ambas clases de MHC. En el Gorrión Común un estudio donde se evaluó la supervivencia y reclutamiento en relación a la diversidad de alelos del MHC y otras variables, los resultados mostraron que covariables de historia de vida como el peso en diferentes etapas de crecimiento o la fecha de eclosión, son más importantes para explicar la supervivencia y reclutamiento que la diversidad genética del MHC (Karlsson *et al.* 2015). La supervivencia en etapas tempranas de vida en aves y mamíferos suele estar influenciada por condiciones ambientales y calidad de cuidado parental (Klug y Bonsall 2014), al igual que el genotipo de los individuos (Ellegren y Sheldon 2008). Sin embargo, se suele omitir covariables no genéticas en este tipo de estudios, y esto podría estar arrojando resultados sesgados o erróneos, puesto que variables no genéticas parece estar explicando en mayor medida la supervivencia y reclutamiento en poblaciones naturales. En el Chorlo nevado, sus crías son precociales y altamente móviles a las pocas horas después de que eclosionaron, y son capaces de alimentarse por sí solas. Sin embargo, durante las primeras semanas de vida son incapaces de regular su temperatura corporal y dependen de los padres para mantenerse en un óptimo de temperatura corporal. Por otro lado, existe alta competencia y peleas constantes por zonas de alimentación entre conespecíficos y otras especies de aves playeras que se reproducen en la misma área. Dos padres pueden proveer mejor cuidado parental (termorregulación y defensa de crías) dando ciertas ventajas a sus crías, respecto a las familias donde uno de los padres abandona muy temprano. Es por lo antes mencionado, que la intensidad de cuidado bi-parental y no el número de alelos encontrados en las crías, podría estar explicando mejor la supervivencia de crías de Chorlo nevado en etapas tempranas de vida.

Estudios previos en poblaciones naturales han demostrado que alelos específicos podrían estar relacionados con la supervivencia. En el Chipe de las Seychelles, se encontró que crías que presentaban el alelo *Ase-ua4*, tenían hasta cinco veces mayor supervivencia que aquellos que no presentaban este alelo (Brouwer *et al.* 2010). Mientras que, en el Gorrión Común (*Passer domesticus*), se reportó que no existe una relación del número de alelo de MHC clase I con la supervivencia de crías, juveniles o reclutamiento (Karlsson *et al.* 2015). No obstante, alelos específicos estuvieron asociados positivamente a la supervivencia de crías y tasas de reclutamiento (Karlsson *et al.* 2015). Asimismo, dos alelos mostraron una relación negativa con la supervivencia de crías en este estudio (Karlsson *et al.* 2015). Nuestros resultados, nos indican que crías del Chorlo nevado que presentan el supertipo-1 (S1), muestra una tendencia a presentar una menor supervivencia (Tabla 2). La aparente asociación estadística con crías que presentan el S1 podría ser un mero efecto estocástico. El modelo que muestra un efecto potencial de la supervivencia del S1, no resultó ser el mejor modelo de predicción de la supervivencia de las crías en el contexto de la diversidad del MHC clase I (Tabla 1). Consistente con esta interpretación, observamos que la frecuencia del S1 aumentó durante el periodo de nuestro estudio. Si S1 conduce a una menor supervivencia en crías, uno esperaría el resultado opuesto, a menos que la supervivencia en etapas de vida posteriores aumente para los individuos portadores de S1. Por ejemplo, Paterson y colaboradores (1998) encontraron que los alelos del MHC muestran diferentes asociaciones en diferentes etapas de vida en la Oveja de Soay.

Por otro lado, el S1 parece mantenerse cercano a las frecuencias a las cuales se les considera como un supertipo raro (con excepción del año 2012 y 2013 que presenta frecuencias por arriba del 10%) y no muestra un gran incremento en sus frecuencias (Figura 2). En el Carricero Tordal se encontró que la variación en frecuencias de ciertos alelos del MCH clase I, ocurrió en apenas nueve años (Westerdahl *et al.* 2004). Incluso, este trabajo demostró que cambios en frecuencias de estos alelos pueden darse de un año a otro. Recientemente, un alelo raro (OLADRB 263) descrito por Paterson y colaboradores (1998), que incrementa la resistencia a parásitos y la supervivencia en la Oveja de Soay, en etapas de juveniles. Incrementó su frecuencia a lo largo de los 23 años que se ha estudiado su población (Huang *et al.* 2022). En ambos estudios (Westerdahl *et al.* 2004 y Huang *et al.* 2022), concluyen que estos cambios en frecuencias de alelos a través del tiempo podrían deberse a procesos de selección balanceadora, derivada de presiones selectivas causadas por cambios en la comunidad de patógenos a través del tiempo en una determinada población. Incorporar más datos provenientes de nuevas cohortes y clases de edades (juveniles y adultos) e indagar si patógenos intracelulares (ej. *Plasmodium Sp*.) ocurren en nuestra población, podría ayudarnos a identificar si el aumento y/o mantenimiento en el S1, al igual que, la gran variación observada en las frecuencias de cierto supertipos se debe a procesos de selección mediados por patógenos o bien procesos demográficos. Una posibilidad es que algunos de los nuevos individuos de Chorlo nevado que inmigran cada año a la población de Ceuta como reproductores, estén incorporando estos supertipos raros (ej. S1) a la población, donde sus crías tienden a sobrevivir menos. Con una tasa constante de inmigrantes cada año incorporando estos supertipos raros, es posible que se logre mantener en baja frecuencia estos supertipos, los cuales se llegan a considerar como raros, pero no brindan una ventaja en términos de adecuación biológica local.

A pesar que diversos estudios han demostrado la importancia de la diversidad o alelos específicos del MHC clase II en tasas de supervivencia (Paterson *et al.* 1998; Bateson *et al.* 2016). En nuestro estudio no encontramos una relación o tendencia con la supervivencia (Tabla 4) o reclutamiento (Tabla 8) asociado con la diversidad del MHC clase II. Un estudio previo donde se caracterizó el MHC del Chorlo nevado a través de su distribución geográfica (incluyendo la población de este estudio) reveló que el MHC clase II, presenta una baja diversidad de alelos y los individuos genotipificados presentaron de uno a dos alelos (Cruz-López *et al.* 2020). El MHC clase II, está asociado a una respuesta inmune contra patógenos extracelulares (Sommer 2005). La baja diversidad encontrada en el Chorlo nevado (seis alelos a nivel poblacional, con individuos llevando de uno a dos alelos) nos hace pensar que esta especie presenta bajas exposición a patógenos extracelulares (Ver Cruz-López *et al.* 2020). Esto nos podría explicar la poca o nula relevancia del MHC clase II en la supervivencia del Chorlo nevado.

Finalmente, disminuir el número de datos incluidos (197 crías genotipificadas para periodo 2006 al 2012) en los análisis pudo haber afectado los resultados de cómo la diversidad del MHC (número de alelos y supertipos) influye en la supervivencia del Chorlo nevado. Un estudio reciente habla de la necesidad de tener tamaños de muestra grandes (>200) para tener una mayor certeza de que los efectos del MHC sobre características de adecuación biológica (ej. supervivencia) son reales (Gaigher *et al.* 2019). Incluso, los autores concluyen que un mayor número de muestra (>200 a 1,000) es requerido cuando se busca investigar los efectos de alelos específicos del MHC y su relación con la adecuación biológica de los individuos (Gaigher *et al.* 2019). (Gaigher *et al.* 2019).

Conclusión

Podemos concluir que nuestro estudio aporta evidencia de que no existe una relación de la diversidad de alelos o supertipos específicos con la supervivencia y tasas de reclutamiento en crías del Chorlo nevado de la Bahía Ceuta. Esto a pesar de que se observó una tendencia negativa de un supertipo específico afectando la supervivencia de crías. Si este supertipo, no brindan una ventaja en la supervivencia, esperaríamos que su frecuencia fuera baja y con tendencia a desaparecer de la población. Sin embargo, un buen número de Chorlos nevados inmigran cada año hacia nuestra población de estudio, y éstos podrían mantener a los supertipos en la población. Se esperaría que adultos reproductores con estos supertipos y bajo éxito reproductivo, emigraran a otros sitios en busca de incrementar su éxito reproductivo. Esto nos deja abierta la posibilidad de indagar en un futuro, la posibilidad de evaluar cómo un bajo éxito reproductivo podría estar influyendo en movimientos o cambios demográficos en nuestra población. Por otro lado, nuestro estudio deja en evidencia la importancia de controlar por factores de confusión como lo son características de historia de vida o morfológicas en este tipo de estudios. El no incluirlos en los análisis podría desestimar la importancia de estos factores en la adecuación biológica de poblaciones. También limitaría nuestra comprensión sobre los procesos ecológicos más relevantes para conservar poblaciones que se encuentran disminuyendo o amenazadas por otros factores ambientales, de historia de vida, fisiológicos o conductuales. O bien, una combinación de éstos con factores asociados a la diversidad genética. Lo anterior es importante, dado que programas de conservación podrían estar enfocando esfuerzos de manejo y conservación en los factores más relevantes para lograr una conservación efectiva en una especie o población amenazada. Finalmente, a pesar que nuestros resultados muestran que no existe una relación significativa de parámetros del MHC con la supervivencia de crías del Chorlo nevado, creemos necesario incrementar el tamaño de muestras. Esto podría ayudarnos a despejar estas dudas sobre la tendencia observada en algunos de los supetipos (ej. S1). Además, comprender mejor cómo este tipo de correlaciones entre genes del MHC con componentes de adecuación biológica, pueden estar ocurriendo y la magnitud en la cual ocurren estas relaciones. O bien, plantear un diferente escenario de cómo nuestra especie de estudio podría estar manteniendo la diversidad del MHC. Debido a que el MHC clase I presenta una fuerte firma de presión selectiva en el Chorlo nevado (Cruz-López *et al.* 2020), es probable que patógenos intracelulares estén jugando un papel importante en procesos de selección y evolución de este grupo de genes inmunes en nuestra especie de estudio. Sería interesante examinar la comunidad de patógenos intracelulares (ej. malaria o hemoparásitos) y sus afectaciones al Chorlo nevado a través de sus poblaciones e investigar cómo estos podrían estar afectando o moldeando cambios en su demografía, o bien, los mecanismos detrás de la evolución y mantenimiento de la diversidad genética del MHC en poblaciones del Chorlo nevado.

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Tablas y figuras

MHC clase I_supervivencia

Tabla 1. Selección de modelos de las variables asociadas al MHC clase I y la supervivencia en el Chorlo nevado. CuidadoBip= Cuidado biparental; S1 a S7= Supertipos 1 al 7; Sraros= Supertipos raros (incluye S1 y S2); DivMHCI= Diversidad de alelos del MHC clase I; DivS= Diversidad de supertipos.

Modelo	GL	LogLik	AICc	Δi	ωi
CuidadoBip + S2 + S3 + S6 + S7 +Sraros	36	-311.4	715.1	0.0	0.51
CuidadoBip + S1 + S2 + S3 + S4 + S6 + S7	37	-311.7	717.0	1.8	0.20
CuidadoBip + DivMHCI	37	-311.9	718.3	3.1	0.10
CuidadoBip + DivS	36	-313.2	719.1	3.9	0.07
Fecha_eclosión + CuidadoBip + Sexo	37	-312.5	720.2	5.0	0.04
CuidadoBip + Fecha_eclosión*DivMHCI	39	-310.6	720.5	5.3	0.03
CuidadoBip + Sexo * DivS	41	-307.7	721.4	6.3	0.02
CuidadoBip + Sexo*DivMHCI	39	-311.7	723.7	8.5	0.00

Tabla 2. Factores asociados a la supervivencia del Chorlo nevado. Se muestran los modelos que mostraron mejor ajuste durante la selección de modelos (AIC_C <2). CuidadoBip= Cuidado biparental; S1-S7= Supertipos del 1 al 7; Sraros= Supertipos raros (incluye al S1 y S4).

Variable	Exp(coef)	EE (coef)	Valor Z	Valor p
Modelo con supertipos individuales VS supertipos raros				
CuidadoBip	0.92	0.03	-2.89	**<0.01**
S2	1.54	0.41	1.05	0.29
S3	0.97	0.37	-0.07	0.95
S6	0.75	0.35	-0.81	0.42
S7	1.80	0.36	1.64	0.10
Sraros	5.10	0.86	1.88	0.06*
Modelo con supertipos individuales				
CuidadoBip	0.92	0.03	-2.87	**< 0.01**
S1	5.51	0.87	1.96	0.05*
S2	1.43	0.44	0.82	0.41
S3	0.89	0.41	-0.26	0.79
S4	3.70	1.15	1.14	0.25
S6	0.72	0.36	-0.89	0.37
S7	1.71	0.37	1.46	0.14

MHC clase II_supervivencia

Tabla 3. Selección de modelos de las variables asociadas al MHC clase II y la supervivencia en el Chorlo nevado. CuidadoBip= Cuidado biparental; HeterMHCII= Heterocigocidad del MHC clase II; Alraros= Alelos considerados raros (incluye Alelo 2, Alelo 3 y Alelo 6).

Modelo	**GL**	**LogLik**	**AICc**	**Δi**	**ωi**
CuidadoBip + HeterMHCII	35	-314.4	717.50	0.00	0.61
Fecha_eclosión + CuidadoBip + Sexo	37	-312.4	719.10	1.60	0.27
CuidadoBip + MHCII_1 + MHCII_4 + MHCII_5 + Alraros	37	-314.0	721.90	4.40	0.06
CuidadoBip + HeterMHCII*Sexo	37	-313.7	723.00	5.50	0.03
CuidadoBip + MHCII_1 + MHCII_2 + MHCII_3 + MHCII_4 + MHCII_5 + MHCII_6	39	-313.0	725.70	8.20	0.01

Tabla 4. Factores asociados a la supervivencia del Chorlo nevado. Se muestran los modelos que mostraron mejor ajuste durante la selección de modelos (AIC_C <2). CuidadoBip= Cuidado biparental; HeterMHCII= Heterocigocidad del MHC clase II.

Modelo MHCII con Zigocidad				
Variable	**Exp(coef)**	**EE (coef)**	**Valor Z**	**Valor p**
CuidadoBip	0.92	0.03	-2.84	**< 0.01**
HeterMHCII	0.87	0.28	-0.51	0.61
Modelo nulo 0 (Fecha_eclosión + CuidadoBip + Sexo)				
Fecha_eclosión	1.24	0.17	1.29	0.20
CuidadoBip	0.91	0.03	-3.00	**<0.01**
Sexo	1.18	0.28	0.60	0.55

MHC clase I_reclutamiento

Tabla 5. Selección de modelos de las variables asociadas al MHC clase I y reclutamiento en el Chorlo nevado. CuidadoBip= Cuidado biparental; DivS= Diversidad de supertipos; Sraros= Supertipos raros; DivMHCI= Diversidad de alelos del MHCI.

Modelo	GL	LogLik	AICc	Δi	ωi
Fecha_eclosión + CuidadoBip + Sexo + DivS	8	-58.1	133.10	0.00	0.73
Fecha_eclosión + CuidadoBip + Sexo*DivS	9	-58.2	135.40	2.30	0.22
Fecha_eclosión + CuidadoBip + Sexo + S3 + S4 + S6 + S7 + Sraros	12	-57.5	141.00	7.90	0.01
Fecha_eclosión + CuidadoBip + Sexo + DivMHCI	8	-62.3	141.50	8.40	0.01
Fecha_eclosión + CuidadoBip + Sexo	7	-63.6	141.80	8.70	0.01

Tabla 6. Factores asociados al reclutamiento del Chorlo nevado. Se muestran los modelos que mostraron mejor ajuste durante la selección de modelos (AIC_C <2). CuidadoBip= Cuidado biparental; DivS= Diversidad de supertipos

Modelo con diversidad de supertipos

Variable	Estimado	EE	Valor Z	Valor p
Intercepto	-6.40	2.75	-2.33	0.02
Fecha_eclosión	-0.01	0.75	-0.01	0.99
CuidadoBip	0.04	0.10	0.36	0.71
Sexo	2.00	1.17	1.70	0.08
DivS	-1.47	1.14	-1.30	0.19

MHC clase II_reclutamiento

Tabla 7. Selección de modelos de las variables asociadas al MHC clase II y reclutamiento en el Chorlo nevado. CuidadoBip= Cuidado biparental; HeterMHCII= Heterocigocidad del MHC clase II

Modelo	**GL**	**LogLik**	**AICc**	**Δi**	**ωi**
Fecha_eclosión + CuidadoBip + Sexo	7	-54.1	123.00	0.00	0.44
Fecha_eclosión + CuidadoBip + Sexo*HeterMHCII	9	-52.3	123.80	0.80	0.29
Fecha_eclosión + CuidadoBip + Sexo + HeterMHCII	8	-53.5	124.00	1.00	0.26
Fecha_eclosión + CuidadoBip + Sexo + MHCII_1 + MHCII_2 + MHCII_3 + MHCII_4 + MHCII_5 + MHCII_6	13	-53.7	135.70	12.70	0.00

Tabla 8. Factores asociados al reclutamiento del Chorlo nevado. Se muestran los modelos que mostraron mejor ajuste durante la selección de modelos (AIC_C <2). CuidadoBip= Cuidado biparental; HeterMHCII= Heterocigocidad del MHC clase II;

Variable	**Estimado**	**EE**	**Valor Z**	**Valor p**
Modelo nulo 0 (Fecha_eclosión + CuidadoBip + Sexo)				
Intercepto	1.01	2.16	4.68	< 0.01
Fecha_eclosión	-6.80	7.30	-0.10	0.92
CuidadoBip	-0.01	1.17	-0.01	0.99
Sexo	1.20	1.24	1.77	0.07
Modelo con interacción (Sexo*HeterMHCII)				
Intercepto	-14.00	4.38	-3.20	<0.01
Fecha_eclosión	-0.15	0.81	-0.19	0.84
CuidadoBip	-0.01	0.13	-0.02	0.98
Sexo	4.91	3.34	1.45	0.14
HeterMHCII	5.04	3.77	1.33	0.18
Sexo2:HeterMHCII	-4.50	3.80	-1.20	0.23
Modelo MHCII con Zigocidad				
Intercepto	-11.05	2.47	-4.46	<0.01
Fecha_eclosión	-0.05	0.73	-0.07	0.94
CuidadoBip	-0.01	0.12	-0.02	0.98
Sexo	1.98	1.24	1.60	0.11
HeterMHCII	1.43	1.44	0.99	0.32

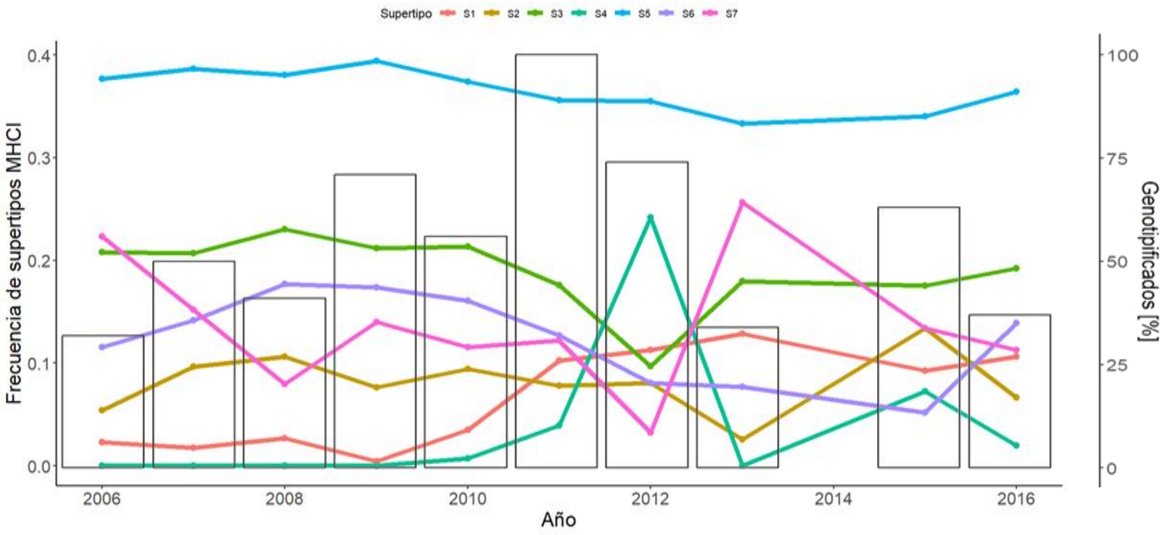

Figura 1. Frecuencia de los siete supertipos del MHC clase I entre las diferentes cohortes. Las frecuencias fueron calculadas como el número de ocasiones que un supertipo fue observado en cada cohorte y dividido por el número total de supertipos observados para esa cohorte. Consideramos el número total de individuos genotipados que pasaron los filtros de calidad (562 individuos) para el periodo 2006 al 2016 (no se cuentan con datos para el año 2014). Las columnas (eje secundario) representan el porcentaje de crías que fueron genotipificadas por año.

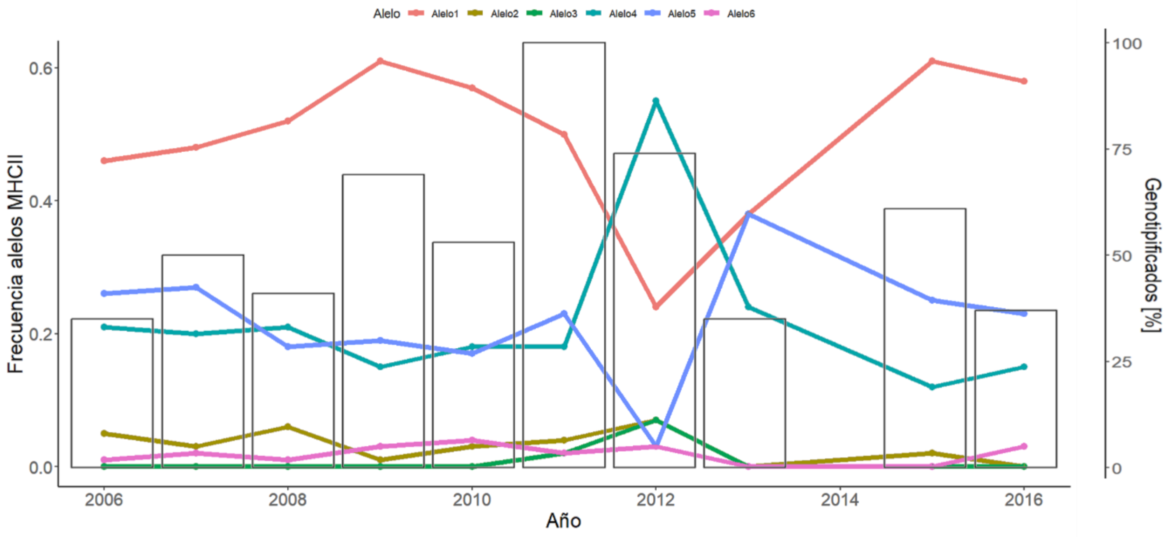

Figura 2. Frecuencia de los seis alelos del MHC clase II observado entre las diferentes cohortes de crías del Chorlo nevado. Las frecuencias fueron calculadas como el número de ocasiones que un alelo fue observado en cada cohorte y dividido por el número total de alelos observados para esa cohorte. Consideramos el número total de individuos genotipados (558 individuos) para el periodo 2006 al 2016 (no se cuentan con datos para el año 2014). Las columnas (eje secundario) representan el porcentaje de crías que fueron genotipificadas por año.

Capítulo V. Discusión general

El propósito de este estudio fue investigar como factores demográficos, de historias de vida y genéticos están relacionados con la viabilidad y supervivencia de una especie de ave playera amenazada en Norteamérica. En primer lugar, se evaluó la viabilidad de una población subtropical de Chorlo nevado (*Charadrius nivosus*) en el Noroeste de México y las tasas vitales involucradas en cambios en el tamaño de una de las poblaciones más importantes para esta especie en México. Al mismo tiempo, se determinó la variación de la supervivencia en diversas etapas de vida y el éxito reproductivo del Chorlo nevado basados en datos de monitoreo detallados y colectados durante un período inicial de siete años. En segundo lugar, se caracterizaron ocho poblaciones de Chorlo nevado, representantes de las tres subespecies reconocidas a lo largo de su área de distribución, con base a los genes del MCH clase I (exón 3) y clase II (exón 2). Finalmente, se investigó como la diversidad genética del MHC clase I y clase II, se encontraba relacionada con la supervivencia y tasas de retorno de las crías de Chorlo nevado considerando la variación ambiental y conductual de una población de la región subtropical con un historial de monitoreo de más de diez años.

Los análisis de viabilidad poblacional, suelen ser utilizados para evaluar el riesgo de extinción y los factores demográficos o tasas vitales prioritarias para la investigación, la planificación e implementación de acciones de manejo orientadas a la conservación de especies o poblaciones en riesgo (Chaudhary y Oli, 2019). El análisis de viabilidad de la población de Chorlo nevado de Bahía de Ceuta predice una probabilidad de extinción de la población del 99.8%, dentro de los siguientes 25 años a partir del 2012. No obstante, en años subsecuentes al 2012, se observó que la población de reproductores se mantuvo estable e incluso la población duplicó su número de individuos reproductores en años recientes (2019 al 2023).

Existen varias razones por las que la trayectoria proyectada por el modelo no coincide con la tendencia poblacional actualmente observada. En primera instancia, hemos presenciado un incremento sustancial en la inmigración, la cual no fue originalmente considerada en el modelo. De manera regular, en la población de estudio se observan nuevos individuos cada año (marcamos alrededor del 90% de los reproductores y el 95% de las crías cada año), lo cual, podría estar explicando que la población se mantenga e incluso que algunos años incremente el número de reproductores. Se sabe que, en poblaciones de aves silvestres, los inmigrantes pueden ser la fuente de un rescate demográfico en poblaciones que muestran déficits demográficos locales (Millon *et al.* 2019). En una población del Cárabo Común (*Strix aluco*) que experimenta bajas tasas reproductivas, el tamaño de su población se mantuvo estable a través de 15 años de estudio. Esto debido, a que la baja tasa de retorno local (causado por las bajas tasas reproductivas) se ve compensada por la duplicación en la proporción de inmigrantes entre los nuevos reclutas (Millon *et al.* 2014).

La afluencia observada de inmigrantes en el estudio presentado aquí, puede deberse a dos razones. Cabe la posibilidad de que existan poblaciones fuentes cercanas que producen muchas más crías de las que pueden ser reclutadas en la población, o bien podría deberse a la continua pérdida del hábitat a lo largo de la costa mexicana del Pacífico, lo que significa, que un mayor número de reproductores tienen que moverse a un menor número de sitios protegidos como nuestra área de estudio. Durante censos y monitoreos realizados en 2019, al ampliar la búsqueda de reproductores en la Bahía de Ceuta se descubrieron sitios nuevos de anidación y crianza, localizados aproximadamente a 3 km del sitio principal (antigua salinera), que están siendo utilizados por individuos de la población reproductora principal. Gracias al seguimiento que se les ha dado a estos nuevos sitios, sabemos que existe intercambio de individuos reproductores entre sitios. Los nuevos sitios ofrecen a las familias un acceso directo a los cuerpos de agua y son utilizados mayormente hacia el final de la temporada, cuando el sitio principal se encuentra completamente secos. Este tipo de inmigración entre poblaciones o red de pequeños sitios reproductivos, puede prevenir las extinciones locales. Esto, en función de la conectividad entre un sitio principal y la red de sitios secundarios que constituyen las poblaciones fuentes-sumideros (Hanski, 2001).

Este intercambio de individuos del Chorlo nevado entre sitios dentro de la Bahía de Ceuta, podría explicar porque la población principal se ha mantenido estable a través de los años, a pesar de la proyección del modelo de viabilidad. Otro factor a considerar es la pérdida de hábitat como salitrales (sitios preferidos por Chorlos para anidar) a causa del desarrollo en sistemas productivos como la acuacultura. Lo que obliga a los reproductores que usaban esas zonas a moverse a otros sitios y podría ser una fuente de inmigrantes para sitios como la antigua salinera de Bahía de Ceuta.

Otro aspecto a considerar, es que se observaron fluctuaciones ambientales considerables que influyeron en las tasas vitales de la población. Por ejemplo, en las temporadas reproductivas en los que en la población principal (antigua salinera) hay abundancia y disponibilidad de agua, el número de reproductores y la supervivencia de crías fue mayor que años con ausencia de agua en el sitio (Gómez del Angel, 2023). Esto puede ser particularmente importante para especies longevas como el Chorlo nevado, ya que la población puede incluso mantenerse estable, si el éxito reproductivo bajo en varias temporadas es compensado por un alto éxito reproductivo en otras temporadas. En Europa, algunos estudios han demostrado que la distribución de aves playeras durante su reproducción, está estrechamente relacionada a sitios que presentan cierto grado de humedad, y el drenaje o escases de agua en áreas de anidación provoca la pérdida de hábitat en extensión y calidad (Milsom *et al.* 2002; Eglington *et al.* 2009).

Un tercer punto para resaltar lo representan las acciones de manejo que se implementaron en respuesta a las predicciones poco alentadoras sobre el destino y la disminución poblacional observada. Aunque menores (limitar el acceso al sitio durante la temporada reproductiva, señalamientos, brindar fuentes de agua durante la temporada seca), estas acciones han sido aparentemente efectivas en detener o revertir la tendencia negativa de la población. Muy probablemente estas acciones no sean suficientes para garantizar el bienestar de la población de aves que se reproducen en la Bahía de Ceuta, por lo que acciones a otro nivel, como restablecer el flujo hidrológico hacia la antigua salinera, son necesarias para abordar los retos a largo plazo que enfrenta la población.

Para concluir, es posible que alguno de estos factores mencionados o la combinación de alguno de ellos, podría estar contribuyendo a que la población reproductora del Chorlo nevado en Bahía de Ceuta, se mantenga estable a través del tiempo. Sin embargo, no fuimos capaces de incluir estos factores en nuestro modelo inicial de riesgo de extinción, debido a que, desconocíamos estos sitios o parches reproductivos y contamos con pocos datos de la calidad del hábitat o la cantidad de agua disponible a través de los años y temporadas reproductivas del sitio. Esto brinda una oportunidad para reevaluar el modelo de viabilidad poblacional e incluir variables de la calidad del ambiente y factores demográficos a nivel meta-poblacional, para tener un análisis más robusto que represente de una mejor manera los procesos involucrados en la permanencia o viabilidad de esta población de Chorlo nevado.

Una parte importante de los modelos demográficos y análisis de viabilidad, es conocer la sensibilidad de las diferentes tasas vitales que podrían afectar las tasas de crecimiento poblacional. En nuestro análisis, la supervivencia de adultos, mostró una mayor sensibilidad a los cambios demográficos. Esto nos indica que las acciones de manejo y conservación deberían estar enfocados en áreas donde la supervivencia de adultos es limitada. Un estudio donde se evaluó las tasas vitales de seis especies de aves playeras que anidan en el Ártico, indicó que la supervivencia de adultos es la tasa vital más relevante que podría conducir a cambios demográficos en estas especies, con la supervivencia de juveniles como el segundo parámetro de importancia, actuando en los cambios de las tendencias poblacionales de estas seis especies de aves playeras (Weiser *et al.* 2020). La mayoría de estos análisis utilizan supervivencia aparente de adultos (producto de dos probabilidades, supervivencia verdadera y fidelidad al sitio). El monitoreo continuo de la población de Chorlo nevado en la Bahía de Ceuta, nos permite hipotetizar que la disminución en supervivencia aparente, es el resultado de una emigración permanente en esta población (disminución de fidelidad al sitio). Muy pocos individuos adultos han sido encontrados muertos en el sitio, a pesar de un monitoreo intensivo durante reproducción. Esta emigración permanente, podría deberse al bajo éxito reproductivo (ej. baja supervivencia de crías y juveniles) o la degradación del hábitat a través de la temporada reproductiva, debido principalmente a la escases de agua, en el tiempo adecuado, a través de los años.

Un estudio realizado por Eberhart-Phillips y colaboradores (2017), demostró que los cambios en el tamaño o crecimiento de esta población están influenciados por la supervivencia aparente de las hembras adultas (Eberhart-Phillips *et al.* 2017). Un menor número de hembras en la población, es la razón por la cual, es un factor limitante en el crecimiento de esta población y las acciones de manejo deberían estar enfocadas en retener un mayor número de hembras reproductivas dentro de la población. Sin embargo, la diferencia en la proporción sexual de adultos sesgada hacia machos observada en la Bahía de Ceuta, es mayormente influenciada por la supervivencia de machos en etapas tempranas de vida (crías y jóvenes). Ello sugiere, que la supervivencia en etapas tempranas de vida puede afectar la viabilidad de una población, a través de la variación de la proporción sexual de adultos (Eberhart-Phillips *et al.* 2017). En familias consideradas como tempraneras durante la temporada reproductiva, las hembras desertan más rápido que en familias tardías, ofreciendo la posibilidad de iniciar una segunda nidada (Kupán *et al.* 2021). No obstante, se confirmó que la deserción no se traduce en la producción de un mayor número de volantones. En aquellas hembras que proporcionan cuidados por más tiempo alcanzan un éxito reproductivo (número de volantones) similar al de las hembras que desertan y tienen una segunda nidada, pero en un tiempo significativamente menor (Kupán *et al.* 2021). Este ahorro de tiempo de las hembras que cuidan por más tiempo (9 días o más) dando una ventaja en supervivencia de sus crías, se traduce en tener suficiente tiempo para establecer una segunda nidada y, por tanto, pueden aumentar aún más su éxito reproductivo. Este comportamiento tan plástico, permite a las hembras del Chorlo nevado, maximizar su éxito reproductivo a través del uso de la poliandria secuencial como un segundo plan que compensa la baja supervivencia de las crías de la primera familia, en un ambiente estocástico (Kupán *et al.* 2021).

Si determinados factores (determinísticos o estocásticos) afectan las tasas vitales, esto podría impactar la viabilidad de una población al afectar su tamaño poblacional (Reeds 2008). Poblaciones pequeñas suelen ser más vulnerables a la extinción al disminuir su potencial adaptativo. Siendo el potencial adaptativo de una especie determinado por la cantidad de variación genética (aditiva) de las características asociadas a la adecuación biológica, dentro y entre poblaciones (Funk *et al.* 2018). La conservación del potencial adaptativo es fundamental para que especies y poblaciones puedan responder antes cambios ambientales. Los genes del Complejo Mayor de Histocompatibilidad (MHC) están directamente asociados con la adecuación biológica (supervivencia, éxito reproductivo, resistencia contra patógenos, etc.) en los vertebrados. Para evaluar si estos genes estaban relacionados con la adecuación biológica del Chorlo nevado, se caracterizó el exón 3 del MHC clase I y el exón 2 del MHC clase II.

Dentro del orden Charadriiforme (aves playeras, gaviotas y otros) han sido pocos los estudios que han caracterizado el MHC (Ekblom *et al.* 2007; Cloutier *et al.* 2011; Buehler *et al.* 2013; Burri *et al.* 2014; Vásquez-Carrillo *et al.* 2014; Pardal *et al.* 2017; Mancilla-Morales *et al.* 2022) en comparación con otros grupos de aves, por ejemplo, los paseriformes. Estos estudios han demostrado, que existe una gran variación en la arquitectura genética del MHC a través de las diferentes familias de aves. En primer lugar, aves no-paseriformes presentan una arquitectura compacta del MHC con un bajo número de loci (Minias *et al.* 2018). Para el Chorlo nevado, amplificamos hasta cuatro loci para el MHC clase I. Otros estudios en Charadriiformes han encontrado un número similar de loci para el MHC clase I (Buehler *et al.* 2013; Pardal *et al.* 2017). En contraste, en paseriformes se han reportado hasta 65 alelos por individuo para el MHC clase I, lo que representa hasta 33 loci (Biedrzycka *et al.* 2017). En el caso del MHC clase II, otros estudios dentro del orden de los Charadriiformes reportan de manera consistente hasta dos loci por individuo (Mancilla-Morales *et al.* 2022) y un máximo de cuatro alelos por individuo. Para el Chorlo nevado, se encontró un máximo de dos alelos por individuos (un locus). Esto indica que los primers/iniciadores diseñados para el Chorlo nevado y otros miembros de la familia Charadriidae, probablemente amplificaron un solo locus para nuestra especie de estudio. Es posible que los primers solo fueron capaces de amplificar un solo gen dentro del MHC clase II. Otra posibilidad, es que el MHC clase II en el Chorlo nevado no presente eventos de duplicación y que este sea una característica ancestral dentro de la especie o bien de la familia Charadriidae.

En un trabajo realizado por Minias y colaboradores (2018), reporta que existe una fuerte presión selectiva en el MHC clase II en aves no-paseriformes que en paseriformes (Minias *et al.* 2018), con una mayor diversidad alélica dentro del MHC clase II dentro los no-paseriformes. Esto lo atribuye a las presiones selectivas impuestas por patógenos intra y extracelulares actuando de manera diferente entre paseriformes y no-paseriformes. En el caso de los Charadriiformes, estos parecen no seguir el patrón propuesto por Minias y colaboradores. Por ejemplo, en aves playeras (Scolopacidae y Charadriidae) presentan una mayor firma de selección en el MHC clase I respecto al clase II. Particularmente, en el Chorlo nevado se encontró hasta 40 alelos para el MHC clase I en ocho poblaciones muestreadas, con individuos portando hasta siete alelos. Mientras, que solo se registraron seis alelos para el MHC clase II y de uno a dos alelos por individuo.

Minias y colaboradores (2018), proponen dos posibles mecanismos para explicar esta presiones selectivas y diferencias entre paseriformes y no-paseriformes. En primer lugar, hace mención que la diferencia en el tamaño entre paseriformes y no-paseriformes es un factor determinante para que se presente una mayor diversidad de patógenos o parásitos extracelulares. Por lo regular, no-paseriformes suelen ser de mayor tamaño y esto brinda un mayor número de nichos y pueden soportar más patógenos extracelulares que aves pequeñas (ej. muchas paseriformes). Segundo, entre estos dos grupos de aves existe diferencias en el uso o el hábitat que utilizan, exponiéndose de manera diferencial al tipo de patógenos que podemos encontrar entre estos hábitats. Por lo común, se asocia a las aves no-paseriformes con cuerpos de agua dulce y este tipo de ambientes acuáticos, suelen presentar una mayor diversidad de patógenos extracelulares. En el caso del Chorlo nevado, es una especie de tamaño pequeño (15 cm y masa corporal entre 35 a 50 gr) más similar a la mayoría de los paseriformes. Además, suele usar hábitats salinos e hipersalinos durante su ciclo anual, este tipo de hábitat se ha demostrado presenta una menor diversidad de patógenos extracelulares (Figuerola, 1999). Esto concuerda con lo propuesto por Minias y colaboradores para explicar estas diferencias entre los patrones de selección del MHC clase I y II para paseriformes y no-paseriformes "la variación en patrones de selección entre grupos de aves probablemente es dirigida por características ecológicas y de historia de vida que regulan la exposición a diferentes tipos de patógenos" (Minias *et al.* 2018).

Diez alelos del MCH clase I parecen ser privados o particulares para tres de las ocho poblaciones muestreadas en este segundo capítulo. En el caso de los cuatro alelos "privados" encontrados para la población de Ceuta y cuatro en la población de Utah. Estos se presentan en bajas frecuencias y es posible que se trate de alelos raros, más que alelos privados. Por otro lado, la población de Puerto Rico presentó dos alelos "privados", presentes en mayor frecuencia, lo que podría indicar una variante o alelo privado real para esta población. Ekblom y colaboradores, encontraron que hasta el 78% de los alelos eran privados para una de las regiones muestreadas para la Agachona Real (Ekblom *et al.* 2007). En el Chorlo nevado, de las tres poblaciones con alelos "privados", dos de ellas presentan un constante flujo genético (D'Urban Jackson *et al.* 2020), lo que sugiere que es poco probable que estos alelos sean privados para estas poblaciones. En el caso de la población isleña de Puerto Rico, esta presentó la diversidad de alelos más baja entre las poblaciones con apenas nueve alelos para el MHC clase I y solo un alelo para el MHC clase II. Sabemos que la población de Puerto Rico ha sido afectada recientemente por una reducción en su tamaño poblacional, afectando su diversidad genética (D'Urban Jackson *et al.* 2020). Cabe la posibilidad que la diversidad genética de marcadores adaptativos, como lo son los genes del MHC, se vea afectados de igual manera por las extinciones locales a la cual ha estado sometida esta población. Provocando la pérdida de alelos y conservando aquellos que podrían darle una ventaja contra patógenos locales.

Gracias a la caracterización del exón 3 y exón 2 del MHC, se investigó si la diversidad del MHC se encontraba relacionada con la supervivencia y tasas de retorno en crías de Chorlo nevado, en una población que había sido monitoreada a largo plazo. No se encontró evidencia que apoyara que la diversidad del MHC (medida como número total de alelos por individuo, número total de supertipos funcionales, alelos, supertipos raros y la heterocigosidad) estuviera asociada con la supervivencia y tasas de retorno en crías del Chorlo nevado. Sin embargo, los modelos nos muestran que crías con supertipos raros tienden a presentar una menor supervivencia. Estudios similares muestran resultados mixtos al respecto de la supervivencia y la diversidad del MHC. Con alelos o supertipos específicos confiriendo una mayor supervivencia tanto en etapas tempranas de vida (Brouwer *et al.* 2010) como en adultos (Sepil *et al.* 2013). Una explicación adicional del porque se conservan alelos del MHC que tienden a ser desventajosos en una población, es que la supervivencia podría tener diferentes asociaciones con alelos del MHC en diferentes etapas de edad, como lo sugiere Paterson y colaboradores (1998), lo que evidencia la compleja interacción entre los patógenos y el sistema inmune del huésped. Por otro lado, Lukasch y colaboradores (2017) reportan que características como peso y tarso que se asocian a la supervivencia de crías del Gorrión Común (*Passer domesticus*), estuvieron relacionadas negativamente con un alelo específico del MHC, afectando la supervivencia de aquellas crías que tenían dicho alelo. En la misma especie en una población relativamente cerrada y que ha sido monitoreada a largo plazo, se encontró que dos alelos en particular afectan la supervivencia de etapas juveniles (Karlsson *et al.* 2015).

La mayoría de otros estudios le dan poca relevancia a factores que no sean los asociados a la diversidad genética del MHC. Como se demostró en uno de los estudios aquí presentes, factores relacionados con la historia de vida (ej. intensidad de cuidado biparental) explican en gran medida la variación en la supervivencia de crías del Chorlo nevado en la población de Ceuta. Sepil y colaboradores (2013), demostraron que factores demográficos como lo es la densidad de reproductores locales, explicaba en gran medida la tasa de retorno y el éxito reproductivo en el Carbonero Común, más que factores asociados a la diversidad del MHC. Mientras que, en el Gorrión Común encontraron que características de historia de vida como en peso y la fecha de eclosión explicaban mejor la supervivencia y reclutamiento, mientras que la diversidad del MHC no contribuye en la supervivencia de esta especie en la población de estudio (Karlsson *et al.* 2015). Estos resultados muestran la importancia de incluir variables de historia de vida y demográficas en este tipo de análisis. Es posible, que otras variables no genéticas, expliquen mejor estos procesos de supervivencia en diferentes etapas de vida. De incluir este tipo de variables y aun así encontrar una correlación significativa con las variables de diversidad genética, ejemplo del MHC, nos podría indicar que la relación encontrada es real y no ha sido enmascarada por otras variables de mayor relevancia para la supervivencia o tasas de retornos.

Si la diversidad genética del MHC en el Chorlo nevado está relacionada con la supervivencia y tasas de retorno, no es del todo claro. En las últimas dos décadas, un gran número de estudios se han realizado para tratar de explicar la magnitud con la que la diversidad genética del MHC está correlacionada con la adecuación biológica de los individuos de una población, así como la forma en la que esto podría orientar los esfuerzos de conservación de la biodiversidad (O´Connor *et al.* 2019). Desde una perspectiva de conservación, los patógenos son una de las principales fuerzas evolutivas que influyen en la adecuación biológica de los individuos. Es de esperarse que si una población está sujeta a presiones selectivas por patógenos, la diversidad de genes asociados con una respuesta inmune se mantenga y que presenten una cierta variabilidad. La población de Chorlo nevado de Ceuta, presenta una diversidad alélica promedio (34 alelos en total) del MHC clase I y un bajo número de alelos (seis alelos) para el MCH clase II (pero dentro el promedio para el orden Charadriiforme). Esto nos podría indicar que, en el Chorlo nevado los patógenos intracelulares son la fuerza selectiva principal actuando. Sin embargo, no se han realizado estudios para conocer la diversidad y exposición a patógenos en esta especie, lo que complica el buscar una posible explicación del rol que desempeñan los patógenos en la adecuación biológica de esta especie. Es posible, que la diversidad genética del MHC en el Chorlo nevado tenga una mayor relevancia para la supervivencia en juveniles o adultos, más que, para las crías, donde, los factores de historia de vida como el cuidado biparental influye más en la supervivencia durante la primera semana de vida. Aunque, existe una tendencia de ciertos supertipos específicos o raros a explicar la supervivencia en crías del Chorlo nevado. El tamaño de muestra podría ser una limitante para definir la relación del MHC con la supervivencia y tasas de retorno. Sobre todo, cuando se busca investigar si un alelo o supertipo específico presentan una relación con alguna variable como la supervivencia, se espera que el tamaño de muestra sea entre 200 a 1,000. En el trabajo aquí presentado, el tamaño de muestra está por debajo del umbral sugerido (197 muestras) para investigar tales efectos. Por lo que, incrementar el tamaño de muestra nos traería un mayor poder de análisis y nos podría ayudar a definir con mayor certeza la relación de la diversidad del MHC con componentes de adecuación biológica en el Chorlo nevado en la Bahía de Ceuta.

Trabajos futuros

A pesar de que el modelo de viabilidad predijo una alta probabilidad de extinción local del Chorlo nevado a mediano plazo, la población reproductora en Ceuta se mantiene estable, sin llegar a los números de reproductores que se registraron durante los primeros años de este proyecto. Buenas condiciones ambientales, parece ser fundamentales para mantener una población sana y en crecimiento en Ceuta. Comprender mejor la dinámica y uso del hábitat durante la temporada reproductiva es fundamental para plantear acciones de manejo y conservación. De manera observacional, parece ser que la falta de agua es un factor de gran importancia para la supervivencia de crías. En los años que se han presentado la entrada de agua por eventos meteorológicos como mar de fondo o más recientemente por la escorrentía de agua de la agricultura, las crías presentan mayores tasas de supervivencia. En este sentido, es importante iniciar con el registro de datos de la calidad del hábitat y de la disponibilidad de posibles fuentes de alimento para las aves playeras que se reproducen en Ceuta. Con estos datos, podríamos evaluar como la variación en la calidad del hábitat influye en la supervivencia de crías y tasas de retorno de reproductores. Al mismo tiempo, esto sería relevante para poner a prueba la hipótesis que los reproductores emigran de la población de Ceuta cuando su éxito reproductivo es bajo y si esta emigración se traduce en una segunda puesta exitosa en sitios aledaños. Esto último, sería en gran medida apoyado por datos obtenidos recientemente de los movimientos locales y uso de hábitat, obtenidos mediante el uso de dispositivos GPS o bien con el uso de la red MOTUS que se ha establecido en el Noroeste de México y que podría ser una herramienta más a explorar, para conocer el movimiento, el tiempo de permanencia en un sitio o las tasas de retorno. Integrar el conocimiento generado hasta la fecha y futuros trabajos, permitirá desarrollar un plan de manejo y conservación para el Chorlo nevado y su hábitat en Ceuta, beneficiándose otras especies con requerimientos similares y que comparten la zona.

Es claro que el MHC tiene una función importante contra la resistencia de patógenos, por lo que, para una mejor comprensión de como las fuerzas selectivas por patógenos moldean la diversidad del MHC, es necesario, estudiar la comunidad de patógenos y su papel como agentes selectivos. Durante el desarrollo del presente estudio, no fue posible evaluar la comunidad de patógenos en la población reproductora del Chorlo nevado en Ceuta. Incluso, de manera general la comunidad de patógenos y sus enfermedades ha sido poco estudiada en el Chorlo nevado. Un primer acercamiento de patógenos que podrían estar afectando a esta especie, es examinar si las poblaciones monitoreadas del Chorlo nevado, presentan parásitos de la sangre (Haematozoa). Tenemos muestras de sangre de cientos de individuos de la población de Ceuta y algunas decenas de otras poblaciones de América. Mediante, el uso de técnicas moleculares sería posible examinar la presencia de este tipo de patógenos. Sobre todo, cuando se tiene en mente los hábitats que utiliza esta especie y la presencia de uno de los vectores más comunes para este tipo de patógenos, como lo es el mosquito. Por otro lado, actualmente vivimos un brote de gripe aviar altamente patogénica que ha afectado a miles de aves alrededor del mundo. El monitoreo continuo de aves silvestres puede ayudarnos a detectar de manera temprana este tipo de enfermedades e iniciar con planes o protocolos para minimizar el impacto en aves silvestres y de importancia comercial (ej. pollos). Además, se sabe que las aves playeras suelen ser un reservorio natural del virus causante de la gripe aviar y muchas de estas especies no presentan síntomas, pero son vectores y excelentes dispersores del virus. Si los Charadriiformes y particularmente las aves playeras, han sido expuesta a través de su historia evolutiva a estos virus causantes de la gripe aviar, es posible que tengan la capacidad para combatir y disminuir los efectos de la enfermedad, de esta manera, los genes asociados a respuestas inmunes podrían estar jugando un rol principal en mantener a las poblaciones sin mayores daños como muertes.

Es posible que la diversidad genética del MHC sea más importante para la supervivencia durante la etapa de adultos para los Chorlos nevados. En este trabajo no se incluyeron a los adultos, pero queda abierta la posibilidad de evaluar si el MHC es importante para la supervivencia en adultos, así como para su éxito reproductivo y/o selección de parejas. Por otro lado, incrementar el tamaño de muestra en crías sería importante para descartar o confirmar si existe alguna asociación de la diversidad del MHC con componentes de adecuación. Con los iniciadores/primers que se desarrollaron para la familia Charadriidae, existe la posibilidad de genotipar el MHC para especies cercanamente emparentadas con el Chorlo nevado. En Ceuta, al menos otras dos especies de *Charadrius* (*C. wilsonia* y *C. vociferus*) utilizan el área para reproducirse. La exposición a una comunidad similar de patógenos, podría dar como resultado que se compartan alelos del MHC entre especies filogenéticamente cercanas. Este tipo de estudios es de gran interés para comprender los procesos evolutivos y la relación de genes del MHC con la presión mediada por patógenos.

Finalmente, a través del desarrollo de este trabajo se investigó a uno de los genes del sistema inmune que ha sido ampliamente estudiado. Si bien los genes del MHC han sido caracterizados y estudiados a profundidad en decenas de especies de vertebrados modelos y no-modelo, existe una gran complejidad en el sistema inmune con cientos de genes actuando y teniendo un papel fundamental para montar una respuesta inmune. Por ejemplo, trabajos recientes han explorado la importancia de la respuesta inmune innata en animales silvestres, donde los Receptores Tipo Toll o TLR's (Toll-Like Receptors) tienen un papel fundamental para que los hospederos puedan detectar una infección e iniciar la respuesta inmune no-específica (Bateson *et al.* 2016; Minias y Vinkler, 2022). Complementar este tipo de trabajos con otros genes que son parte del sistema inmune es relevante desde el punto de vista evolutivo, de igual forma, al ejercer una influencia en parámetros demográficos como la supervivencia y reproductivos, es importante caracterizar los diferentes componentes genéticos del sistema inmune, de esta manera, lograr una mejor comprensión de las vías por las cuales se da la resistencia a enfermedades infecciosas y los efectos sobre poblaciones en riesgo (Acevedo-Whitehouse y Cunningham, 2006). Sobre todo, en la actualidad que brotes de diferentes enfermedades ha puesto en peligro a poblaciones de organismos silvestres e incluso al humano, provocando graves disminuciones demográficas en poblaciones silvestres, incluyendo algunas especies amenazadas.

Conclusión

Es evidente que la población reproductora del Chorlo nevado en Bahía de Ceuta ha disminuido hasta en un 70%. A pesar que en recientes años (2021-2023) el número de reproductores ha aumentado, persiste un número bajo de reproductores (~50%) respecto al primer año del estudio (2006). Si bien el modelo de viabilidad poblacional estimó una probabilidad de extinción del 99.8% a partir del 2012 y dentro los siguientes 25 años. Datos recientes de esta población nos indica que la proyección del modelo no se está cumpliendo. La inmigración podría estar explicando el incremento y estabilidad en el número de reproductores en la población de Bahía de Ceuta en los últimos años.

Frecuentemente, las poblaciones pequeñas son propensas a disminuir su potencial evolutivo (determinado por la cantidad de variación genética aditiva de características asociadas con la adecuación biológica). Dentro los genes con mayor potencial de influir en adecuación biológica de los individuos de una población, se encuentran los genes del Complejo Mayor de Histocompatibilidad. La caracterización de estos genes inmunes, en ocho poblaciones a través del rango de distribución del Chorlo nevado, mostró una diversidad alélica similar a otras aves del orden de los Charadriiformes. Un total de 41 alelos fueron registrados para el MHC clase I y seis alelos para el MHC clase II. Las diferencias tan marcadas en el número de alelos para el MHC clase I y el MHC clase II. Podrían ser una respuesta a la presión selectiva impuesta por la exposición a patógenos dependientes de los hábitats que usa el Chorlo nevado en la región subtropical y tropical (un mayor número de posibles vectores de patógenos intracelulares). Al igual que, características morfológicas como un tamaño menor, lo que implica una menor variedad de nichos para parásitos extracelulares. La población de la subespecie *tenuirostris* presentó las mayores diferencias en cuanto al número de alelos total (nueve alelos). Esto podría estar relacionado a la historia demográfica de esta subespecie, debido a que en los últimos 200 años *tenuirostris* ha presentado extinciones locales en su rango de distribución en el Caribe (D'urban Jackson *et al.* 2020). Por su parte, las otras dos subespecies (*nivosus* y *occidentalis*) presentan un número similar de alelos. Consistente con el elevado flujo de genes observado entre estas poblaciones continentales.

Finalmente, la interacción entre la dinámica poblacional y los genes del Complejo Mayor de Histocompatibilidad fue abordada investigando la relación de estos genes adaptativos con componentes de adecuación biológica como la supervivencia y tasas de retornos de crías del Chorlo nevado. No se encontró una relación significativa de las diferentes medidas de diversidad del MHC con la supervivencia y tasas de retorno en crías. Por el contrario, los modelos nos indican que otras variables no genéticas son de mayor relevancia para la supervivencia de las crías en el Chorlo nevado. La intensidad del cuidado biparental (medido como el número de días que ambos padres cuidan de las crías) mostró ser importante para la supervivencia de las crías. Esto expone que, en etapas tempranas de vida los genes del MHC no representan una ventaja en temas de supervivencia para el Chorlo nevado. Mientras que factores de historia de vida y patrones conductuales son de relevancia para la dinámica poblacional del Chorlo nevado en la Bahía de Ceuta.

Siendo estos cambios en la dinámica de una población (ej. cambios en tasas vitales y tamaño poblacional) los que repercuten directamente en la viabilidad de las poblaciones y su riesgo de extinción. Comprender cómo la calidad y cambios en el ambiente, la calidad genética de los individuos, el potencial evolutivo de una población y la dinámica en la demografía interactúan para mantener poblaciones que se encuentran disminuyendo, es fundamental para que las acciones de conservación, recursos asignados y planes de manejo sean enfocadas en aquellas acciones que puedan optimizar una tasa de crecimiento poblacional con fines de recuperar poblaciones o especies que se encuentren disminuyendo.

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Anexo I

Sex-specific early survival drives adult sex ratio bias in Snowy Plovers and impacts mating system and population growth

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Sex-specific early survival drives adult sex ratio bias in snowy plovers and impacts mating system and population growth

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Adult sex ratio (ASR) is a central concept in population biology and a key factor in sexual selection, but why do most demographic models ignore sex biases? Vital rates often vary between the sexes and across life history, but their relative contributions to ASR variation remain poorly understood—an essential step to evaluate sex ratio theories in the wild and inform conservation. Here, we combine structured two-sex population models with individual-based mark–recapture data from an intensively monitored polygamous population of snowy plovers. We show that a strongly male-biased ASR (0.63) is primarily driven by sex-specific survival of juveniles rather than adults or dependent offspring. This finding provides empirical support for theories of unbiased sex allocation when sex differences in survival arise after the period of parental investment. Importantly, a conventional model ignoring sex biases significantly overestimated population viability. We suggest that sex-specific population models are essential to understand the population dynamics of sexual organisms: reproduction and population growth are most sensitive to perturbations in survival of the limiting sex. Overall, our study suggests that sex-biased early survival may contribute toward mating system evolution and population persistence, with implications for both sexual selection theory and biodiversity conservation.

ASR | *Charadrius nivosus* | mark–recapture | sex allocation | two-sex matrix model

Sex ratio variation in wild populations has important consequences for population dynamics and hence, biodiversity conservation (1). Because reproduction in sexual organisms involves both males and females, a shortage of either sex could compromise population viability (2). A reduction in the number of breeding females directly reduces birth rates and hence, population productivity (3), whereas an overabundance of males may increase violence and aggression, such that both male and female survival rates are reduced (4). Although a small number of males can potentially fertilize many mates, females in male-biased populations may need to compete for breeding opportunities with high-quality males, which can induce additional mortality (5). If males are in short supply, fathers also tend to reduce their parental investment, which could negatively impact offspring survival (6, 7). Additionally, a biased sex ratio in either direction will decrease effective population size, which has adverse consequences for genetic diversity (8). Therefore, depending on mating system, populations with biased sex ratios may be more vulnerable to extinction than unbiased populations (9).

Recent studies also suggest that the adult sex ratio (ASR; the proportion of the adult population that is male) impacts breeding strategies because the limiting sex has the advantage in mating and parental decisions (10–12). For example, male-biased avian populations tend to have polyandrous mating systems and male-biased parental provisioning (13). Although the theory linking ASR to breeding system is relatively new, there are already supporting studies: parental cooperation is associated with an unbiased ASR in birds (14), whereas ASR is a strong predictor of sex-specific sexual activity and divorce rates in humans (15, 16).

Despite the importance of ASR in population biology, biodiversity conservation, and breeding system evolution, the origins of ASR biases remain unclear. Biases in the ASR can emerge via a number of mutually nonexclusive demographic pathways (11, 17, 18). For instance, sex biases may occur at conception or birth (19), or the survival of male and female juveniles may differ to the extent that fewer of one sex reach adulthood (20). Furthermore, sex differences in adult survival or maturation rates could create a shortage of the sex that has higher mortality (4) or slower maturation (18), and if emigration is not compensated

Significance

Sex biases are widespread in nature and represent a fundamental component of sexual selection and population biology—but at which point in life history do these biases emerge? We report a detailed individual-based demographic analysis of an intensively studied wild bird population to evaluate origins of sex biases and their consequences on mating strategies and population dynamics. We document a strongly male-biased adult sex ratio, which is consistent with behavioral observations of female-biased polygamy. Notably, sex-biased juvenile rather than adult survival contributed most to the adult sex ratio. Sex biases also strongly influenced population viability, which was significantly overestimated when sex ratio and mating system were ignored. Our study, therefore, has implications for both sexual selection theory and biodiversity conservation.

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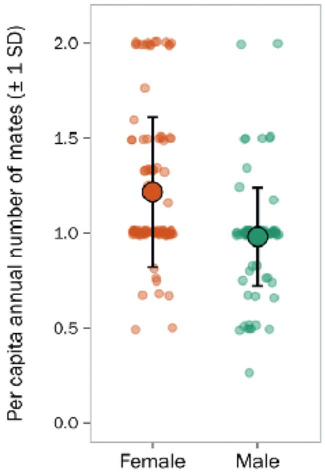

Fig. 1. Sex bias in mating system illustrated as the per capita annual number of mates acquired by male and female snowy plovers (Mann–Whitney–Wilcoxon test: $W = 3{,}264$, $P < 0.001$).

for by immigration, sex differences in dispersal behavior could create local biases in ASR (21).

A number of studies of wild vertebrate populations have evaluated the independent contributions of the above pathways to ASR bias (22–24). However, to fully understand ASR bias requires these components to be modeled simultaneously to quantify their relative contributions. In practice, large empirical datasets from natural populations incorporating stage- and sex-specific vital rates are uncommon (25–27). Furthermore, males and females often have different behaviors or ecological niches (28), which can make one or the other easier to detect (9, 11). Fortunately, these sources of sampling bias can be accounted for using mark–recapture methods (29).

Here, we investigate the demographic origins of ASR bias in a polygamous bird using 7 years of individual-based sex- and stage-specific life history data. Polygamous species have a special significance in sex ratio studies because they are predicted to be at higher risk of extinction (1, 30). We studied a small ground-nesting shorebird, the snowy plover [*Charadrius nivosus* (31)], which is endangered in parts of its Nearctic range and has a sequentially polygamous mating system (32, 33). Using a two-sex matrix model, we show that the ASR of this species is substantially more male-biased than previously reported (34). Sex differences in chick and juvenile survival contribute most to ASR bias, suggesting that ASR variation is particularly susceptible to factors that influence early life history stages. Furthermore, we show that population growth is most sensitive to adult female survival under a male-biased ASR, signifying that sex-specific early survival can affect population viability via ASR variation. Importantly, our study suggests that sex-biased survival in early life has ramifications for mating system variation and knock-on effects for population growth.

Results

We conducted this study at Bahía de Ceuta, a subtropical lagoon on the coastal plain in northwestern Mexico (23°54′ N, 106°57′ W). Between 2006 and 2012, we uniquely marked and monitored 1,259 individuals (436 females and 390 males initially marked as chicks and 221 females and 212 males initially marked as adults). Although our marking methods were limited to breeding adults and chicks, we detected no sex difference in the proportion of this marked population that was nonbreeding (paired t test: $t = 0.429$, $df = 4$, $P = 0.69$) (Fig. S1). Therefore, this marked subset of the population represents a broadly representative sample from which to draw inferences about the dynamics of the population at large and elucidate the contributions of sex- and stage-specific survival toward ASR bias.

Mating System. To understand the ASR in the context of mating system, we quantified sex-specific mating strategies of snowy plovers at our study site. Although both sexes can be polygamous, female snowy plovers desert broods to seek serial mates, leaving males to provide parental care alone (33). Thus, we expected that females would acquire, on average, a greater number of mates per year than males. Based on behavioral observations of 456 families with known identities of both parents, female-biased polygamy is precisely what we found (Fig. 1) (Mann–Whitney–Wilcoxon test: $W = 3{,}264$, $P \leq 0.001$). As such, the mating system index in the mating function (Eq. **4**) was polyandrous ($h = 0.82$).

Sex-Biased Survival and ASR Bias. We estimated stage- and sex-specific survival rates using mark–recapture analysis to control for imperfect detection in the field. Mark–recapture modeling revealed sex differences in encounter probability for juveniles and adults that would confound simple estimates of survival and ASR based solely on return rates or uncorrected counts of males and females (Table 1). Apparent survival was strongly male-biased across all life history stages, with male survival being 11.5% higher than female survival at the chick stage, 51% higher for juveniles, and 0.5% higher at the adult stage (Fig. 2*A*). Hatching sex ratio was slightly female-biased but did not significantly deviate from parity [average $\rho = 0.486$ (95% CI = 0.435–0.536), $P = 0.588$, $n = 340$ hatchlings from 116 full broods]. Overall, our model indicated a strongly male-biased ASR [mean = 0.632 (95% CI = 0.460–0.785)] (Fig. 2*B*).

Contributions to Male-Biased ASR. To elucidate the stage-specific contributions of sex differences in survival to ASR bias, we conducted a life table response experiment (LTRE), which revealed that all vital rates contributed in the same direction (i.e., male-biased) but differed in magnitude. A sex difference in juvenile survival made the largest overall contribution to ASR bias (Fig. 2*C*). Specifically, the contribution of sex-biased juvenile survival toward ASR was 3.3 times higher than sex-biased chick survival and 17.6 times higher than sex-biased adult survival. Hatching sex ratio and mating system made negligible contributions (Fig. 2*C*).

Table 1. Summary statistics of sex- and stage-specific estimates of the snowy plover population

Sex and stage	*N*	ϕ	*p*
Female			
Chick	416	0.48 (0.41–0.55)	0.56 (0.53–0.58)
Juvenile	234	0.15 (0.11–0.19)	0.48 (0.34–0.62)
Adult	221	0.68 (0.62–0.74)	0.52 (0.40–0.65)
Male			
Chick	372	0.53 (0.47–0.60)	0.55 (0.53–0.58)
Juvenile	243	0.22 (0.18–0.27)	0.66 (0.48–0.74)
Adult	212	0.69 (0.63–0.74)	0.66 (0.54–0.77)

N indicates the number of individual encounter histories used for mark–recapture modeling, ϕ is apparent survival, and *p* is encounter probability. Estimates are shown as the medians and 95% confidence intervals of each bootstrapped distribution. ϕ and *p* are expressed as daily rates in the case of chicks, and annual rates for juveniles and adults.

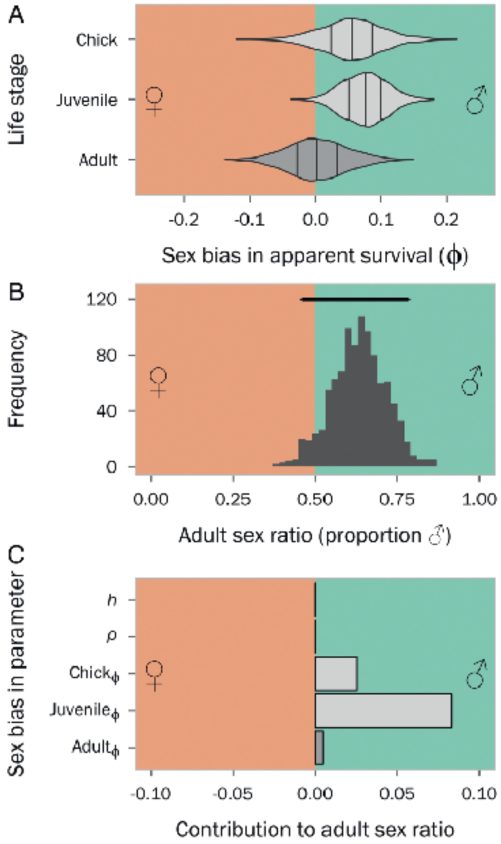

Fig. 2. (*A*) Bootstrap distributions of sex-biased apparent survival (i.e., difference between males and females). Vertical lines indicate medians and interquartile ranges, and shades of gray correspond to first-year (light gray) or adult (dark gray) parameters shown in Fig. 6. (*B*) Bootstrap distribution of the derived ASR. The horizontal bar indicates the 95% confidence interval of the ASR estimate based on 1,000 iterations [mean ASR = 0.632 (95% CI: 0.460–0.785)]. (*C*) Relative contributions of model components to ASR in our LTRE comparing the empirically derived sex-specific model with a theoretical model with no sex bias (notation: h = mating system index, ρ = hatching sex ratio, ϕ = apparent survival). ASR is expressed as the proportion of the adult population that is male; thus, changes in female-biased parameters have a negative effect on ASR, and hence, their LTRE statistics are negative.

Consequences of ASR Bias and Polygamy on Population Viability. Biased ASR and polygamy create conditions whereby reduced survival of the limiting sex can compromise population viability, which has important implications for conservation. Our perturbation analysis showed that population growth was most sensitive to adult survival under all hypothetical scenarios of ASR and mating system (Fig. 3). Adult female survival elasticities were highest under scenarios of male-biased ASR. As expected, there was no sex-specific sensitivity of vital rates under an unbiased ASR and monogamous mating system. However, elasticity was highest for adult males under an unbiased ASR and polyandry.

To elucidate the conservation consequences of disregarding sex biases, we compared the predictive accuracy of a detailed two-sex model incorporating polygamy with a conventional one-sex model. Over the 7-year study period, average population growth was below replacement ($\lambda = 0.859 \pm 0.28$ SD) (Fig. 4). This observed rate of decline was captured by the uncertainty distribution of the two-sex model [$\lambda = 0.849$ (95% CI: 0.802–0.897)] (Fig. 4). In contrast, the one-sex version of the model exhibited greater uncertainty and significantly overestimated population growth [$\lambda = 0.947$ (95% CI: 0.883–1.01)] (Fig. 4).

Discussion

We present a comprehensive demographic model based on detailed individual-based life history data from an intensively monitored bird population. By incorporating sex-specific feedbacks between survival and frequency-dependent reproduction,

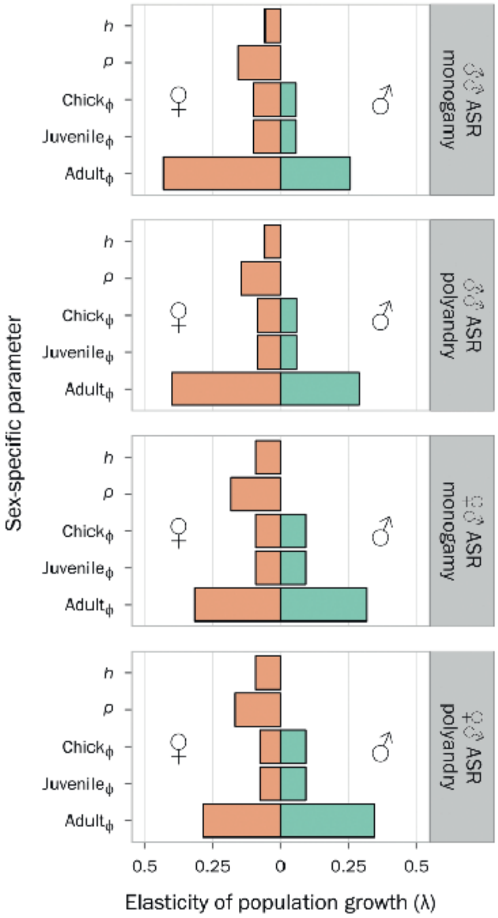

Fig. 3. Sex-specific sensitivity analysis of population growth (λ) under four scenarios of ASR and mating system: h = mating system index, ρ = hatching sex ratio, and ϕ = apparent survival.

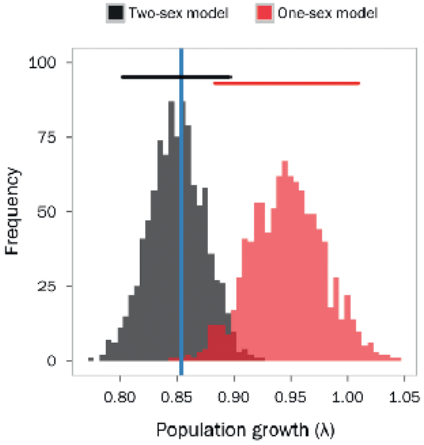

Fig. 4. Distributions of deterministic population growth under a two-sex frequency-dependent matrix model (black) and a conventional one-sex matrix model (red) compared with the observed average annual population growth over the 7-year study period (blue vertical line). Horizontal bars above each distribution represent the 95% confidence intervals of the bootstrap simulation for each model.

our model predicted a strongly male-biased ASR. This result was complemented by our behavioral observations of a polyandrous mating system. Therefore, our findings build on recent empirical and theoretical studies linking ASR to the evolutionary origins and consequences of mating system variation (10–12) and also provide insights into the sex- and stage-specific demographic components that contribute to ASR bias. Males had consistently higher apparent survival than females across all life stages, but a sex difference in the apparent survival of juveniles had the largest impact on ASR bias. Furthermore, population growth was most sensitive to perturbations in adult female survival under the male-biased ASR. Taken together, our results uncover the demographic pathways linking individual-level variation in survival and sex roles to population-level dynamics.

Obtaining reliable survival estimates from natural populations is challenging because of sex differences in behavior and life history. Our study addressed this uncertainty through mark–recapture models and bootstrapping. A central assumption of our model is that our marked subset of the population is representative of the entire population. This assumption is appropriate given that we marked the vast majority of chicks and breeding adults in the population. Furthermore, we did not find a sex difference in the proportion of breeders vs. nonbreeders (Fig. S1), indicating that our ASR estimate is not confounded by an excess number of unmarked nonbreeding females.

Females had higher rates of polygamy than males, which is in line with most published records from other snowy plover populations (32, 35). This female-biased mating system complemented a strongly male-biased ASR (0.63). ASR in this species has previously been reported to be less extreme (34) than found here, although the previous study was unable to incorporate sex-specific chick and juvenile survival, which made the greatest contributions to the ASR bias in this population (this study) and others (36). ASR bias is a widespread phenomenon in wild vertebrate populations, with mammals typically being female-biased (mean ASR = 0.37 ± 0.15 SD) and birds typically being male-biased (mean ASR = 0.55 ± 0.09 SD) (37). Our ASR estimate for snowy plovers, therefore, is within the natural variation observed in other avian taxa (9).

Importance of Early Sex-Specific Demographic Processes. Several hypotheses can be put forward to explain the observed pattern of male-biased early survival. Focusing first on the chicks, male hatchlings are significantly larger than their sisters in this population (38), potentially providing males with an advantage during early development. Another mutually nonexclusive possibility is that male chicks could achieve faster growth rates, which has been observed in Kentish plovers *Charadrius alexandrinus* (38), for example, either by virtue of sex-specific parental care (39) or as a consequence of sex-specific immunocompetence (40). Alternatively, predation could act sex-specifically, although male and female chicks do not differ appreciably in appearance and behavior, and we did not detect a sex difference in encounter rates (Fig. S2). Lastly, the sexes might differ in premature investment in sexual traits (41), although this seems unlikely: sexual ornamentation is moderate, and body size differences at maturity are small (31) (about 4%).

The sex bias during the juvenile stage made the largest contribution to the ASR bias. This finding corroborates the results of earlier avian studies showing that sex-specific first-year survival may lead to an ASR bias (25, 36). Our study goes further than previous works by decomposing the contributions from the first-year stage into chick and juvenile contributions. Juvenile survival contributed most toward ASR bias, probably because these naïve individuals face multiple challenges during the transition from parental independence to sexual maturity, potentially including predation, harsh winter climates, and food shortages, all of which could disproportionately affect either sex. For example, in sexually sized dimorphic species, including red deer *Cervus elaphus* and great bustards *Otis tarda*, male young are less able to cope with severe winter weather (42) and food shortage (43), probably owing to the metabolic demands of large body size. In snowy plovers, such a mechanism is unlikely given the moderate size differences between males and females (31).

Another possibility is that sex-biased dispersal behavior could contribute toward sex differences in apparent juvenile survival because natal dispersal is typically female-biased in birds (44), with snowy plovers being no exception (45, 46). However, dispersal and survival are not necessarily independent phenomena because dispersal often entails survival costs, such as increased predation risk and unfamiliarity of novel environments (47). Moreover, chicks are unable to disperse beyond the breeding site, and therefore, their survival estimate approximated true survival and thus, implies a role of intrinsic sex differences in early survival. In addition, over the 7 years of this study, few adults were resighted in adjacent populations, and these sightings are unbiased with respect to sex. Finally, an independent study of snowy plovers in Monterey Bay, California found that survival was male-biased, even after accounting for sex-specific dispersal (34).

Negligible Effect of Sex Allocation. The hatching sex ratio, based on 340 hatchlings, was unbiased and served as a proxy for the secondary sex ratio. Despite popular interest in sex allocation theory (48, 49), relatively few studies have convincingly shown offspring sex ratio biases in wild populations (50). Düsing (51), Fisher (52), and others (53–55) reasoned that, if sex biases in survival emerged after the period of parental investment, sex allocation should not deviate from parity. This proposition is precisely what we found, with ASR being strongly influenced by the sex-biased survival of independent juveniles rather than deviations in the hatching sex ratio (Fig. 2). Furthermore, although the sex-biased survival of dependent chicks provided a noteworthy contribution to ASR bias (Fig. 2), fathers provide uniparental care of chicks in this species, and therefore, the period of maternal investment typically ends at hatching. Given this parental care system, our result further confirms theoretical expectations of an unbiased hatching sex ratio.

Evolutionary Feedbacks Between ASR and Mating System. Mating systems are influenced by the availability of mates (11). A biased ASR creates conditions whereby one sex is in limited supply, thus forcing the other sex to compete for access to mates (7, 10). In shorebirds, ASR is a strong predictor of mating and parental strategies (13), with the limiting sex tending to have greater mating opportunities and reduced parental investment. These sex differences in the costs and benefits of parental care may facilitate polygamy (6).

However, the relationship between sex ratio and mating system represents a causality dilemma because of the positive feedback that polygamous mating systems impose on ASR bias and vice versa (56). On the one hand, polygamy entails sex-specific costs caused by sexual selection that could drive the ASR bias, whereas on the other hand, ASR bias creates uneven mating opportunities and thus, facilitates polygamy. We show that sex biases originate before maturity (Fig. 5*i*) and are, therefore, likely influenced by natural selection. For example, genotype–sex interactions could impact chick survival during development (57) rather than during adulthood, when sexual selection is expected to have a strong impact on survival. Consequently, ASR bias seems to drive the mating system rather than vice versa (Fig. 5*ii*). We cannot discount the possibility that sexual selection contributes toward sex biases in early survival [for instance, via differential early investment in secondary sexual traits (41)]. However, this explanation seems unlikely because sexual dimorphism in plovers is negligible, and if anything, males are more ornamented than females (31, 58).

Consequences of Mating System and ASR on Population Growth. In vertebrates, adult survival is often the most important parameter influencing population growth because adults have the greatest reproductive potential (59). This phenomenon was the case in our population, but the effect of adult survival was sex-specific because of ASR bias and polygamy. Under a male-biased ASR, adult female survival had the highest elasticity to population growth, meaning that a small perturbation to adult female survival has a larger effect on population growth than an equivalent perturbation in all other parameters. Attempting to dissect the effects of ASR and mating system, we found that sex-specific elasticities of adults were greatest under scenarios of male-biased ASR compared with scenarios of polyandry (Fig. 5*iii*). Conversely, in scenarios of unbiased ASR, both sexes had the same elasticities for all parameters under monogamy, but under polyandry, adult male survival had the highest elasticity. These contrasts highlight the reproductive constraints imposed on populations with a biased ASR. When ASR is male-biased, polyandrous mating strategies optimize individual fecundity and thus, maximize population growth (1). However, reproduction is dependent on the availability of gametes, and thus, a population with female-biased ASR and a polygynous mating system may not be limited in the same way because a single male can produce many more gametes than a single female and hence, father more offspring. Therefore, similar studies of polygynous populations are needed to critically test the predictions of mating system, ASR, and population growth.

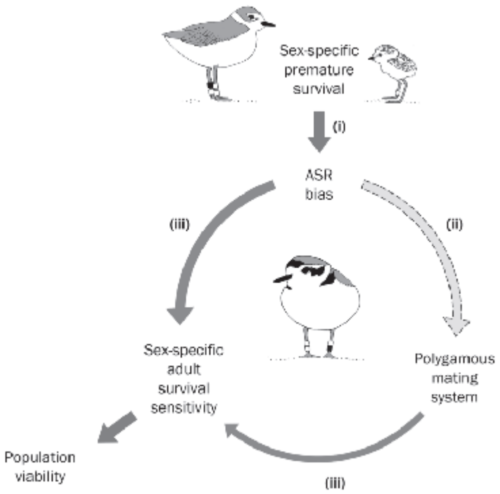

Fig. 5. Schematic representation of the sex- and stage-specific demographic feedbacks between ASR, mating system, and population viability. In our study system, ASR bias (*i*) is driven by sex-specific survival of early life history stages and (*ii*) likely facilitates a polygamous mating system. (*iii*) Both ASR bias and polygamy contribute to sex-specific elasticities of population growth, with ASR having the strongest effect as illustrated by arrow width.

Implications of Two- Versus One-Sex Modeling for Biodiversity Conservation. Modeling population trajectories is an important tool for conservation and management (60). Sex biases could potentially play an important role in population dynamics, particularly for polygamous species, but sex-structured models remain uncommon. Here, we compare a conventional one-sex model with a two-sex model incorporating frequency-dependent reproduction and sex-specific survival. We show that our two-sex model provides a better fit to the observed data than a one-sex model. Moreover, although both models indicate negative population trajectories, the one-sex model substantially underestimates the rate of population decline and exhibits greater uncertainty. This finding has important conservation implications, especially for sex-biased populations of threatened or endangered species, where failing to incorporate sex-specific vital rates could lead to erroneous conclusions and overestimated population viability.

Conclusions. With the rise of conservation-based monitoring of individually marked populations (61), a wealth of demographic data presents new scientific horizons for ecologists and evolutionary biologists interested in sex-specific modeling. By combining extensive individual-based sex- and stage-specific vital rates and structured population models, we show that a male-biased ASR in a natural population is driven by male-biased survival of early life history stages (Fig. 5*i*). Our results indicate that ASR likely drives the mating system (Fig. 5*ii*), although additional experimental and/or comparative studies are needed to establish the causal link. Both ASR and mating system facilitate sex-specific sensitivities to population growth (Fig. 5*iii*), with ASR having the strongest effect. Male-biased survival in snowy plovers is consistent with recent comparative studies (56, 62) suggesting that many bird species may exhibit male-biased ASR (9, 37). Thus, our study makes an important contribution to understanding a widespread phenomenon in natural populations and highlights that ASR variation likely acts as an important catalyst of mating system dynamics and population viability.

Materials and Methods

Field and Laboratory Methods. Fieldwork permits (01717/10, 01367/11, 02898/12) were granted by the Secretaria de Medio Ambiente y Recursos Naturales (SEMARNAT) to M.C.-L. and C.K. Our study was performed in accordance with the approved guidelines outlined by SEMARNAT. Over the 7-year study period, we collected mark–recapture and individual reproductive success data during daily surveys of the study site over the breeding season that typically spanned from mid-April to mid-July. Plover chicks were captured by hand and adults were captured using funnel traps on broods or nests (63). We assigned adults a unique combination of three color rings and an alpha-numeric metal ring, allowing the use of both captures and noninvasive resightings to estimate survival. Regular brood resightings combined with regular recaptures aided analyses of daily survival for chicks. Given our intensive nest search and capture efforts, we are confident that we ringed the vast majority of chicks (>95%) and breeding adults (>85%) in the local population. Nests and broods were frequently monitored every 2–7 days to assess daily survival and identify tending parents. During captures, ~25–50 µL of blood was sampled from the metatarsal vein of chicks and the brachial vein of adults for molecular sex typing with the Z-002B marker (64) and verification with the Calex-31 marker located on the W chromosome (65). For our PCR conditions see ref. 38.

Quantifying Mating System. We evaluated the mating system of the population using a dataset that only included individuals for which we (*i*) were confident of the identity of their mates and (*ii*) had observed them in at least two reproductive attempts that were either within the same season or in different seasons. Sex differences in the per capita number of annual mates were evaluated using a nonparametric Mann–Whitney–Wilcoxon test.

Estimation of Sex- and Stage-Specific Survival. Our structured population model considered sex-specific survival across three key stage classes in avian life history: chicks, juveniles, and adults (Fig. 6). The chick stage was defined as the 25-day period between hatching and fledging, during which offspring are dependent on parental care (66). The juvenile stage was defined as the 1-year transition period spanning from fledging to recruitment into the adult population. The adult stage represented a stasis stage in which individuals were annually retained in the population.

We used mark–recapture models to account for sex, stage, and temporal variation in encounter (p) and apparent survival (ϕ) probabilities because they allow for imperfect detection of marked individuals during surveys and the inclusion of individuals with unknown fates (29). We use the term "apparent survival," because true mortality cannot be disentangled from permanent emigration in this framework (29). Furthermore, only a few nearby populations are regularly monitored, and we have limited evidence that marked individuals disperse. *SI Materials and Methods* has additional details of our survival analysis.

Matrix Model Structure. We built a two-sex postbreeding matrix model for the population that incorporated all three stages of plover life history into two annual transitions denoting first-years and adults (Fig. 6). Transitions of projection matrices are required to have equal temporal durations (67), and thus, the chick stage (25 days) was combined with the juvenile stage (~11 months) as lower-level matrix elements to describe the transition of premature individuals to adulthood (Fig. 6). The projection of the matrix for one annual time step (t) is given by

$$\mathbf{n}(t+1) = \mathbf{M}\mathbf{n}(t), \qquad [1]$$

where $\mathbf{n}$ is a 4×1 vector of the population distributed across the two life stages and two sexes:

$$\mathbf{n} = \begin{bmatrix} ♀_{\text{1st year}} \\ ♀_{\text{Adult}} \\ ♂_{\text{1st year}} \\ ♂_{\text{Adult}} \end{bmatrix}, \qquad [2]$$

and $\mathbf{M}$ is expressed as a 4×4 matrix:

$$\mathbf{M} = \begin{bmatrix} 0 & R_♀(1-\rho) & 0 & R_♂(1-\rho) \\ \phi_{♀C}\phi_{♀J} & \phi_{♀A} & 0 & 0 \\ 0 & R_♀(\rho) & 0 & R_♂(\rho) \\ 0 & 0 & \phi_{♂C}\phi_{♂J} & \phi_{♂A} \end{bmatrix}, \qquad [3]$$

where transition probabilities (ϕ) between life stages are the survival of chicks (C), juveniles (J), and adults (A) for females (♀) and males (♂). The hatching sex ratio (ρ) describes the probability of hatchlings being either male (i.e., ρ) or female (i.e., $1-\rho$). Per capita reproduction of females ($R_♀$) and males ($R_♂$) is expressed through sex-specific mating functions used to link the sexes and produce progeny for the following time step of the model given the relative abundances of each sex (67). Here, we use the harmonic mean mating function, which accounts for sex-specific frequency dependence (68):

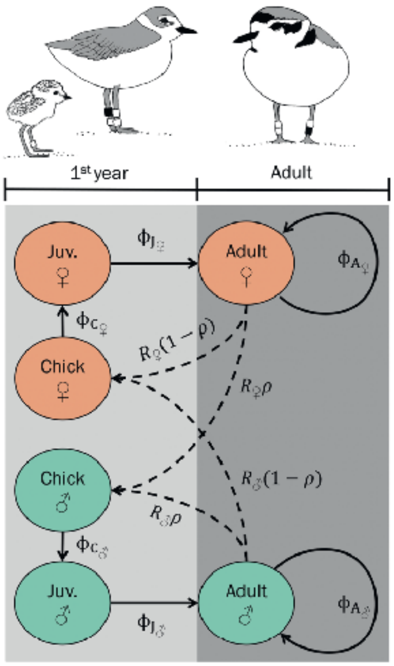

Fig. 6. Snowy plover lifecycle flow diagram illustrating sex-specific survival (ϕ) among three life stages (chick = C, juvenile = J, and adult = A) and the link between the sexes via the frequency-dependent mating function (R) (Eq. **4**). The hatching sex ratio (ρ, proportion of male hatchlings) serves as a proxy for the primary sex ratio and allocates progeny to the male or female chick stage. Lower-level parameters (i.e., chick and juvenile survival) constitute the transition of first-year individuals (illustrated in light gray).

$$R_♀ = \frac{kn_♀}{n_♂ + n_♀ h^{-1}}$$
$$R_♂ = \frac{kn_♂}{n_♂ + n_♀ h^{-1}}, \qquad [4]$$

where k is the modal clutch size (three in the case of snowy plovers), h is an index of the mating system ($h > 1$ signifies polygyny, $h = 1$ signifies monogamy, and $h < 1$ signifies polyandry), and $n_♀$ and $n_♂$ are the densities of adult females and males, respectively, in each time step of the model. In accordance with the predominantly polyandrous mating system, h was defined as the inverse of the average annual number of mates per female:

$$h = \frac{1}{\frac{1}{N_♀}\sum_{i=1}^{N_♀}\frac{m_i}{b_i}} \qquad [5]$$

where $N_♀$ is the number of breeding females in the marked population, b is the total number of years female i was seen breeding, and m is the total number of mating partners female i had over b years. To account for potential sex biases arising before the chick stage (i.e., sex allocation), we evaluated if the hatching sex ratio deviated significantly from parity (*SI Materials and Methods* has details).

Estimation of the ASR. We estimated ASR from the stable stage distribution (w) of the two-sex matrix model:

$$\text{ASR} = \frac{w_{♂A}}{w_{♂A} + w_{♀A}}, \qquad [6]$$

where $w_{♂A}$ and $w_{♀A}$ give the proportions of the population composed of adult males and females, respectively, at equilibrium. To evaluate uncertainty in our estimate of ASR caused by sampling and process variation in our survival parameters, we implemented a bootstrapping procedure that resampled our mark–recapture data (*SI Materials and Methods* has details).

Life Table Response Experiment of ASR Contributions. Perturbation analyses provide important information about the relative effect that each component of a matrix model has on the population-level response (in our case, ASR). To assess how influential a sex bias in parameters associated with each of the three life stages was on ASR dynamics, we used an LTRE. An LTRE decomposes the difference in response between two or more "treatments" by weighting the difference in parameter values by the parameter's contribution to the response (i.e., its sensitivity) and summing over all parameters (67). Here, we compared the observed scenario (**M**) with a hypothetical scenario ($\mathbf{M}_0$), whereby all female survival rates were set equal to the male rates and the hatching sex ratio was unbiased (i.e., $\rho = 0.5$). Thus, our LTRE identifies the drivers of ASR bias by decomposing the difference between the ASR predicted by our model and an unbiased ASR (25).

The contributions (C) of lower-level demographic parameters (θ) were calculated following Veran and Beissinger (25)

$$C(\theta) = (\theta_{♂} - \theta_{♀}) \times \frac{\partial \mathrm{ASR}}{\partial \theta}, \qquad [7]$$

where $\partial \mathrm{ASR}/\partial \theta$ is the sensitivity of ASR to perturbations in the demographic rate θ in matrix $\mathbf{M}'$, which is a reference matrix "midway" between the two scenarios (67):

$$\mathbf{M}' = \frac{\mathbf{M} + \mathbf{M}_0}{2}. \qquad [8]$$

The two-sex mating function makes our model nonlinear in the sense that the projection matrix and specifically, the fertility elements (Eq. **4**) depend on sex-specific population structure. Perturbation analyses must, therefore, accommodate the indirect effects of parameter perturbations on population growth via their effects on population structure. To estimate the sensitivities of the vital rate parameters to ASR, we used numerical methods that independently perturbed each parameter of the matrix, simulated the model through 1,000 time steps, and calculated ASR at equilibrium. These computations produced parameter-specific splines, from which $\partial \mathrm{ASR}/\partial \theta$ could be derived. Our approach appropriately accounts for the nonlinear feedbacks between vital rates and population structure, although it does not isolate the contribution of this feedback (26, 69).

Population Growth Consequences to ASR Bias and a Polygamous Mating System. Biased ASR and polygamous mating systems can restrict the reproductive potential of a population because of a scarcity of the limiting sex (70). Thus, population viability can be indirectly affected by ASR and mating system via the sex-specific effects that vital rates have on population growth under a biased ASR, a polygamous mating system, or both (71). To investigate the relative influence that a biased ASR or a polygamous mating system has on population growth, we conducted a sensitivity analysis of all sex-specific parameters using four scenarios of the two-sex model: (*i*) polyandrous and male-biased ASR (i.e., the observed scenario), (*ii*) polyandrous and unbiased ASR, (*iii*) monogamous and male-biased ASR, and (*iv*) monogamous and unbiased ASR. In polyandrous scenarios, h was set to the value from field observations, whereas in monogamous scenarios, $h = 1$. In scenarios of unbiased ASR, male survival rates were assigned to both sexes (i.e., $\mathbf{M}_0$ above), whereas the original sex-specific structure was retained in male-biased scenarios.

Under each scenario, sensitivities of λ to perturbations in each parameter (θ) were estimated numerically as described above. Sensitivities were rescaled into elasticities (e), which describe the proportional response of λ to a proportional perturbation of a demographic parameter (67). This way, the sensitivity of parameters becomes directly comparable. Elasticities were calculated as

$$e(\theta) = \frac{\theta}{\lambda} \times \frac{\partial \lambda}{\partial \theta}, \qquad [9]$$

where $\partial \lambda/\partial \theta$ is the sensitivity of λ to perturbations in parameter θ.

Comparison of Two- Versus One-Sex Models. Two-sex population models are rarely used in conservation biology because of the detailed data required to correctly parameterize them (70). As such, vital rates are typically estimated for only one sex or generalized across both sexes. However, in polygamous species, reproductive success varies according to the relative abundances of mates (71), which is dictated by ASR and sex-specific survival. Therefore, ignoring sex-specific vital rates in polygamous species could misinform conservationists and wildlife management of population viability.

To explore how population growth varies under a two-sex model and a conventional one-sex model, we compared deterministic population growth of the two-sex model (**M**) with that of a one-sex model in which rates were averaged over both sexes (**A**):

$$\mathbf{A} = \begin{bmatrix} 0 & F \\ \phi_C \phi_J & \phi_A \end{bmatrix}, \qquad [10]$$

where F is the average annual per capita fecundity of females (expressed as hatchlings), and ϕ is the sex-averaged survival of chicks (C), juveniles (J), and adults (A). Deterministic growth (λ) was calculated as the dominant eigenvalue of **A** and the asymptotic value of $\sum \mathbf{n}_t / \sum \mathbf{n}_{t-1}$ for **M**. To acknowledge uncertainty, we used the bootstrapped survival analysis described above by estimating the λ of each iteration under the structure of **A** or **M**. We contrasted the central tendency and spread of these distributions to one another and the arithmetic average λ of the actual population trend over the 7-year study period.

All of our modeling and statistical analyses were conducted using R version "You Stupid Darkness" (72), with significance testing evaluated at $\alpha = 0.05$. We provide all computer code and documentation as a PDF file written in Rmarkdown together with all of the raw datasets needed to reproduce our modeling and analyses (Dataset S1).

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Anexo II

CeutaOPEN, individual-based field observations of breeding snowy plovers *Charadrius nivosus*

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DATA DESCRIPTOR

CeutaOPEN, individual-based field observations of breeding snowy plovers *Charadrius nivosus*

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Shorebirds (part of the order Charadriiformes) have a global distribution and exhibit remarkable variation in ecological and behavioural traits that are pertinent to many core questions in the fields of evolutionary ecology and conservation biology. Shorebirds are also relatively convenient to study in the wild as they are ground nesting and often occupy open habitats that are tractable to monitor. Here we present a database documenting the reproductive ecology of 1,647 individually marked snowy plovers (*Charadrius nivosus*) monitored between 2006 and 2016 at Bahía de Ceuta (23°54N, 106°57W) – an important breeding site in north-western Mexico. The database encompasses various morphological, behavioural, and fitness-related traits of males and females along with spatial and temporal population dynamics. This open resource will serve as an important data repository for addressing overarching questions in avian ecology and wetland conservation during an era of big data and global collaborative science.

Background & Summary

Longitudinal data on individuals living in the wild represent the gold standard for research in organismal ecology, as subjects are sampled repeatedly over multiple stages of their life-history while being exposed to the natural evolutionary pressures of their native environments[1]. These types of data have offered evolutionary ecologists valuable insights into the selective processes that affect species over multiple generations such as, for example, the role of stochastic climate events shaping the beak morphologies of Darwin's Finches[2], the predator-prey cycles of mammal communities on the Serengeti[3], or the demographic dynamics of alpine plants[4] and animals[5] in response to climate change. However, collecting field data over many consecutive years while following standardized methods requires substantial labour and consistent funding. Due to these challenges, raw longitudinal field data from wild populations are rarely made open to the public[6] – thus limiting the transparency and reproducibility of published research methods and results in evolutionary ecology. Furthermore, releasing raw data has the potential benefit of stimulating more substantive discussion and criticism within the scientific community, which can advance research topics and forge productive collaborations. Here, we offer an open access database of our raw field observations over an 11-year period of 1,647 uniquely marked individuals from an important breeding population of snowy plovers (*Charadrius nivosus*) in Mexico.

Charadrius plovers are small ground-nesting shorebirds that occur worldwide. As a group, plovers present a model system for investigating fundamental and applied topics in organismal biology as they occupy open habitats that are easy to monitor and experimentally manipulate, and they exhibit intra- and interspecific variation in several behavioural, ecological, and demographic traits. For example, plovers display remarkable diversity and plasticity in breeding tactics with sex roles during courtship, mating, and parental care varying appreciably among populations both between and within species[7]. The snowy plover is native to North America[8] and is one

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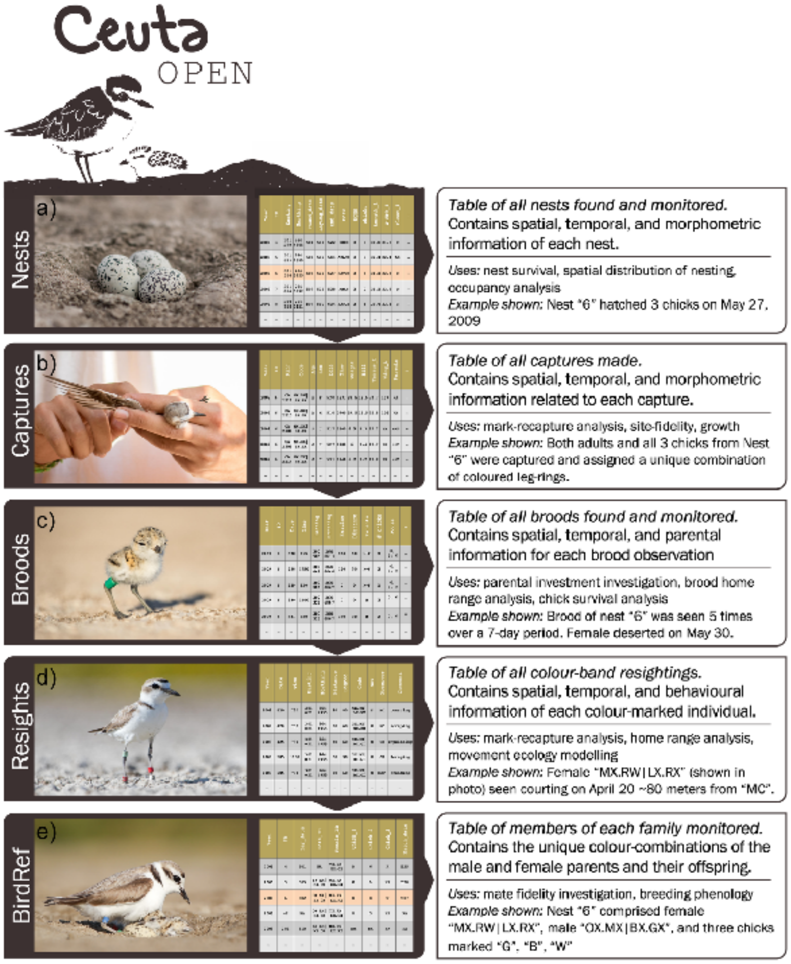

Table of all nests found and monitored. Contains spatial, temporal, and morphometric information of each nest.

Uses: nest survival, spatial distribution of nesting, occupancy analysis
Example shown: Nest "6" hatched 3 chicks on May 27, 2009

Table of all captures made. Contains spatial, temporal, and morphometric information related to each capture.

Uses: mark-recapture analysis, site-fidelity, growth
Example shown: Both adults and all 3 chicks from Nest "6" were captured and assigned a unique combination of coloured leg-rings.

Table of all broods found and monitored. Contains spatial, temporal, and parental information for each brood observation

Uses: parental investment investigation, brood home range analysis, chick survival analysis
Example shown: Brood of nest "6" was seen 5 times over a 7-day period. Female deserted on May 30.

Table of all colour-band resightings. Contains spatial, temporal, and behavioural information of each colour-marked individual.

Uses: mark-recapture analysis, home range analysis, movement ecology modelling
Example shown: Female "MX.RW|LX.RX" (shown in photo) seen courting on April 20 ~80 meters from "MC".

Table of members of each family monitored. Contains the unique colour-combinations of the male and female parents and their offspring.

Uses: mate fidelity investigation, breeding phenology
Example shown: Nest "6" comprised female "MX.RW|LX.RX", male "OX.MX|BX.GX", and three chicks marked "G", "B", "W"

Fig. 1 Schematic of the CeutaOPEN database. During fieldwork, data collection was divided across five main tasks: (**a**) nest monitoring, (**b**) captures of adults and chicks, (**c**) brood monitoring, (**d**) resightings of individually colour ringed adults, and (**e**) determining the identity of all breeding pairs and their offspring. The data obtained during each of these activities are structured in our database as the five tables shown here that contain a common variable such as a nest "ID" or a bird "code", that can be utilized by the user for relational queries.

of the least abundant shorebirds on the continent (estimated population size: 25,869) with many populations in decline and requiring intensive management[9]. Apart from being a public icon of avian conservation, snowy plovers have also increasingly captured the spotlight for their intriguing ecology and life-history. Their unusual biology features a rare breeding behaviour characterized by highly dispersive polyandry and male-biased uniparental care[10,11].

In this data descriptor we present CeutaOPEN – an open-access database containing the raw data from our fieldwork between 2006 and 2016 monitoring a breeding population of snowy plovers at Bahía de Ceuta, a subtropical lagoon on the coastal plain of north-western Mexico (23°54′N, 106°57′W). The database includes individual-based observations of reproductive effort, movements, morphometrics, and social behaviour (Fig. 1). Previously, we have used subsets of these data to report on a wide variety of topics in organismal biology, including sex ratio variation[12], population viability[13], courtship behaviour[14], incubation behaviour[15], parental care[16], ontogeny[17], chronobiology[18], camouflage mechanisms[19], offspring desertion[20] and mating system dynamics[21]. The

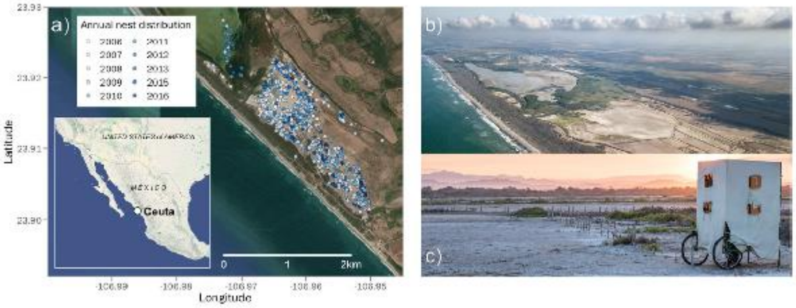

Fig. 2 (a) Map of the Bahía de Ceuta study site and photos of the (b) salina breeding habitat and (c) a mobile hide in which observers conduct non-invasive field work.

motivation for making our database open is to provide evolutionary ecologists with an accessible resource that will serve as an important repository for addressing overarching questions in organismal biology and conservation. Here we describe our field methods for collecting the observations presented in the database, we summarize the contents of the database, and we provide a code-based tutorial demonstrating how to import and query the database within the R environment and conduct, for example, a simple analytical workflow to investigate sex-specific ontogeny.

Methods

Study area.

Plovers breeding in Bahía de Ceuta mainly concentrate their activities on 200 ha of salt flats that contain several abandoned evaporation ponds. This habitat (hereafter "salina") is surrounded by red mangrove (*Rhizophora mangle*) and characterized by sparse vegetation and open substrates. Nesting typically commences in late March or early April when flooding from spring tides and high precipitation recedes. By mid-July the breeding season concludes when rains and spring tides resubmerge the salina. Throughout the remainder of the year, the flooded salina and surrounding lagoons are used as important wintering habitats for plovers and other migratory shorebirds, with the region being protected by the Ramsar convention[22]. Our monitoring effort throughout the 11-year study period was focused on the largest contiguous section of salt flats in the study area where the vast majority of known breeding activity occured (Fig. 2a,b). However, in drought years or at the peak of the breeding season when tidewaters had maximally retreated, we made observations of plovers nesting and tending broods in several small pockets of salina adjacent to the main study site (Fig. 2a).

Data collection.

Over the 11-year study period, we monitored the population daily between April and July, and once every month or two during the remainder of the year. We used a car and mobile hides[23] (Fig. 2c) to search for nests, broods, and determine the identity of breeding plovers with binoculars and scopes. During fieldwork, our data collection was divided across four main field tasks: (1) nest monitoring, (2) captures of adults and chicks, (3) brood monitoring, and (4) resights of individually colour ringed adults. The data obtained during each of these activities are structured in our database as tables (Fig. 1) containing a common variable such as a nest "ID" or a bird "code", that can be utilized by the user for relational queries. The basic format of these tables was taken from ref. [24]. Fieldwork permits to collect the data presented in CeutaOPEN were granted by the Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT). All of our field activities were performed in accordance with the approved ethical guidelines outlined by SEMARNAT. Here we explain the details of our data collection pertinent to the database.

Nest data. We regularly searched for nests (Fig. 1a) and incubating plovers by traversing the salina on foot, by car or in a mobile hide[19] (Fig. 2c). Upon discovery, we recorded the nest's geographic location, the found date and time, and measured the width and length of each egg in the clutch with calipers. To estimate the initiation-date of the clutch (i.e., date when the first egg was laid), we floated each egg in a jar of water and scored the embryonic stage of development according to a calibrated table[25]. For hatched clutches that were initially discovered more than 10 days after laying, we estimated initiation-date by subtracting 25 days (i.e., the mean incubation time in our population[18]) from the hatching date and subtracting an additional 5 days to account for a 2-day egg laying interval[11]. We checked nests every 2–7 days to assess survival and identify tending parents.

Capture data. We captured plover chicks by hand and adults using mist nets or funnel traps on broods or nests. To individually identify members of the population, we assigned adults a unique combination of three to four colour leg rings and an alpha-numeric metal ring (see photo in Fig. 1d). Likewise, we marked chicks less than 2 weeks old with a single colour ring and a metal ring (see photo in Fig. 1c). Given our intensive nest search and capture efforts, we are confident that we ringed the vast majority of chicks (>95%) and breeding adults (>85%) in the local breeding population every year. During captures, we sampled the metatarsal vein of chicks or the brachial vein of adults and drew ~25–50 μL of blood for subsequent genetic analyses. Additionally, we measured

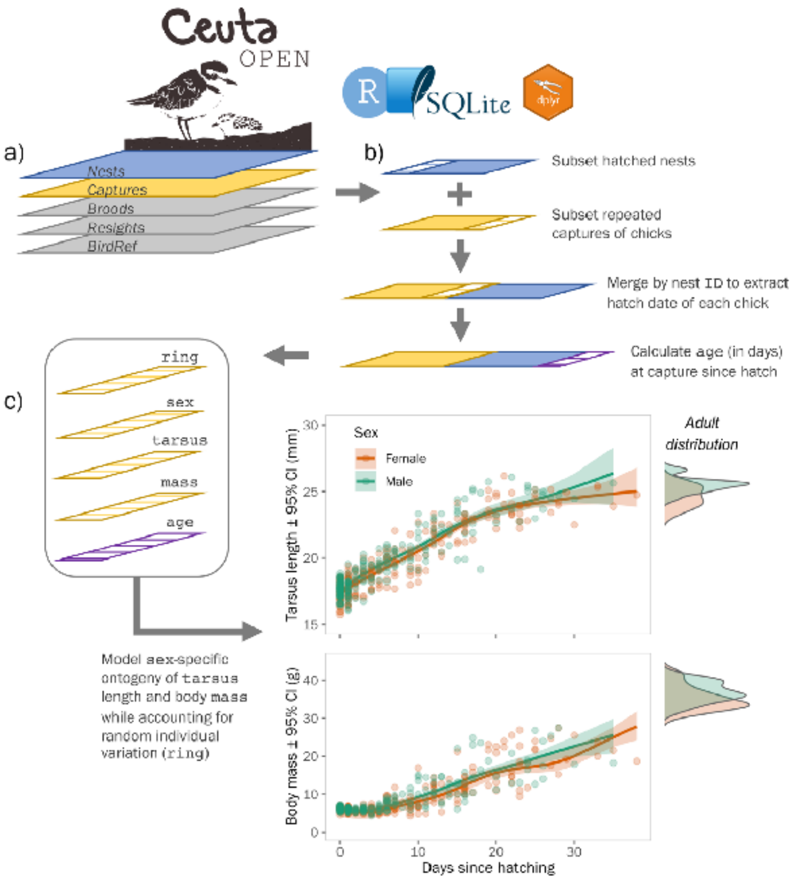

Fig. 3 Schematic of an example analytical workflow using CeutaOPEN within the R environment to investigate how chick morphometric data may be used to study growth and ontogeny. (**a**) Import the database into R using the RSQLite[31] package. (**b**) Use the dplyr[32] package to join the '*Captures*' table with the '*Nests*' table by nest "ID" to determine the "hatch_date" of each captured individual. Subset the result to individuals that have repeated captures and calculate the "age" at capture by subtracting the "hatch_date" by the capture "date". (**c**) Use the bamlss[33] package (i.e., "Bayesian Additive Models for Location, Scale, and Shape") to determine the sex-specific growth trends while controlling for repeated measures within individuals and random annual variation. Plot the fitted values to visualise the trends (see Supplementary File 1 for more details).

body mass, bill length, tarsus length, and wing length for all captured individuals (Fig. 3). As snowy plovers exhibit only minor sexual dimorphisim in plumage and body size, we molecularly determined sex using the Z-002B marker[26] and verification with the Calex-31 marker located on the W chromosome[27] for all adults and chicks captured before 2014. For PCR conditions see ref. [17].

Brood data. Similar to our data collection of nests, we resighted broods (see photo in Fig. 1c) every 1–7 days to assess chick survival and determine sex-specific patterns of parental care and desertion. Each brood observation includes the time, distance and azimuth from the observer to the brood, geographic location of the observer, number of chicks seen, and the identity of them and their parents.

Resight data. We typically resighted colour ringed individuals (see photo in Fig. 1d) opportunistically while in the field. Since 2009 we surveyed the entire salina within a single day at least once during the breeding season

Column	Data name	Description of data
1.	species	species of plover (all snowy plover 'SNPL' in this case)
2.	population	population at which nest was monitored (all 'Ceuta' in this case)
3.	year	year during which nest was monitored
4.	site	site at which nest was monitored
5.	nest	unique identifier of nest (unique within year and within site)
6.	ID	a concatenation of year, site, and nest to make a unique nest identifier across sites and years
7.	easting	UTM easting of nest
8.	northing	UTM northing of nest
9.	utm	UTM zone of nest
10.	found_date	date nest was discovered (stored in the internal 'Date' format of R and represents the number of days since January 1, 1970, the 'Unix epoch'. Converted easily in R using 'as.Date(found_date, origin = "1970-01-01")')
11.	found_time	time nest was discovered (24 h format, e.g. '1633')
12.	nest_initiation_date	estimated date when the first egg of the nest was laid (i.e., its 'initiation'). The estimate is calculated by subtracting the age in days of the oldest egg (determined by the floatation scores 'float1', 'float2', and 'float3' defined below) and a 5-day laying period for three-egg clutches or a 3-day laying period for two-egg clutches or a 1-day laying period for one-egg clutches (egg-laying intervals are based on ref. [24]. Determining initiation dates of clutches found at stage 'F' is imprecise, and thus we estimated the initiation date by subtracting 25 days from the hatch date (i.e., the average length of incubation in this population) and an additional 5, 3, or 1 days for the laying period depending on the clutch size. For nests found at stage 'F' that failed before hatching, the nest initiation date is 'NA'. Stored in the internal 'Date' format of R and represents the number of days since January 1, 1970, the 'Unix epoch'. Converted easily in R using 'as.Date(nest_initiation_date, origin = "1970-01-01")')
13.	end_date	date nest ended (cause specified in 'fate' column; stored in the internal 'Date' format of R and represents the number of days since January 1, 1970, the 'Unix epoch'. Converted easily in R using 'as.Date(end_date, origin = "1970-01-01")')
14.	last_observation_alive	date nest was last observed active. Stored in the internal 'Date' format of R and represents the number of days since January 1, 1970, the 'Unix epoch'. Converted easily in R using 'as.Date(last_observation_alive, origin = "1970-01-01")')
15.	fate	fate of nest (e.g., 'Hatched', 'Predated', 'Abandoned', etc.)
16.	male	colour-ring combination of male confirmed tending nest. The scheme can be noted as XX.XX|XX.XX where X indicates a possible position for a color (or metal) ring, the full-stop marks the position of the knee-joint and the pipe divides the left and right leg. Thus the readout is "left above. left below | right above. right below". See page 9 of ref. [25] for more details.
17.	female	colour-ring combination of female confirmed tending nest. The scheme can be noted as XX.XX|XX.XX where X indicates a possible position for a color (or metal) ring, the full-stop marks the position of the knee-joint and the pipe divides the left and right leg. Thus the readout is "left above. left below | right above. right below". See page 9 of ref. [25] for more details.
18.	no_chicks	number of chicks hatched from nest
19.	clutch_size	number of eggs found in nest
20.	length1	length in millimeters of egg #1
21.	width1	width in millimeters of egg #1
22.	float1	float score of egg #1 as defined on page 5 of ref. [25]
23.	length2	length in millimeters of egg #2
24.	width2	width in millimeters of egg #2
25.	float2	float score of egg #2 as defined on page 5 of ref. [25]
26.	length3	length in millimeters of egg #3
27.	width3	width in millimeters of egg #3
28.	float3	float score of egg #3 as defined on page 5 of ref. [25]
29.	photo	indication if a photo of nest was taken (1) or not (0)
30.	observer	initials of observer who found nest
31.	comments	miscellaneous comments pertinent to nest's observation

Table 1. Nests data of snowy plovers breeding in Bahia de Ceuta, Mexico, between 2006 and 2016. This dataset contains information on egg dimensions, laying phenology, nest fate, geographic location, and the identity of incubating parents. These data can be used to assess individual reproductive effort and success, mate and site fidelity, and senescence, for example.

to record all colour ringed individuals present. As with our brood data, each resight includes the distance and azimuth to the individual, the geographic location of the observer, and any noteworthy comments pertaining to the individual's behaviour.

Data Records

Our database and all other files described in this manuscript are stored in a publicly available OSF repository[28]. The file Ceuta_OPEN_vX-X.sqlite contains the SQL (Structured Query Language) database of four tables containing our raw observations collected during routine fieldwork (Nests, Captures, Broods, and Resights), and a fifth table (BirdRef) that uses relational information to summarize the identities of the parents and offspring belonging to each nest and subsequent brood. The structure of these tables is defined in Tables 1–5 below. This Data Descriptor is based on version 1.4 of the CeutaOPEN database.

In summary, the CeutaOPEN database contains information on 794 surveyed nests, 2,824 captures of 1,647 marked individuals, 415 monitored broods, and 6,939 resightings of colour-marked individuals. Over the 11-year

Column	Data name	Description of data
1.	species	species of plover (all snowy plover 'SNPL' in this case)
2.	population	population at which capture was made (all 'Ceuta' in this case)
3.	year	year during which capture was made
4.	site	site at which capture was made
5.	nest	unique identifier of nest at which capture was made (unique within year and within site). If capture was made at a brood originating from an unknown nest, the identifier is negative (e.g., ' 2').
6.	ID	a concatenation of year, site, and nest to make a unique nest identifier across sites and years
7.	ring	alpha-numeric code of metal ring assigned to captured individual
8.	code	color-ring combination assigned to captured individual. The scheme can be noted as XX.XX|XX.XX where X indicates a possible position for a color (or metal) ring, the full-stop marks the position of the knee-joint and the pipe divides the left and right leg. Thus the readout is "left above. left below | right above. right below". See page 9 of ref. [25] for more details.
9.	age	age of captured individual ('J' – juvenile (chicks and first-years), 'A' – adult (second-years and older))
10.	field_sex	sex of individual determined in the field based on ornamentation and other clues (e.g., time of capture, parental care, etc.), where 'F' – female, 'M' – males, and 'J' – unknown sexed juvenile
11.	mol_sex	sex of individual determined in the lab with the P2/P8 and Calex-31 markers (for our PCR conditions see ref. [17]), where 'F' – female, 'M' – males, 'U' – insufficient molecular evidence (e.g., markers failed), and 'NA' – individual not molecularly sex-typed. Note: all birds initially captured in years after 2013 have not yet been molecularly sex-typed
12.	sex	sex of captured individual ('F' – female, 'M' – males, 'J' – juvenile of unknown sex)
13.	easting	UTM easting of capture
14.	northing	UTM northing of capture
15.	utm	UTM zone of capture
16.	date	date capture was made (stored in the internal 'Date' format of R and represents the number of days since January 1, 1970, the 'Unix epoch'. Converted easily in R using 'as.Date(date, origin = "1970-01-01")')
17.	time	time capture was made (24 h format, e.g. '1633')
18.	parents	parents attending captured individual (if age = 'J') at time of observation ('0' – no parent present; '1' – one parent (not identified whether male or female); '2' = female only ('2+' when female identified, whilst male's identity was uncertain); '3' – male only ('3+' when male identified, whilst female's identity was uncertain, i.e., opposite of '2+'); '4' – both present)
19.	weight	weight in grams of captured individual
20.	bill	length in millimeters of upper mandible of captured individual. Measured as the distance between the tip of the forehead feathering at the base of the upper bill, along the ridge of the culmen, and the tip of the bill (also known as the "exposed culmen" measurement; *sensu* page 8 of ref. [31]
21.	left_tarsus	length in millimeters of left tarsus of captured individual. Measured as the distance between the notch at the end of the lateral condyle of the tibiotarsus on the backside of the leg, to the last tarsal scute on the front of the leg at the base of the foot (also known as the "outside tarsus" or "diagonal tarsus" measurement; *sensu* page 11 of ref. [30]
22.	right_tarsus	same as 'left_tarsus' measurement above but for right leg of captured individual
23.	left_wing	length in millimeters of left wing of captured individual. Measured as the distance from the carpal joint (the bend of the wing) to the longest primary feather whilst flattening the wing and straightening the primaries (also known as the "maximum flat" or "flattened and straightened" measurement; sensu page 6 of ref. [31]
24.	right_wing	same as 'left_wing' measurement above but for right wing of captured individual
25.	blood	indication if blood from captured individual was collected ('1') or not ('0')
26.	moult	primary molt score of captured individual. Scored as the stage of the moult and the number of feathers at that stage. See ref. [26] for more details.
27.	fat	fat score of captured individual, scored as the amount of visible fat in the furcular region or tracheal pit. See ref. [26] for more details.
28.	lice	indication if feather lice from captured individual were collected ('1') or not ('0')
29.	faecal	indication if faeces from captured individual was collected ('1') or not ('0')
30.	photo	indication if a photo of captured individual was taken ('1') or not ('0')
31.	observer	initials of observer making capture
32.	comments	miscellaneous comments pertinent to capture event

Table 2. Captures data of snowy plovers breeding in Bahía de Ceuta, Mexico, between 2006 and 2016. This dataset contains information on bird morphology, age, sex, capture time and location, and the identity of the individual. These data can be used to assess apparent survival with mark-recapture models, site fidelity, and growth rates of chicks, for example.

study period, we spent 927 days collecting these data in the field – amounting to over 20,000 hours of observational effort.

CeutaOPEN is one of only a few open-access databases to provide raw field observations of an individually-marked wild vertebrate species (for other examples, see refs. [29,30]). We therefore believe our database will provide a valuable model for future field biologists to consult when structuring their data and deciding whether to provide public access.

Column	Data name	Description of data
1.	species	species of plover (all snowy plover 'SNPL' in this case)
2.	population	population at which brood was observed (all 'Ceuta' in this case)
3.	year	year during which brood was observed
4.	site	site at which brood was observed
5.	brood	unique identifier of brood (unique within year and within site). Broods originating from known nests retain the nest identifier found in the *Nests* table, whereas broods hatching from unknown nests have a negative identifier (e.g., '−2')
6.	ID	a concatenation of year, site, and nest to make a unique brood identifier across sites and years
7.	easting	UTM easting of brood observation
8.	northing	UTM northing of brood observation
9.	utm	UTM zone of brood observation
10.	date	date brood observation was made (stored in the internal 'Date' format of R and represents the number of days since January 1, 1970, the 'Unix epoch'. Converted easily in R using 'as.Date(date, origin = "1970-01-01")')
11.	time	time brood observation was made (24 h format, e.g. '1633')
12.	distance	estimated distance in meters between brood and observer
13.	degree	estimated bearing of brood relative to observer (i.e., the number of degrees in the angle measured in a clockwise direction from the north line to the line joining the observer to the brood)
14.	parents	parents attending brood at time of observation ('0' = no parent present; '1' = one parent (not identified whether male or female); '2' = female only ('2+' when female identified, whilst male's identity was uncertain); '3' = male only ('3+' when male identified, whilst female's identity was uncertain, i.e., opposite of '2+'); '4' = both present)
15.	male	color-ring combination of male observed tending brood. The scheme can be noted as XX.XX|XX.XX where X indicates a possible position for a color (or metal) ring, the full-stop marks the position of the knee-joint and the pipe divides the left and right leg. Thus the readout is "left above. left below | right above. right below". See page 9 of ref. [25] for more details
16.	female	color-ring combination of female observed tending brood. The scheme can be noted as XX.XX|XX.XX where X indicates a possible position for a color (or metal) ring, the full-stop marks the position of the knee-joint and the pipe divides the left and right leg. Thus the readout is "left above. left below | right above. right below". See page 9 of ref. [25] for more details
17.	chicks	number of chicks observed in brood. Because of temporary or permanent brood adoption, number of chicks can be larger than initial brood size at subsequent observations
18.	chick_codes	color ring combinations of all chicks observed (individuals seperated by a comma). The scheme can be noted as XX.XX|XX.XX where X indicates a possible position for a color (or metal) ring, the full stop marks the position of the knee-joint and the pipe divides the left and right leg. Thus the readout is "left above. left below | right above. right below". See page 9 of ref. [25] for more details.
19.	brood_photo	indication if a photo of the brood was taken ('1') or not ('0')
20.	observer	initials of observer making brood observation
21.	comments	miscellaneous comments pertinent to brood's observation

Table 3. Broods data of snowy plovers breeding in Bahia de Ceuta, Mexico, between 2006 and 2016. These data contains information on the time and location of a brood observation, the identity and number of chicks seen alive, and the identity of the parents tending chicks. These data can be used to assess parental investment, brood home range, and chick survival, for example.

Technical Validation

During each field season of the snowy plover project at Bahía de Ceuta, observers receive comprehensive training on our sampling protocol[25] and general avian field methodology. In all 11 years of data collection, at least one of us was present in the field to oversee fieldwork and assess the quality of observations. Moreover, field assistants usually aided us with fieldwork for academic purposes (e.g., as part of a bachelor, master, or doctoral project), which encouraged personal interest in maximizing the quality of their data collection. All breeding data from the 2014 breeding season was lost, which is why this year is missing the nest and brood data (Table 6). Likewise, 2015 does not include brood data because broods were not resighted in this year (Table 6).

During the data processing and development of the final database, verification and validations were made at several stages: during fieldwork we would regularly check each other's notes for unusual observations, during the digitization of field data in spreadsheets we would scrutinize outlier measurements, and throughout the assembly of the SQL database we conducted thorough data cleaning (e.g., removing white space from strings, enforcing consistent notation and symbology, etc.). These data quality checks were run annually before merging new observations with the master database.

Usage Notes

The CeutaOPEN database is available under a Creative Commons Attribution 4.0 International Public License, whereby anyone may freely use and adapt our data, as long as the original source is credited, the original license is linked, and any changes to our data are indicated in subsequent use. The database has undergone multiple rounds of curation to purge inconsistencies and errors. Any further errors that are spotted by us or brought to our attention by users will be corrected and documented in future version releases of the database. When using any of the CeutaOPEN materials presented here, please cite this Data Descriptor in addition to the version of the database that was used. Furthermore, for all projects making considerable use of the CeutaOPEN database, we encourage users to reach out to us to offer the opportunity to comment prior to the publication of their work.

Column	Data name	Description of data
1.	species	species of plover (all snowy plover 'SNPL' in this case)
2.	population	population at which resighting was made (all 'Ceuta' in this case)
3.	year	year during which resighting was made
4.	site	site at which resighting was made
5.	easting	UTM easting of observer's location while resighting
6.	northing	UTM northing of observer's location while resighting
7.	utm	UTM zone of observer's location while resighting
8.	date	date resighting was made (stored in the internal 'Date' format of R and represents the number of days since January 1, 1970, the 'Unix epoch'. Converted easily in R using 'as.Date(date, origin = "1970-01-01")')
9.	time	time resighting was made (24 h format, e.g. '1633')
10.	distance	estimated distance in meters between resighted bird and observer
11.	degree	estimated bearing of resighted bird relative to the observer (i.e., the number of degrees in the angle measured in a clockwise direction from the north line to the line joining the observer to the brood)
12.	code	color-ring combination of the resighted individual. The scheme can be noted as XX.XX|XX.XX where X indicates a possible position for a color (or metal) ring, the full stop marks the position of the knee-joint and the pipe divides the left and right leg. Thus the readout is "left above. left below | right above. right below". See page 9 of ref. [25] for more details.
13.	sex	sex of individual determined in the field based on ornamentation and other clues (e.g., capture history, parental care, etc.), where 'F' — female, 'M' — males, and 'J' — unknown sexed juvenile
14.	census	indication if the resighting was conducted as part of a census count ('1') or not ('0')
15.	observer	initials of observer making resighting
16.	comments	miscellaneous comments pertinent to the resighting

Table 4. Resights data of snowy plovers breeding in Bahia de Ceuta, Mexico, between 2006 and 2016. This dataset contains information on the time and location of a colour-ringed adult, the identity of the individual, and behavioural information recorded during the observation. These data can be used to assess apparent survival with mark-recapture models or investigate space-use through home range analysis or movement ecology models.

Column	Data name	Description of data
1.	species	species of plover (all snowy plover 'SNPL' in this case)
2.	population	population at which family was observed (all 'Ceuta' in this case)
3.	year	year during which family was observed
4.	site	site at which family was observed
5.	family	unique identifier of family (unique within year and within site). Families found as nests retain nest identifier found in *Nests* table, whereas families found as broods hatching from unknown nests have a negative brood identifier (e.g., '−2') found in *Broods* table)
6.	ID	a concatenation of year, site, and nest to make a unique family identifier across all sites and years
7.	nest_initiation_date	estimated date when the first egg of the nest was laid (i.e., its 'initiation'). The estimate is calculated by subtracting the age in days of the oldest egg (determined by the floatation scores 'float1', 'float2', and 'float3' defined in the *Nests* table) and a 5-day laying period for three-egg clutches or a 3-day laying period for two-egg clutches or a 1-day laying period for one-egg clutches (egg-laying intervals are based on ref. [34]. Determining initiation dates of clutches found at stage 'F' is imprecise, and thus we estimated the initiation date by subtracting 25 days from the hatch date (i.e., the average length of incubation in this population) and an additional 5, 3, or 1 days for the laying period depending on the clutch size. For nests found at stage 'F' that failed before hatching, the nest initiation date is 'NA'. Stored in the internal 'Date' format of R and represents the number of days since January 1, 1970, the 'Unix epoch'. Converted easily in R using 'as.Date(nest initiation_date, origin = "1970-01-01")')
8.	hatching_date	date nest hatched (stored in the internal 'Date' format of R and represents the number of days since January 1, 1970, the 'Unix epoch'. Converted easily in R using 'as.Date(hatching_date, origin = "1970-01-01")'; 'NA' if nest fate was other than 'hatch' in *Nests* table)
9.	male	metal ring alpha-numeric code of male parent observed with nest/brood
10.	female	metal ring alpha-numeric code of female parent observed with nest/brood
11.	chick1	metal ring alpha-numeric code of first chick assigned to brood
12.	chick2	metal ring alpha-numeric code of second chick assigned to brood
13.	chick3	metal ring alpha-numeric code of third chick assigned to brood
14.	exp	indication if family was part of an experiment
15.	type	indication of type of experiment conducted
16.	manip	date of possible experimental manipulation (stored in the internal 'Date' format of R and represents the number of days since January 1, 1970, the 'Unix epoch'. Converted easily in R using 'as.Date(manip, origin = "1970-01-01")')

Table 5. Bird Reference ("BirdRef") data of snowy plovers breeding in Bahia de Ceuta, Mexico, between 2006 and 2016. This dataset is a relational table of the *Nests, Captures*, and *Broods* tables (Tables 1, 2, and 3) summarizing the identity of all members in a family (i.e., metal ring alpha-numeric codes of both parents and all chicks, if applicable). These data can be used to quantify mating system and assess individual variation in breeding phenology, for example.

Year	Captures	Nests	Broods	Resights
2006	456	158	69	19
2007	655	140	102	593
2008	229	76	30	577
2009	335	82	48	1257
2010	438	96	69	913
2011	225	70	35	806
2012	92	34	12	154
2013	87	31	16	491
2014	10	—	—	500
2015	148	50	—	812
2016	149	57	34	817

Table 6. Annual summary in data of captures conducted, nests surveyed, broods monitored, and resights of colour-marked individuals.

We recommend that users employ R to access and wrangle the CeutaOPEN database for their study. To help this process, please refer to the accompanying RMarkdown document (Supplementary File 1) to follow our suggested analytical workflow for utilizing CetuaOPEN with the RSQLite[31] and dplyr[32] packages in the R environment.

Code availability

To assist users with accessing and querying our database, we have written an accompanying RMarkdown document (Supplementary File 1) that provides a commented workflow for utilizing CetuaOPEN with the RSQLite[31] and dplyr[32] packages in R.

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Author contributions

L.E.-P. wrote the Data Descriptor, constructed the database, and coded the accompanying RMarkdown document (Supplementary File 1). M.C.-L. collected and curated data, and managed field operations. L.L.A. collected and curated data, and managed field operations. S.G.d.A. collected and curated data, and managed field operations. W.R.-A. collected and curated data, and managed field operations. M.B.-P. managed field operations. C.K. initiated the study, collected and curated data, and managed field operations. All authors helped revise the paper.

Competing interests

The authors declare no competing interests.

Additional information

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Anexo III

Offspring desertion with care? Chick mortality and plastic female desertion in Snowy Plovers

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Original Article

Offspring desertion with care? Chick mortality and plastic female desertion in Snowy Plovers

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Offspring desertion is often a plastic behavioral strategy that requires precise timing as the termination of parental care may have profound consequences for the fitness of parents and offspring. However, the decision process involved with termination of care is still poorly understood. Snowy Plovers *Charadrius nivosus* show highly flexible brood care with some females deserting the brood early and re-mate, whereas others provide extended care until the young are independent. Using a dynamic modeling framework, we investigated the effect of multiple factors on the decision-making process of female brood care in Ceuta, Mexico over a 7-year period. Females were more likely to stay with larger broods, while their probability of care was lower at the beginning of the season, when re-mating opportunities are higher than later in the season. Offspring condition at hatching did not influence the length of female care. Chick death and offspring desertion frequently coincided, suggesting that deteriorating offspring condition may trigger female desertion. Females deserted broods with high survival prospects when their absence did not impact negatively chick survival. Conversely, females deserted broods with low survival prospects when chick mortality despite female care reduced the value of the brood and re-mating was still possible. This suggests that female Snowy Plovers are sensitive to the needs and the value of their broods and adjust their parental care strategy accordingly. Taken together, we conclude that offspring desertion is a highly plastic behavior that allows females to maximize their reproductive success in a stochastic environment.

Key words: **behavioral plasticity, offspring desertion, parental care strategies, polyandry, reproductive success, trade-off**

INTRODUCTION

Parental care increases parental fitness by improving offspring growth, condition, and ultimately survival (Klug and Bonsall 2014). At the same time, parental care adds major costs to reproduction that may compromise adult survival and/or future fecundity of the parent (Gross and Sargent 1985; Smith and Wootton 1995; Zink 2003; Buzatto et al. 2007; Royle et al. 2012). In particular, multiple breeders of long-lived species face a trade-off between improving the prospects of the current brood through care and the reduction of their own future reproductive success and/or survival through extended care (Williams 1966; Trivers 1972; Westneat and Sargent 1996; Webb et al. 1999; Ackerman et al. 2003; Magrath and Komdeur 2003).

One possible way of escaping the strains of parental duty is premature care termination through offspring desertion. Originally considered as maladaptive or "abnormal behavior" (Fujioka 1989; Hrdy 1999), theoretical and experimental studies have shown that offspring desertion is often beneficial for the parents. When survival of the current brood is not compromised, desertion followed by re-mating can lead to increased lifetime reproductive success for the deserter (Trivers 1972; Maynard Smith 1977; Smith and Wootton 1995; Székely et al. 1996; Webb et al. 1999; Ward et al. 2009; Royle et al. 2012). In order to make an adaptive decision over desertion, parents should consider the following five key parameters: 1) the needs of the current offspring, 2) the value and

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prospects of the current brood, 3) re-mating opportunities, 4) their own condition, and 5) behavior of the other parent in biparental systems (Kelly and Kennedy 1993; Székely 1996; Webb et al. 2002; Houston et al. 2005).

First, the needs of the current brood may depend on the environment in which the offspring will grow up. For example, in a harsh environment one parent may not be able to raise the young alone, whereas in a benign environment the offspring may fare as well with one parent as with both, leaving one parent free to desert (Beissinger and Snyder 1987; Székely et al. 1996; Blanken and Nol 1998; Amat et al. 1999a; Magrath and Komdeur 2003; Eldegard and Sonerud 2009; Kosztolányi et al. 2009).

Second, the value and prospects of the current brood are primarily determined by brood size and offspring condition. Brood size is the most obvious predictor of desertion with parents usually caring longer for larger than for smaller broods (e.g., Beissinger and Snyder 1987; Fujioka 1989; Steinhart et al. 2008; Ward et al. 2009). Brood condition and prospects may chiefly depend on environmental conditions, thus, parents need to assess offspring survival with or without their contribution in the current environment (Steinhart et al. 2008).

Third, the deserting partner must assess its re-mating opportunities. Re-mating opportunities determine who gains more from deserting the family and who gains more from care (Keenleyside 1985; Fujioka 1989; Balshine-Earn and Earn 1998; Pilastro et al. 2001; Roulin 2002; Eldegard and Sonerud 2009; Thomson et al. 2014). Recent studies have shown that reproductive behavior is often tied to sex ratios of the adult population (Kokko and Jennions 2008; Liker et al. 2013; Grant and Grant 2019) with biased sex ratios altering potential for sequential polygamy in each sex. In that case, the rarer sex has a higher opportunity for polygamy and is more likely to desert the brood (Eberhart-Phillips et al. 2018).

Fourth, the parent should consider its future reproductive prospects. Parents need to assess their own condition and survival prospect in this process. When parental care depletes energy reserves or increases the risk of predation, desertion may improve the prospect of adult survival and enable them to reproduce again (Osorno 1999; Currie et al. 2001; Jamieson 2012).

Fifth, a deserting parent will leave dependent offspring behind. The deserter needs to consider the ability and behavior of the remaining parent, who will be left with the young alone. When the remaining parent is able to fully compensate for the lack of the second carer, continued care by both parents is not necessary (Fujioka 1989; Székely et al. 1996; Roulin 2002; Osorno and Székely 2004; Harrison et al. 2009; Ward et al. 2009).

Importantly, all of these factors change through the brood care period. Furthermore, they are often intertwined and not independent from each other. It is essential to understand the relationship between these factors in order to establish the motive behind, and the adaptivity of early termination of care. For example, the brood size might decrease over the brood care period apparently decreasing the value of the current brood. However, the value for the parents often also depends on the re-mating opportunities; if brood size decreases in early broods when re-mating opportunities are still high, parents may be better off prioritizing future reproduction over their current offspring. By contrast, if late broods decrease in size, re-mating opportunities are often low and, consequently, parents may be better off to continue caring. Similarly, brood age may be related to the length of care. Older chicks usually have better survival opportunities than young chicks. Therefore, parents of older broods may decide to complete care even if mortality occurs. When desertion happens during an early stage of care, the young are still vulnerable and the remaining parent might not be able to fully compensate. In this case, early termination of care will only be adaptive if the gains from additional matings are higher than the losses incurred in the deserted brood (Ezaki 1988; Eldegard and Sonerud 2009; van Dijk 2009).

It is often not clear whether low survival prospects of the offspring are cause or consequence of desertion. In the former case, the deserting parent may essentially abandon a sinking ship as continued care will not improve the offspring survival prospects. The deserter then chooses to make the best of a bad job by starting a fresh mating attempt to offset its initial low reproductive success (Székely et al. 1996; Ackerman et al. 2003; Klug and Bonsall 2007). In the latter case, the deserter already takes into account lower future survival of the deserted offspring but expects to obtain an overall higher reproductive success through desertion and re-mating than when remaining with the first brood (Osorno 1999).

Plovers (Charadriinae) are small shorebirds with high variation and flexibility in parental care (e.g., Vincze et al. 2017; Eberhart-Phillips et al. 2018). They typically lay small clutches with two to four eggs, which implies that reproductive success can only increase through producing multiple clutches (or double brooding, Blomqvist et al. 2001). Precociality of the offspring has facilitated the evolution of plastic brood care systems that may feature bi- or uniparental brood care (Székely and Reynolds 1995; Houston et al. 2005; Thomas and Székely 2005; Eberhart-Phillips 2019). The frequency of desertion varies not only between species but also between and within populations (Eberhart-Phillips et al. 2018). Several predictors of the length of biparental care have been identified previously including initial brood size (also referred to "current brood size," Székely and Cuthill 2000; Ward et al. 2009), biotic environment (such as population density and/or predation pressure, Amat et al. 1999a; Kosztolányi et al. 2006), and abiotic environment (e.g., temperature, AlRashidi et al. 2010). Desertion is strongly linked to re-mating opportunities that are influenced by adult sex ratios (Székely et al. 1999; Amat et al. 1999a; Stenzel et al. 2011; Parra et al. 2014; Carmona-Isunza et al. 2017; Eberhart-Phillips et al. 2017; Eberhart-Phillips et al. 2018; Eberhart-Phillips 2019).

In many plover populations, desertion appears positively related to chick mortality (Székely and Williams 1995; Székely et al. 1996; Székely and Cuthill 1999; Amat et al. 1999b; Cruz-López et al. 2017). It was previously reported that the majority of deserted Snowy Plover *Charadrius nivosus* families were characterized by low chick survival, which might imply that brood desertion is maladaptive (Cruz-López et al. 2017). However, whether desertion is cause or consequence of reduced chick survival remained unclear. Following up on these results, we investigated the phenology and fitness consequences of brood care in Snowy Plovers breeding at Bahía de Ceuta, Mexico (hereafter "Ceuta"). We focused on female care as this parental behavior has the highest plasticity in this population (Cruz-López et al. 2017). The male-biased sex ratio that in the population emerges during the juvenile stage (Eberhart-Phillips et al. 2017) presumably provides better re-mating opportunities to females. Consequently, brood desertion by males is extremely rare, whereas brood desertion by females is frequently observed (Cruz-López et al. 2017). Using the fates of 262 families collected over seven breeding seasons we assessed the adaptive value and the dynamics of female brood care and offspring desertion. We used a dynamic modeling method (Schmidt et al. 2010) to identify both static and dynamic predictors of female desertion. We considered present brood size, that is, the brood size on a specific day, and brood age as dynamic variables that represent changing values and needs of the brood. We used hatching date, male, female and brood condition as static predictors. Specifically, we 1) described the pattern of female brood care strategies in this population, 2) compared reproductive success of the different female strategies, 3) tested which of the dynamic and static social and environmental variables predict the length of female care and, 4) examined the temporal association of chick mortality and termination of female care. With this we explored whether and how chick mortality influences the decision of the females to continue or terminate care.

METHODS

Field work and data collection

We studied breeding Snowy Plovers at Ceuta, a coastal wetland in Northwest Mexico between April and July from 2006 to 2012. During the study period, about 50–100 Snowy Plover pairs nested annually on tidal salt flats of an abandoned salt extraction site surrounded by mangrove forests and agricultural fields (Cruz-López et al. 2017; Eberhart-Phillips et al. 2017; Plaschke et al. 2019). We monitored Snowy Plover families daily using the methodology described in Székely et al. (2008).

We searched for nests and families by scanning the salt flats for incubating plovers with binoculars and scopes from a mobile hide or a car. When a nest was found, we recorded the location and established laying and hatching dates based on the floating technique assuming a 25-day incubation period (Plaschke et al. 2019). We caught incubating parents on the nests with a funnel trap and marked them with a unique color-metal-ring combination consisting of three color rings and a numbered metal ring. We revisited the nests approximately every 2–4 days until we heard the chicks calling or pecking inside the eggs and afterwards daily to capture, measure and mark chicks before they leave the nest scrape (Cruz-López et al. 2017; dos Remedios et al. 2015). We marked the chicks with a metal and a single color ring. This allowed us to follow the individual fates of most chicks. We took body measurements and a blood sample of adults and chicks for molecular sex identification. We re-sighted families if possible at least once every 2 days until the brood had reached an age of 5 days and approximately every 3 days until the brood age reached 25 days at which brood age Snowy Plovers become independent (Cruz-López et al. 2017). At each sighting, we observed broods for at least 15 minutes or until each previously attending parent had been seen to identify all family members present (Székely and Cuthill 1999). If a bird was not seen during this period, we recorded it as "missing" for that day.

Data processing and statistical analysis

We collected brood attendance data for a total of 367 broods. These broods are part of the CeutaOPEN core data set by Eberhart-Phillips et al. (2020). From these, 12 (3.3%) did not have hatching date information, 38 (10.4%) had two or fewer observations, in 7 (2%) broods the male deserted or disappeared and in a further 48 (13.1%) broods the female deserted in a period between two brood observations that were longer than 3 days apart. After removing these broods, we included 262 broods for further analyses. The number of broods varied annually, paralleling a strong population decline throughout the study period (Cruz-López et al. 2017).

In case broods disappeared or failed before fledging age, we noted the day the brood was last seen. We assumed that both parents were present with the clutch until hatching as incubation in Snowy Plovers is biparental (Vincze et al. 2013; Vincze et al. 2017). We calculated the duration of female and male care for each brood in days from the hatching of the first chick. In 2007–2009 and 2011, we performed brood manipulations such as cross-fostering of freshly hatched chicks and eggs ($N = 112$). The manipulations either increased brood size (up to five chicks, $N = 11$), left brood size unchanged with either eggs or chicks swapped between broods ($N = 65$), or reduced brood size ($N = 36$). We repeated our main model i) using only data from unmanipulated broods and ii) with an additional "manipulation" predictor with manipulation types as factorial levels to test whether manipulation effected female care behavior. The results of these additional models were qualitatively similar (data and results are available on GitHub, see Data availability statement).

For each family member (parent or chick), we defined the last day of presence as the mid-point between the day the bird was last observed with the family and the first day the focal bird was either observed permanently without the family, or the family was observed permanently without it. For parents, permanent absence meant that they deserted the broods. We considered temporarily absent family members, that is, those that were subsequently re-sighted with the family, as "present." The maximum duration of care was 26 days (from day 0, when the first chick hatched in the brood, until day 25, the fledging age). To test whether our estimation of the desertion date would somehow impact our results, we created a stricter data set where we only included broods for which the exact day of female desertion was known ($N = 114$). Again, in this analysis, we obtained similar results (data and results are available on GitHub, see Data availability statement).

Female brood care

We considered four scenarios to describe the termination of female care. 1) Desertion: the female left the brood while at least one chick was still attended by the male. 2) Brood failure: either both parents were observed without the chicks, or the male was seen alone within 3 days of the last observation of the brood attended by both parents. 3) Full term care: the female stayed until at least one chick fledged. 4) Unfinished observations: unfledged broods with unknown fate that were attended by the female at the last observation.

Reproductive success and breeding time

To assess the fitness consequences of female brood care strategies, we compared the seasonal reproductive success and breeding time (i.e., the total number of days a female spent with breeding related activities) of females that for the first clutch either provided full term care or deserted and then re-mated locally (Figure 1). We calculated reproductive success as the total number of fledglings for a certain female parental care strategy within a season. Many females disperse to different breeding sites between breeding attempts (Stenzel et al. 1994). Here, we compared the local breeding time of females that stayed for at least two breeding attempts or provided full term care in Ceuta. The breeding time represents the cumulative number of days of egg formation and incubation (3 + 25 days) and the days from hatching of the first chick to the end of care of the last brood. It also includes the time that the female spent on finding a new mate after desertion (Figure 1). In the full-term care group, all females had only one breeding attempt, whereas in the deserting group, all females had two breeding

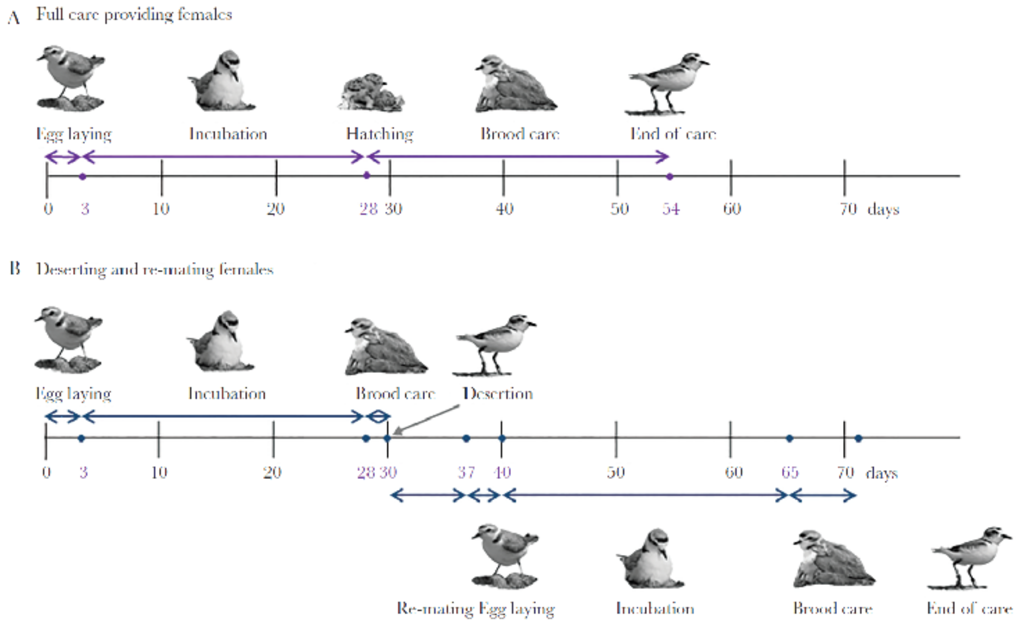

Figure 1
Schematics for calculating breeding time (length of care in days) in full-term care providing ($N = 5$) (a), and deserting and locally re-mating ($N = 9$) females (b).

attempts with hatchlings with different males. We compared breeding time (in days) of the two groups using a Linear Model with "type of care" as predictor. We compared reproductive success (total number of fledglings) between the two groups using a Generalized Linear Model (GLM) with Poisson error distribution.

Predictors of the length of female brood care

As a dynamic measure for the length of female care we calculated a novel response variable: probability of care. Probability of care was derived in a similar manner as the commonly used daily nest survival, an established unbiased measure in ecology to understand fundamental mechanisms of population dynamics (Schmidt et al. 2010; Converse et al. 2013). Probability of care refers to the probability that a female will care on a certain day given the probability of care on the previous day. We adopt this measure to assess the drivers for termination of parental care as it allows us to assess not only static but also dynamic predictors that are changing over the care period. We analyzed the effects of seven biologically meaningful predictor variables on the probability of care. Five of these were static predictors: hatching date, male tarsus length, male condition, female condition, and chick hatching condition.

Hatching date provides a measure for the progress of the breeding season and we predicted that females would be more likely to stay and care for the chicks later in the season as re-mating opportunities diminish (Székely and Cuthill 2000). We used relative hatching date, which is the z-transformation of the Julian hatching dates for each year. We calculated relative hatching date by using all broods with available hatching date information.

As a measure of male size, we used male tarsus length, the mean length of left and right tarsus of the male parent. As males are the main care providers, larger males may be better at protecting their chicks in conflicts with other families or better at thermoregulation. We predicted that females would be more likely to care longer to support smaller mates than larger ones. As another set of quality measures we calculated "male/female/chick condition" using the scaled mass index method by Peig and Green (2009). This method fits a linear regression between tarsus and weight data points and for each individual calculates a relative weight value according to the mean tarsus length of the population using the slope value of the regression estimates. Positive values refer to individuals with better than population average condition and negative values refer to individuals with worse than average condition. Chick condition was calculated using the hatching mass and size values of the chick in best condition in a brood within 1 day after hatching. This variable highly correlated with the mean condition value of the brood (R = 0.93). Similarly, to male tarsus length we predicted that females would stay longer with males in lower condition. We predicted that females would desert chicks with better condition faster as they have higher survival prospects than chicks with worse condition that might require more care. Alternatively, females might stay and care longer for chicks in better condition as their survival prospects (and hence their reproductive value) are higher.

Present brood size and brood age are two dynamic variables whose values were determined every day over the brood care period. Present brood size, the brood size on a given day, allowed us to test whether females would take into account the present number of their chicks when deciding whether to desert or continue to care. We predicted that females would be more likely to continue to care for bigger than for smaller broods. Finally, for brood age we predicted that older broods are more likely to be deserted as chicks are more independent (Gratto-Trevor 1991; Currie et al. 2001). However, this relationship may be modified by seasonality. Because of reducing re-mating opportunities through the season, some females especially at the end may stay longer and care until fledging. Therefore, we also included the second polynomial of brood age (brood age^2). Present brood size, the brood size on a certain day, may provide a more precise estimate on the effect of brood size on care than initial brood size. Importantly, present brood size differs from the commonly used current brood size (Székely and Cuthill 2000; Ward et al. 2009), which refers to the initial brood size of the current breeding attempt and is fixed for a given breeding attempt. By contrast, present brood size may change during the care period, that is, it decreases with chick mortality. Basing our models on present rather than initial brood sizes allowed us to investigate the female decision to continue or terminate care without assuming the "Concorde fallacy" (Dawkins and Carlisle 1976), that is, that past investment instead of future prospect has the strongest impact on the parental care decision (Ackerman et al. 2003).

We set up a binomial generalized linear mixed model to estimate the effect of the predictors on probability of care using a Markov-chain Monte Carlo (MCMC) algorithm in a Bayesian framework (Korner-Nievergelt et al. 2015). We used STAN (Stan Development Team 2018) through the R packages rstan, rstanarm (Stan Development Team 2018) and arm (Gelman and Hill 2007). We fitted two random intercepts: year and female ID since females can have multiple breeding events within and between years. We modeled probability of care of each female with Bernoulli errors.

The likelihood to continue care for a given day was formulated as

$$
\begin{aligned}
&y_{\text{care}}[i,t] \sim \text{bernoulli}\,(y_{\text{care}}[i,t-1],\ Si,t)\,;\\
&\text{logit}\,(Si,t) = \beta_0 + \beta_1\ \text{present brood size}_{i,t} + \beta_2\ \text{hatching date}_i\\
&\quad + \beta_3\ \text{chick condition}_i + \beta_4\ \text{male tarsus length}_i\\
&\quad + \beta_5\ \text{female conditioni} + \beta_6\ \text{male condition}_i + \beta_7\ \text{brood age}_{i,t}\\
&\quad + \beta_8\ \text{brood age}_{2i,t} + \sigma\ \text{year} * u\ \text{year}_i + \sigma\ \text{female ID} * w\ \text{female ID}_i;
\end{aligned}
$$

where y_{care} represents female care for each day as binary variable ("1" for care and "0" for no care) with dimensions i as the number of broods ($N = 262$) and t as the length of the brood care period (0–25 days). S is the daily care continuation probability, which relates to brood and day specific predictors of the model. All fixed variables were z-transformed with mean = 0 and standard deviation (SD) = 1. We used normal priors with mean = 0 and SD = 5 on fixed effects, normal priors (mean = 0, SD = 1) on random effects and Cauchy (0,5) priors on the sigma parameters (year, female ID).

Since STAN currently does not accept missing values for the estimations, we substituted missing values with the population average for male tarsus length and condition variables ($N_{\text{females}} = 25$, $N_{\text{males}} = 23$, $N_{\text{chicks}} = 8$). Importantly, the model can distinguish between different end of care events. The last value the model considered for a certain brood was always the last observation day, that is, the first day the female did not provide care anymore in deserted and failed broods, therefore had the value of 0. Alternatively, for broods with unknown fate or full term biparental care the value was 1 and referred to the last day the female still provided care (Korner-Nievergelt et al. 2015). We obtained samples of the posterior distribution of the model parameters from five independent Markov chains with 4000 iterations each. We discarded the first 2000 values of the burn-in period of each chain and then calculated posterior parameter estimates from the remaining iterations. For analytics and visual inspection to determine convergence of the model we used *shinystan* package (Stan Development Team 2018). All models had fully converged with the $\hat{R}$ values = 1 (Gelman et al. 2004). For visualization of the results, we used the *bayesplot* (Gabry and Mahr 2017) and *MCMCvis* packages in R (Youngflesh 2018).

Termination of care and chick mortality

In this analysis, we examined the relationship between female termination of care and chick mortality. First, we tested whether females were more likely to terminate care on days when one or all chicks died than when the brood size did not change. We included all broods belonging to the desertion, brood failure and full term care categories, excluding unfinished broods. Second, we examined whether females were more likely to desert on days when chick mortality happened. For this, we only considered deserted broods where at least one chick died and tested whether desertion occurred randomly or coincided with chick loss. We included all broods for which the last pre- and first postdesertion observations were no more than 3 days apart. Deteriorating chick condition, because of starvation, injury or disease, may precede chick loss by 1 or 2 days. Females may be able to anticipate chick death and leave such broods before the actual reduction of brood size as we often observed chicks moving sluggishly and lagging behind the family shortly before they disappeared (C.K., M.C.L., personal observations).

We used co-occurrence models (conditional logistic regression) to test whether female desertion and chick mortality co-occurred more frequently than expected by chance. We coded both desertion (i.e., the change in probability of care) and chick mortality as "1," and continued female care and no chick loss as "0." We added Nest ID as a grouping variable for the model. We used the "clogit" function of the *survival* R package for the analysis (Therneau and Lumley 2015). For all statistical analyses, we used R version 3.5.3. (R Development Core Team 2010).

RESULTS

Female brood care

Among the 262 Snowy Plover broods examined, desertion was the most frequent terminal event of female care with 185 (71%) of all broods deserted. Desertion typically happened during early brood care, that is, in 93% of the deserted broods, the female left within 10 days after hatching. The peak of desertion occurred 2 days after hatching. In stark contrast, only a minority of five (2%) females cared for the brood until fledging. Fifteen (5%) broods failed before the female deserted. Finally, for 57 (22%) broods the terminal fate was not known and these females were still seen with the brood at the last observation.

Reproductive success and breeding time

Deserting females that re-mated locally at our breeding site and successfully hatched chicks from both attempts ($N = 9$) did not have higher reproductive success than full time caring females (N = 5) (LM: Estimate: −0.17 ± 0.35, confidence interval [CI]: −0.87 to 0.52, $P = 0.677$, Figure 2a). Both groups fledged on average 2.5 chicks. However, deserting females showed a higher variance in reproductive success than caring females with the most successful female having up to five fledglings.

The breeding time of deserting females was, however, substantially longer than that of caring females (Figure 2b). Deserting females spent on average two and a half weeks (33%) longer at reproduction than females that cared for their chicks until fledging (GLM: Estimate: −17.1 ± 2.9, CI: −23.3 to −10.8, $P < 0.001$).

Predictors of the length of female brood care

Hatching date, present brood size, and brood age^2 were all associated with female "probability of care" (Figure 3a). Early in the season, females terminated care quickly to desert the brood. By the end of the season, females continued to care longer and probability of care increased from 0.45 (95% CrI: 0.23–0.67) to 0.97 (95% CrI: 0.94–0.99) over the season (Figure 3b). The probability of care for females caring for large broods were higher than those for females caring for small broods (Figure 3c). Mean probability of care for the broods with one chick was 0.7 (95% CrI: 0.5–0.83) and increased by approximately 0.03 for every additional chick to 0.83 (95% CrI: 0.7–0.9) for a five chick brood, the largest brood size in our sample. Brood age^2 also showed some association with length of female care. Rates of probability of care decreased from 0.8 (95% CrI: 0.76–0.88) at hatching to 0.75 (95% CrI: 0.62–0.84) at a brood age of 8 days and then increased to 0.95 (95% CrI: 0.76–0.99) for females that stayed until the age of 25 days (Figure 3d).

Neither male tarsus size nor any of the condition variables were statistically clearly related to the probability of care as CrIs overlapped with zero (Figure 3a). Similarly, brood manipulations had no clear effect on female probability of care. The analysis without the manipulated broods did not change the results qualitatively (data and results are available on GitHub, see Data availability statement).

Termination of care and chick mortality

In 55 (26.7%) broods, the female terminated care on the day a chick died (Figures 4 and 5). The conditional logistic regression model showed a clear association between chick mortality and female termination of care ($P < 0.001$). Co-occurrence of desertion and chick mortality was influenced by the present brood size at termination of care. When a brood had one chick nearly all females deserted before that chick died or fledged (co-occurrence for broods with one chick: 14.3%, $P = 0.49$) meaning that there were few co-occurrences of chick death and termination of care. However, co-occurrence became statistically clear in broods with more than one chick at the time of termination of care (two chicks: 29%, $P < 0.001$; three chicks: 32.7%, $P < 0.001$; more than three chicks: 40%, no P value as model did not converge since all chick death events coincided with (or after) female desertion, Figure 6). Restricting the analysis to include only those broods that were deserted (i.e., excluding one chick broods where desertion and mortality cannot co-occur by definition) resulted similar co-occurrence between female care termination and chick mortality (26.6%, $P < 0.001$).

DISCUSSION

How do parents decide whether to continue care or desert their offspring? Our analysis of female brood care in Snowy Plovers suggests that females constantly assess the needs, value, and survival prospects of their offspring while they provide care. This ability is a precondition for an adaptive decision about whether or not to continue care. Deserting and re-mating Snowy Plovers attempt to

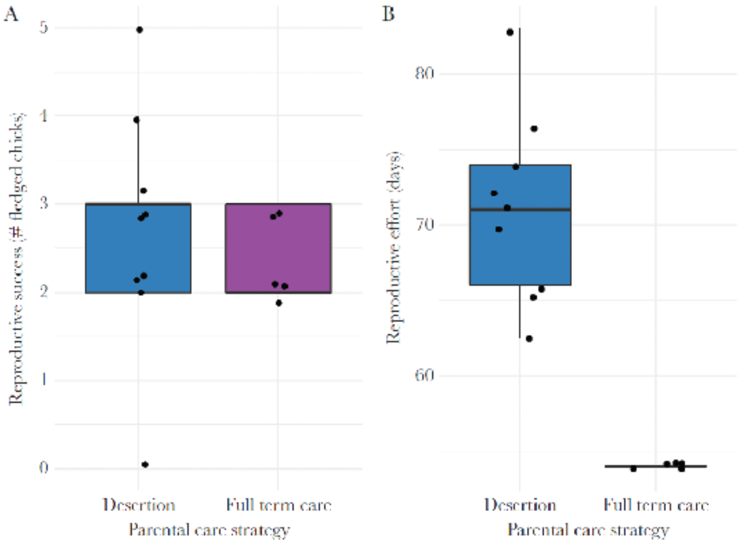

Figure 2
Reproductive success (a) and breeding time (b) of deserting and locally re-mating (N = 9) and full-term caring (N = 5) Snowy Plover females at Ceuta.

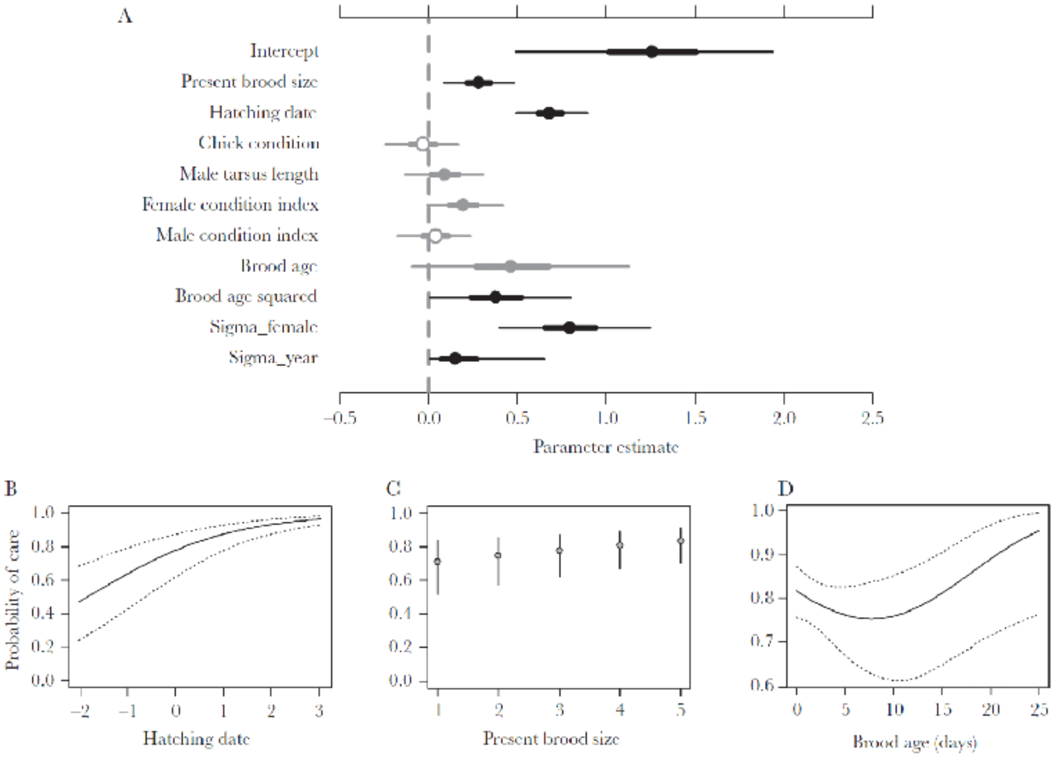

Figure 3
Predictors of the probability of female care in 262 Snowy Plover broods at Ceuta when all other predictors are kept at the mean. (a) Summary of all model predictors. *Sigma_female* and *Sigma_year* are random effects. Dots represent means, thick lines standard deviations and thin lines are the 95% credibility intervals (CrIs). Black symbols indicate no CrI overlap with zero, for grey symbols CrIs overlap with zero. (b,c,d) Details for fixed effects with a statistically clear impact on the probability of female care: hatching date (standardized to the annual mean) (b), present brood size (c), and brood age (d). Continuous lines refer to mean estimates and dotted lines represent the CrIs (b, d), circles represent the mean and continuous lines represent CrIs (c). For clarity, we plotted the predicted values for discrete observed present brood size categories whereas the statistical model was run on scaled values.

maximize their reproductive success by rapid divorce after hatching as divorced females produce a higher number of hatchlings than females that retain their mates (Halimubieke et al. 2019). Yet, we confirmed that desertion does not necessarily translate into producing more fledglings (Cruz-López et al. 2017). Our findings suggest that full term care providing females reach a similar reproductive success (measured as number of fledglings) as deserting and locally re-mating females but in significantly shorter time. Moreover, occasionally, caring females will still have enough time left to establish a new clutch and may hence increase their reproductive success further. The low number of locally re-mating females with successfully hatched chicks ($N = 9$) suggests that many deserting females disperse further to find new partners. However, it also shows that chick survival is generally low. Some of the dispersing females may reach higher reproductive success than locally re-mating females (Halimubieke et al. 2019). However, the breeding time of dispersers must be even higher than that of caring or locally re-mating females as some females disperse to great distances, therefore, breeding dispersal must take up further time (Stenzel et al. 1994). On the other hand, our results show that providing full term care is not a common strategy of the females; we recorded only six females staying until fledging, whereas the large majority of females deserted before. In spite of the low numbers, our analysis shows the potentials of the two strategies and illustrates that re-mating does not necessarily provide advantages to females that chose this strategy.

When analyzing variables associated with the length of female care, we found both static and dynamic predictors that related to the probability that females will continue care. The dynamic modeling approach allowed us to analyze the consequences of changes in these predictors on individual decisions of females. Our study identified three either static or dynamic predictors for the length of female care. First, the hatching date of a brood was positively associated with the length of female brood care. In early broods, females cared for a shorter time and deserted the broods faster than in late broods. This is consistent with desertion for sequential polygamy and has also been observed in many other plover populations (Warriner et al. 1986; Székely and Williams 1994; Amat et al. 1999a; Székely and Cuthill 2000; Kosztolányi et al. 2009). Re-mating opportunities decrease with every day, so fast desertion will maximize the reproductive potential of early breeding females whereas late breeding females may be better off providing care.

Second, we found that the present brood size had an effect on the probability of female care. Females were less likely to stay when brood size decreased during the brood care period. This suggests that for females the value of the brood is not fixed and determined by initial brood size and/or the breeding season (Székely and Cuthill 2000) but rather is assessed regularly over the brood care period. Therefore, Snowy Plover females avoid the "Concorde fallacy" (Dawkins and Carlisle 1976; Armstrong and Robertson 1988; Ackerman et al. 2003; Magalhães and Geoffrey White 2016). That survival prospects of the brood, particularly chick mortality, eminently affected the female's decision whether to continue or terminate care is shown by the close temporal association between chick mortality and desertion. The co-occurrence of chick mortality and desertion was much stronger than expected by chance. Chick death seemed to be an important trigger for females to terminate brood care albeit our data did not allow us to firmly determine whether the mortality necessarily preceded desertion. Instead some females may already desert the brood if

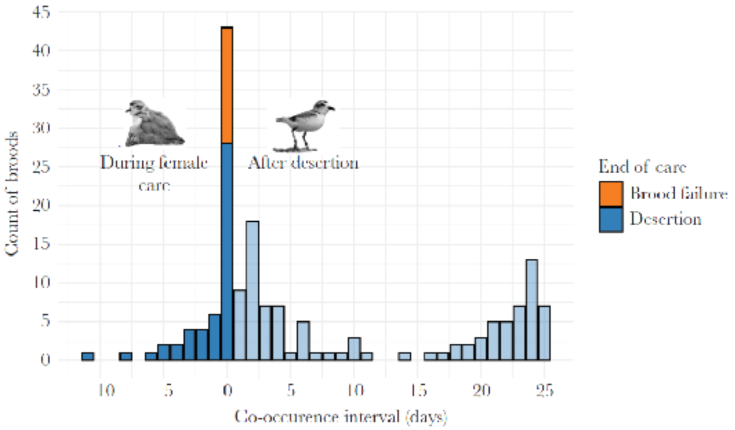

Figure 4

Co-occurrence of chick mortality and female care termination in 165 Snowy Plover broods with known dates of chick death or fledging. The diagram shows the interval between the most recent chick death and female termination of care within broods. Mortality and care termination coincide on the same day when the co-occurrence interval is zero. Negative values refer to mortality while the female still cared (dark blue). Positive values refer to cases when chick mortality happened after the female had deserted (light blue).

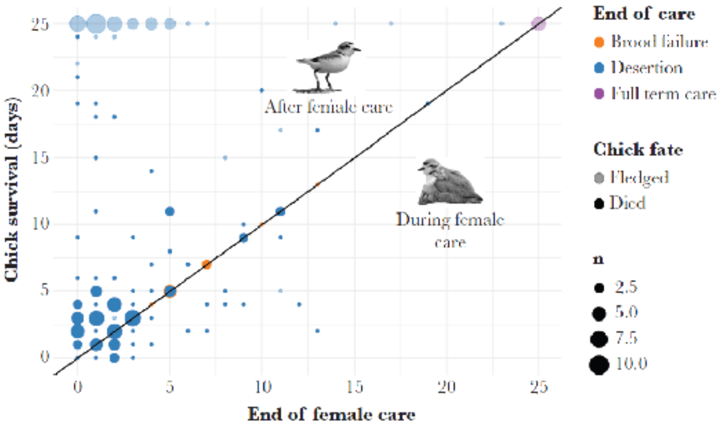

Figure 5

Chick survival from the first day of brood care in relation to length of female care for 170 Snowy Plover broods with known dates for chick death or fledging. Only one chick per brood, whose fate was most closely associated with the end of female care, is plotted. Dot sizes refer to number of broods. Coordinate (0,0) indicates the day of hatching and hence the start of female brood care. The diagonal line indicates co-occurrence of chick fate (died or fledged) and female care termination on the same day. Below the diagonal are broods in which the female continued to care after one chick had died, above the diagonal are broods where the female deserted before the first chick had died.

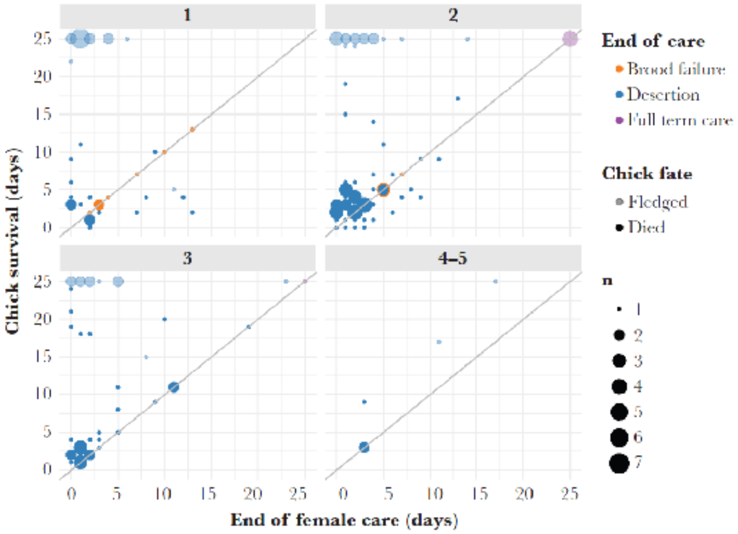

Figure 6

Chick survival from the first day of brood care and length of female care according to present brood size for 170 Snowy Plover broods, for which the date for mortality (or fledging) was exactly known. For each brood, only the chick whose fate was most closely associated with the end of female care is plotted. Dot sizes refer to number of broods. Coordinate (0,0) indicate the day of hatching and hence the start of female brood care. The diagonal line indicates co-occurrence of chick fate and female care termination on the same day. Below the diagonal are broods where the female continued to care after one chick had died, above the diagonal are broods where the female deserted before the first chick died. Present brood size refers to the number of chicks for the day the female ended care, which may differ from the initial brood size.

one chick is in bad condition due to sickness or starvation and, therefore, likely to die regardless of female care. We found that for broods with two or more chicks the co-occurrence of female care termination and chick mortality was particularly high. At the same time, nearly all broods with one chick were deserted by the female before the chick had died or fledged. This suggests that one-chick broods do not have enough reproductive value for the female to stay and care.

Third, we found that the probability of female care changed with brood age. Snowy Plover chicks are most vulnerable during the first 3 days of their lives (Colwell et al. 2007) and the peak of desertion fell into this period. We observed an initial decline in the probability to care until the age of 9 days. This is consistent with our expectations based on the needs of the brood. As chicks grow older, they improve their thermoregulation and require less brooding, one of the most time consuming care behaviors (Székely and Cuthill, 2000). Hence, the presence of the second carer becomes less important. Interestingly, after the age of 9 days, the relationship changed and the probability to continue to care increased until fledging. In precocial species such as the Snowy Plover, chick survival typically increases with age (Colwell et al. 2007; Cruz-López et al. 2017). For females that already cared for an extended period, care continuation may also be the best option as the value of the current brood is high, especially at the end of the season when the re-mating opportunities are low (Figure 3b, Székely and Cuthill 2000).

Similar to other studies, neither parent condition nor male size was clearly associated with the length of female care (Székely and Williams 1995; Amat et al. 1999b; Amat et al. 2000). As desertion rates declined with season, desertion does not seem to be a response to energy depletion as in other species (Gratto-Trevor 1991; Osorno 1999; Currie et al. 2001). Chick condition at hatching had no clear effect on the length of female care either. Yet, the significant co-occurrence of desertion and chick mortality suggest that females are sensitive to changes in chick condition over the brood care period.

Parental brood care in plovers mainly consists of thermoregulation, warning chicks of predators and defending them from attacks of competitors (Carmona-Isunza et al. 2017). However, chick survival is also strongly dependent on the availability of high quality habitat with invertebrate food, which declines over the season (Kosztolányi et al. 2006; dos Remedios et al. 2015; Cruz-López et al. 2017). In a stochastic environment plastic care behavior that follows the changing value and needs of their brood can be highly advantageous. In Snail Kites *Rostrhamus sociabilis*, for example, one parent tends to desert the brood once a single parent can care for the chicks alone. In this system chick survival is generally low, therefore, parents do not compromise offspring survival by desertion. Rather desertion happens once the needs of the brood reached the "monoparental threshold" either due to brood size reduction or to advanced brood age (Beissinger 1986, 1990). In other studies the value of broods has been shown to be important for desertion. Female ducks tended to desert their clutches once the number of depredated eggs reached a certain proportion (Ackerman et al. 2003). Our results suggest that for many Snowy Plover females in this population desertion may have two different motives: 1) increase the reproductive success through sequential polygamy or 2) make the best of a bad job. Both scenarios are represented by distinct clusters in Figure 5. Early breeding females may utilize the good environmental conditions and desert to increase their reproductive success by producing a second clutch quickly without jeopardizing the survival of the deserted brood (Osorno 1999). Similar to the "monoparental threshold" (Beissinger 1990), the value of the current brood is high while the needs for care are low. By contrast, to make the best of a bad job, females desert when their parental effort cannot prevent chick mortality. This is the main reason for desertion in our population where chick survival decreases with season (Cruz-López et al. 2017). Chick starvation and flooding, which are the main threats for offspring failure in the Ceuta population (Cruz-López et al. 2017; Plaschke et al. 2019), cannot be mitigated by the parents. After the death of one or more chicks the value of the brood will become too low for the female to continue care (similar to the "value threshold," Ackerman et al. 2003). Although the needs of the brood are still high, they cannot be fulfilled by the female. By contrast, continued care is only expected when both the needs and the value of the brood are high, that is, the resulting biparental care fulfils the offspring needs, for example, through protection.

In conclusion, the examination of high-resolution real-time data with a dynamic modeling approach revealed multiple hidden facets of the decision-making process of a species with a highly plastic parental care behavior. Our study demonstrates that for Snowy Plover females the decision over care or desertion is dynamically changing and responds to both the current needs and the value of the offspring, adjusted to the seasonally changing mating opportunities. Deserting females represent a heterogeneous group. They include some successful females that pursue polyandrous matings to multiply their reproductive success. Yet most deserting females use sequential polyandry as a plan B to compensate for low chick survival in their current brood. Our dynamic modeling approach allowed us to gain novel insight into the variety of plastic parental care strategies. The observed high behavioral plasticity may enable females to maximize their reproductive success in highly fluctuating environments through multiple adaptive reproductive strategies, which deserves further investigation.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

Data availability: Analyses reported in this article can be reproduced using the data provided by Kupán et al. (2020). All data and R codes are available on GitHub repository: https://github.com/kupankrisztina/Snowy-Plover-Desertion.

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