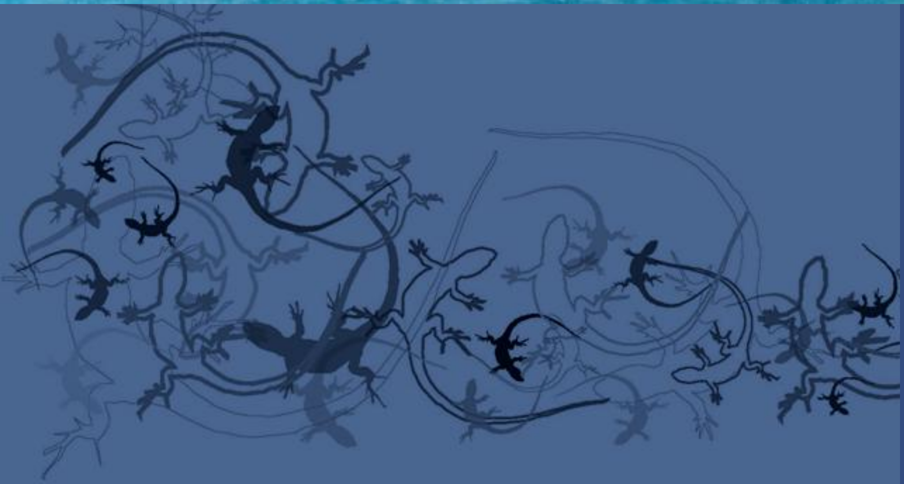
Tesis doctoral

Evaluación del estado de poblaciones naturales con cuatro indicadores: Aplicación a una especie insular



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TESIS DOCTORAL

Evaluación del estado de poblaciones naturales con cuatro indicadores: Aplicación a una especie insular

Memoria presentada por ***Mario Garrido Escudero*** *para optar al título de Doctor en Ciencias Biológicas*

Salamanca, 2013

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Certifica que la presente Tesis Doctoral titulada "**Evaluación del estado de poblaciones naturales con cuatro indicadores: Aplicación a una especie insular**", que se presenta para optar al título de Doctor por la Universidad de Salamanca, ha sido realizada por **Mario Garrido Escudero**, licenciado en Biología, bajo mi dirección en el Departamento de Biología Animal, Parasitología, Ecología y Edafología-Química Agrícola de la Universidad de Salamanca, y considero que reúne los requisitos necesarios, por lo que autorizo su presentación para ser evaluada.

Dr. Valentín Pérez Mellado

Tesis doctoral presentada por **Mario Garrido Escudero** para la obtención del título de Doctor por la Universidad de Salamanca

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"Ningún animal fue maltratado durante el rodaje de esta película."

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Capítulo 1

Introducción

Introducción general

1. ISLAS

“. . . no es exagerado decir que cuando hayamos dominado las dificultades presentadas por las peculiaridades de la vida en las islas nos resultará comparativamente más sencillo hacer frente a los problemas más complejos y menos definidos de la distribución continental. . .”

(A.R. Wallace, 1883, p. 242)

1. La necesidad de las islas

1.1. Tipos de islas

Estrictamente, una isla es una porción de tierra firme rodeada de agua por todas partes. En el ámbito de la biogeografía y de la ecología insular, ‘isla’ se podría definir como la fracción o fracciones de territorio adecuado para el desarrollo de una especie y que, además, está rodeado por territorio no apto para el desarrollo de dicha especie. Esta definición trasciende a las islas *sensu estricto* para contemplar situaciones continentales que pueden asimilarse a este marco conceptual. A la luz de esta definición, se introdujo en los años 80 el concepto de ‘islas de hábitat’ para definir aquellos hábitats terrestres o acuáticos rodeados de otros hábitats muy diferentes. En éstos, el contraste entre la "isla" y la matriz circundante es menos fuerte, aunque todavía suficiente para representar una barrera o filtrar los movimientos de población (Whittaker y Fernández-Palacios, 2007).

Mucho antes del desarrollo de la teoría de la tectónica de placas y en un momento en que los científicos estaban sólo empezando a comprender la importancia de los eventos de glaciación, Alfred Russel Wallace ofreció una primera clasificación de las "verdaderas" islas. En su libro titulado ‘Island life’ (3ª ed. en 1902, publicado por primera vez en 1880) las agrupa según su origen geológico y sus propiedades biológicas en 3 grandes grupos:

1. Islas oceánicas

Habitualmente, se encuentran situadas lejos de los continentes y separadas de éstos por grandes profundidades. Surgidas del fondo del mar debido a la actividad volcánica de los fondos marinos, en general las islas oceánicas aparecen y desaparecen para siempre en lapsos de tiempo del orden de 10 a 20 millones de años. La biota de estas islas suele ser singular, y está caracterizada por la ausencia de especies que no disponen de capacidad de dispersión a larga distancia (Wallace, 1902; Whittaker y Fernández-Palacios, 2007).

2. Islas continentales


Figura 1. Alfred Russell Wallace. Fuente: Wikimedia Commons.

O islas continentales recientes (*sensu* Wallace). Son islas incluidas en la plataforma continental pero, en la actualidad, separadas de ésta por brazos de mar poco profundos y estrechos. Estas islas continentales recientes han estado separándose y uniéndose de los respectivos continentes en numerosas ocasiones, en función del aumento y disminución del nivel del mar asociados a las glaciaciones. Presentan una biota prácticamente idéntica a la de su continente más cercano, lo que avalaría una separación reciente de éste (Wallace, 1902; Whittaker y Fernández-Palacios, 2007).

3. Fragmentos continentales o micro-continentes

Denominadas por Wallace islas continentales antiguas, están caracterizadas por estar situadas lejos de los continentes. Son fragmentos que se separaron hace decenas de millones de años de sus continentes respectivos por la formación de nuevas dorsales centro-oceánicas, y que pueden eventualmente colisionar con nuevos continentes perdiendo así su estatus insular, tal y como le ocurrió a la India. La singularidad de su biota, especialmente de su fauna, es resultado del aislamiento de estos fragmentos durante largos períodos de tiempo (Wallace, 1902; Whittaker y Fernández-Palacios, 2007).

Actualmente, Whittaker y Fernández-Palacios (2007) proponen una modificación de la clasificación de Wallace acorde a los conocimientos de la actual Biogeografía Insular. Sin embargo, advierten sobre la dificultad de establecer los límites entre los distintos tipos de islas a la hora de clasificarlas. Según estos autores, en una primera división estarían las 'islas *sensu estricto*', totalmente rodeadas de agua, y las 'islas de hábitat', que constituyen otro tipo de hábitat insulares (ver más arriba). A su vez, subdividen las 'islas *sensu estricto*' en islas continente (Australia), islas oceánicas, fragmentos continentales, islas continentales e islas en lagos o ríos.

Tipos de isla	**Ejemplos**
Islas *sensu stricto*	
Islas continente	- Australia
Islas oceánicas	- Hawaii, Canarias
Fragmentos continentales	- Madagascar, Nueva Caledonia
Islas continentales	- Gran Bretaña, Groenlandia
Islas en lagos o ríos	- Gurapá (Amazonas), Islas Royal (Lago Superior, USA)
'Islas de hábitat'	
Parches de hábitat terrestre diferenciado y aislado por una matriz hostil	- Fragmento forestal rodeado por zonas agrícolas
Marinas	- Arrecife aislado alrededor de una isla oceánica - Los arrecifes de coral separados - Los montes submarinos sumergidos

Tabla 1. Clasificación simple de los tipos de islas: (1) los tipos clásicos, "verdaderas" islas; (2) Islas de hábitat (Fuente: Modificado del original de Whittaker y Fernández-Palacios, 2007).

Las islas oceánicas son aquellas se han formado a través de las placas oceánicas y nunca han estado conectadas a masas de tierra continentales.

- Los fragmentos continentales son islas que por su ubicación pasan por ser islas oceánicas pero, en realidad, respecto a su origen son fragmentos antiguos de la masa continental varados en los océanos por procesos de tectónica de placas.
- Las islas continentales son las islas emplazadas en la plataforma continental. Muchas de estas islas se comunicaron con el continente durante las eras glaciares del Cuaternario ya que durante esos períodos los niveles del mar eran significativamente más bajos. El período más reciente de conexión finalizó tras la transición del Pleistoceno al Holoceno. El Holoceno comenzó hace unos 11.500 años, pero los mares y océanos tardaron varios miles de años en alcanzar su nivel actual.
- Por último, las islas que se producen dentro de las masas de agua dulce, en lagos y en ríos suficientemente grandes, que al ser más comparables a las islas marinas que a las islas de hábitat pueden ser considerados ‘verdaderas’ islas.

Alcover et al. (1998) definen un nuevo grupo de islas en relación al tiempo de aislamiento y la especificidad de su fauna y flora, a las que denomina ‘islas para-oceánicas’. Como hemos visto, se consideran islas oceánicas aquellas que nunca se han conectado con el continente y, además, su fauna muestra una alta proporción de

Figura 2. Cuenca del Mediterráneo durante el Messiniense. Se aprecia la desecación de la cuenca así como la conexión existente entre islas mediterráneas actuales, como las Baleares, con zonas continentales adyacentes. Fuente: F. Ledesma Rubio, 2004.

endemismos, están desequilibradas en comparación con faunas continentales, y presentan una baja diversidad de especies de grupos taxonómicos de niveles superiores. Por el contrario, las islas continentales son aquellas que han estado varias veces conectadas con los continentes y sus faunas son submuestras de las faunas continentales, con pocos endemismos y una mayor diversidad de especies (Alcover et al., 1998 y referencias incluidas). Las islas para-oceánicas se situarían entre ambos tipos. Son las que han estado previamente conectadas al continente, pero son similares faunísticamente a islas oceánicas (debido a que la conexión de la isla-continente se produjo en un pasado distante, fue de corta duración, o no promovió la transferencia de fauna). Algunos ejemplos de islas para-oceánicas, lo constituyen la mayor parte de las islas del Mediterráneo, como Baleares. Éstas estuvieron aisladas durante el Pleistoceno, aunque unidas durante el Messiniense (hace 5,3 millones años) a zonas continentales circundantes, eso sí, aisladas como macizos montañosos por profundos desiertos salinos (Alcover et al., 1998).

1.2. Las islas como modelo: el paradigma del laboratorio natural

Los fenómenos de especiación y subespeciación insular han suscitado el interés de los investigadores. El aislamiento insular genera procesos sumamente rápidos de diferenciación feno- y genotípicos en las poblaciones animales como consecuencia del denominado ‘efecto fundador’ (Dobzhansky et al., 1980) que influye en la variabilidad genética inicial de las mismas. A partir de distintas poblaciones aisladas por el mar, se produce pues una evolución posterior en la que intervienen procesos de tipo estocástico (Kimura y Ohta, 7971) y/o selectivos (Dobzhansky et al., 1980; Ayala y Valentine, 1979).

Resulta complicado manipular los ecosistemas experimentalmente; debido a ello, gran parte de la ecología moderna ha tenido que depender de la explotación de los ‘experimentos naturales’: situaciones en las que dos (o más) ecosistemas se diferencian entre sí en uno (o unos pocos) de los factores que afectan a una comunidad. En este sentido, el papel de las islas ha sido crucial en el avance de determinadas disciplinas científicas como la Biogeografía, la Ecología y la Evolución. Las islas constituyen espacios cuyos límites -la línea costera- están netamente definidos, en contraposición a los ecosistemas continentales. Además, su tamaño es muy limitado en comparación con el de los continentes y por tanto muy fácil de abarcar, especialmente si atendemos sólo a las islas oceánicas. Su aislamiento característico, ya sea transitorio o permanente, ha posibilitado que en ellas ocurran a corto plazo una serie de fenómenos basados en la simplicidad de sus comunidades, al participar menos elementos en ellos que en áreas continentales comparables (efectos ecológicos); y otros, a largo plazo, fundamentados en la imposibilidad que tienen algunas especies, que por eventos azarosos colonizaron las islas, de intercambiar material genético con las poblaciones de origen (efectos evolutivos). Debido a todo ello, en sus comunidades participan menos elementos que en áreas continentales comparables.

Esta relativa simplicidad permite deducir de modo más sencillo las interacciones entre los componentes de estas comunidades, mientras que el contexto más complejo del continente puede ocultarlas (Simberloff, 1974). Ello es así en la medida en que las islas constituyen un universo más homogéneo y simple que el de los continentes, de tal forma que se pueden evidenciar fenómenos biológicos con una claridad que sólo se obtendría en tubos de ensayo. Según Mayr (1967), es como si la naturaleza hubiera diseñado toda una serie de experimentos y nuestra labor se limitara a analizar dichos resultados. De hecho, cada biota insular constituye un experimento en sí mismo, puesto que es el resultado de una serie de características físicas e históricas inherentes a la isla en la que se encuentra.

Las islas que constituyen un archipiélago exhiben habitualmente distintas combinaciones de especies que las que vemos en zonas continentales adyacentes. En los archipiélagos, encontramos una serie de zonas aisladas con similares condiciones climáticas y geológicas que han sido colonizadas de forma independiente, de manera simultánea o en un orden particular, y sobre cada una de las cuales se han desarrollado una particular fauna y flora. De este modo, las islas que constituyen los archipiélagos pueden ser vistas como réplicas repetidas, discretas (alopátridas) y relativamente simples de un experimento natural en la evolución (MacArthur y Wilson, 1967; Whittaker, 1998; Whittaker y Fernández-Palacios, 2007); gracias a ello, los investigadores son capaces de observar las respuestas, como los cambios de nicho de las distintas especies en relación a la presencia o ausencia de otras especies, o factores tanto bióticos como abióticos. Hay varios modelos que pueden ilustrar esta afirmación; un ejemplo paradigmático sería el de los trabajos de Schoener (1975), en los que compara la altura de los lugares en árboles y arbustos empleados por *Anolis carolinensis* en diferentes islas en relación a la presencia/ausencia de otra especie competidora, *Anolis sagrei*.

Las islas también pueden ser aprovechadas como experimentos ecológicos naturales desde otras perspectivas. En este caso, ya que suelen presentar un menor número de especies depredadoras que en áreas continentales similares, las islas pueden servir de modelo para estudiar los efectos de la ausencia/presencia de especies depredadoras. De igual manera, al existir una menor riqueza de especies, permiten la realización de un análisis sobre los efectos que la competencia interespecífica tiene sobre la ecología de las especies que pueblan esas islas.

Por todas estas razones, la admiración que sienten los naturalistas por las islas, especialmente por las islas oceánicas, y por su particular fauna y flora, ha cristalizado científicamente en resultados de gran valor. Éstas han servido como modelo y fuente de inspiración a biólogos evolutivos o ecólogos para elaborar sus teorías, confirmarlas o refutarlas. Basten los trabajos de Darwin en Galápagos (1859), los de Wallace (1881, primera ed.) en Nueva Zelanda o, más recientemente, los elaborados por MacArthur y Wilson y su *Teoría de la Biogeografía Insular* (1967), Jonathan Losos (2009) en el Caribe o Rosemary y Peter Grant (1999, 2011) en Galápagos.

Figura 3. Viaje de Darwin por las Galápagos embarcado a bordo del Beagle.
Fuente: https://campus.digication.com/darwin/Gal_pagos_Islands

2. Especie y zona de estudio

2.1. Zona de estudio

Como se ha señalado más arriba, las pequeñas islas son escenarios ideales para desarrollar estudios ecológicos. En este contexto, las poblaciones insulares de lagartijas mediterráneas han sido objeto de numerosos estudios, desde los ya clásicos trabajos de Martin Eisentraut (Pérez-Mellado et al., 2008). En el área mediterránea, existen numerosas poblaciones insulares endémicas de lacértidos que son excelentes ejemplos de adaptación y pueden haber sufrido evoluciones paralelas. Las poblaciones insulares de Baleares se desarrollan en islas e islotes con diferentes historias de aislamiento, tamaño y edad, estando además sometidas a distintas condiciones ecológicas, las cuáles son de vital importancia en el proceso de diferenciación de una población (Mayr, 1963). Se considera incluso que las islas poseen ambientes ecológicos crecientemente diferentes cuanto menor sea su superficie (MacArthur y Wilson, 1967). Las Baleares están localizadas en el oeste del mar Mediterráneo y se consideran islas para-oceánicas (Alcover et al., 1998), es decir, biológicamente similares a las oceánicas aun habiendo estado conectadas a zonas continentales en el pasado. Son islas, pues, que presentan una desarmonía importante respecto a la flora y fauna continental, con muchos grupos totalmente ausentes, y además con un número relativamente alto de especies endémicas. Sin embargo, las únicas especies de vertebrados terrestres que sobreviven de la antes rica fauna plio-pleistocénica balear son el ferreret (*Alytes muletensis*) y dos especies endémicas del género *Podarcis*: la lagartija balear (*Podarcis lilfordi*) en las islas Gimnésicas (Mallorca, Menorca, Cabrera y sus correspondientes islotes), en el este, y la lagartija de las Pitiusas, (*Podarcis pityusensis*) en las Pitiusas (Ibiza y Formentera y los islotes costeros), en el oeste.

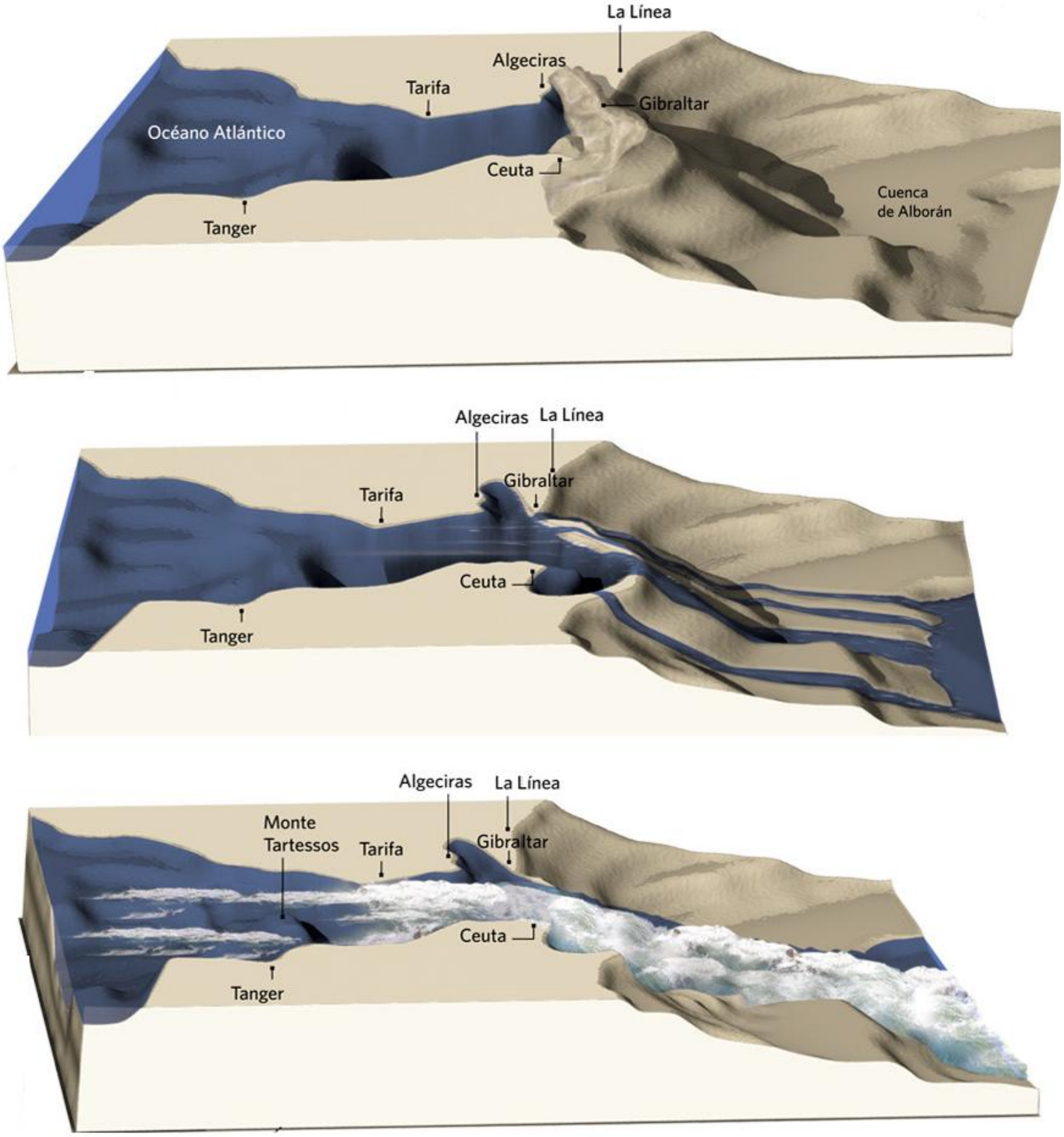


Figura 4. Tras la crisis del Messiniense se produjo la re-inundación del Mediterráneo a través del actual estrecho de Gibraltar. Fuente: Diario de Sevilla

Ambas especies de lacértidos son endémicas de las Baleares y, se trata de dos especies genéticamente bien diferenciadas (Terrasa et al., 2004). Presentan un alto grado de parentesco al proceder de un antecesor común que, probablemente, colonizó las áreas comunes de Baleares durante el Messiniense (Arnold et al., 2007).La subida del nivel del mar tras la crisis Messiniense, produjo el aislamiento de las Baleares respecto a zonas continentales adyacentes y su separación en los grupos orientales (Gimnésicas) y occidentales (Pitiusas). Además, la profundidad del canal intermedio entre Gimnésicas y

Pitiusas, ha impedido probablemente cualquier contacto entre *P. lilfordi* y *P. pityusensis* ulterior, lo que también parece haber dado lugar a linajes evolutivos independientes dentro de otros varios grupos taxonómicos (Terrasa et al., 2008). Sin embargo, entre las poblaciones aisladas de *P. lilfordi* de las Gimnésicas han existido repetidas conexiones. Por ejemplo, durante la últimas grandes glaciaciones, a saber, la de Riss (hace 200.000 años) y la de Würm (25.000 años atrás), se produjo una disminución en el nivel del mar de 130 y 110 m., respectivamente. Gracias a estas bajadas del nivel del mar, Mallorca, Menorca y Cabrera se convirtieron en una gran isla (la 'Gran Balear') (Cuerda, 1989; Gracia y Vicens, 1998). En contraste, los períodos cálidos interglaciares produjeron que los niveles del mar aumentaran hasta 11 m. por encima de los niveles actuales, haciendo que la masa de tierra aérea se redujese a la mitad, fragmentando así las poblaciones animales y vegetales (Cuerda, 1989; Ginés y Ginés, 1993; Goy et al., 1997). Estos eventos han modificado el contenido y estructura genética de dichas poblaciones, y han dejado una huella perceptible a través de la acumulación de mutaciones, en el sentido en que éstas reflejan el tiempo de separación (Terrasa et al., 2008).

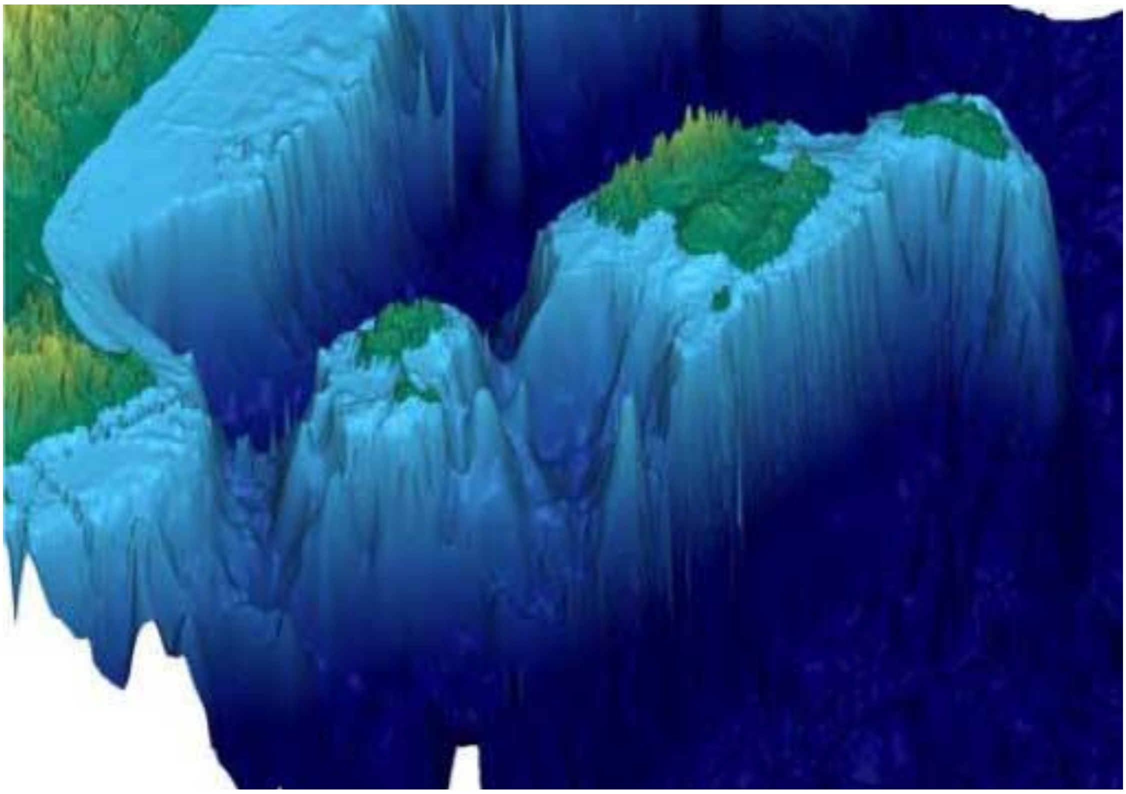


Figura 5. Mapa batimétrico del Mediterráneo en las Islas baleares. Se observa la profundidad del canal intermedio entre Gimnésicas y Pitiusas. Fuente: http://www.vistaalmar.es/medio-ambiente/cambio-climatico/939-investigacion-del-clima-a-traves-de-la-sal-del-mar.html.

El clima es bastante similar en todas las Islas Baleares; se trata del meso-mediterráneo templado, caracterizado por la estacionalidad de las precipitaciones, que son especialmente intensas en otoño y sumamente escasas en verano. También por una estacionalidad en las temperaturas aunque no es tan acusada, las temperaturas extremas raramente superan los 35 º C en verano y no bajan de los 0 º C en invierno (Lluch i Dubon, 1997). Lluch i Dubon (1997) habla de un clima en el que predomina la irregularidad, con fuertes variaciones entre años, sobre todo en las precipitaciones. En Menorca, la temperatura media anual de 16,7 °C. En cuanto al régimen de precipitaciones, la media anual es de 625 mm (450-650 mm), aunque la mayoría de las lluvias se producen en otoño en forma de fuertes tormentas. Además, se dan marcadas diferencias entre distintos años, de forma que se alternan largos períodos de sequía con otros más lluviosos. Otra característica digna de mención es el viento, que predominantemente sopla del norte (conocido como *tramuntana*), aunque de abril a junio los vientos del sur también son notables. Según el Pla d'Ordenació de Recursos Naturals (PORN de l'Illa de l'Aire, 2004), la tramontana lleva un importante contenido de partículas salinas, las cuales tienen un gran impacto sobre la vegetación, especialmente en el norte de Menorca (PORN de l'Illa de l'Aire, 2004). La tramontana es, de hecho, uno de los factores climáticos que tienen mayor incidencia. Este viento del norte sopla con fuerza durante los meses de invierno, pudiendo superar con relativa facilidad los 100 km/h en los temporales más fuertes, y se suaviza durante el verano. Como consecuencia de estas características, únicamente aquellas especies que han sido capaces de adaptarse a veranos largos y secos, así como a las oscilaciones en el régimen de lluvias, han podido sobrevivir (PORN l'Illa de l'Aire, 2004).

Durante el presente estudio se visitó la casi totalidad del área de distribución conocida para la lagartija balear en los islotes costeros de Menorca (Figura 6). Aunque se han muestreado todas las poblaciones donde existen lagartijas baleares, básicamente nos hemos centrado en estudiar la relación entre los cuatro indicadores de calidad y sus efectos en la población de la isla del Aire, y puntualmente en Colom, S'en Tosqueta y Binicodrell; únicamente en estas islas ha sido posible llevar a cabo estudios con tamaños de muestra suficientes. A pesar de ello, los tamaños de muestra manejados en cada uno de los análisis no son idénticos, ya que no en todas las poblaciones fue posible obtener la totalidad de la información precisa.

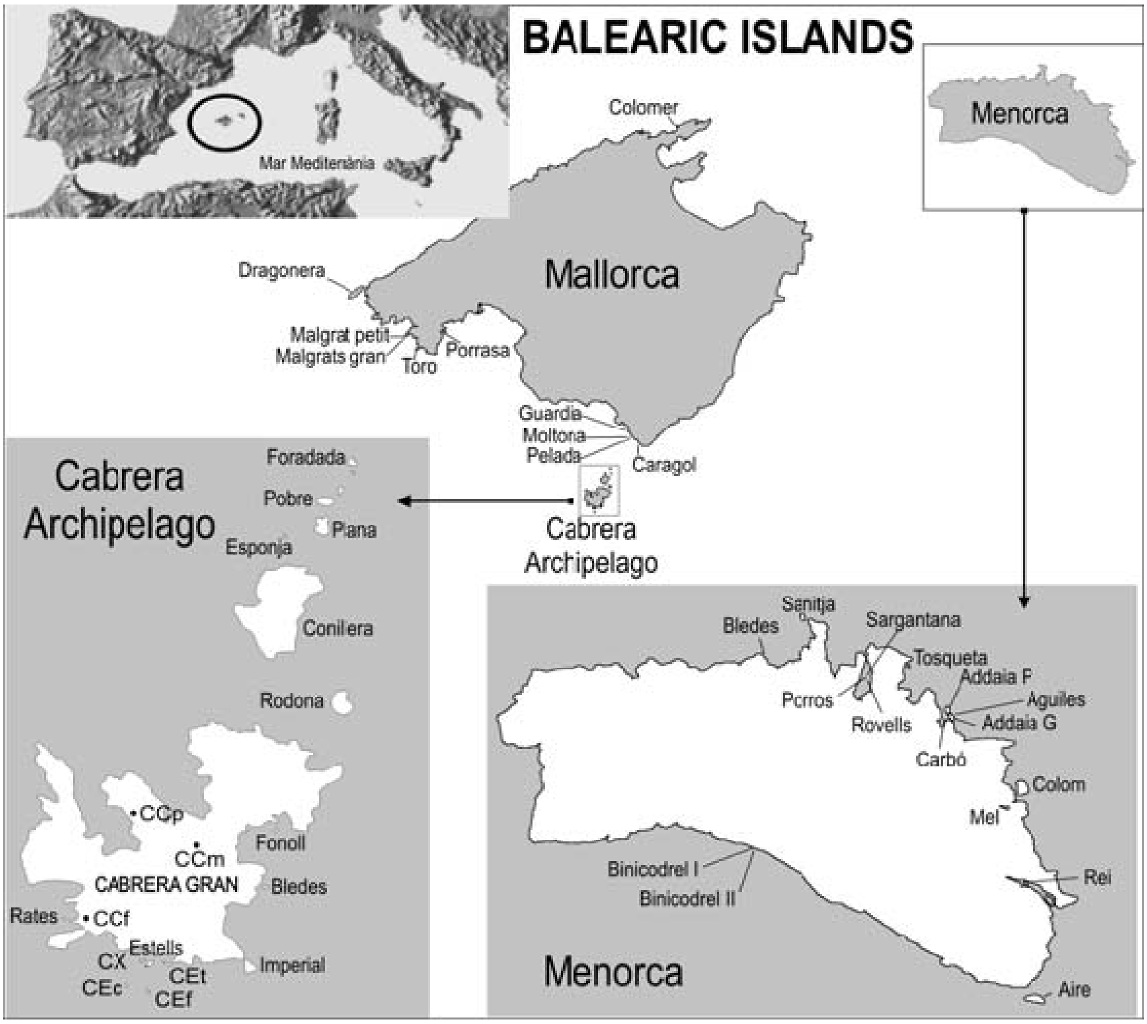


Figura 6. Localización geográfica de las poblaciones de *P. lilfordi*. (Fuente: Terrasa et al., 2008).

2.1.1. Isla del Aire

La isla del Aire data de hace 8.000 años, y es probablemente el islote más antiguo de Menorca (Pérez-Mellado, 1989). Su superficie es de 342.500 m², con un perímetro de unos 3.300 metros, y posee un relieve llano que transcurre en suave pendiente en dirección norte-sur, de modo que la zona de máxima altitud alcanza aproximadamente los 12 metros sobre el mar en forma de abruptos acantilados. La isla cuenta con un muelle ubicado en la zona norte, que permite el amarre de pequeñas embarcaciones. En la zona septentrional y próxima al muelle encontramos una pequeña caseta propiedad de la Autoridad Portuaria y cerca de ella algunas ruinas de piedra de edificaciones anteriores. De la caseta arranca un camino de 465 metros de longitud que atraviesa la isla terminando en un faro en la zona sur, cerca del cual se disponen unas edificaciones abandonadas. La mayor parte de la isla es de naturaleza calcárea, como el sur de Menorca (Pérez-Mellado et al., 2007).

Figura 7. La Isla del Aire. Al fondo, la isla de Menorca. Se observa el camino que une el faro, en el sur, con el embarcadero de la parte norte. Fuente: www.masmar.net.

El clima en la Isla del Aire sigue básicamente el mismo patrón que el de Menorca. Sin embargo, sí se dan algunas diferencias, especialmente en lo que se refiere al viento. De hecho, la fuerza de éste aquí es especialmente elevada, y ello afecta, lógicamente, a su ecología y sus características físicas. No por nada recibe el nombre de Isla del Aire (PORN l'Illa de l'Aire, 2004).

La diversidad vegetal de la isla está condicionada por su historia, morfología, topografía y factores ecológicos como la alta salinidad, el fuerte viento, los largos períodos de sequía y la gran abundancia de excrementos de aves marinas en el terreno, de manera que la mayoría de las plantas son xeronitrohalófitas (Pérez-Mellado, 1989). El terreno está cubierto por arbustos bajos mediterráneos (entre los que destaca *Suaeda vera*) y una gran variedad de especies herbáceas (*Pancratium maritimum*, *Crithmum maritimum*, *Limonium* spp., entre otras); especial mención merece la rapa mosquera, *Dracunculus muscivorus*, que es un endemismo tirrénico, es decir, perteneciente al Mediterráneo occidental (Pérez-Mellado, 1989). La primavera es la época de floración de *D. muscivorus*, momento en que estas plantas son excelentes lugares para las lagartijas, ayudando a su termorregulación y facilitando el forrajeo (Pérez-Mellado et al., 2007). Durante el verano, cuando la escasez de alimentos es más acusada, sus frutos son un recurso extremadamente importante, las lagartijas consumen frutos siguiendo la progresiva maduración de éstos. Hay una pequeña comunidad de porte arbóreo que está formada por un bosquete de *Tamarix africana* en la zona norte a ambos lados del camino asfaltado. Encontramos también algún ejemplar de *Pistacia lentiscus y Phillyrea media* var. *rodriguezii* (endemismo menorquín) en formaciones arbustivas moldeadas por el viento que conforman ‘cojinetes’ muy compactos. La cobertura vegetal varía dependiendo de las precipitaciones y la presencia de conejo, *Oryctolagus cuniculus*, de modo que en 1997 llegó a un máximo del 90 % debido a la ausencia del lagomorfo en el islote (PORN l’Illa de l’Aire, 2004).

La isla es interesante desde el punto de vista de su avifauna, ya que es un importante lugar de paso para multitud de aves migradoras (en su mayoría Paseriformes). Además, residen todas las especies de aves marinas presentes en Menorca: hay una pequeña colonia de gaviotas de Audouin (*Ictyaetus audouini*) en la península occidental; una población de gaviota patiamarilla (*Larus michaellis*) de unas 300 parejas reproductoras que se extiende por casi todo el territorio insular; cormoranes moñudos (*Phalacrocorax aristotelis*) hallados recientemente en 2003; aproximadamente 40 parejas de pardela cenicienta (*Calonectris diomedea*); una par de colonias de paíños (*Hydrobates pelagicus*) en la parte más rocosa; y entre 15 y 30 parejas reproductoras de la amenazada pardela balear (*Puffinus mauretanicus*). También reside en el islote una pareja de halcones peregrinos (*Falco peregrinus*), que anida en un acantilado de la zona sur cercano al faro. Otras rapaces como el aguililla calzada (*Aquila pennata*) y el cernícalo vulgar (*Falco tinnunculus*) visitan a menudo la isla del Aire, pero no nidifican en ella (PORN l’Illa de l’Aire, 2004).

2.1.2. Isla de Colom

Colom es el islote más grande de los que rodean la isla de Menorca, tiene una superficie de unas 59 hectáreas y su intrincada línea de costa completa un recorrido de casi 12 km. La costa noreste (la que da a mar abierto) es rocosa y escarpada, pero en cambio, la costa que encara la isla grande, alberga dos playas bien resguardadas (Pérez-Mellado, 1989). Históricamente, ha sufrido diversas vicisitudes desde su remota ocupación humana. Encontramos restos de una basílica paleocristiana del siglo V, ha pasado por una corta etapa como lazareto en el siglo XVIII, y en el s. XX, fue permanentemente ocupada y explotada desde el punto de vista agrícola y ganadero (Fernández et al., 1999; Pérez-Mellado et al., 2003).

En la isla d'En Colom predomina la maquia litoral, una formación densa y sombría formada por arbustos altos (1,5-4 m.) de tipo esclerófilo , es decir, adaptada a la sequía, así como una gran variedad de especies herbáceas. La vegetación está formada por una comunidad de *Launaea cervicornis*, especie que forma pequeños cojinetes espinosos. Entre las plantas de porte arbustivo más interesantes destaca el arbusto endémico *Daphne rodriguezii*; el tamarindo (*T. africana*) es otra planta representativa de la isla, una especie de arbusto de origen africano; también es bastante abundante *P. lentiscus*, y en la zona norte, *S. vera* y *Erica* sp (Pérez-Mellado, 1989).

En el conjunto de islotes de Menorca, Colom destaca por ser el único que posee una cierta variedad de vertebrados potencialmente depredadores de la lagartija balear, incluyendo a la culebra de escalera, *Rhinechis scalaris* (Esteban et al., 1994), la rata negra, *Rattus rattus*, una floreciente colonia reproductora de la gaviota patiamarilla y alguna pareja nidificante de cernícalo vulgar (Pérez-Mellado, 1989).

Figura 8. Ortofotografía de la isla de Colom. Se observa al este la isla principal de Menorca. Fuente: Google maps.

2.1.3. Binicodrell

Este pequeño islote tiene una altura máxima de 6 metros sobre el nivel del mar. La parte superior del islote es plana y los bordes exteriores, acantilados y fuertemente erosionados. La zona superior se halla cubierta por diversas plantas, aunque la vegetación puede considerarse densa únicamente en la mitad occidental (Pérez-Mellado, 1989). A excepción de algún ejemplar de *S. vera*, a refugio en pequeñas oquedades, *Arthrocnemum sp.* y *C. maritimum* la vegetación está casi exclusivamente formada por plantas herbáceas, entre las que destacan por su abundancia: *Allium ampeloprasum*, *Limonium* spp. y *Asparagus stipularis*. Posee algunas especies de pequeñas aves paseriformes nidificantes (Pérez-Mellado, 1989).

Figura 9. Ortofotografía de los escull de Binicodrell (izquierda) y la Illa d'en Tosqueta (derecha). Se observa en ambos casos la isla principal de Menorca. Fuente: Google maps.

2.1.4. Tosqueta

Se halla al este de la Mola de Fornells y al sur de Punta Rodona. La parte superior es plana y los bordes muy escarpados y erosionados. Al estar situada en la costa norte de Menorca, su vegetación está fuertemente afectada por los fuertes vientos y la salinidad (Pérez-Mellado, 1989). Casi la totalidad de la isla está cubierta por vegetación herbácea, de hecho, un 60% de su superficie se halla cubierta por *A. ampeloprasum*. Encontramos también: *Daucus carota*, *A. stipularis*, *C. maritimum*, *Beta* sp., y *Anthemis* sp. Hay otras plantas de porte arbustivo, como *P. lentiscus* y *Phillyrea angustifolia*, que han sido moldeadas por el viento de Tramontana, y con aspecto de 'cojinete' en la parte occidental y meridional. Hallamos algunas especies de pequeñas aves nidificantes, y una población introducida de rata negra, *R. rattus* (Pérez-Mellado, 1989).

2.2. Especie en estudio

La lagartija balear, *P. lilfordi* (Günther, 1874) es un pequeño lacértido endémico de las Islas Baleares. Los abundantes restos fósiles y subfósiles que se encuentran en Mallorca y Menorca atestiguan que esta especie habitaba en las Baleares mayores (Mallorca y Menorca), mientras que actualmente su distribución se ha reducido a los islotes que circundan las costas de estas islas y al archipiélago de Cabrera. Se cree que la extinción de la lagartija balear en Mallorca fue posterior al año 300 a.C. y en Menorca alrededor del 50 a.C., o incluso más tardía, ya que las revisiones más recientes muestran la presencia de la especie en el registro fósil de ambas islas hasta el Holoceno (Bailón, 2004). Hay diversas teorías acerca de las causas de la desaparición de la lagartija balear en las islas mayores, como un efecto directo de los depredadores, de especies competidoras, o la consecuencia de la reducción de mecanismos antidepredadores esenciales, como la autotomía caudal. De cualquier modo, existe consenso actualmente a la hora de señalar a los depredadores introducidos por el hombre, especialmente los carnívoros de pequeño tamaño, como los responsables de la extinción (Eisentraut, 1949; Mertens, 1957; Salvador, 1993; Pérez-Mellado et al., 1997).

P. lilfordi es una especie propia del bosque mediterráneo y sus diferentes etapas de sustitución. Muestra preferencia por el matorral aclarado y las formaciones florísticas de las áreas costeras, incluyendo zonas caracterizadas por su pobreza faunística y florística y, en general, por la ausencia de agua dulce, como los islotes, los cuales forman sistemas aislados (Pérez-Mellado, 1989, 1998a). Cada uno de ellos presenta peculiaridades propias y difieren enormemente en tamaño, topografía, distancia a la isla grande más cercana, y vegetación. Del mismo modo, la situación demográfica y de conservación de las distintas poblaciones es muy diferente; en algunas de las islas se pueden encontrar densidades extremadamente altas, mientras que en otras los efectivos poblacionales se reducen a unos pocos individuos (Pérez-Mellado, 1989, 1998a; Pérez-Mellado et al., 2008).

Se han descrito 28 subespecies de *P. lilfordi* de acuerdo a las diferencias en biometría, diseño, coloración y folidosis, no todas ellas aceptadas. En las más recientes revisiones (Pérez-Mellado, 1997, 1998) se recogen 23, ocho de ellas para las costas de Menorca, seis para Mallorca y el resto presentes en Cabrera. Hay una población extinguida: la población de la isla de Ses Rates (Pérez-Mellado, 1989). Sobre la base de la ecología de *P. lilfordi*, los islotes costeros de Mallorca y Menorca se caracterizan por una disponibilidad de recursos bastante escasa, un bajo número de competidores terrestres y una presión de depredación variable, pero, de modo general, reducida (Pérez-Mellado et al., 2007, 2008). Debido a tales particularidades, estas poblaciones manifiestan unas cualidades excepcionales para realizar variados estudios de ecología, como son las elevadas densidades de población (Pérez-Mellado et al., 2008), que facilitan la toma de muestras estadísticamente relevantes. Otra característica condicionada por esta situación es la reproducción, con unos tamaños de puesta reducidos, una extensión del período reproductor durante varios meses al año, múltiples puestas por hembra, una existencia de conductas de vigilancia de pareja (ver, por ejemplo, Perera y Pérez-Mellado, 2004) y otros rasgos que son típicos de poblaciones sometidas a condiciones extremas.

Cada uno de los cuatro islotes estudiados en este trabajo presenta una subespecie propia de lagartija Balear. En la Isla del Aire encontramos a la subespecie *P. lilfordi lilfordi*, una subespecie grande y de color melánico. El dorso y los costados son uniformemente negros y la zona ventral es de una coloración azul ultramar. Presenta valores altos de escamas del collar, gulares y ventrales. Las patas posteriores son relativamente largas, sobre todo en las hembras (Pérez-Mellado, 1998).

P. lilfordi codrellensis es una subespecie presente en los esculls de Binicodrell, pequeña, con dorso pardo oscuro, de cabeza relativamente estrecha y con patas posteriores largas. Tiene un bajo número de escamas en el collar y la zona gular y un alto número de escamas ventrales (Pérez-Mellado, 1998).

En Tosqueta, Bleda Mayor y otros islotes de la bahía de Fornells (Sargantana y Rovells) está *P. lilfordi sargantanae*. Es una subespecie de tamaño medio con valores también medios para la folidosis y biometría. Generalmente presenta líneas dorsolaterales relativamente bien marcadas. Muchos individuos, incluso los adultos, poseen un neto contraste entre el dorso pardo verdoso y la cola verde (Pérez-Mellado, 1998).

Por último, *P. lilfordi brauni* (Müller, 1927), habita la isla de Colom. Es de talla mediana, con el dorso de color verde oliva, azul en machos viejos y con el vientre gris claro (Pérez-Mellado, 1998).

Figura 10. Diferentes subespecies de la lagartija balear *P. lilfordi*, presentes en cada uno de los cuatro islotes estudiados en este trabajo.

3. Objetivos y antecedentes

El objetivo de este trabajo es evaluar el estado de salud de las distintas poblaciones de *P. lilfordi*, una lagartija endémica de las Islas Baleares. El estado de salud y el potencial estrés ambiental al que están sometidas dichas poblaciones se estimaatendiendo a cuatro indicadores independientes, poniendo a la vez a prueba su fiabilidad y buscando la posible relación entre ellos. Estos cuatro indicadores son: el llamado estado de condición de los individuos, la carga parasitaria, la respuesta inmune y el grado de asimetría fluctuante observado en caracteres bilaterales. Tales indicadores han sido empleados con éxito en reptiles de pequeño tamaño (ver referencias más abajo). Asimismo, se establecen las relaciones con las respuestas adaptativas en términos de rasgos de la ecología del comportamiento, que incluyen: las estrategias de obtención del alimento, la capacidad de defensa y las tácticas de escape de los depredadores.

3.1. Condición física

La condición corporal de un animal refiere a su estado energético. Se emplea como un índice aproximado de la condición física del individuo, de su estado nutricional, y de la salud en general (Bonnet et al., 1994; Green, 2000). En vertebrados, existen numerosos métodos no destructivos para estimar la condición corporal que se basan en la masa corporal en relación con las medidas lineales de tamaño corporal (revisiones en Blem, 1990 y Brown, 1996). El principal objetivo de estos métodos consiste en separar los aspectos de la masa corporal que son debidos al tamaño estructural de los aspectos debidos las grasas y otras reservas energéticas (Green et al., 2001). O dicho de otro modo, estos índices de condición corporal intentan determinar la masa del individuo asociada con las reservas de energía después de corregir por el tamaño del cuerpo (Schulte-Hostedde et al., 2005). En este estudio hemos aplicado uno de los métodos más comunes que se utilizan para medir la condición implica una regresión de la masa corporal sobre algún índice lineal del tamaño del cuerpo, usando los residuales de esta regresión (mediante el método de los mínimos cuadrados ordinarios o lineales) como un índice de la condición corporal. De este modo, un individuo con un valor residual positivo se considera que está en mejores condiciones que un individuo con un residual negativo (Schulte-Hostedde et al., 2001, 2005). En ocasiones, para comparar la condición corporal entre diferentes categorías (edad, sexo, áreas,…) hemos utilizado el análisis de covarianza (ANCOVA), introduciendo el peso como variable de respuesta, algún índice lineal del tamaño del cuerpo como covariable y dicha variable categorizadora como factor. El análisis de la condición corporal mediante el uso de ANCOVA ya ha sido usado y recomendado por diversos autores (García-Berthou, 2001; Hayes y Shonkwiler, 2001).

Está establecido, por tanto, que un animal en buen estado tiene más reservas de energía que un animal en mal estado y, por consiguiente, aquellos individuos con mejor condición corporal deberían ser menos susceptibles a la inanición (Pond, 1981; Laurie y Brown, 1990; Civantos y Forsman, 2000). Quiere decirse que, en los reptiles, los índices de condición corporal son un buen indicador de la tasa de consumo previo de alimentos y el tamaño de las reservas energéticas (Bonnet et al., 1994; Forsman y Lindell, 1996). Su influencia en las aptitudes de los individuos está ampliamente estudiada para pequeños lacértidos, aunque en ocasiones con diferentes resultados. Así, la velocidad de carrera es dependiente de la condición en *Crotaphytus collaris* según algunos estudios (Schluter et al., 1991; Clobert et al., 2000; Jennions et al., 2001); lo mismo sucede en *Zootoca vivipara* (Oppliger et al., 1996). Teniendo esto en cuenta, la mala condición corporal puede estar asociada a la disminución de la velocidad o la resistencia y puede también afectar a la capacidad de escape, lo que requiere que la presa en malas condiciones deba iniciar la huida antes, para así tener una probabilidad de fuga igual o casi igual a la de los individuos con mejor condición corporal (Hawlena et al., 2009). Además, en otras especies de pequeños lacértidos, parece que la velocidad de carrera está débilmente relacionada, o no hay relación alguna, con la condición corporal (Amo et al, 2007; Vervust et al., 2008).

Sin embargo, el comportamiento antidepredador sí que parece ser dependiente de la condición corporal de los individuos (Beale y Monaghan, 2004). Con estas premisas, los sujetos en mejor condición serían más capaces de asumir los costes asociados a la suspensión de la alimentación o el traslado a otras áreas que los individuos en peor condición, para quienes la necesidad de seguir alimentándose es la prioridad (Hawlena et al., 2009). Los individuos en peor condición pueden no tener más opción que seguir alimentándose durante el tiempo que les sea posible; ello es lo esperado según el denominado ‘principio de protección de activos’ (Clark, 1994). Teniendo en cuenta que la reproducción es esencial en la aptitud biológica de los individuos, el ‘principio de protección de activos’ (Clark, 1994) establece que cuando los individuos han de tomar de decisiones frente a los depredadores, el valor reproductivo es un factor esencial a tener en cuenta. Debido, además, a que el valor reproductivo suele ser dependiente de la edad y la condición, el comportamiento antidepredador óptimo también depende a menudo de estas variables (Clark, 1994). Esta asociación variaría entre las especies que viven en ambientes diferentes y presentan distinto comportamiento social (Cabido et al., 2009). De un modo más general, se podría decir asimismo, que los riesgos y los costes que asume un individuo están sujetos a una variación como consecuencia de la propia variación en su condición corporal (Stankowich y Blumstein, 2005). Hay otras pautas de comportamiento cuya relación con el estado corporal ha sido establecida. López et al. (2005), analizando *Iberolacerta monticola*, descubrieron que los individuos más ‘atrevidos’ tenían mejor estado de condición y un mejor estado de condición que aquéllos más ‘conservadores’. Otro ejemplo lo proporcionan Meylan et al. (2002), quienes descubrieron que, entre los juveniles de *Z. vivipara*, la dispersión se ve favorecida por una mejor condición corporal.

La condición también se ha relacionado con parámetros de la aptitud biológica, como la reproducción y la supervivencia en reptiles y en otros muchos taxones (Schulte-Hostedde et al., 2005 y referencias incluidas). Las hembras se sienten atraídas por machos en mejor condición corporal, bien porque, presuntamente, existe una relación entre dicha condición y la calidad del macho y su mejor dotación genética (Cooper y Vitt, 1993), bien para evitar el acoso de otros machos, lo que permitiría a las hembras aumentar el tiempo de forrajeo (Censky, 1997). También los niveles de hormonas del estrés están a menudo negativamente relacionados con la condición corporal (Moore y Jessop, 2003), que es a su vez una señal fiable para las hembras que están en busca de un macho de 'alta calidad' (Andersson, 1994). El otro componente de la *aptitud biológica*, la supervivencia, también está vinculada al estado de condición de los individuos. Laurie y Brown (1990) y Civantos (2003), trabajando durante varias temporadas con juveniles de iguana marina (*Amblyrhynchus cristatus*) y de lagartija colirroja (*Psammodromus algirus*) respectivamente, encontraron una clara relación entre condición corporal y las tasas de supervivencia anuales. De acuerdo con ello, la forma particular en que la mortalidad depende de la condición corporal puede tener importantes consecuencias para la estructura y la dinámica de las poblaciones (Civantos y Forsman, 2000).

3.2. Respuesta inmune

Un rasgo de rendimiento que puede estar relacionado con la aptitud biológica de los individuos es la capacidad de desarrollar una respuesta inmune eficiente, puesto que un buen mecanismo de defensa inmune podría ser crucial en la prevención de invasiones parasitarias y enfermedades (Janeway et al., 2004). El sistema inmunológico de los vertebrados ha evolucionado como una defensa compleja contra la amenaza constante de la enfermedad y al parasitismo (Kaufmann et al., 2002), que puede privar al hospedador de recursos vitales y nutrientes, impactando de este modo negativamente en su supervivencia (Behnke y Barnard, 1990; Gulland, 1995).

En términos generales, el sistema inmune comprende dos componentes que difieren en función e historia evolutiva: el componente adaptativo y el innato. El sistema inmune adaptativo es exclusivo de vertebrados mandibulados y consta de dos ramas: la humoral (células B) y la celular (células T) (Martin et al., 2008b). La inmunidad humoral es responsable del control extracelular del patógeno, mientras que la inmunidad celular es generalmente responsable del control intracelular del patógeno (células T citotóxicas) y / o la gestión de las células B y otras respuestas inmunes (células T auxiliares). Aunque estas defensas puede proporcionar memoria inmunológica de infecciones previas, que son de desarrollo más lento y costoso para alcanzar eficacia en términos de control de patógenos con respecto al otro sistema principal de la defensa inmune, la inmunidad innata (Martin et al., 2008b). El sistema inmune innato se compone de un conjunto diverso de tipos de células y de sustancias secretadas por éstas. Las defensas innatas son eficaces para controlar varios tipos de parásitos, y son mucho más rápidas que las defensas adaptativas (Martin et al., 2008b). Sin embargo, son a menudo más costosas y pueden ser más perjudiciales para el propio individuo que las defensas adaptativas (Klasing, 2004). Además, a menudo son las defensas innatas las que determinan la naturaleza y la intensidad de la respuesta inmune adaptativa (Janeway et al., 2004). Hasta la fecha, muchas técnicas se han utilizado para medir la respuesta inmune innata y adaptativa.

Es esta variación en la resistencia a patógenos, dependiente del sistema inmune, la que puede conducir a diferencias en las probabilidades de supervivencia (Saino et al., 1997) y en el éxito reproductivo (Hamilton y Zuk, 1982; Saino et al., 1997) de los individuos. Asimismo, un creciente número de estudios ha demostrado que la función inmune está sometida a un proceso de selección en una amplia variedad de grupos, incluyendo a las lagartijas (Svensson et al., 2001). No obstante, algunos autores defienden que el conocimiento de los mecanismos del sistema inmune en animales en su hábitat natural es aún muy escaso, pese al creciente interés de los biólogos evolutivos (Zuk, 1994; Sheldon y Verhulst, 1996; Schmid-Hempel, 2003).

En la literatura científica, no hay uniformidad en el estudio de la respuesta inmune. Se han usado distintas técnicas y se ha estimado la capacidad de reacción de varios componentes del sistema inmune (revisión en Demas et al., 2011). Así, por ejemplo, French et al. (2008) analizaron la proporción de leucocitos heterófilos respecto linfocitos para evaluar la inmunidad innata. En su caso, emplearon esta técnica con el fin de verificar las relaciones entre el sistema inmune y los nuevos agentes estresantes a los que está sometida una población urbana de *Urosaurus ornatus*. Sin embargo, en pequeños lacértidos, la capacidad de respuesta del sistema inmune adquirido es la medida más utilizada para evaluar la capacidad del sistema inmune. Mediante la inyección subcutánea de un antígeno se puede obtener una estimación de la capacidad de respuesta del componente celular. El antígeno más usado ha sido la fitohemaglutinina (PHA, en sus siglas en inglés, Svensson et al., 2001; López et al., 2005; Amo et al., 2007), y el LPS, en un menor número de ocasiones (Bonneaud et al., 2003; López et al., 2009). El PHA es un antígeno de origen vegetal que no provoca alteraciones en los niveles de proteínas de estrés u otros parámetros hematológicos (Merino et al., 1999), y la reacción que induce desaparece generalmente a las 48 horas de la inyección. Este antígeno provoca la acumulación perivascular de linfocitos T en la zona de inyección, seguida por la infiltración de macrófagos (Goto et al., 1978).

Más recientemente, se ha usado el ensayo llamado ‘Bacterial killing’ (BK), que determina la respuesta inmune innata mediante la estimación de la capacidad de destrucción bacteriana del plasma; se evalúa la capacidad del complemento de la sangre para matar una colonia bacteriana de *Escherichia coli* in vitro. Este ensayo se está revelando como una herramienta muy valiosa para medir, en una amplia variedad de organismos, la inversión realizada en el sistema inmune innato (Chester et al, 2010; Matson et al, 2006; Zimmerman et al, 2010). Siguiendo la recomendación de Demas et al. (2011), se decidió evaluar la respuesta inmune de los individuos mediante la cuantificación de la capacidad de destrucción bacteriana del plasma sanguíneo. BK proporciona una evaluación más relevante de la función inmune que los ensayos de componentes aislados inmunes y también es preferible a las técnicas de monitorización que pueden variar cuantitativamente y cuya interpretación es un tanto problemática (Norris & Evans, 2000; Demas et al, 2011). Además, este ensayo proporciona una evaluación pertinente de la función inmunitaria del huésped y la determina sin la necesidad de inducir una respuesta externa mediante la inyección de antígenos o mitógenos relativamente artificiales (Demas et al., 2011). Por estas razones, BK encaja adecuadamente con nuestro propósito de conocer el rendimiento del sistema inmune en las poblaciones naturales.

Para realizar la prueba BK, las muestras de sangre para el análisis inmunológico se recogieron *in situ* en un microcapilares heparinizados. Las muestras de sangre se almacenaron con hielo hasta que se centrifugaron a 3600 rpm durante 12 minutos. El plasma fue separado dentro de las ocho horas posteriores a la recogida. De cada individuo hemos adquirido aproximadamente 30 µl. de plasma que se mantuvo congelado a -20 º C hasta que se llevaron a cabo los ensayos inmunológicos. Hemos seguido un protocolo similar al desarrollado por Ruiz et al. (2010, 2011). Se preparó una solución madre bacteriana diluyendo $2x10^{-6}$ veces una concentración saturada de *E. coli* en un medio CO_2-independiente con 2,34 mg de L-glutamina (Sigma-Aldrich, St Louis, MO). Las pruebas piloto (resultados no publicados) mostraron que esta concentración era adecuada para conseguir controles positivos cuantificables (evitando el césped bacteriano), pero con las colonias suficientes para estimar la capacidad bactericida del plasma. A continuación, se diluyeron las muestras de plasma en 200 µl. de la solución bacteriana previamente diluida. Estos cócteles de plasma / bacterias se incubaron durante 30 min a 37 ° C. Posteriormente, se sembraron las muestras sobre placas de agar, incluyendo dos controles positivos (solo con solución bacteriana) y un control negativo (sin solución bacteriana). Las placas se incubaron durante la noche a 37 ° C para permitir el crecimiento de colonias. Al día siguiente se contó el número de colonias en cada placa y se compararon con los controles positivos con el fin de calcular el porcentaje de bacterias muertas por cada muestra de plasma [% de bacterias muertas = 100 - (# promedio de colonias en la placa de muestra / media # de colonias de las placas de control positivo) * 100]. Fueron descartadas las placas de Petri en las que detectamos contaminación por colonias de otras bacterias. En ningún caso se formaron colonias en las placas de control negativo.

En reptiles, el funcionamiento del sistema inmune ha sido estudiado considerando sus distintos componentes; pero un factor común que une a todas las categorías de la función inmunológica es que su funcionamiento requiere recursos que, de otra forma, podrían haber sido usados para alguna otra necesidad. La hipótesis de que la función inmunitaria es costosa para el anfitrión parece razonable (Martin et al., 2008b y referencias dentro). Sin embargo, poco se sabe acerca de los costes energéticos directos de elaborar una respuesta inmune, aunque algunas de las respuestas inmunitarias (fiebre, por ejemplo) deben tener considerables costes energéticos. Mucho mejor documentada está la evidencia indirecta de que la función inmune es costosa para los anfitriones. Obviamente, la asignación óptima de los recursos para la función inmune dependerá de las demandas de otros requerimientos y de los beneficios que proporcionen (Sheldon y Verhulst, 1996).Esto es, como la inversión en la función inmune puede ser costosa (Svensson et al., 2001; Bonneaud et al., 2003), una mayor inversión en ella puede conducir a compromisos (*trade-off*) con otras necesidades que son importantes para la aptitud biológica de los individuos. Se ha demostrado que montar una respuesta inmune puede provocar un aumento significativo de la tasa metabólica en reposo, lo que sugiere que existen importantes requerimientos energéticos que limitarían los recursos para procesos tales como la reproducción (Martin et al., 2008b). El reciente interés de los investigadores en estudiar la respuesta inmune se debe, en gran parte, a estos potenciales compromisos con otras necesidades.

Aparte del bien conocido compromiso entre inmunidad e inversión en reproducción (revisión en Norris y Evans, 2000), otros han sido descritos en lacértidos; por ejemplo, el que existe entre el sistema inmune y los caracteres sexuales secundarios. Los resultados encontrados por López et al. (2009), trabajando con machos de *Podarcis hispanica*, sugieren que hay un potencial equilibrio entre la regulación fisiológica del sistema inmune y la asignación de los nutrientes esenciales (vitaminas) a las señales sexuales químicas, aunque también es cierto que estos compromisos pueden ser dependientes del contexto. Así, el consumo de recursos altamente energéticos puede eliminar la necesidad de decidir entre sistema inmune o esfuerzo reproductivo (Ruíz et al., 2011). Por consiguiente, sólo los individuos en mejor condición son capaces de producir buenos ornamentos sexuales sin sacrificar la respuesta inmune. Otros compromisos importantes se producen en el cuidado parental (Moreno et al., 1999) o el comportamiento de apareamiento (McKean y Nunney, 2001).

3.3. Carga parasitaria

La ecología evolutiva indica que los parásitos pueden ser importantes agentes selectivos que afectan a la biología de la población hospedadora, la ecología y la evolución (Salkled y Schwarzkopf, 2005 y referencias incluidas).Dichos parásitos compiten con los hospedadores por los recursos, causando efectos adversos en diversos aspectos, como el crecimiento de la población y su regulación (Holmes, 1995; Hudson et al, 1998), la distribución espacial (Price, 1980; Van Riper et al., 1986), la fisiología (Schall et al., 1982), el comportamiento (revisión en Moore, 2002), la dominancia social (Schall y Houle, 1992), la fecundidad de los hospedadores, su apareamiento y su éxito reproductivo (Dunlap y Schall, 1995; Martín et al., 2008), o la capacidad competitiva (Park, 1948). Los hospedadores pueden incluso verse favorecidos por ciertos parásitos, ya que reducen más el rendimiento de un competidor conespecífico: si los parásitos son perjudiciales para los individuos en mejores condiciones para resistir la infección tendrán una mayor aptitud. De acuerdo con ello, los individuos con mejores capacidades serían más capaces de capturar a sus presas, huir de los depredadores o establecer un dominio social (Garland et al., 1990). Por todo esto, los patrones de parasitismo nos ayudan a entender cuáles son los procesos que mantienen las asociaciones parásito-hospedador y, además, son esenciales para comprender la dinámica de la población del hospedador y su ecología.

A pesar del profuso conocimiento que tenemos acerca de los parásitos que causan enfermedades en los seres humanos, o aquéllos que tienen un impacto directo sobre la economía, sabemos muy poco tanto de las relaciones parásito-hospedador en las poblaciones naturales, como de las causas y las consecuencias para el anfitrión, sobre todo si los efectos no son letales. Hay numerosos estudios orientados a conocer el grado de parasitismo en poblaciones del género *Podarcis* en Europa. La mayoría se centran en el ectoparasitismo, por ejemplo, en *Podarcis muralis* (Strijbosch et al., 1980; Amo et al., 2005), *Podarcis melisellensis* (Huyghe et al, 2009, 2010), *Podarcis sicula* (Ouboter, 1981; Biaggini et al, 2009) o *Podarcis erhardii* (Hurston, 2007), entre otras. En cuanto a los parásitos internos, los gastrointestinales han sido ampliamente estudiados en *Podarcis vaucheri* (Carretero et al., 2011), *P. erhardii* (Roca et al., 2009), *Podarcis bocagei* y *P. carbonelli* (Galdón et al., 2006; Roca et al., 2006), *P. lilfordi* y *P. pityusensis* (Hornero y Roca, 1992; Roca y Hornero, 1994; Roca, 1996), *P. muralis* (Kirin, 2002) y en poblaciones introducidas de *P. muralis* y *P. sicula* en Estados Unidos (Burke et al, 2007). Sin embargo, menos atención se ha prestado al estudio de parásitos sanguíneos, que se ha estudiado, por ejemplo, en *P. bocagei* y *P. carbonelli* (Roca y Galdón, 2010), y en *P. muralis* (Amo et al., 2005); pero tan sólo en un caso relativo a poblaciones insulares: *P. melisellensis* (Huyghe et al., 2010).En nuestro estudio, nos centramos en cuantificar los patrones de parasitismo (prevalencia e intensidad) de dos diferentes parásitos, ectoparásitos (ácaros) y parásitos sanguíneos (hemogregarinas), así como sus posibles efectos sobre distintos componentes de la aptitud biológica.

3.3.1. Ectoparásitos

Los parásitos hematófagos como las garrapatas o los ácaros están presentes en muchas especies animales. Dentro de éstos, los ácaros del género *Ophionyssus* (Acari; Mesostigmata; Macronyssidae) son parásitos esencialmente de lagartos escamosos (Squamata). Este género comprende 15 especies; siendo 13 de ellas parásitas de lagartos (Fain y Bannert, 2000). En esta categoría, los lacértidos son parasitados por seis especies de *Ophionyssus*, dos de ellas -*O. galloticolus* y *O. setosus*-, parásitos del género *Gallotia* de las Islas Canarias (Bannert et al., 2000). No hemos encontrado ningún estudio taxonómico sobre los ectoparásitos del género *Podarcis* en España o en la cuenca mediterránea. No obstante, el género *Ophyonissus* está presente en muchas especies de lagartos de Europa (Strijbosch et al., 1980), incluyendo algunas especies de la Península Ibérica (Moraza et al, 2009; Bannert et al., 2000; Majlathova, 2010). Los ectoparásitos observados en este estudio fueron asignados tentativamente a este género.

Además del daño directo causado a través de la succión de sangre, las especies de *Ophionyssus*, que bajo condiciones de laboratorio pueden ser letales para sus huéspedes (Frye, 1991), sirven también como vectores potenciales de endoparásitos (Reichenow, 1913, 1919, 1921 y Bannert et al., 1995 en Bannert et al, 2000; Camin, 1948; Svahn, 1975; Álvarez-Calvo, 1978). Los ectoparásitos del género *Podarcis* se estudiaron en varias poblaciones, pero mayoritariamente en relación a las consecuencias sobre rasgos vitales de los anfitriones (Huyghe et al., 2009; Biaggini et al., 2009; Oppliger et al, 2004). Desde nuestro punto de vista, son pocos los estudios realizados en las poblaciones insulares de la cuenca mediterránea, a excepción del trabajo realizado por Hurston (2007) en *P. erhardii*.

En términos generales, los daños causados por ectoparásitos hematófagos, como los ácaros, están relacionados con distintos componentes de la historia natural del hospedador. En un aspecto puramente fisiológico, pueden dañar los tejidos, vaciar los fluidos internos o desencadenar la respuesta inmune, con los costes energéticos derivados de ello (Wakelin, 1996). Actúan también como vectores de enfermedades y endoparásitos (ver más arriba). En lacértidos, se ha encontrado una relación indirecta entre el ectoparasitismo y la supervivencia. La descendencia de hembras altamente parasitadas de *Z. vivipara* presentaba unas menores probabilidades de supervivencia (Sorci et al., 1994). En el aspecto genético, la heterocigosis se ha vinculado con la resistencia a los ácaros (Brown et al., 1995). También, se ha establecido una relación con el esfuerzo reproductor, de modo que los machos con mayores niveles de testosterona albergan más ectoparásitos (Salvador et al., 1996). Los ácaros pueden afectar negativamente la aptitud y la condición corporal (Sorci y Clobert, 1995), incluso las capacidades locomotoras del hospedador. Main y Bull, (2000), trabajando con juveniles de *Tiliqua rugosa* infectadas con ectoparásitos, encontraron una clara correlación entre la carga parasitaria y la velocidad de carrera, e incluso con el comportamiento de las lagartijas. En *Z. vivipara*, los efectos negativos causados por los ectoparásitos durante la gestación afectan a la capacidad de dispersión de los juveniles (Sorci et al., 1994).

En este trabajo, los ectoparásitos se contaron en el lugar donde se capturaron los individuos. Se realizó la inspección de toda la superficie del cuerpo de las lagartijas con un monóculo de 5x. Con el fin de facilitar el conteo de los ectoparásitos, dividimos la superficie del cuerpo en 6 partes: 1) la zona rostral, dorsalmente hasta las escamas parietales y occipital, ventralmente hasta las submaxilares; 2) la zona gular, que comprende las escamas gulares hasta la zona del collar incluida. Dentro del tronco consideramos dos partes, 3) la zona dorsal, hasta el primer verticilo caudal, y los flancos laterales, hasta las escamas ventrales, y 4) la zona ventral, la zona de las escamas ventrales, que en los lacértidos son más grandes y bien diferenciadas; 5) la zona pericloacal, alrededor de la escama anal hasta el primer anillo caudal. Por último, 6) la zona caudal, a partir del primer anillo caudal; en caso de que haya sido regenerada, distinguimos entre ambas secciones de la cola, la parte original y la regenerada. La intensidad de parasitismo se estimó por el total de ectoparásitos contados en toda la superficie corporal y, la prevalencia, como el porcentaje de individuos infectados en la zona de estudio.

3.3.2. Hemogregarinas

En el caso de pequeños lacértidos mediterráneos, los parásitos sanguíneos más comunes son las hemogregarinas intraeritrocíticas (Amo et al., 2004, Pérez-Mellado, datos inéditos en *P. pityusensis*). Las hemogregarinas (Apicomplexa: Adeleorina) son protozoos parásitos y están presentes en especies de muy distintos taxones, incluidos los reptiles. El término ‘hemogregarina’ se refiere a coccidios parásitos de la sangre, de la familia Haemogregarinidae, que contiene los géneros *Hepatozoon*, *Haemogregarina* y *Karyolysus* y *Hemolivia* (Telford, 2008).Las especies de hemoparásitos de lagartijas baleares, como ocurre con muchas otras especies de lacértidos de la Península Ibérica, no han sido aún descritas. En nuestro caso, sólo hemos encontrado una tipo de parásito sanguíneo pero, debido a que los géneros de esta familia (hemogregarinas *sensu lato*) sólo pueden ser caracterizados por las distintas etapas de ooquistes presentes en el huésped invertebrado (Levine, 1982; Barnard y Upton, 1994), solamente podemos identificar estos parásitos como hemogregarinas, sin llegar a concretar el género. Lo mismo ocurre en otros trabajos sobre la carga parasitaria en lacértidos (Amo et al, 2005; Huyghe et al, 2010), aun cuando el objetivo fundamental de alguno de estos estudios es la identificación sistemática del parásito (Roca y Galdón, 2010). Son estos mismos autores (Roca y Galdón, 2010) los que sugieren que los parásitos sanguíneos de los lacértidos de la Península Ibérica y las Islas Canarias sean actualmente clasificados dentro de los géneros *Hepatozoon* o *Hemolivia*. Las hemogregarinas observadas en esta investigación son tentativamente asignadas a estos dos géneros, debido a que una identificación más profunda presenta serias dificultades (Roca y Galdón, 2010). Para una correcta identificación se requeriría observar su presencia en un hospedador intermediario y/o estudiar una fase enquistada del parásito en otros órganos de las lagartijas (Carbonell y Ferrer, com. pers.)

Las hemogregarinas muestran un ciclo indirecto, incluyendo, al menos, dos hospedadores: un huésped final, vertebrado, en el que aparecen en la forma de gametocitos en las células de la sangre, principalmente en los eritrocitos (en muchos casos, esta es la única fase conocida del parásito; ver Telford, 2008); y un vector invertebrado. En lacértidos, los vectores más comunes son las garrapatas y los ácaros (Telford, 2008), adquiridos por los individuos cuando comparten lugares adecuados para tomar el sol, forrajear u ocultarse. Otro método aceptado de transmisión de hemogregarinas es el de la ingestión, ya sea a través de la depredación sobre otros huéspedes vertebrados infectados o por ingestión directa del vector invertebrado (véase, por ejemplo, Telford, 2008).

Aunque los hematozoos son comunes en reptiles (Mackerras, 1961; Smith, 1996), existe poca información sobre cómo afectan al hospedador. En general, los efectos de las hemogregarinas en sus hospedadores han sido hasta el momento poco estudiados y, al parecer, sólo unos pocos trabajos han evaluado la carga parasitaria en lagartijas insulares del género *Podarcis* (Galdón et al., 2006; Roca et al., 2009; Huyghe et al., 2010). En un aspecto puramente fisiológico, los gamontes (etapa de su ciclo de vida involucrada en la reproducción sexual) de las hemogregarinas pueden desplazar el núcleo de los eritrocitos e inhibir la función normal de las células (Smith, 1996), destruyendo los glóbulos rojos y causando anemia (Caudell et al., 2002). Los individuos infectados con hematozoos responden liberando eritrocitos inmaduros en la circulación, presumiblemente para contrarrestar la disminución en la capacidad de transportar oxígeno debido al daño causado a los eritrocitos maduros (Schall et al., 1982; Schall, 1983; Oppliger et al., 1996; pero ver también Brown et al., 2006). Además, la presencia de gametocitos en células de la sangre indica también la presencia de esquizontes en órganos internos, donde causan daños adicionales (Svahn, 1974).En infecciones intensas o en huéspedes no bien adaptados o que padecen un estrés adicional, se manifiesta la enfermedad clínica, ya sea debido a la patología causada por las distintas etapas de la infección en los tejidos o debido a la anemia hemolítica (Keymer, 1981; Smith et al., 1996; Wozniak et al., 1996).En condiciones de laboratorio, los parásitos de la sangre pueden, incluso, causar la muerte de las lagartijas (Schall, 1996), aunque no tenemos suficiente información para determinar si esto ocurre en las poblaciones naturales. Así, por ejemplo, en un estudio en *Z. vivipara* los parásitos de la sangre parecen no afectar a la supervivencia (Sorci et al., 1996). También han sido descritas otras consecuencias puramente fisiológicas del endoparasitismo, entre las que destacamos una mayor tasa metabólica en reposo (Oppliger et al., 1996).

El impacto de los parásitos sanguíneos sobre el hospedador ha sido analizado sobre distintos componentes de la aptitud biológica y sobre distintos rasgos vitales. Se han relacionado las hemogregarinas con un aumento del esfuerzo reproductivo en *Z. vivipara* (Sorci et al., 1996). También hay relación negativa entre el parasitismo y la velocidad máxima de carrera (Oppliger et al., 1996), aunque otros autores no fueron capaces de hallar dicha relación (Schall et al., 1982). Sin embargo, Oppliger et al. (1996) sí que hallaron una influencia negativa del parasitismo sobre la resistencia en carrera. Oppliger y Clobert (1997) también en *Z. vivipara* detectaron una disminución de la capacidad de regeneración de la cola ligada al parasitismo. De igual manera, se han documentado sutiles cambios de comportamiento relacionados con la carga parasitaria (Schall et al., 1982). Las hembras de *Z. vivipara* que estaban parasitadas durante la temporada de primavera tuvieron una tasa de supervivencia similar pero una menor tasa de recaptura que las no parasitadas durante el verano (Sorci et al., 1996). Bouma et al., (2007) encontraron una relación entre el grado de parasitación de los individuos y su distribución espacial. Los machos infectados muestran un reducido estatus social, un menor número de cortejos y exhiben un comportamiento menos territorial que los no infectados (Dunlap y Schall, 1995).

Para obtener las muestras de sangre, realizamos un pequeño corte longitudinal en el lado dorsal y proximal de la cola con un bisturí estéril. Con la gota de sangre que se obtuvo, se llevó a cabo un frotis sanguíneo. A veces, las muestras de sangre se obtuvieron tras cortar una pequeña porción de las puntas de cola (que se utilizó posteriormente para la extracción de ADN). Los frotis de sangre se colocaron en portaobjetos y se secaron al aire en el campo. En el laboratorio, los portaobjetos se fijaron con metanol absoluto durante 10 minutos y después se tiñeron con Giemsa modificado durante 20 minutos. Las muestras se analizaron usando un microscopio óptico a 400x. Los parásitos de la sangre sólo fueron identificados como hemogregarinas (véase más arriba). La intensidad de parasitismo se estimó en un total de 2.000 células contadas por muestra y la prevalencia, como el porcentaje de individuos infectados en la zona de estudio.

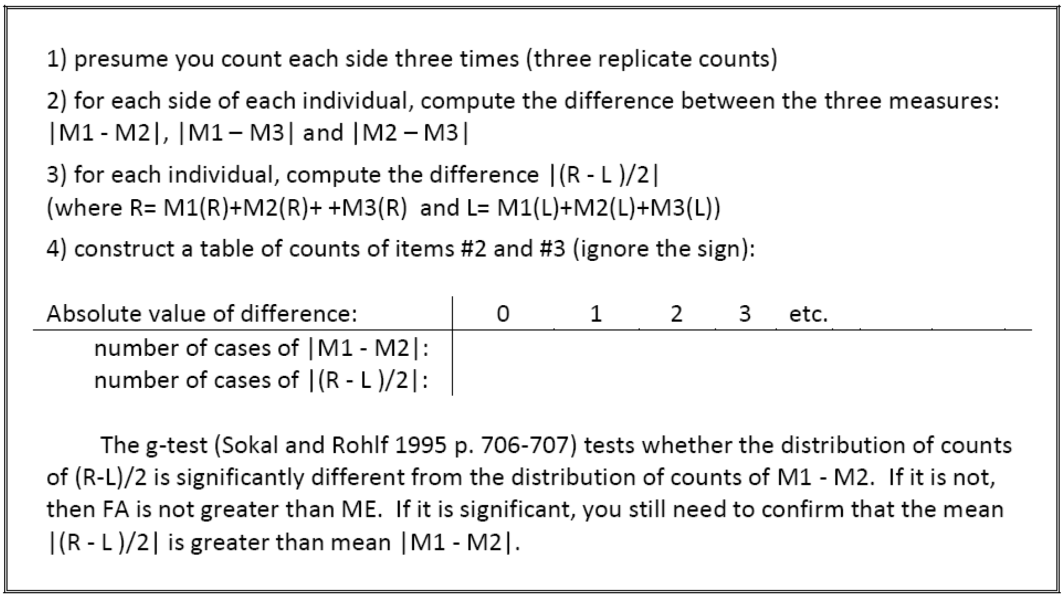
3.4. Asimetría fluctuante

Lerner (1954) define homeostasis genética como “la propiedad de una población para equilibrar su composición genética y resistir así los cambios repentinos” siendo, de este modo, el investigador que estableció las bases de lo que hoy conocemos como estabilidad en el desarrollo. Actualmente no hay consenso en el uso de una sola definición de la estabilidad de desarrollo y están en uso varias definiciones (Debat y David, 2001). En 1992, Zakharov propone que es “la capacidad de un determinado genotipo para producir el mismo fenotipo en ambos lados del cuerpo bajo un amplio rango de condiciones ambientales”, ya en el 2000, Van Dongen y Lens definen la estabilidad del desarrollo como “la capacidad de un individuo para amortiguar su desarrollo frente a perturbaciones aleatorias” (Debat y David, 2001). Entre ambas definiciones, Palmer (1994), Møller y Swaddle (1997), Clarke (1998) o Auffray et al. (1999) dan otras (Debat y David, 2001). Se supone, en general, que la inestabilidad podría surgir como consecuencia de una imperfecta adaptación al medio ambiente, debido a fenómenos como la mutación, la coevolución huésped-parásito, la evolución sexual antagónica u otros procesos (Møller y Swaddle, 1997). Desde otro punto de vista, la función de la estabilidad del desarrollo es modular los efectos de las perturbaciones durante la ontogenia de un rasgo mediante un número de mecanismos diferentes. Éstos incluyen mecanismos de retroalimentación y otros que pueden estabilizar los procesos de crecimiento (Møller, 2006). Se han propuesto varias medidas para estimar el impacto de la inestabilidad en el desarrollo; entre ellas, la que actualmente centra más la atención de los investigadores es la asimetría fluctuante (FA, en sus siglas en inglés) (Møller y Swaddle, 1997).

La FA corresponde a las diferencias pequeñas y aleatorias entre las expresiones derecha e izquierda de un carácter o rasgo, que de otro modo sería bilateralmente simétrico (Van Valen, 1962). La simetría de cualquier estructura bilateral refleja, presumiblemente, la estabilidad del desarrollo de un organismo y, por lo tanto, su habilidad para compensar las perturbaciones genéticas y ambientales (Møller y Swaddle, 1997). Así, la asimetría fluctuante puede considerarse una medida de la inestabilidad del desarrollo expresada como desviaciones no direccionales a partir de una simetría bilateral (Van Valen, 1962) o como la habilidad de un genotipo para mostrar el desarrollo estable a pesar del estrés ambiental o genético (Møller y Swaddle, 1997). La FA debe distinguirse de otras formas de asimetría, como la asimetría direccional (DA), que se define como y la llamada antisimetría (AS). La DA se produce cuando un lado de un rasgo es propenso a desarrollarse más que el otro (Van Valen 1962). En los casos de asimetría direccional es posible predecir qué lado del rasgo será más grande antes, por lo que debe haber algún elemento genético que lo controle (revisión en Palmer y Strobeck, 1986). Por lo tanto, dentro de una población, existe un sesgo para que un lado de un rasgo sea más grande que el otro. En un histograma de frecuencias de asimetrías (valor derecho menos izquierdo) de los valores de la población, observaremos una gran inclinación hacia la izquierda o la derecha, la dirección de la inclinación se da en función dl rasgo estudiado y de la especie. La AS se produce cuando un lado de un carácter es más grande que el otro, pero no hay ningún sesgo en cuanto a qué lado será más grande (Timofeeff-Ressovsky, 1934 en Møller y Swaddle, 1997). Por lo tanto, no es posible predecir qué lado del carácter se desarrolle más que el otro La antisimetría muestra una distribución de frecuencias platicúrtica (es decir, de amplio pico) o bimodal dentro de una población.

Los tres términos, FA, DA y AS refieren a los patrones de variación en un rasgo particular que exhibe una muestra de individuos. Son necesarias técnicas estadísticas adecuadas para diferenciar entre los tres tipos de asimetría, así como para establecer qué rasgos cumplen los requisitos de la asimetría fluctuante. La aplicación de estas técnicas estadísticas es variada en la literatura y aún continúa evolucionando. Igualmente, mientras hace unos años se trabajaba con el valor de asimetría de un solo rasgo, o de varias rasgos de forma separada, hoy se aconseja el uso de índices compuestos, ya que los análisis que combinan la información de varios rasgos demuestran ser detectores más fiables del stress (Leung et al., 2000).

En este estudio, para los rasgos métricos y merísticos donde las diferencias absolutas entre los lados derecho e izquierdo (| R - L |) son grandes (4-5 unidades), aplicamos el test propuesto por Palmer (1994) y Palmer y Strobeck (1986) para determinar si la variación entre lados es significativamente mayor que el error de medida (ME). La prueba es un ANOVA de dos vía (lados x individuos), y debe llevarse a cabo de forma rutinaria como parte de cualquier estudio de FA (Palmer y Strobeck, 1986). Esto procedimiento prueba la significación de la variación de dicho rasgo entre ambos lados con respecto al error de medida. Una ventaja del procedimiento de ANOVA factorial es que, al mismo tiempo, se puede examinar la significación de la DA. Para los rasgos merísticos, donde la diferencia entre los lados es pequeña (1-2 unidades) y donde puede existir un bajo error medida, la técnica del ANOVA no funciona correctamente (Palmer, 1994). Palmer (1994 y com. pers.) recomienda realizar una prueba de máxima verosimilitud o test de la G (Sokal y Rohlf, 1995). Para obtener una estimación de la significación estadística de la variación entre ambos lados en relación al error de medida se han de comparar los recuentos de asimetría dentro de cada nivel (por ejemplo, -1, 0, +1) con los correspondientes recuentos del error entre repeticiones (por ejemplo, -1, 0, +1) (Tabla 2). Si la prueba es estadísticamente significativa, es decir, la variación entre lados y el error de medida son distintos, todavía es necesario confirmar que la media de la diferencia entre todas las medidas (|Mx-Mi|/n) es menor que la media de las diferencias entre ambos lados del carácter (|(R-L)/n|), donde "n" es el número de réplicas. En estos casos, DA debe ser estimado con un test de la t de la media de la diferencia entre ambos lados (R-L) (Palmer, 1994).



1) presume you count each side three times (three replicate counts)

2) for each side of each individual, compute the difference between the three measures:
|M1 - M2|, |M1 – M3| and |M2 – M3|

3) for each individual, compute the difference |(R - L)/2|
(where R= M1(R)+M2(R)+ +M3(R) and L= M1(L)+M2(L)+M3(L))

4) construct a table of counts of items #2 and #3 (ignore the sign):

Absolute value of difference:	0	1	2	3	etc.
number of cases of |M1 - M2|:					
number of cases of |(R - L)/2|:					

The g-test (Sokal and Rohlf 1995 p. 706-707) tests whether the distribution of counts of (R-L)/2 is significantly different from the distribution of counts of M1 - M2. If it is not, then FA is not greater than ME. If it is significant, you still need to confirm that the mean |(R - L)/2| is greater than mean |M1 - M2|.

Tabla 2. Desarrollo del test de la g para comprobar la significación de FA vs ME en valores merísticos en los que la diferencia entre los lados es pequeña (1-2 unidades) (Palmer, 1994 y com pers.).

Típicamente, AS muestra una distribución de frecuencias platicúrtica o bimodal en una población dada, mientras DA presenta un sesgo (Møller y Swaddle, 1997). Para comprobar el sesgo y la curtosis usamos las pruebas de Anscombe-Glynn y D'Agostino, respectivamente (Zar, 2010). Por último, Palmer y Strobeck (1986) sugieren buscar la dependencia de FA con el tamaño del cuerpo, y la corrección de ésta dependencia si es necesario. Pero, sin embargo, Palmer (1994) observó que el tamaño corporal puede reflejar algún aspecto de la condición. De este modo, al corregir FA de acuerdo al tamaño de un rasgo podemos estar modificando una FA que sea quizá sea independiente del tamaño del rasgo. De acuerdo con ello, se optó por evitar la corrección de la dependencia del tamaño, porque éste podría estar correlacionado con los factores en estudio y la corrección puede encubrir su asociación con la FA (Palmer y Strobeck, 1986; Bancila et al, 2010).

Inicialmente, la FA se definió como un parámetro poblacional, aunque actualmente se aplica también como un indicador de la calidad del individuo (Møller y Swaddle, 1997). A nivel poblacional, las pequeñas islas del Mediterráneo constituyen el escenario perfecto para estudiar los efectos que los factores genéticos y ecológicos tienen sobre la estabilidad del desarrollo, dado que hospedan poblaciones aisladas en ecosistemas relativamente sencillos. Además, la inestabilidad de desarrollo medido como FA es, probablemente, más evidente en poblaciones de pequeñas islas, ya que soportan más estrés medioambiental (MacArthur y Wilson, 1967) y más estrés genético, debido a sus altos niveles de consanguinidad genético (Frankham, 1998), siendo por ello, además, más susceptibles al estrés ambiental. Las poblaciones consanguíneas pueden sufrir determinados cambios en el medio ambiente que las poblaciones no endogámicas perciben como no estresantes, lo que indica que el estrés genético y el ambiental pueden interactuar (Kristensen et al., 2006). Hay pruebas de que dicha interacción puede conducir a la sinergia entre ambos tipos de estrés, provocando que condiciones ambientales que normalmente son benignas puedan ser perjudiciales (Kristensen et al., 2006). Como grupo, los ectotermos son ideales para estimar estos dos tipos de estrés como potencial generador de FA (Leary y Allendorf, 1989). Dentro de éstos, las lagartijas combinan rasgos métricos (por ejemplo, las patas traseras o las dimensiones de la cabeza) y merísticos (escamas, poros femorales, laminillas subdigitales) relativamente fáciles de medir, (Soulé, 1967) y son excelentes modelos para los estudios ecológicos o eco-morfológicos.

Desde un punto de vista genético, la teoría supone una correlación positiva entre la FA y los niveles de endogamia, puesto que aumenta la homocigosis (Møller y Swaddle, 1997). En ausencia de intervención humana, el flujo genético entre las islas es limitado o inexistente. Crnobrnja-Isailovic et al. (2005) argumentaron que las condiciones intrínsecas de poblaciones aisladas (por ejemplo, los efectos de cuello de botella derivados de su fundación, una fuerte selección a largo plazo, el pequeño tamaño efectivo de la población o los efectos del hacinamiento), pueden conducir a altos niveles de endogamia, a períodos de intensa selección direccional y a un grado de homocigosis muy elevado. En consecuencia, el equilibrio genómico podría romperse y la capacidad del organismo para amortiguar la aparición de errores aleatorios en su expresión se reduciría. En definitiva, la teoría sugiere una correlación positiva entre la FA y los niveles de endogamia, porque ésta aumenta la homocigosis (Møller y Swaddle, 1997).

El grado de asimetría fluctuante puede emplearse como indicador del estrés ambiental al que está sometida una población. De hecho, Soulé (1979) demostró que, en poblaciones insulares, la asimetría fluctuante parecía directamente relacionada con el grado de estrés ambiental (ver también Gilligan et al., 2000; Valentine et al., 1973). Incluso, se ha llegado a afirmar que ciertas medidas individuales de la estabilidad del desarrollo, como la asimetría fluctuante, brindan una estimación fiable de la calidad del hábitat (Carbonell y Tellería, 1999), mayor si cabe que la derivada del tamaño de la población (Clarke, 1992). Entre los factores no genéticos, la densidad ha sido una de las más estudiadas. Møller y Swaddle (1997) y Møller y Manning (2003) presentan evidencias de un diverso grupo de taxones y concluyen que la densidad de población se traduce en mayores niveles de FA, presuntamente generados porque la disponibilidad de recursos se reduce según aumenta la densidad y debido a que la competencia depende de ella. Las enfermedades también parecen estar estrechamente relacionadas con el grado de asimetría (Møller, 2006). Asimismo, algunos estudios relacionan la FA con el desarrollo a una temperatura subóptima (Ji et al., 2002; Qualls y Andrews, 1999). También parece existir una estrecha relación con la escasez de recursos o con contaminantes ambientales como determinados compuestos químicos. Se pueden ver más ejemplos de éstas y otras causas ambientales generadoras de stress en Møller y Swaddle (1997).

De acuerdo a la literatura, la asimetría afecta a determinadas aptitudes relacionadas con, como la velocidad de escape (Martín y López, 2001; López y Martín, 2002); en *P. sicula*, se encontró una disminución significativa en la capacidad de esfuerzo (tiempo hasta el agotamiento corriendo de forma continua), en individuos con mayores valores de asimetría fluctuante (Vervust et al., 2008; pero véase también Warner y Shine, 2006). De igual manera, el apareamiento y el éxito reproductivo parecen guardar relación con la inestabilidad en el desarrollo (Martín y López, 2000; Martín y López, 2006) y Møller y Swaddle (1997) lo relacionan también con los mecanismos de señalización animal. En ese mismo trabajo se pueden consultar otros muchos ejemplos.

Sin embargo, determinados aspectos de la FA están sujetos a un intenso debate. La polémica más importante surge en torno a su heredabilidad (Møller y Thornhill, 1997 y las respuestas de otros autores incluidas en el mismo número de la revista). Otros aspectos también despiertan dudas. Por ejemplo, algunos autores no fueron capaces de encontrar la relación establecida entre densidad y FA, parcialmente (Wiener y Rago, 1987) o totalmente (Kruuk et al., 2003). Bjorksten et al. (2001) concluyeron que la asimetría fluctuante es un mal indicador de estrés en el desarrollo y la calidad genética. Warner y Shine (2006) no lograron hallar un efecto negativo de la FA sobre las capacidades locomotoras. Es decir, la literatura sobre asimetría fluctuante está llena de ejemplos en los que los supuestos efectos deletéreos sobre de los individuos no se encuentran, o en los que los supuestos factores ambientales o genéticos no conllevan un aumento del grado de asimetría de las poblaciones o de los individuos. La FA es, sin duda, y de acuerdo con la literatura, el indicador menos fiable de los cuatro usados en este estudio, probablemente debido a las amplias expectativas generadas inicialmente y por el corto recorrido científico de este campo.

3.5. Relación entre indicadores

Aparte de las citadas relaciones establecidas entre estos cuatro indicadores y sus efectos sobre distintos componentes y rasgos vitales, la literatura revela varios ejemplos de interrelación entre ellos. En lacértidos, Sorci y Clobert, (1995) relacionan las elevadas tasas de parasitismo con una condición corporal más pobre. Basándonos en el hecho de que los parásitos compiten con el anfitrión por los recursos, a esa relación también se puede incorporar la respuesta inmune. La pérdida de condición corporal implica una disminución de los recursos destinados a la defensa contra infecciones parasitarias, ya que el estado nutricional puede influir en la capacidad de las lagartijas para desarrollar una respuesta inmune eficiente (Cooper et al., 1985; Smallridge y Bull, 2000). Asimismo, el sistema inmune y los niveles de parasitación parecen relacionados indirectamente mediante distintos mecanismos. La hipótesis del hándicap de la inmunocompetencia (Zahavi, 1975; Folstad y Karter, 1992; Wedekind y Folstad, 1994) sugiere que los andrógenos circulantes (u otros componentes bioquímicos) regulan la expresión de los caracteres sexuales secundarios y reducen la eficacia del sistema inmune y, por tanto, suprimen la capacidad de los individuos para montar una respuesta inmune adecuada contra los parásitos. Por otra parte, es bien sabido que las condiciones estresantes tienen un efecto inmunosupresivo y pueden afectar a la resistencia a los parásitos (Oppliger et al., 1998). Las conexiones entre el estrés, los glucocorticoides, y la inmunidad están bien establecidos; los receptores de glucocorticoides están presentes en los tejidos linfáticos y leucocitos en todo el cuerpo, y el estrés induce modificaciones del sistema inmune (French et al., 2010 y referencias incluidas). No obstante, las relaciones específicas entre el estrés y la inmunidad varían considerablemente según el contexto. Así, por ejemplo, un gran número de estudios ha demostrado inmunosupresión bajo condiciones de estrés crónico, mientras que otros revelan que el estrés agudo puede en realidad aumentar la eficacia de la respuesta inmune Moore, 2008 y French, 2010 y referencias incluidas). De esta forma, la noción de la inmunosupresión inducida por el estrés es demasiado simplista y los efectos exactos varían ampliamente dependiendo de varios factores: el tipo de agente estresante, la duración del estrés, la respuesta inmune específica medida, y la condición energética y/o reproductiva de los individuos (Moore, 2008 y French, 2010 y referencias incluidas).

De entre todos los indicadores, la relación de la asimetría con los otros tres es la menos obvia y no cuenta con tanto apoyo experimental. Recientemente, se ha propuesto que la FA puede ser un estimador la calidad individual (revisión en Møller y Swaddle, 1997; pero véase también Palmer, 1999 y Simmons et al., 1999). Esta propuesta se basa en la suposición de que la FA es una medida sensible de estrés ambiental y genético, y ha llevado a la predicción de que debe covariar negativamente con la condición corporal (Møller, 1990; Møller y Pomiankowski, 1993; Watson y Thornhill, 1994). Diversos autores relacionan negativamente la FA con el parasitismo (Møller, 1996; Brown y Brown, 2002 y referencias incluidas) y la función inmune, alegando al menos tres razones diferentes (Møller, 1996). En primer lugar, una mala condición puede conducir tanto a un elevado nivel de asimetría como a una alta prevalencia de infecciones parasitarias (tabla 1 en Møller, 1996). En segundo lugar, si la capacidad competitiva de un individuo depende de la condición corporal, en igualdad de condiciones, los individuos que han sufrido inestabilidad durante su desarrollo a menudo se verían desplazados a ambientes más pobres con un mayor riesgo de encontrar parásitos. En tercer y último lugar, los parásitos pueden explotar los recursos limitados de sus anfitriones alterando de esta forma el óptimo desarrollo, causa directa del aumento en los niveles de asimetría.

De este modo, la función inmune puede estar negativamente relacionada con la FA en la población, basándonos en la pérdida de condición corporal (van Noordwijk y de Jong, 1986; Møller y Saino, 1994). Esta correlación negativa se ha encontrado tanto en rasgos bilaterales de carácter ornamental de mamíferos e insectos (Lagesen y Folstad, 1998; Rantala et al., 2000), como en rasgos relacionados función locomotora en insectos (Rantala et al., 2004). Estudios previos confirman que tanto el parasitismo en diversos taxones (Brown y Brown, 2002; revisión en Møller, 1996) como la activación del sistema inmune en aves (Fair et al., 1999, Whitaker y Fair, 2002) aumentan el nivel de FA en los individuos. Møller, (1996) y Møller y Swaddle (1997) concluyen que la regulación del sistema inmune puede desestabilizar el desarrollo de los individuos parasitados, explicando así la asociación encontrada entre el parasitismo, la aptitud biológica y el grado de asimetría.

La relación directa entre la defensa inmune y la estabilidad de desarrollo no ha sido profusamente investigada, aunque algunos autores han encontrado una correlación negativa. Un nexo entre ambos se daría a través del grado de homocigosis, que afectaría a ambos indicadores, aumentando el nivel de asimetría y perjudicando la capacidad de respuesta inmune (Møller y Thornhill, 1997; Møller, 2006 y referencias en ellos). Otra alternativa lo explica a través de las malas condiciones ambientales que aumentan la asimetría fluctuante y otras medidas de la inestabilidad del desarrollo. Las condiciones ambientales adversas también son conocidas por reducir la inmunocompetencia (Chandra y Newberne, 1977; Gershwin et al., 1985), y una asociación directa entre la inestabilidad en el desarrollo y el parasitismo es así probable. Hay otras muchas teorías, pero ninguna cuenta con una sólida demostración empírica. Por ejemplo, Møller y Thornhill (1997) defienden que es posible que las hormonas sexuales sean un factor mediador de las relaciones entre la defensa inmune y la estabilidad de desarrollo.

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Capítulo 2

Prevalencia e intensidad de parásitos sanguíneos en lagartijas insulares

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Prevalencia e intensidad de parásitos sanguíneos en lagartijas insulares

Resumen

Los parásitos son importantes para comprender la dinámica poblacional y la ecología de los hospedadores. En muchas islas del Mediterráneo, poco se sabe acerca de los parásitos sanguíneos de las lagartijas. En este trabajo estudiamos las relaciones entre los hemoparásitos y su hospedador, la lagartija endémica *Podarcis lilfordi* (Günther, 1874) en la isla del Aire (Menorca, Islas Baleares, España). La intensidad de la infección fue aproximadamente del 1% de los eritrocitos de la sangre y la prevalencia del 95%. Estos valores son más altos que en otras poblaciones continentales del género *Podarcis*. Proponemos que la reducida variabilidad genética y / o la alta densidad de población en las islas explican estas diferencias. Una mayor proporción de adultos que de jóvenes estaban infectados, probablemente porque estuvieron más expuestos a los parásitos durante su vida. Entre las lagartijas adultas, la prevalencia fue mayor entre los machos, lo que puede ser la consecuencia de presentar unos niveles de actividad más altos y por el mayor número de contactos físicos durante las interacciones macho-macho. También encontramos que los individuos infectados eran más grandes que los no infectados. La mayor tasa de prevalencia en adultos es responsable de estas diferencias. La intensidad de parasitación fue similar entre los distintos grupos de edad y sexo. La prevalencia se mantuvo estable entre temporadas, mientras que la intensidad de parásitos aumenta en verano, cuando la disponibilidad de recursos y la condición corporal disminuyen. Hemos encontrado una relación entre la prevalencia, la intensidad y la condición corporal, presentando una mayor prevalencia e intensidad parasitaria aquellos individuos con peor condición.

Prevalence and intensity of blood parasites in insular lizards

Abstract

Parasites are important in understanding population dynamics and ecology of hosts. In several Mediterranean islands, little is known about lizards' blood parasites. We studied the relations between haemoparasites and an endemic lizard host, *Podarcis lilfordi* (Günther, 1874) on Aire Island (Menorca, Balearic Islands, Spain). The infection intensity was about 1% of the red blood cells and 95% of the individuals were infected. These values are higher than in continental populations of the genus *Podarcis*. The reduced genetic variability and/or the high density in islands are proposed to explain such differences. More adults than juveniles were infected, probably because they were more often exposed to parasites during their lifetime. In adult lizards, prevalence was higher in males, may be as a consequence of their higher activity levels and more frequent physical contacts during male-male interactions. We found that infected individuals were larger than uninfected ones. A higher prevalence in adults is responsible of these differences. The intensity of infection was similar between age and sex classes. Prevalence remains stable between seasons, while parasite load increases in summer, as the availability of resources and body condition decreases. We found a relation between prevalence, intensity and body condition, with a higher prevalence and parasite load in individuals with lower condition.

Keywords: lizard; haemogregarines; island; host-parasite system; body condition.

1. Introduction

Parasites compete with hosts for resources, causing adverse effects on many aspects as their population growth and regulation (Holmes, 1995; Hudson et al., 1998), spatial distribution (Price, 1980; Bouma et al, 2007), reproductive success (Schall, 1996) and sexual selection (Hamilton and Zuk, 1982). The parasite-host system shows a temporal and spatial flexibility reflected in the variation of prevalence and parasite infestation within a species or population. The parasitism on *Podarcis* lizards has been studied in several populations, but in few of them on blood parasites [*Podarcis bocagei* (Seoane, 1884) and *Podarcis carbonelli* Pérez-Mellado, 1981 (Roca and Galdón, 2010), *Podarcis muralis* (Laurenti, 1768) (Amo et al., 2005) and some introduced populations of *P. muralis* and *Podarcis sicula* (Rafinesque-Schmaltz, 1810) in North America (Burke, 2007)].

The haemogregarines (Apicomplexa: Adeleorina), are protozoan parasites of a wide variety of species, including reptiles. Blood parasites from lacertid lizards of the Iberian Peninsula and Canary Islands are currently classified within the genera *Hepatozoon* or *Hemolivia* (Roca and Galdón, 2010). The haemogregarines observed in this study are tentatively assigned to these two genera, because a deeper identification presents serious difficulties (Roca and Galdón, 2010). Haemogregarines shows an indirect cycle, including, at least, two hosts: a final vertebrate host, where they appear in the form of gametocytes in the blood cells, mostly in erythrocytes (in many cases, this is the only known phase of the parasite, see Telford, 2008) and an invertebrate vector. In lizards the most common vectors are ticks and mites (Telford, 2008), acquired when lizards share suitable places to bask or hide. The effects of haemogregarines in their carriers have been little studied, but, at least, we know that they destroy red blood cells causing anemia (Caudell et al., 2002). Furthermore, the presence of gametocytes in blood cells indicates the presence of schizonts in internal organs, causing additional damages (Svahn, 1974). Under laboratory conditions, blood parasites can even cause the death of the lizards (Schall, 1996). We do not have enough information to determine whether this occurs in natural populations.

Apparently only few studies have assessed parasite load on insular *Podarcis* lizards (Galdón et al., 2006; Roca et al., 2009; Huyghe et al., 2010). However, insular areas are ideal scenarios to study the effects of ecological factors on life-history traits of organisms. In the absence of human intervention, gene flow between islands is limited or absent. Some lizard populations of the Balearic lizard, *Podarcis lilfordi* (Günther, 1874) in Menorca (Balearic Islands, Spain) live in small islets, with limited trophic resources and lacking predators (Pérez-Mellado, 1989). Thus, insular populations can attain higher densities than continental ones and, therefore, individuals can interact more frequently with conespecifics. We expect to find higher rates of parasitism in insular habitats than in continental populations. But, also, parasitism should be higher than in other insular populations coexisting with predators. Since the conditions are less favourable in summer at Mediterranean islands (Pérez-Mellado and Corti, 1993), we expect to find higher infection rates than during spring because individuals have fewer resources to cope with infestation. During four consecutive years, we studied the prevalence and intensity of haemoparasites in the Balearic lizard from Aire Island (Menorca). As in other small coastal islets, the parasite-host equilibrium probably suffered less disruption than in larger islands or continental areas.

2. Material and methods

P. lilfordi is an endemic lizard of the Balearic Islands. It is a medium-sized lacertid lizard (snout-vent length, SVL, to 81 mm. for males and to 75 mm. for females; Pérez-Mellado, 1998). The study was conducted during the spring and summer of years 2007, 2008, 2009 and 2010 in Aire Island, a small islet of Menorca. It is a typical Mediterranean coastal islet, with a vegetation highly influenced by its environmental characteristics (strong winds, high salinity and long drought periods). Vegetal cover is dominated by low shrubs and a variety of herbaceous species. Lizard densities can be very high, over 4,000 individuals per hectare (Pérez-Mellado et al., 2008b).

Lizards were collected by noosing. For each individual, we recorded age, sex, SVL, tail length and weight. Body condition was estimated from the residuals of the regression of log transformed weight versus log transformed SVL (e.g. Schall and Pearson, 2000 or García-Ramírez, 2005 and references therein). Following Schall and Pearson (2000), we grouped individuals according to their condition, creating a ranking variable of body condition of individuals above (positive residuals) or below (negative residuals) average body condition. Schall and Pearson (2000) refer to individuals as healthy or unhealthy, as their condition was "positive" (above average of the residuals) or "negative" (below average of the residuals).

To obtain blood samples, we made a slight cut in the dorsal side of the tail with a sterile scalpel. With the detached drop blood we obtained a blood smear *in situ*.

Sometimes, blood samples were obtained by clipping off the tail tip, using tail tips to extract DNA for other purposes. Specimens were always released at the site of capture. Blood smears on microscopic slides were air dried in the field. In the lab, slides were fixed in absolute methanol for 10 minutes and then stained in Giemsa for 20 minutes. Samples were analyzed using an optical microscope at 400X. The only blood parasites identified were haemogregarines (see above). The intensity of parasitism was estimated on a total of 2,000 cells. Prevalence was estimated as the percentage of infected individuals.

The statistical analyses have been carried out in R environment (ver. 2.12.1, R Development Core Team, 2010). To compare the prevalence of infestation among different groups of age and sex classes, body condition or seasons we used G or Fisher's exact tests, according to Zar (2010). Comparisons of the intensity of infection of lizards were done with one-way ANOVAs or Kruskal-Wallis tests.

3. Results

The prevalence of infestation by haemogregarines in *P. lilfordi* from Aire Island was 95.05% (499 of 525 individuals). We found significant differences in prevalence between adults and juveniles (G-test, G = 5.98, df = 1, p = 0.01), with a higher prevalence in adults than in juveniles (uninfected / infected: 20/463 and 6/36, respectively). Among adults, prevalence was also significantly different between sexes (G-test, G = 3.93, df = 1, p = 0.047, with a higher prevalence in males (uninfected / infected males and females: 8/289 and 12/174, respectively). There were no differences in prevalence between females and juveniles (G-test, G = 2.50, df = 1, p = 0.11; Fig. 1).

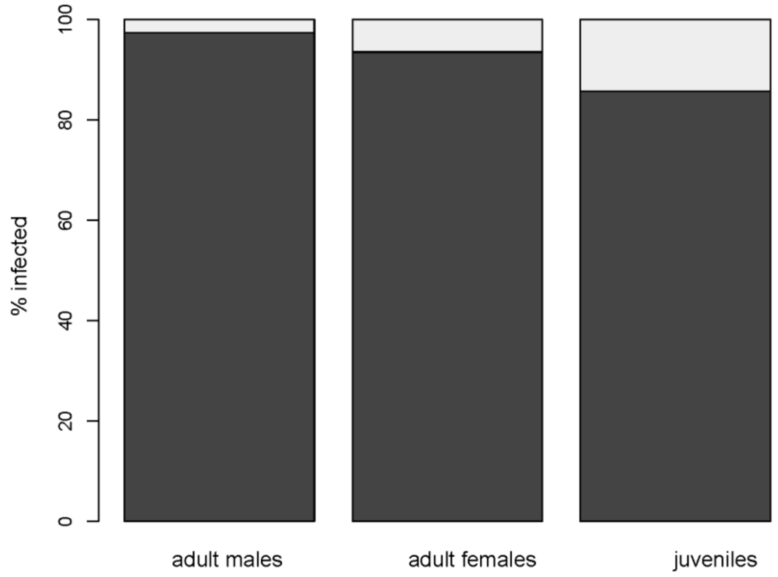

Figure 1. Percentage of adult males, adult females and juveniles infected and uninfected

In addition, we do not detected differences in the prevalence between spring and summer samples (Fig. 2), either for the whole set of individuals under study (G-test, G = 0.11, df = 1, p = 0.74), or within each age and sex category (males: Fisher's test, p = 0.49, females: Fisher's test, p = 0.56, juveniles, Fisher's test, p = 0.66). In addition, no differences were found among years (Fisher's test, p = 0.06).

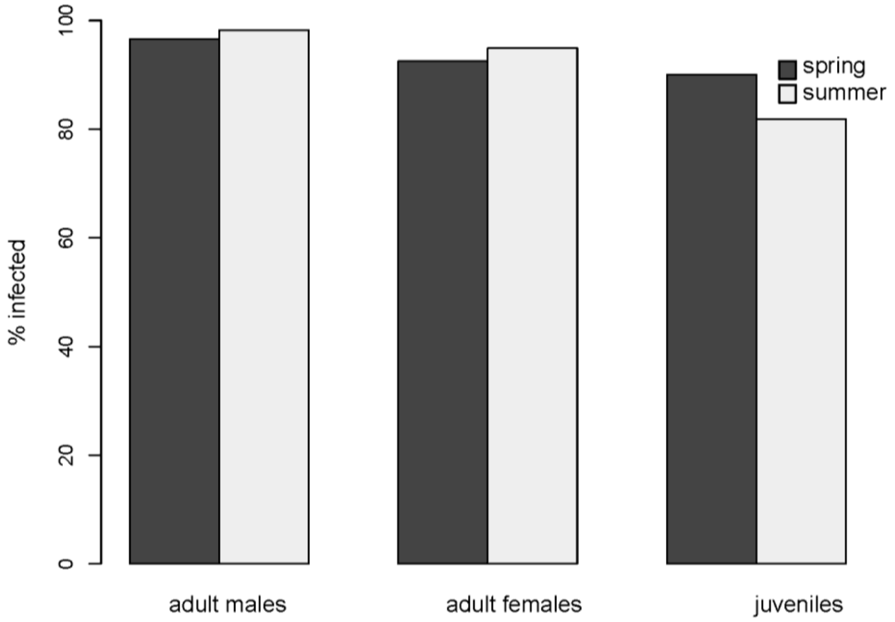

Figure 2. Percentage of adult males, adult females and juveniles infected in each season

The intensity of infection ranged from 0 to 351 infected cells in 2,000 observed red cells ($\bar{x}$±SE = 17.51±1.19). There were no differences in the intensity of infection between adults and juveniles (adults: $\bar{x}$±SE = 17.73±1.28; juveniles: $\bar{x}$±SE = 15.00±2.35; one-way ANOVA, $F_{1,\ 523} = 0.40$, $P = 0.53$), nor between each age and sex category (adult males: $\bar{x}$±SE = 17.93±1.33; adult females: $\bar{x}$±SE = 17.36±2.54; juveniles: $\bar{x}$±SE = 15.00±2.35; one-way ANOVA, $F_{2,\ 523} = 0.20$, $p = 0.82$). Preliminary comparative data for only two seasons showed that parasite load varied significantly between seasons (Fig. 3), being higher during summer (spring: $\bar{x}$±SE = 14.45±1.05; summer: $\bar{x}$±SE = 21.80±2.42; Kruskal-Wallis test, $\chi^2 = 7.28$, $p = 0.007$). In fact, the overall differences between seasons were due to those of adult males (Kruskal-Wallis test, $\chi^2 = 4.53$, $p = 0.03$). In females (one-way ANOVA, $F_{1,\ 184} = 1.95$, $p = 0.16$) and juveniles we did not find significant differences (Kruskal –Wallis test, $\chi^2 = 1.66$, $p = 0.20$).

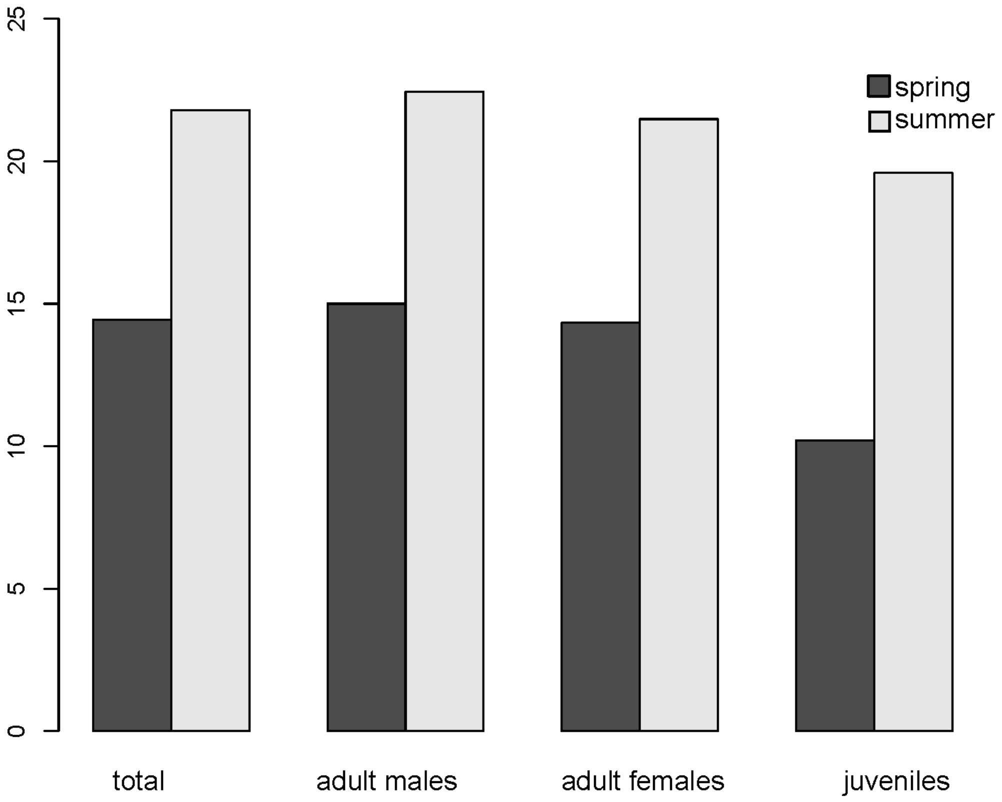

Figure 3. Mean of infected cells (in 2,000 erythrocytes) by season and age-sex categories

Infected individuals are larger than uninfected ones (SVL: infected $\bar{x}$±SE = 67.55±0.25, non infected $\bar{x}$±SE = 63.65±1.03; one-way ANOVA, $F_{1,\ 523} = 12.13$, $p <$

0.001; Fig. 4). Differences in prevalence between age and sex groups might be responsible for the differences in SVL between infected and non infected lizards. For this reason, we studied the relationship between body size and prevalence in males, females and juveniles separately. In this case, we found no relation between body size and prevalence, with only marginal differences in adult males (adult males: one-way ANOVA, $F_{1, 295} = 3.78$, $p = 0.05$; adult females: one-way ANOVA, $F_{1, 184} = 1.41$, $p = 0.32$; juveniles: one-way ANOVA, $F_{1, 40} = 0.20$, $p = 0.66$). Similarly, we found no relation between body size and intensity ($r^2 = 0.005$; $F_{1, 523} = 2.86$, $p = 0.09$).

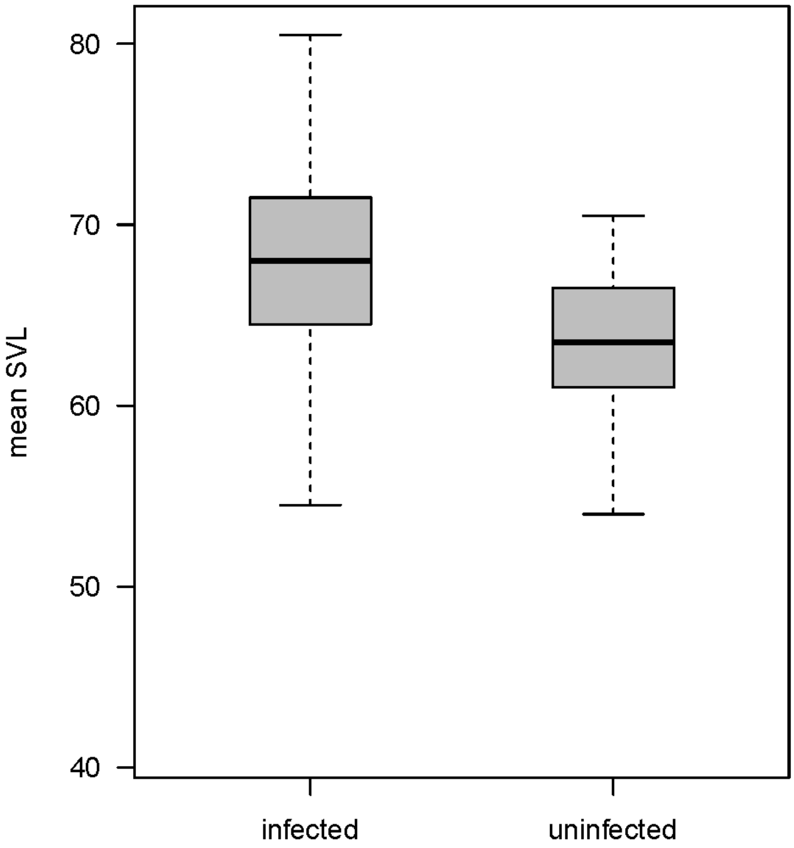

Figure 4. Mean (±SE) body size (mm) of infected and uninfected lizards

After removing the effect of SVL ($r^2 = 0.81$; $F_{2, 509} = 804$, $p < 2.2\text{x}10^{-16}$), body condition was significantly different between groups ($F_{2, 509} = 804$, $p < 2.2\text{x}10^{-16}$; Fig. 5), and the condition of lizards was lower in summer for the whole group of lizards under study ($F_{1, 503} = 177.06$, $p < 2\text{x}10^{-16}$), as well as for adult males ($F_{1, 288} = 50.07$, $p = 1.134\text{x}10^{-11}$), adult females: ($F_{1, 177} = 45.58$, $p = 2.02\text{x}10^{-10}$) and juveniles ($F_{1, 38} = 18.94$, $p = 9.82\text{x}10^{-5}$). Analyzing prevalence and condition category (see Material and

Methods), we found that prevalence was lower among individuals with better condition (positive residuals of the body condition) than from those lizards with a lower condition (negative residuals of the body condition), both in adult males and females (for adult males: Fisher's test, p = 0.01; for adult females: Fisher's test, p = 0.04). We also found differences in the intensity of infestation for all individuals (Kruskal-Wallis test χ^2 = 17.22, df = 1, p = 3.3x10^{-05}). We detected higher parasitemia among individuals with lower body condition. These differences were also found in adult males (Kruskal-Wallis test χ^2 = 13.36, df = 1, p < 0.001), but not separately in females and juveniles, although the number of infected cells was also higher in individuals with lower body condition.

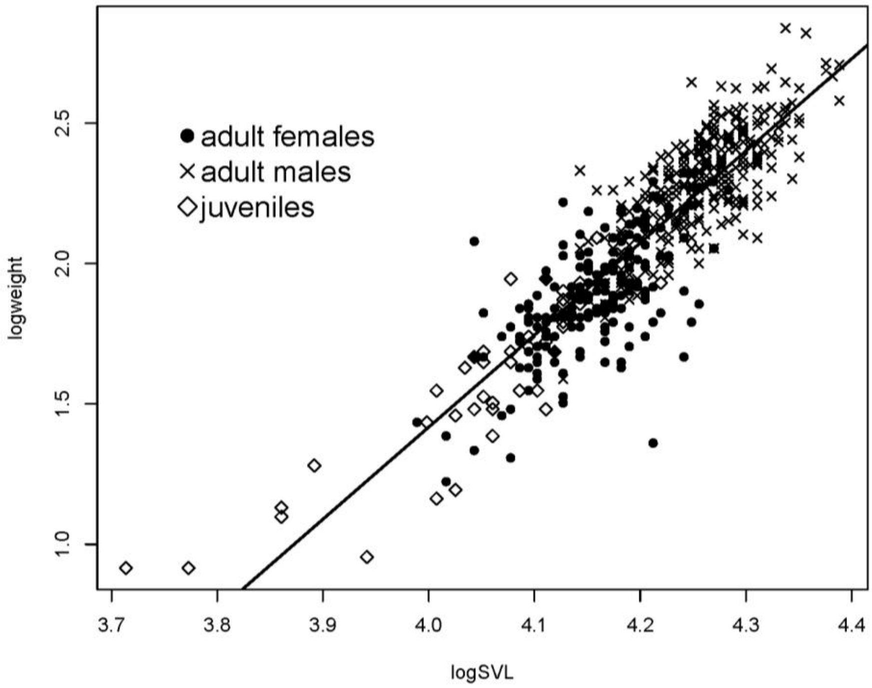

Figure 5. Relation between body size (mm.) and weight (gr.)

4. Discussion

This is the first study on blood parasites of the Balearic lizard. In fact, the Mediterranean lacertid lizards have been little studied from this viewpoint, especially in insular species. Prevalence of the Balearic lizard from Aire Island reached 95%, one of the highest even recorded (see references below). Only in the case of *Gallotia atlantica* (Peters and Doria, 1882) from Alegranza Island (Canary Islands, Spain), a lizard-blood parasite system with a prevalence of 100% was described (García-Ramírez et al., 2005).

Thus, the prevalence in *P.lilfordi* is higher than prevalence found in continental populations of lacertid lizards as *P. muralis*, (Amo et al. 2005), *P. bocagei*, or *P. carbonelli*, (Roca and Galdón, 2010, see Table 1). In Tenerife Island, Oppliger et al. (1999) found in *Gallotia galloti* (Oudart, 1839) a low prevalence of hematozoans (Table 1). Burke (2007) found no blood parasites in North American introduced populations of *P. muralis* and *P. sicula*, probably due to the absence of specific vectors and the recent introduction of these populations.

Specie	***origin***	***Prevalence (%)***	***Authors***
Podarcis lilfordi	*Insular*	*95%*	*Present study*
Gallotia atlantica	*Insular*	*100%*	*García-Ramírez et al., 2005*
Podarcis muralis	*Continental*	*58%*	*Amo et al., 2005*
Podarcis bocagei	*Continental*	*74.7%*	*Roca and Galdón, 2010*
Podarcis carbonelli	*Continental*	*69.7%*	*Roca and Galdón, 2010*
Gallotia galloti	*Continental*	*17.8 - 20.0%*	*Oppliger et al. 1999*
Podarcis muralis	*Continental*	*0 %*	*Burke, 2007*
Podarcis sicula	*Continental*	*0 %*	*Burke, 2007*

Table 1. Prevalence of infestation by blood parasites in different lacertid lizards' species

The higher prevalence observed in Aire Island could be related with the bottleneck effect and the degree of inbreeding, intrinsic to the origin of many insular populations. That is, the loss of genetic variability was proposed as one of the causes that can erode the resistance to parasitism (Hamilton et al., 1990). Particularly, in populations where individuals are closely related, the expected low level of genetic variability would facilitate a parasite found lower genetic resistance in the host (Shykoff and Schmid-Hempel, 1991). The lack of predation and competitors allow high lizard densities in several Mediterranean islets. In Aire Island the density reaches over 4,000 individuals per hectare (Pérez-Mellado et al., 2008b). Lizards' density can be an important factor, as it increases the exposure to mites of infected conespecifics, although the critical factor would be the density of the intermediate hosts (Svahn, 1974). The Balearic lizard lacks social organization, but the high density could act as a promoter of higher probabilities of infection, even if in some studies no relation between density and prevalence was found (Godfrey et al., 2006).

The prevalence of adults was higher than in juveniles. This result agrees with those obtained in *Iberolacerta cyreni* (Müller and Hellmich, 1937) by Amo et al. (2004), *Tiliqua rugosa* (Gray, 1825) (Smallridge and Bull, 2000) and *G. galloti* (Oppliger et al., 1999). Adults monopolize the best foraging and basking places where, probably, they are more exposed to vectors of blood parasites. Juveniles are normally located at less favorable areas. In fact, on Aire Island, the proportion of different age classes is significantly different at different areas of the islet (Pérez-Mellado et al., 2007; Garrido and Pérez-Mellado, submitted). In addition, parasite prevalence is significantly different between males and females (see similar results in Klein, 2004). Two hypotheses have been proposed to explain these differences. First, the higher prevalence in males of *P. lilfordi* may be related to an increased activity of males in comparison with females (Pérez-Mellado et al., unpublished data) as it was proposed for *Sceloporus occidentalis* Baird and Girard, 1852 (Tälleklint-Eisen and Eisen, 1999). Second, a higher prevalence may also be related to aggressive behaviour and male-male interactions, frequently observed in Aire Island. Such encounters often end up with males bite each other's the tail and even caudal autotomy and tail consumption by the opponent may occur (Pérez-Mellado et al., 1997). This behaviour would increase the chances of transmission of blood parasites between males, as in the case of *Gallotia stehlini* (Schenkel, 1901) (Matuschka and Bannert, 1989). The level of aggressiveness is mediated by high levels of testosterone. Thus, males are more likely to be infected than females, because of the immunosuppressive effects of testosterone (Klein, 2004). We found that infected lizards were larger than the uninfected ones. Amo et al. (2004) also found that prevalence was higher in larger *I. cyreni*. In a study conducted exclusively with adult individuals of *P. muralis*, no differences were found (Amo et al., 2005). In our case, observed differences were clearly due to a higher prevalence in adult males.

We found no significant differences in prevalence among the four years of study. The stability of prevalence for long periods of time was also detected in other species of reptiles (Smallridge and Bull, 2000; Salkled and Schwarzkopf, 2005). Between spring and summer, prevalence remained also stable for males, females and juveniles. No differences between seasons were found in other species (Amo et al., 2004, 2005; Godfrey et al., 2006). We suggest that some individuals were infected in previous years and maintained parasite infection, while new infestations occurred at the beginning of the activity period and then remained stable. The absence of differences between years and seasons would reflect the stability of parasite-host interaction in the system under study.

Regarding parasite load, the intensity was reduced to about 1% of the cells, lower than in other insular populations (García-Ramírez et al., 2005) and higher than in continental areas (Smallridge and Bull, 2000; Amo et al., 2005). Blood parasites do not reproduce within lizard red blood cells (Olsen, 1977). Therefore, as Bouma et al. (2007) suggest, hosts replace infected erythrocytes gradually and higher infection intensity might result from multiple reinfections. Thus, the high densities in islands may be the underlying reason because parasitic load in *P. lilfordi* is higher than intensity observed in *Podarcis* species from continental areas. Oppliger et al. (1998) showed that in captivity, after four weeks, parasite load of individuals living in overcrowded terraria was three times higher than in individuals from lower density terraria. Between groups, we found a similar intensity of infection by blood parasites in adults and juveniles, as it was in adult males and females. Amo et al. (2006) observed a similar situation in *Timon lepidus* (Daudin, 1802).

Body condition of individuals declined in summer for each category. Parasite load changed significantly for the overall population and for adult males separately, with higher values in summer. Juveniles and adult females showed a slight tendency to an increase in the intensity of infection. In lizard of continental populations, differences in parasite load between seasons were related with reproductive effort (Salvador et al., 1996). During the breeding season, males invest a large amount of energy in mating strategies, regulated by testosterone levels. Thus, males can experience an immunocompetence handicap during the breeding season, as suggested by Folstad and Karter (1992). However, in coastal islet around Menorca, mating period starts in February and can lasts until the end of July (Perera and Pérez-Mellado, 2004) and daily activity is almost continuous throughout the year. Therefore, another factor, other than reproductive effort, could be also responsible for the change in parasitic load and the loss of body condition.

In Mediterranean islets, trophic availability is limited (Pérez-Mellado and Corti, 1993; Pérez-Mellado, 1989) and the climate is characterized by strong short-term and seasonal fluctuations (Fuentes, 1984), leading to significant variations in food resources. During summer, the drought is strong at the islet under study, imposing a dramatic decrease in terrestrial arthropods. As a result, individuals could not have enough available food resources to maintain a suitable body condition to resist parasites, as they do during spring. In addition, during summer, the diet changes and plant matter consumption increases (Pérez-Mellado and Corti, 1993). In gut and intestinal parasites, the degree of herbivory is related with parasitism (Roca, 1999). There is no evidence of a direct relation in blood parasites and herbivory. But parasite vectors (acari) are more abundant in soils covered with vegetation (Resh and Cardé, 2003), thus, the exposure of lizards to vectors of haemogregarines is higher while feeding on plants where they are more abundant (Biaggini et al., 2009 and references therein). Consequently, temporal changes in parasite load may be due to changing levels of host immunity as a result of a lower body condition and/or a seasonal variation in exposure to vectors and subsequent reinfections (Telford, 1984 in Brown et al., 2006). Males show higher mobility (Pérez-Mellado et al., 2007) and interact more frequently with conspecifics (Salvador et al., 1996), probably leading to an increase in exposure to parasite's vectors from infected conspecifics (Klein, 2004). In spring, these contacts are also usual, but in summer, as the condition of lizards is lower, the ability to cope with reinfestation declines and, consequently, the virulence of the new infestations may become more evident.

According with categories proposed by Schall and Pearson (2000), we found a higher prevalence in lizards with a lower condition, probably due to their reduced ability to fight against infection (see similar results in Cooper et al., 1985; Smallridge and Bull, 2000; Amo et al., 2006). These differences did not appear in juveniles, probably they were less exposed to parasites because of a shorter time to be infected. In addition, juveniles are normally located at less favourable areas. We found a higher intensity infection in individuals of lower body condition only in adult males, reinforcing the hypothesis that male-male interactions increases their probability of reinfection.

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Capítulo 3

Patrones de parasitismo en lagartijas insulares

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Patrones de parasitismo en lagartijas insulares: efectos del tamaño corporal, el estado de condición y la disponibilidad de recursos

Resumen

Estudiar los patrones de parasitismo puede dar una idea de los procesos que mantienen las asociaciones parásito-hospedador y que son esenciales para entender la dinámica de las poblaciones de hospedadores y de su ecología. La distribución espacial de las lagartijas dentro de una población se ve influenciada por la distribución de recursos y por las capacidades individuales para acceder a ellos. En varias islas del Mediterráneo, donde la escasez de alimentos y la imprevisibilidad en la disponibilidad de recursos son la norma, algunos recursos particulares pueden ser muy importantes durante ciertos períodos del año. Este es el caso de rapa mosquera, *Dracunculus muscivorus*, en la isla del Aire, España. En este estudio, se analizó la infestación parasitaria por parásitos sanguíneos en un lagartija endémica, *Podarcis lilfordi*, en relación con la distribución de *D. muscivorus* durante la primavera. Se encontró una mayor prevalencia de parasitismo en los machos de la zona central de la isla, donde la planta era significativamente más abundante. También, en esta área, la proporción de machos era mayor y éstos más grandes que en las otras dos áreas con densidades inferiores de la planta. Además, aparecieron diferencias en la intensidad de infección parasitaria, siendo más alta en los individuos de la zona central, y se halló una relación entre el tamaño corporal y la carga de parásitos, teniendo los individuos más grandes mayor proporción de células infectadas. Proponemos a la desigual distribución espacial de los individuos, debido a la disponibilidad diferencial de la rapa mosquera, como causa para explicar las diferencias observadas. En este sentido, hemos detectado cómo la habilidad para luchar y tener acceso a los recursos podría influir en los patrones de parasitismo en la isla del Aire.

Patterns of parasitism in insular lizards: Effects of body size, condition and resource availability

Abstract

Patterns of parasitism can provide insight into processes that maintain host–parasite associations and is essential to understand host's population dynamics and its ecology. Spatial distribution of lizards within a population is influenced by the distribution of resources and the individual's skills to gain access to them. In several Mediterranean islands, with food scarcity and unpredictability of resource availability, some particular resources can be extremely important during some periods of the year, as it is the case of dead horse arum, *Dracunculus muscivorus*, on Aire Island, Spain. In this study, we analyzed parasite infestation by blood parasites in an endemic lizard host, *Podarcis lilfordi*, in relation to the distribution of *D. muscivorus* during spring. We found higher prevalence of parasitism in males from a central area, where the plant was significantly more abundant. Also, in this area, males were more abundant and larger than in two areas with lower plant densities. Moreover, overall differences appeared in the intensity of infection, being higher in individuals from central area, and we found a relation between body size and parasite load, with more infected cells in larger individuals. Spatial distribution of individuals due to differential availability of the dead horse arum is proposed as an explanation for observed differences. In this sense, we detected how ability to fight for and access resources could influence patterns of parasitism in Aire Island.

Keywords: Blood parasites; Host–parasite dynamics; Islands; Resources; *Podarcis lilfordi*

1. Introduction

Animals are not distributed randomly, because some places are better than others in terms of resource availability when balanced against the risks or costs of acquiring the resource (Zug et al., 2001). If all resources were spread uniformly in the environment or even across different environments, it would be difficult for individuals or species to segregate spatially (Zug et al., 2001). In the case of lizards, Hews (1993) suggested that food resources influence their abundance, moreover, that sexes differ in social behaviour and spatial distribution in response to food abundance. Even, territory acquisition is influenced by its quality in some lizard species, which affects motivation to fight and defend territories (Hews 1993). To that effect, individuals are assumed to gain possession of areas where they win fights or encounters, while conspecifics that lose agonistic interactions leave areas where they were defeated. In this sense, body size and aggressiveness are two of the most important traits that determine dominance status, and hence, priority access to resources (Archer, 1988 in Civantos, 2000). Shifts in niche exploitation within a population may also arise as a result of ontogenic constraints imposed by differences in body size, physiological capacities and relative allocation to age-associated behaviour (Durtsche et al., 1997). Thus, most dominant individuals secure the territory or a given resource, often excluding subordinates from optimal habitats (Krebs, 1971; King, 1973). Consequently, the proportion of those dominant individuals in resource rich habitats increases, as well as the probabilities of aggressive encounters between them. Stamps (1977 and references therein) argued that intraspecific aggression is a result of critical limited resources, and aggressive encounters may involve risk of damage, even death. Thus, individuals fighting to access resources are more exposed to stressful conditions and regarding parasites, it is well known that stressful conditions have an immunosuppressive effect and may affect disease resistance (Oppliger et al., 1998). Stressed individuals may be less efficient to fight against parasites and prevalence and intensity of infection could rise. Accordingly, parasites could be considered as a cost for individuals inhabiting resource rich habitats. In addition, many studies in lizards show fitness-related effects of parasites, such as poorer body condition (Merino et al., 2000). The most infected individuals would be less able to compete for the access to resources. However, parasite impact seems to be spatially dependent. In favourable habitats, the host can accept the costs of being parasitized by gaining access to more favourable resources (Bull and Burzacott, 1993;

Weatherhead et al., 1995). Thus, in these habitats hosts are able to maintain higher parasite intensities (Bouma et al., 2007). In addition, parasites can alter hosts' spatial distribution (Price, 1980; Van Riper et al., 1986), as it occurs for Australian sleepy lizard, *Tiliqua rugosa*, where infected individuals present smaller home ranges than uninfected ones (Bouma et al., 2007).

Some lizard populations of the Balearic lizard, *Podarcis lilfordi* (Günther, 1874), in Menorca (Balearic Islands, Spain) live in small islets, lacking terrestrial predators and with very limited trophic resources (Pérez-Mellado, 1989). In small Mediterranean islets, food scarcity and unpredictability of resource availability is almost the norm (Pérez-Mellado and Corti, 1993). In this situation, some particular resources can be extremely important during some periods of the year. This is the case of the dead horse arum, *Dracunculus muscivorus* (Araceae). During spring, this plant has a strong interaction with the Balearic lizard at Aire Island. There is the blooming period of *D. muscivorus*, when plants are excellent perching sites for thermoregulation and ambush foraging of lizards, because of their thermogenic capacity and the attraction of potential flying pollinators, mainly bow flies. In addition, a small proportion of those pollinators are captured by basking lizards or directly from floral chambers of the plant (Pérez-Mellado et al., 2000, 2007). Despite that the Balearic lizard, is a non-territorial species (Pérez-Mellado, 1998), the exploitation of this resource leads lizards to compete for blooming plants, almost monopolized by larger adult males (Pérez-Mellado et al., 2007). So, as it happens in other non-territorial species living in areas with scarce resource availability (see for example the cases of *Darevskia* and *Podarcis muralis* in Steward, 1965 and Pianka and Vitt, 2003), the spatial distribution of lizards at Aire Island is strongly influenced by dead horse arum density (Pérez-Mellado et al., in press).

In a previous work (Garrido and Pérez-Mellado, submitted) we described the natural levels of prevalence and intensity of a parasitic infection of the Balearic lizard, *P. lilfordi*, in Aire Island (Menorca, Spain). In this study we focus our attention in two delimited areas of Aire Island characterized by a different availability of the dead horse arum during spring. Our hypothesis is that the distribution of this resource affects the proportion of adult males in the area of higher plant density and lizard's exposure to parasitic infection. We expect a raise in parasitic infection in the area of higher density of *D. muscivorus*, with lizards subjected to a higher stress and an increased competition for access to the dead horse arum.

2. Material and methods

P. lilfordi is an endemic lizard of the Balearic Islands. It is a medium-sized lacertid lizard (snout-vent length, SVL, up to 81 mm. in males and 75 mm. in females; Pérez-Mellado, 1998). The study was conducted during the springs of 2007, 2008, 2009 and 2010 in Aire Island (39°48′3″N 4°17′24″E), a small islet off Menorca (Balearic Islands, Spain). The island has a surface of around 342500 m² and is about one mile far from the south-eastern coast of Menorca. The vegetation is highly influenced by its environmental characteristics, strong winds, high salinity and long drought periods and it is dominated by low shrubs and a variety of herbaceous plant species. Lizard densities can be very high, over 4,000 individuals per hectare (Pérez-Mellado et al., 2008b).

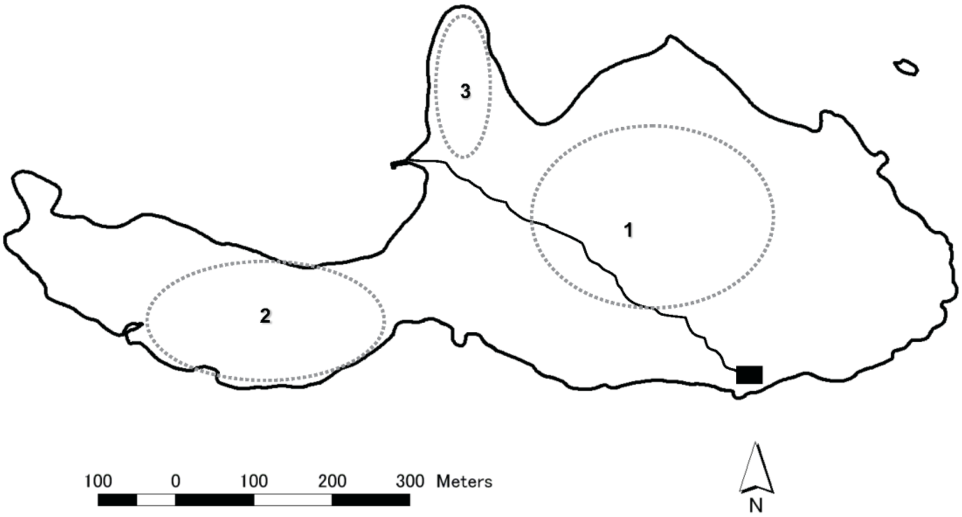

Figure 1. Map of Aire Island according to the zoning used for the study, in which: 1) central area 2) Western peninsula 3) Northern peninsula

The haemogregarines (Apicomplexa: Adeleorina), are protozoan parasites of a wide variety of host species, including reptiles. The species of haemoparasites of Balearic lizards, as it happens with many other lacertid lizards from the Iberian Peninsula, has been not yet described. In our case, we found just one form of blood parasite, but, because the genera of this family can only be characterized by oocyst stages within the invertebrate host (Levine, 1982; Barnard and Upton, 1994), we can only identify these parasites as haemogregarines, as in other papers on parasite load of lacertid lizards (Amo et al, 2005; Huyghe et al, 2010), even when a systematic identification was the main target of the study (Roca & Galdón, 2010). These authors suggest that blood parasites from lacertid lizards of the Iberian Peninsula and Canary Islands are currently classified within the genera *Hepatozoon* or *Hemolivia* (Roca and Galdón, 2010). Haemogregarines shows an indirect cycle, including, at least, two hosts: a final vertebrate host, where they appear in the form of gametocytes in the blood cells, mostly in erythrocytes (in many cases, this is the only known phase of the parasite, see Telford, 2008) and an invertebrate vector. In lizards the most common vectors are ticks and mites (Telford, 2008), acquired when lizards share suitable places to bask, forage or hide. There is another accepted method of transmission of haemogregarines, via ingestion either from predation on another infected vertebrate hosts or by direct ingestion of the infected invertebrates (see for example, Telford, 2008). The effects of haemogregarines in their carriers have been little studied, but, at least, we know that they destroy red blood cells causing anemia (Caudell et al., 2002). Furthermore, the presence of gametocytes in blood cells indicates the presence of schizonts in internal organs, causing additional damages (Svahn, 1974). Even, under laboratory conditions,

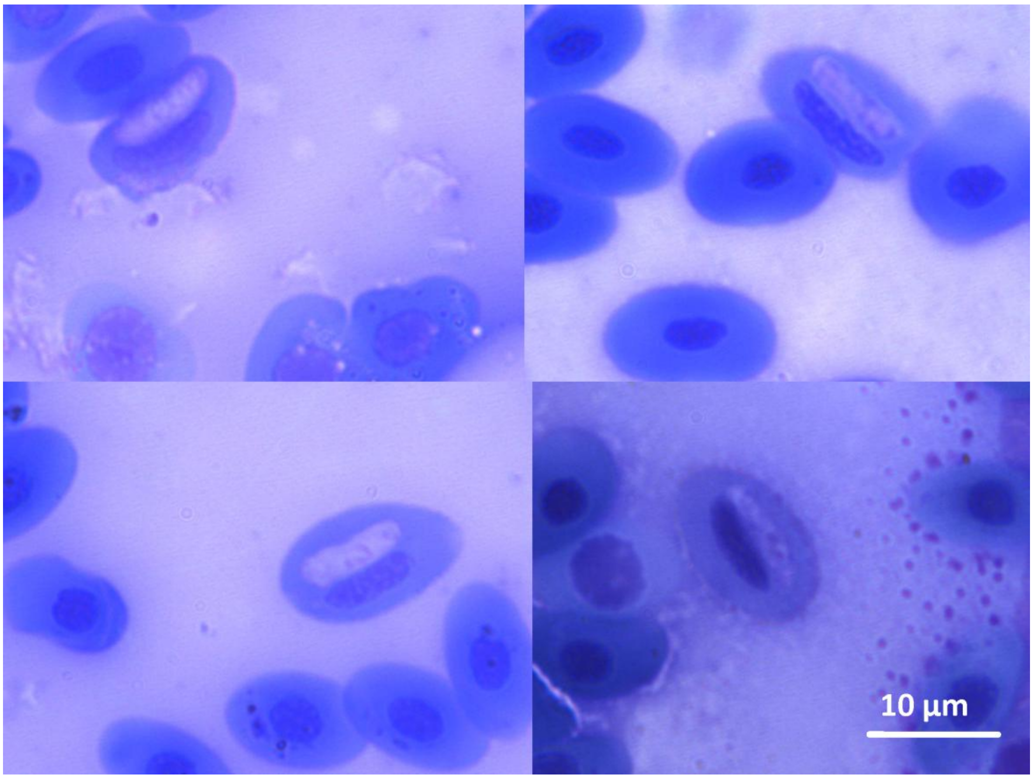

Image 1. Scaled microphotography of infected red blood cells, where the nuclei of cells are displaced by gametocytes of haemogregarine

blood parasites can even cause the death of the lizards (Schall, 1996). As haemogregarines are vector-borne parasites, we also checked for ectoparasites because they could be the component which determines differences.

According to Pérez-Mellado et al. (2007), we delimited two areas in relation to the distribution and abundance of *D. muscivorus*. Taking as a reference the middle track from dock to lighthouse (Fig. 1), the first area, which we designated as "central area", is located within the core of the island to the east of the pathway. The artificial track marks a clear limit of vegetation. East to the track there is the area of densest shrub vegetation, dominated by *Suaeda vera* and with the highest recorded density of *D. muscivorus*, up to almost 11,000 plants per hectare (Pérez-Mellado et al., 2007). It is also the area of the island with less influence of salinity. Moreover, just in central area we got evidence that is where *D. muscivorus* is exploited as a resource during the blooming period, and, probably, due to this reason, the density of the aroid is higher than in any other part of the islet. Northern and western peninsulae of the islet, are considered collectively the second area of study, “peninsulae” (Figure 1). During spring, the key difference in relation to central area is the abundance of dead horse arum. In northern Peninsula, it is very scarce and Pérez-Mellado et al. (2007) did not recorded any individual plant during line transects, even if some plants were present. In western Peninsula density was also significantly lower than in central area (less than 5,100 plants per hectare, see Pérez-Mellado et al., 2007).

Lizards were collected by noosing. For each individual, we recorded age, sex, snout-vent length (SVL), tail length and body weight. We also counted the number of ectoparasites observed on the body surface immediately after capture. To obtain blood samples, we made a slight longitudinal cut in the dorsal and proximal side of the tail with a sterile scalpel. With the blood drop we obtained a blood smear *in situ*. Sometimes, blood samples were obtained by clipping off the tail tips (that were later used for DNA extraction). Specimens were always released at the site of capture. Blood smears were placed on microscopic slides and air dried in the field. In the lab, slides were fixed with absolute methanol for 10 minutes and then stained with modified Giemsa for 20 minutes. Samples were analyzed using an optical microscope at 400X. The only blood parasites identified were haemogregarines (see above). The intensity of parasitism was estimated on a total of 2,000 counted cells per sample. Prevalence was estimated as the percentage of infected individuals at each of the two areas under study.

Individuals were marked with different colour pens for each area. Throughout the study period marked individuals from one area were not seen in other areas. Moreover, relying on results obtained by Pérez-Mellado et al. (in press) about the area of influence of individuals, we captured lizards which home ranges does not extend outside one of the two studied areas (Pérez-Mellado et al., in press). Between years, individuals are easily recognizable because of the mark left in the tail after the cut. So, lizards captured in previous years, were discarded. Thus, all along the period of four years, each individual was only studied once.

Lizard abundances were estimated with line transects during April of 2009 and 2010. The width of transects was 2 meters. Counts were made in sunny days, during the morning hours with highest lizard activity. All transects were made by the same observer (VPM). Each individual was classified as adult male, adult female or juvenile. This method provides a relative abundance of lizards and sex and age ratios for each area under study.

Males, females and juveniles exploited open spathes of dead horse arum in a completely different way (Pérez-Mellado et al., 2007). Thus, we looked for differences between age-sex categories in each variable under study. Then, we applied GLM models for males, females and juveniles separately to understand which factors can have a better predictive value. To compare prevalence of infestation among age-sex classes or areas and the age and sex ratios among different areas or years we applied Fisher's test. To determine changes in prevalence of haemogregarines between areas, years and body size (SVL) we used generalized linear models (GLM), with the parasites prevalence as the dependent variable following a binomial distribution, and including the interactions between the three variables in the model (Crawley, 2007). As endoparasite loads distributions had homogeneous variances, relations with area, year and body size (SVL) were analyzed using a GLM with a Gaussian distribution. In the same way, mite loads relations with age-sex categories and with area and body size (SVL) were analyzed using a GLM with a Gaussian distribution (Crawley, 2007). Ectoparasites intensities were log-transformed when required. Using Generalized Linear Models, the significance of each factor in a GLM was estimated from the analysis of accumulated deviance and the associated statistic.

Sometimes it is necessary to calculate body condition separately for each age-sex category because they present differences in body shape. To test it, we compared initially the body condition of adult males, females and juveniles with ANCOVA analyses, introducing log mass as the response variable, age-sex variable as a factor and log SVL as the covariate. Then, to analyze the effects that area and year have on body condition, we introduced both factors in a two-way ANCOVA, using log of mass as the response variable and log of SVL as the covariate. Following Crawley (2007), factors and interactions of them which had no significance were removed from the GLM and ANCOVA models until we get minimal adequate model, taking into account AIC (Akaike's Information Criterion) at each step. In all cases, *post hoc* Tukey's tests were made using package ´multcomp´ (Hothorn et al., 2008). The statistical analyses were done with R (ver.2.12.1, R Development Core Team, 2010).

3. Results

No differences were found in the sex-ratios of both area among years (G-test: central area, G = 0.03, df = 1, p = 0.86; peninsulae area, G = 0.44, df = 1, p = 0.50). Thus, we pooled data of the two years of study. In peninsulae area, we found a 1:1 sex-ratio (G-test, G = 0.49, df = 1, p = 0.50), while in central area the proportion was significantly skewed towards males (G-test, G = 10.67, df = 1, $p < 0.01$). Accordingly, there were differences in sex-ratio between areas (G-test, G = 44.12, df = 1, $p = 3.085 \times 10^{-11}$; see Table 1). Age ratio also varied among areas (G-test, G = 29.29, df = 1, $p = 6.22 \times 10^{-8}$), being higher the proportion of adults at the central area (Table 1).

Area	*M*	*F*	AD	JUV	Sex-ratio (M/F)	Age-ratio (AD/JUV)
Central area	*373*	*181*	*554*	*64*	*2.06/1*	*8.66/1*
Peninsulae area	*199*	*231*	*432*	*121*	*0.86/1*	*3.57/1*

Table 1. Proportion of adults (AD), adult males (M), adult females (F), juveniles (JUV) and sex- and age ratio at each area

Prevalence of infection was similar between adults and juveniles (Fisher's test, p = 0.53; see Table 2), but in adults we found significant differences between sexes (Fisher's test, p = 0.049; see Table 2), with a higher prevalence in males than in females. For males, GLM binomial model, revealed just differences between areas (χ^2 = 6.08, *df* = 1, *p* = 0.01) with more infected individuals in central area (see Table 2).

	Infected / total	*Percentage*	*Conf. interval*
Males			
Total	***121/124***	***97.58 %***	***93.09 - 99.50***
Central area	*78/78*	*100.0 %*	*95.38 – 100.0*
Peninsula area	*43/46*	*93.48 %*	*82.10 – 98.63*
Females			
Total	***72/79***	***91.14 %***	***82.59 – 96.36***
Central area	*44/46*	*95.65 %*	*85.16 – 99.47*
Peninsula area	*28/33*	*84.85 %*	*68.10 – 94.89*
Adults			
Total	***195/205***	***95.12 %***	***91.21 – 97.64***
Central area	*122/124*	*98.39 %*	*94.30 – 99.80*
Peninsula area	*71/79*	*89.87 %*	*81.02 – 95.53*
Juveniles			
Total	***13/14***	***92.86 %***	***66.13 – 99.82***
Central area	*1/8*	*87.5 %*	*47.35 – 99.68*
Peninsula area	*6/6*	*100.0 %*	*54.07 – 100.0*

Table 2. Prevalence percentages with confidence intervals by sector and age-sex categories

The year (χ^2 = 5.30, *df* = 3, *p* = 0.15), body size (χ^2 = 0.31, *df* = 1, *p* = 0.57) and the interactions between the three response variables had not effect over prevalence (all *p* > 0.05). In females (Table 2), prevalence varied significantly between years (χ^2 = 13.11, *df* = 3, *p* < 0.01) because all non infected females were captured in 2009. Body size had not got any influence in females´ prevalence ($\chi^2 = 0.51$, $df = 1$, $p = 0.47$) and no differences appeared among areas ($\chi^2 = 2.76$, $df = 1$, $p = 0.10$). None of the interactions were significant (all $p > 0.05$). Neither the variables: year ($\chi^2 = 1.79$, $df = 2$, $p = 0.41$), size ($\chi^2 = 0.77$, $df = 1$, $p = 0.38$) and area ($\chi^2 = 1.18$, $df = 1$, $p = 0.28$); nor the interactions seemed to have effect over juveniles´ prevalence (all $p > 0.05$).

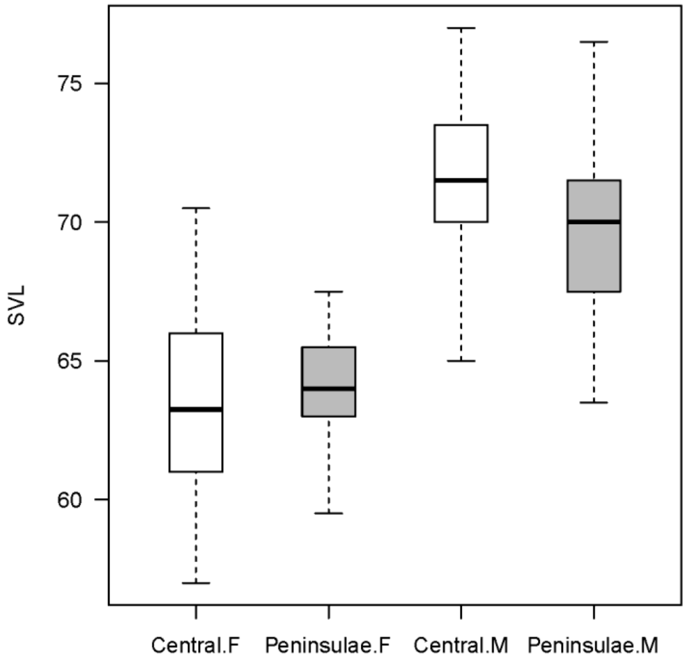

Figure 2. Boxplot of males (M) and females´ (F) SVL (mm) at each area

We did not find differences in the SVL of adult females (central area: $\bar{x}\pm SE$ = 63.51±0.49; peninsulae: $\bar{x}\pm SE$ = 63.79±0.45; GLM, $F_{1,\,77} = 0.16$, $p = 0.69$) or juveniles (central area: $\bar{x}\pm SE$ = 58.62±2.40; peninsulae: $\bar{x}\pm SE$ = 58.33±3.39; GLM, $F_{1,\,12} = 0.005$, $p = 0.94$) between areas. But for adult males, we found that the body size of individuals varied between areas (central area: $\bar{x}\pm SE$ = 71.47±0.31; peninsulae: $\bar{x}\pm SE$ = 69.41±0.44; GLM, $F_{1,\,122} = 14.98$, $p < 0.001$) being larger males from central (Figure 2).

Parasite load was not different for adults and juveniles (GLM, $F_{1,\,217} = 0.61$, $p = 0.43$), nor between adult males and females (GLM, $F_{1,\,204} = 0.07$, $p = 0.79$; see Table 5). The infection intensity among adult males (see Table 3) was significantly different between years (homogeneous variances, Fligner test: $\chi^2 = 9.80$, $df = 81$, $p = 0.21$; GLM, $F_{3,\,120} = 3.50$, $p = 0.02$) and areas (GLM, $F_{1,\,119} = 5.37$, $p = 0.02$), but the interaction between them was not significant (GLM, $F_{3,\,115} = 1.07$, $p = 0.36$), indicating that relation among areas was stable over the years. Thus, males from central area showed higher infection intensity (Table 5), and, regarding years, *post hoc* comparisons revealed than parasite load in 2010 ($\bar{x}\pm SE$ = 21.94±3.39) was statistically higher than in 2008 ($\bar{x}\pm SE$ = 11.67±1.82) (Tukey´s test, $t = 2.875$, $p = 0.02$) and 2007 ($\bar{x}\pm SE$ = 11.94±1.36) ($t = 2.55$, $p = 0.05$), other comparisons between years were not significant (all $p > 0.05$). Body size was not related with infection intensity (GLM, $F_{1,\ 118} = 2.90$, $p = 0.09$). All interactions terms in males' GLM were not significant (all $p > 0.05$). For adult females (see Table 4), none of the interactions terms between SVL, area and year were significant (Fligner test, $\chi^2 = 72.19$, $df = 63$, $p = 0.20$; all $p > 0.05$). No differences between years were found (GLM, $F_{3,\ 75} = 0.24$, $p = 0.87$), but, between areas, females from central area were more parasitized (GLM, $F_{1,\ 77} = 4.18$, $p = 0.04$; see Table 5). Parasite intensity was strongly related with SVL (GLM, $F_{1,\ 76} = 7.20$, $p = 0.01$). For juveniles we were not able to find any relation with the intensity of infection (all $p >$ 0.05), probably due to small sample size.

	Df	Resid. Df	F	Pr(>F)
NULL		123		
Area	1	119	5.37	0.02*
SVL	1	118	2.90	0.09
Year	3	120	3.52	0.02*

Minimal adequate model of parasite intensity for males

	Df	Resid. Df	F	Pr(>F)
NULL		123		
Area:SVL	1	111	1.93	0.17
Area:year	3	115	1.07	0.37
SVL:year	3	112	0.86	0.46
Area:SVL:year	3	108	0.53	0.66

Table 3. Results of the GLM for blood parasite intensities of adult males: Minimal adequate model and F-values, degrees of freedom and P- values of the variables and interactions when they were removed from the model

Ectoparasite load did not differ between adults and juveniles (adults: $\bar{x}\pm SE$ = 125.44±9.99; juveniles: $\bar{x}\pm SE$ = 85.00±19.64; Fligner test, $\chi^2 = 0.81$, $df = 1$, $p = 0.37$; GLM, $F_{1,\ 217} = 1.10$, $p = 0.30$), nor between adult males and females (males: $\bar{x}\pm SE$ = 134.54±14.45; females: $\bar{x}\pm SE$ = 110.00±11.68; Fligner test, $\chi^2 = 0.32$, $df = 1$, $p = 0.57$; GLM, $F_{1,\ 203} = 1.34$, $p = 0.25$). Then, we searched for relations between mites and haemogregarines intensities and for ectoparasite loads between areas. We ran three models, one for males, one for females and a third for juveniles Only for adult males we found differences between areas (Fligner test, males, $\chi^2 = 77.50$, $df = 62$, $p = 0.09$; GLM, $F_{1,\ 123} = 8.30$, $p = 0.005$), with higher mite loads in peninsulae males ($\bar{x}\pm SE$ = 154.26±18.47) than in central area ($\bar{x}\pm SE$ = 116.59±20.05), but in an opposite way to intensity of blood parasites (see above). In addition, we do not find any relation with haemogregarines (GLM, $F_{1,\,123} = 0.006$, $p = 0.94$) and for the interaction (GLM, $F_{1,\,123}$ = 0.006, $p = 0.94$). For females, any relation was statistically significant (Fligner test, χ^2 = 58.63, $df = 48$, $p = 0.14$; GLM, area: $F_{1,\ 76} = 2.64$, $p = 0.11$; haemogregarines: $F_{1,\ 75}$ = 0.0002, $p = 0.99$; interaction: $F_{1,\ 74} = 0.42$, $p = 0.52$), as well as for juveniles (Fligner test, $\chi^2 = 13.00$, $df = 11$, $p = 0.29$; GLM, area: $F_{1,\ 13} = 0.60$, $p = 0.46$; haemogregarines: $F_{1,\,13} = 0.0002$, $p = 0.99$; interaction: $F_{1,\,13} = 0.36$, $p = 0.85$).

	Df	Resid. Df	F	Pr(>F)
NULL		78		
Area	1	77	4.18	0.04*
SVL	1	76	7.20	0.01*

Minimal adequate model of parasite intensity for females

	Df	Resid. Df	F	Pr(>F)
NULL		78		
Year	3	75	0.24	0.87
Area:SVL	1	66	0.90	0.35
Area:year	3	70	1.08	0.36
SVL:year	3	67	0.48	0.70
Area:SVL:year	3	63	1.17	0.33

Table 4. Results of the GLM for blood parasite intensities of adult males: Minimal adequate model and F-values, degrees of freedom and P- values of the variables and interactions when they were removed from the model

In relation to body condition, first we performed an ANCOVA analysis to look for differences between males, females and juveniles (see Material and Methods). As expected, there was a significant and positive correlation between body mass and SVL (one-way ANCOVA, $r^2 = 0.84$, $F_{1,\ 207} = 266.11$, $p < 2.2\text{x}10^{-16}$), and a significant difference between age-sex groups ($F_{2,\ 207} = 218.5$, $p < 2.2\text{x}10^{-16}$).

	Males	**Females**	**Adults**	**Juveniles**
Total	16.90±1.46 (126)	16.11±2.93 (79)	16.60±1.44 (205)	12.57±1.46 (14)
Central area	19.04±1.80 (78)	20.91±4.71 (46)	19.73±2.07 (124)	9.5±3.49 (8)
Peninsula area	13.67±2.53 (48)	9.42±2.07 (33)	11.9±1.71 (81)	16.67±4.54 (6)

Table 5. Mean infection intensity and standard error by area and age-sex categories (sample sizes in parentheses)

Because body condition must be estimated separately for males, females and juveniles, we ran separate ANCOVAs for each group with area and year as factors and using log of mass as the response variable and log of SVL as the covariate. For males, the minimal adequate model showed a significant difference taking into account the three variables. As expected, SVL was positively and strongly correlated with body mass ($F_{1,\ 115} = 123.82$, $p < 2.2\text{x}10^{-16}$).We found a significant influence of area in body condition ($F_{1,\ 115} = 7.79$, $p = 0.006$), that is, individuals from central area showed better body condition than peninsulae ones. Body condition differed significantly between years ($F_{3,\ 115} = 9.03$, $p = 2.03\text{x}10^{-5}$) and Tukey's *post hoc* revealed that during 2008 lizards had a better body condition than during 2007 ($t = 2.65$, $p = 0.04$) and lizards from 2010 had a lower body condition than those from 2008 ($t = -5.11$, $p< 0.001$) and 2009 ($t = -0.33$, $p= 0.008$).

For females, the minimal adequate model also showed significant differences for the three variables. SVL was positively and strongly correlated with body mass ($F_{1,\ 70} = 64.26$, $p = 1.71\text{x}10^{-11}$).We found only a marginal influence of area in body condition ($F_{1,\ 70} = 3.91$, $p = 0.05$), that is, individuals from central area showed better body condition than peninsulae ones. Body condition differed significantly between years ($F_{3,\ 70} = 11.64$, $p = 2.83\text{x}10^{-6}$) and Tukey's *post hoc* revealed that during 2010 females had a worst body condition than during 2007 ($t = -4.85$, $p< 0.001$), 2008 ($t = -3.86$, $p = 0.001$) and 2009 ($t = -2.62$, $p= 0.048$) and individuals from 2009 presented less body condition than those from 2007 ($t = -0.14$, $p = 0.01$).

4. Discussion

The structure of population between areas was very different. In central area, where *D. muscivorus* is very abundant and exploited by *P. lilfordi,* the proportion of juveniles and females were almost 2.5 times lower than in peninsulae. Critically limited resources can be the cause of intraspecific aggression (Stamps, 1977) and, therefore, the structure of population would be temporarily modified. There are other examples where the foraging of lizards on a particular plant species can strongly affect their spatial distributions, as in the case of *Niveoscincus microlepidotus* foraging on blooming shrubs of *Richea scoparia* (Olsson et al., 2000). In Aire Island, Pérez-Mellado et al. (2007) observed that adult males excluded actively juveniles and adult females from open spathes of *D. muscivorus*, indicating a displacement of adult females and juveniles from the optimal area of dead horse arums. Also, they were unable to detect any change in other available resources during spring and summer. Thus, the most parsimonious explanation for their results is that site fidelity of adult males is a consequence of the flowering over spring of the dead horse arums, and, once adult males started to monopolize central area, the exclusion of adult females and juveniles could became stronger. It is demonstrated that the high concentration of adult males had direct consequences in the adult sex ratio due to an increased aggression to adult females, whose survival and emigration rates dropped (Le Galliard et al., 2005). This behaviour, although it seems paradoxical, especially if we think that it occurs during breeding season, is similar to the observations reported by Pérez-Mellado et al. (in press) in a different study made during spring 2004. In this study, it was suggested that females were of lower priority (against food) to the male choice system during the period in which the monopolization of *D. muscivorus* seemed to explain their spatial distribution.

Even, males from central area were larger than peninsulae ones. Differences in SVL between areas were only found in adult males. This is a consequence of intraspecific competition to gain possession of better areas, because body size is an important factor in winning contests (Tokarz, 1985; Olsson, 1992). Thus, individuals acquire their territory (review in Stamps, 1994) and exclude subdominants from optimal habitats (Krebs, 1971; King, 1973). Where the proportion of dominant males increases, it is more likely the raise of aggressive encounters. Thus, the degree of aggressiveness or relative dominance may determine the quality of a territory where an individual lives (Civantos, 2000). For example, more aggressive side-blotched lizards (*Uta stansburiana*) increased the size and quality of their home ranges (Fox, 1983). According to expectations, results showed differences in patterns of parasitism between the two defined areas of Aire Island during spring. From our viewpoint, observed differences in the distribution of *D. muscivorus* between areas are closely related with differential distribution and behaviour of lizards (Pérez-Mellado et al., 2007), and, ultimately, of differences in parasitic load.

Differences in prevalence between areas were just found for adult males. During spring, central area was mostly occupied by adult males, and those males were larger than males from peninsulae area. But, what drives these differences in parasite infestation? Mites act as haemogregarine´s vector, but, for males, mite load was higher in peninsulae sector and no differences between areas appeared for females. Alternatively, infection by haemogregarines could happen by direct ingestion of the infected vector or from predation on another infected vertebrate hosts (see Material and Methods and Telford, 2008). Thus, we proposed another mechanism of infection: parasite transmission between hosts increases with aggressive encounters. It is well known that body size and aggressiveness are two of the most important traits determining the dominance status, and, consequently, a prior access to resources (Archer, 1988 in Civantos, 2000). So, the higher proportion of adult and larger males at central area could imply more male-male aggressive encounters. In addition, the frequency of these encounters would increase if we consider that dominant males show higher activity levels than females and juveniles (Martín and López, 2000b). In male-male interactions, it is common that lizards bite each other's the tail and even caudal autotomy and tail consumption by the opponent can occur (Pérez-Mellado, 1997), eating at the same time the infected blood and the mites hooked to the tail. Therefore, the ingestion of a tail can increase the probabilities of transmission of blood parasites between males.

Within adults, prevalence was higher for males. Differences in the rate of parasitism between sexes are well documented (see, for example, Salvador et al., 1996). Two hypotheses have been proposed to explain these differences: (1) ecological: because males of *P. lilfordi* show an increased activity than females as it was proposed in *Sceloporus occidentalis* (Zuk and McKean, 1996). A higher prevalence may also be related to aggressive behaviour and male-male interactions, frequently observed in Aire Island.; and (2) physiological, usually hormonal in origin as the level of aggressiveness is mediated by high levels of testosterone. Thus, males are more likely to be infected than females, because of the immunosuppressive effects of testosterone (Zuk and McKean, 1996).

As we expected, both males and females from central area showed higher parasitic load. It could be thought that abundance of larger males or females drives the differences in blood parasite intensity among sites, and that the size effect confounds the differences in intensity among areas. Yet, for adult males, body size was not related with parasite load. However, SVL was related with parasite load in females, but average SVL was similar between areas. Neither mites explained those differences between areas as they were not related with haemogregarines load. Moreover, they were more abundant in peninsulae males and not different for females between areas. Other factor that has been related with parasite infection is body condition. Various studies related lizards' fitness with the effects of parasites, as a poorer body condition (Sorci and Clobert, 1995; Merino et al., 2000). However, in our study, males and females of the central area had, respectively, better and slightly better condition than those less infected individuals from peninsulae area. Thus, lizards from central area can access to this valuable resource, the dead horse arum, while individuals with a poor condition were excluded from that area.

From our results, it seems clear that the higher intensity of infection in central area was probably due to an increased competition for access to resources and, consequently, to a higher exposition to aggressive encounters with dominant males than individuals from peninsulae area. These encounters could, *per se*, result in re-infections, in the same way as described for prevalence. Moreover, agonistic interactions derived in social stress, which could be the factor driving differences in the intensity of infection. It´s well known that stressful conditions have an immunosuppressive effect and may affect disease resistance (Oppliger et al., 1998). In central area, agonistic interactions were more frequent, so this social stress can induce an increase in parasite load, as it was observed in *Zootoca vivipara* (Oppliger et al., 1998) or *Iberolacerta monticola* (Amo et al., 2004). There is an alternative but less convincing explanation, according to which the impact of parasite may be spatially dependent, that is, parasitic loads would be higher in more favourable habitats for hosts, as it is the case of central area during spring. At these habitats, hosts could compensate the costs of being parasitized by gaining the access to more favourable resources (Bull and Burzacott, 1993; Weatherhead et al., 1995).

In conclusion, in Aire Island, the temporal availability of a valuable resource, the dead horse arum, strongly influences the structure of lizard population. Dominant individuals, mainly males, monopolized the central area where the resource is, excluding subdominant individual to other parts of the island. Thus, as dominant and more aggressive individuals were concentrated in this area, the probabilities of agonistic encounters raises, leading to an direct increase of parasitism directly, by ingestion of infected tissues or vectors by eating opponent´s tail, or indirectly, as stress increased and suppressed immune function.

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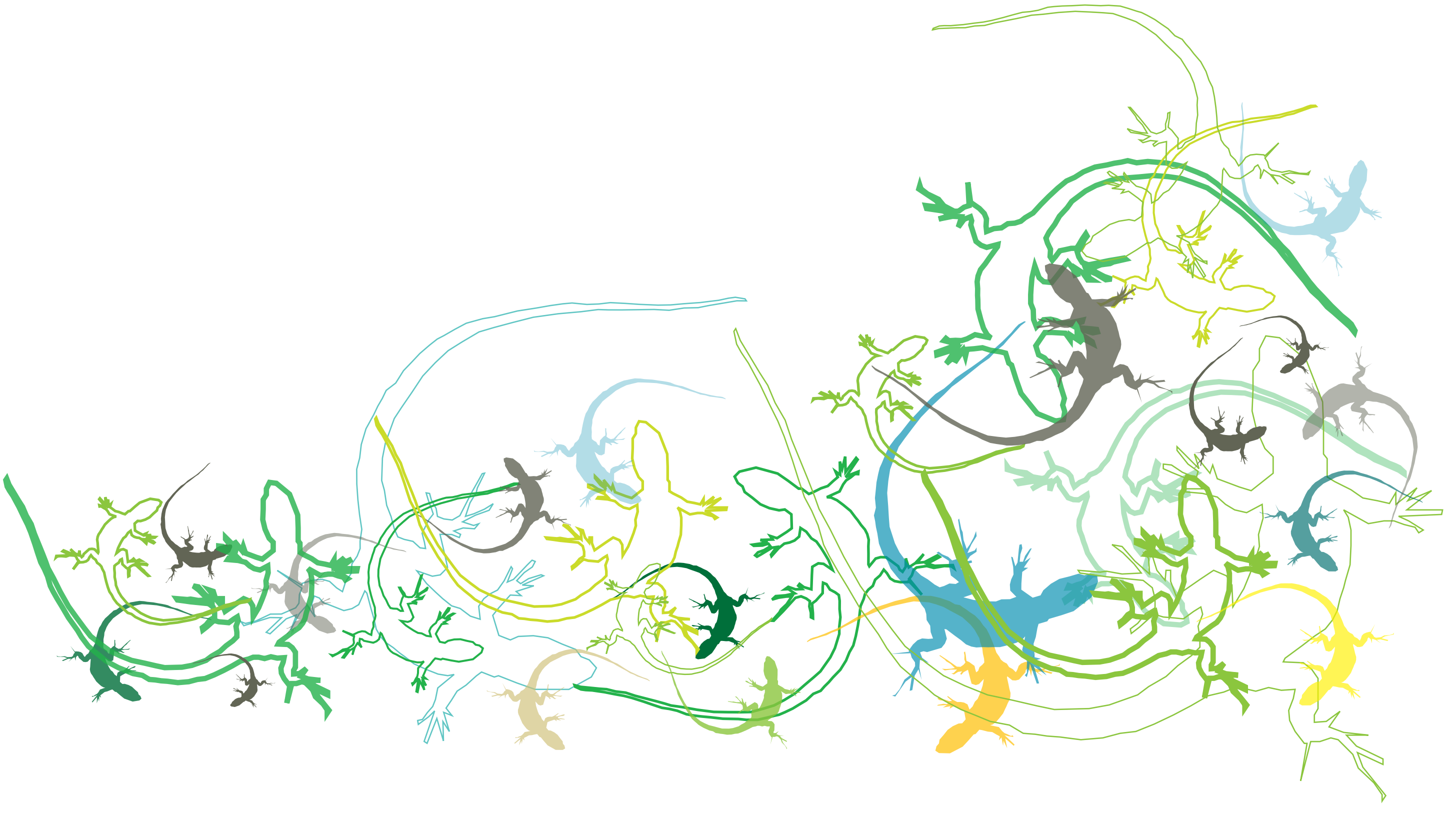
Capítulo 4

La presión humana afecta a los patrones de ectoparasitismo y la condición en una lagartija insular



La presión humana afecta a los patrones de ectoparasitismo y la condición en una lagartija insular, *Podarcis lilfordi*

Resumen

Los parásitos son esenciales para comprender la dinámica poblacional y la ecología de los hospedadores. Por ello, se ha propuesto a los parásitos como una de las fuerzas evolutivas más importantes. En varias islas del Mediterráneo, poco se sabe acerca de los parásitos que infectan a las lagartijas del género *Podarcis*. Los estudios realizados sobre ectoparásitos que infectan a las lagartijas de este género se centran principalmente en estudiar el impacto que tienen sobre los rasgos vitales de los hospedadores. Sin embargo, el conocimiento de los niveles naturales de infestación y prevalencia parasitaria es un primer paso necesario para entender cómo un parásito interactúa con sus hospedadores. En este trabajo estudiamos los factores que afectan el ectoparasitismo en una lagartija endémica, *Podarcis lilfordi* (Günther, 1874), en la isla del Aire (Menorca, Islas Baleares, España). Centramos nuestra atención en la influencia de las perturbaciones humanas sobre el parasitismo. Ambas, la prevalencia y la intensidad de la infección, se encuentran entre las mayores que se han encontrado en las poblaciones de lacértidos mediterráneos. La reducida variabilidad genética y/o la alta densidad, propia de las islas, se han propuesto para explicar tales valores de intensidad y prevalencia. La intensidad de la infección fue mayor en adultos, probablemente debido a que monopolizan los mejores lugares, donde las posibilidades de re-infección son mayores. Como en muchas otras poblaciones, entre los adultos, los machos mostraron mayor carga parasitaria. Las diferencias inmunológicas, genéticas y de comportamiento se proponen para explicar esta variabilidad. Además, las lagartijas reconocen a los humanos como depredadores, realizando conductas de defensa que son costosas en términos fisiológicos. Por lo tanto, la perturbación humana también podría afectar a otros rasgos vitales, como la defensa contra los parásitos. Durante el verano, cuando el turismo es particularmente intenso, los machos y hembras adultos que habitan las zonas visitadas de la isla mostraron una mayor carga de ectoparásitos y una disminución más marcada de la condición corporal en comparación con los individuos de áreas no

visitadas. Proponemos que los costes derivados de realizar conductas de escape, como respuesta a la presencia humana, son responsables de las diferencias observadas. En consecuencia, la presión humana afectaría el equilibrio parásito-hospedador y la condición corporal de los hospedadores, disminuyendo así su estado físico y sus probabilidades de supervivencia.

Human pressure influences patterns of ectoparasitism and condition in an insular lizard, *Podarcis lilfordi*

Abstract

Parasites are essential to understand population dynamics and ecology of hosts. Consequently, parasites are proposed as a major evolutionary force. In several Mediterranean islands, little is known about parasites infecting *Podarcis* lizards. Studies of ectoparasites infecting *Podarcis* lizards are mainly in relation to life history traits of hosts. Anyhow, the knowledge of natural levels of parasite and prevalence of infection is the first step to understand how a parasite interacts with their hosts. We studied factors affecting ectoparasitism in an endemic lizard, *Podarcis lilfordi* (Günther, 1874) on Aire Island (Menorca, Balearic Islands, Spain). We focus our attention in the influence of human disturbance. Both, prevalence and intensity of infection were among the highest ever found in lacertid lizard populations. The reduced genetic variability and/or the high density in islands are proposed to explain such intensities and prevalences. Intensity of infection was higher for adults, probably as they monopolize the best places where probably became repeatedly re-infected. As in many other populations, within adults, males showed higher parasite load. Immunological, genetic and behavioural differences are proposed to explain this variability. Lizards recognize humans as predators, performing defence behaviours, which are costly in physiological terms. Thus, human disturbance may also affect other life-history traits, as defence against parasites. During summer, when tourism is particularly intense, adult males and females from visited areas of the island showed higher ectoparasite load and a stronger decrease of body condition in comparison with individuals from non visited areas. We propose that the costs of eliciting escape behaviours because of human presence are responsible for observed differences. Consequently, human pressure would affects parasite-host equilibrium and body condition of hosts, decreasing their fitness and survival probabilities.

Keywords: ectoparasites; lizard; host-parasite system; island; body condition; human impact.

1. Introduction

Parasites cause adverse effects on many traits of hosts, as their population growth and regulation (Holmes, 1995; Hudson et al., 1998), spatial distribution (Price 1980; Bouma et al., 2007), reproductive success (Schall, 1996) and sexual selection (Hamilton and Zuk, 1982). Also, they have been shown to drive host's behaviour (Moore, 2002) or the evolution of sex (Hamilton, 1980). Given the high risk of infection and fitness reduction caused by parasites, they should be leading hosts to strong selection pressure. Parasites are increasingly seen as major drivers of ecological and evolutionary processes (Schmid-Hempel, 2009). Thus, the study of the relations between parasites and their host populations is essential to understand their role in host's fitness and life-history traits. Often, the first step to understand how a parasite interacts with its hosts is to know the natural levels of parasite and prevalence of infection among individuals within a wild population (Smallridge and Bull, 2000).

Lacertid lizards of the genus *Podarcis* are today widespread across mainland and island of ecosystems of the Mediterranean region (Arnold, 1973). In many cases, *Podarcis* species are the only terrestrial vertebrate in many coastal islets of the Mediterranean basin (Corti et al., 1999). However, little is known about the parasite communities infecting the genus *Podarcis*, and especially from those species occurring on islands (Roca and Hornero, 1994; Roca, 1996; Hurston, 2007; Roca et al., 2009). Moreover, studies of ectoparasites infecting *Podarcis* lizards were almost relative to their consequences on life history traits of hosts (Oppliger et al., 2004; Amo et al., 2006; Biaggini et al., 2009; Huyghe et al., 2009).

During four consecutive years, we studied the prevalence and intensity of ectoparasites in the Balearic lizard (*Podarcis lilfordi*) from Aire Island (Menorca). There are few studies from insular populations in the Mediterranean basin (but see the case of *Podarcis erhardii* in Hurston, 2007). However, island populations are of particular interest as they often display unusual life history characteristics. Small Mediterranean islands, as Aire, are ideal scenarios to study host-parasite interactions because they have isolated lizard populations on relatively simple environments. Thus, the parasite-host equilibrium would probably suffer less disruption than in larger islands or continental areas. Moreover, Aire is a small islet with limited trophic resources, lacking terrestrial predators (Pérez-Mellado, 1989) and having high lizard densities (Pérez-Mellado et al., 2008). In the absence of human intervention, gene flow between islands is limited or absent and, for populations where individuals are closely related, the expected low level of genetic variability would facilitate parasitism, because parasites would found lower genetic resistance in the host (Shykoff and Schmid-Hempel, 1991). According to this hypothesis, we expect to find higher rates of parasitism in insular habitats than in continental populations. In Aire Island, with higher lizard densities than in continental populations, individuals can interact more frequently with conespecifics. Consequently, we expect to find higher rates of parasitism in this island.

The parasite-host system shows a temporal and spatial flexibility reflected in the variation of prevalence and parasite infestation within a species or population. But equilibrium in host–parasite interaction may be broken if any additional factor influence lizard’s condition (Amo et al., 2005), as human disturbance. Small island populations are particularly sensitive ecosystems as may endure more environmental stress (MacArthur and Wilson, 1967) and genetic stress (Frankham, 1998). Even more, due to increased genetic stress, small populations may be more susceptible to environmental stress. Inbred populations may suffer greatly certain changes in the environment that none inbred populations perceive as not stressful, indicating that the genetic and environmental stress can interact (Kristensen et al., 2006). This is the case of several populations of the Balearic lizard, *Podarcis lilfordi* in coastal islets of Menorca and Mallorca (Balearic Islands, Spain). On the other hand, the impact caused by humans on natural populations is currently in the spotlight and the Mediterranean islands, receive the major bulk of visitors throughout the summer. The effect may be especially important because increased tourism has been suggested as possible factor of this decline (Corbett, 1989; see more references in Amo et al., 2006).

Hence, we focus our attention on the impact of human pressure, comparing the dynamics of infestation between areas frequently visited by humans and those undisturbed during spring and summer. Throughout the summer Aire, as other

Mediterranean islands, received the major bulk of visitors. As Amo et al. (2006) showed, human pressure negatively affects reptiles, because many species respond to humans as potential predators, by readily escaping to refuges (e.g. Amo et al., 2003; Martín and López, 1999) and increasing the intensity of antipredatory behaviours (Martín and López, 1999; Pérez-Tris et al., 2004). Antipredatory behaviours are expensive in terms of physiological costs such as a decrease in body condition (Martín and López, 1999; Pérez-Tris et al., 2004) and could be reflected in other fitness-related components, as parasite load. Navarro et al. (2004) showed that over perceived risk of predation, individuals tend to reallocate available resources. Thus, the immune system receives a reduced priority because its elevated costs. Under experimental conditions, the individuals exposed to predators reduced their ability to cope with parasitism (Navarro et al., 2004). Therefore, human disturbance can cause a break in the parasite-host equilibrium of wild populations.

Lizards could expend a great amount of energy on escape behaviour, instead its investment in other physiological requirements. As fewer resources should be available to invest in parasite defence, we expect that the impact of parasitism must be higher in individuals inhabiting areas of higher human pressure. Moreover, in summer drought is particularly strong and resources scarcer (Pérez-Mellado and Corti, 1993). Thus, individuals can hardly compensate for the additional cost of repeatedly performing escape behaviours due to continued human disturbance and those costs would be easily detected in the study. We expect that tourism would affect natural parasite-host equilibrium, and that this disruption should be reflected in parasitism rates. In the last decades many populations of several lizards’ species are in decline in Europe, and increased tourism has been proposed as possible to be determinant in this decline (Corbett 1989; see more references in Amo et al., 2006).

2. Material and methods

2.1. Study area and species

P. lilfordi is a medium-sized lacertid lizard endemic of the Balearic Islands (snout-vent length, SVL, up to 81 mm. in males and 75 mm. in females; Pérez-Mellado, 1998). The study was conducted during the spring and summer of 2007, 2008, 2009 and 2010 in Aire Island (39°48′3″N 4°17′24″E), a small islet off Menorca, which is probably the oldest and most remote coastal islet of Menorca. Its surface is around 34 hectares. It has a Mediterranean mesothermic climate characterized by mild winters and hot dry summers. The vegetation is highly influenced by strong winds, high salinity and long drought periods. Low Mediterranean shrubs and a variety of herbaceous species cover the island (see Pérez-Mellado et al., 2007). Lizard densities can be very high, over 4,000 individuals per hectare (Pérez-Mellado et al., 2008).

Haematophagous parasites as ticks or mites are present in many animal species. Parasites can damage tissues, deplete fluids, trigger the immune response and serve as vectors of diseases (Wakelin, 1996). Mites of the genus *Ophionyssus* (Acari; Mesostigmata; Macronyssidae) are mainly parasites of Squamata. This genus includes 15 species, 13 of them confined to lizards of various genera (Fain and Bannert, 2000). Lacertid lizards are parasitized by six species of *Ophionyssus*, two of them, *O. galloticolus* and *O. setosus*, parasites of the genus *Gallotia* from Canary Islands (Bannert et al., 2000). In the case of the genus *Podarcis,* we did not found any taxonomic study of their ectoparasites from Spain or Mediterranean basin. *Ophyonissus* is present in many lizard species from Europe (Strijbosch et al., 1980), including some species from the Iberian Peninsula (Moraza et al., 2009; Bannert et al., 2000; Majlathova, 2010). The ectoparasites observed in this study are tentatively assigned to this genus. In addition to a direct parasitic influence through blood sucking by mites, that in vivarium can be lethal to their hosts (Frye, 1991), *Ophionyssus* species serve as potential vectors of endoparasites (Álvarez Calvo, 1978; Bannert et al., 1995, Reichenow 1921, 1919, 1913 in Bannert et al., 2000; Camin, 1948; Svahn, 1975).

Aire is a fairly flat island. A jetty, suitable for dockings of small to medium boats, is located in the central zone of the northern coast. The constructions of the island are all in ruin with the exception of a small hut by the jetty and the automatic lighthouse. Between the dock and the lighthouse there is a narrow track of 465 meters, the main accessible area for visitors. Thus, the bulk of visitors and their potential impact are concentrated around this pathway. We divided the island according to human pressure differences. This pressure is particularly intense during summer, while during spring visitors are sporadic. Taking as a reference the middle track from the dock to the lighthouse, we designated a visitors' area covering the entire area under the influence of the track. In practice, this is the only area visited during summer. Then, the rest of the island was almost free of humans. This is the undisturbed area.

Lizards were collected by noosing. For each individual, were recorded age, sex, snout-vent length (SVL), tail length and body weight. Body condition was estimated from the residuals of the regression of log transformed body weight on log transformed SVL. Ectoparasites were counted at the capture place with a 5x monocle, inspecting the whole body surface of the lizards.

2.2. Statistical analyses

Following Schall and Pearson (2000), we grouped individuals according to their body condition, creating a categorical variable of body condition of individuals above (positive residuals) or below (negative residuals) the average body condition. Schall and Pearson (2000) refer to individuals as 'healthy' or 'unhealthy', as their condition was 'positive' (above average of the residuals) or 'negative' (below average of the residuals).

The statistical analyses were carried out in R environment (ver. 2.12.1, R Development Core Team, 2010). To compare prevalence of parasitic infestation among different sectors, age-sex categories and years we applied Fisher´s tests or G tests with Williams' correction (Zar, 2010). Males, females and juveniles may show differences patterns parasitism. Thus, we looked for differences between age-sex categories in each variable under study. Then, we applied Generalized Linear Models (GLZM) for males, females and juveniles separately to understand which factors (age-sex categories, years and islet's sectors) have a better predictive value over parasite load (Crawley 2007). As parasite load are count data and did not show homogeneous variances, we applied GLZM´s with Poisson's model (Grafen and Hails, 2002). To study the relation between body size and parasitism, we applied a binomial GLZM analysis for ectoparasite prevalence. The relation between parasite load and SVL was also analysed using GLZM.

In data analysed using GLZM, the significance of each factor was estimated from the analysis of accumulated deviance and the associated statistic. If we observed overdispersion, we applied quasi-Poisson GLZM's, compensating for overdispersion by refitting the model using quasi-Poisson rather than Poisson errors (Crawley, 2007).

We compared the body condition of adult males, females and juveniles by taking the age-sex variable as factor. Then, body condition was estimated from the residuals of the regression of log transformed body weight versus log transformed SVL. Following Crawley (2007), factors and interactions without significance were removed from the GLZM and ANCOVA models until we get minimal adequate model, taking into account AIC (Akaike's Information Criterion) at each step. Post-hoc comparisons were made using 'multcomp' package (Hothorn et al., 2009). Spearman´s rank correlation test was used to test if parasite load was related with SVL or body condition.

3. Results

The prevalence of ectoparasites in *P. lilfordi* from Aire Island was 96.74% (504 of 521 individuals). We did not detect differences in prevalence between adult males and females (uninfected/infected: 11/285 and 5/178, respectively; G-test, G = 0.33, df = 1, P = 0.56), nor between adults and juveniles (uninfected / infected: 16/463 and 1/41, respectively; Fisher's test, P = 1). Prevalence was fairly stable over years for all age-sex categories (adult males: Fisher's test, P = 0.07; adult females: Fisher's test, P = 1; juveniles: Fisher's test, P = 0.43). Also, no differences appeared between seasons (G-test, G =3.67, df = 1, P = 0.06) or between sectors for the whole sample of individuals (G-test, G = 2.43, df = 1, P = 0.12), neither for juveniles (seasons: Fisher's test, P = 1; sectors: Fisher's test, P = 1) or females separately (seasons: G-test, G = 0.0132, df = 1, P = 0.91; sectors: Fisher's test, P = 0.66). For adult males, we did not find differences between sectors (Fisher's test, P = 0.35), but differences in prevalence between seasons were clear (G-test, G = 4.94, df = 1, P = 0.03), with less infected individuals during summer than in spring (uninfected / infected: 8/109 and 3/176, respectively).

Prevalence is not related with body size in females (one-way ANOVA, $F_{1, 181}$= 0.18, P = 0.67). For males, as they showed differences in prevalence among seasons, we analyzed seasons separately. Anyway, no differences in SVL appeared regarding prevalence in spring (one-way ANOVA, $F_{1,\ 177}$= 1.82, P = 0.18) or summer (one-way ANOVA, $F_{1,\ 115}$= 1.73, P = 0.19).

The intensity of infection ranged from 0 to 1138 ($\bar{x}$±se = 88.63±5.3) ticks per individual lizard. Different intensities appeared between adults and juveniles (adults: $\bar{x}$±se = 92.33±5.69; juveniles $\bar{x}$±se = 46.43±8.89; GLZM, $F_{1,\ 520}$= 6.85, P = 0.01), and marginal differences between sexes (adult males: $\bar{x}$±se = 100.72±7.97; adult females: $\bar{x}$±se = 78.77±7.34; GLZM, $F_{1,\ 478}$= 3.70, P = 0.0552).

	Visitors area	**Undisturbed area**	**Total**
Total	104.86±10.4	111.32±11.3	100.72±7.97
Spring	116.43±14.1	154.26±18.47	129.11±11.58
Summer	81.91±13.15	40.61±5.69	57.3±8.21

Table 1. Ectoparasites ($\bar{x}$±se) load of males by area and season. Mites were counted inspecting the whole body surface of the lizards

We ran separate models for adult males, adult females and juveniles, introducing seasons, sectors and years as factors. For adult males, three-way interaction and the interactions area-year and season-year were not significant (all P > 0.05) and were removed from the model. We found significant differences among seasons ($F_{1,\ 291}$= 26.29, P = 5.42×10^{-7}), showing summer fewer parasite load, and years ($F_{3,\ 288}$= 15.65, P = 1.87×10^{-9}). Differences were due to lower parasite load in 2007 ($\bar{x}$±se = 15.51±4.48) respect 2008 ($\bar{x}$±se = 114.46±19.54) (Z = 4.75, P < 0.001), 2009 ($\bar{x}$±se = 88.57±10.45) (Z = 3.53, P = 0.002) and 2010 ($\bar{x}$±se = 134.31±15.81) (Z = 5.09, P< 0.001).

	Visitors area	**Undisturbed area**	**Total**
Total	81.61±9.07	73.48±12.58	78.77±7.34
Spring	94.49±12.35	133.47±20.09	106.37±10.64
Summer	61.15±12.46	13.5±2.71	41.6±7.87

Table 2. Ectoparasites ($\bar{x}$±se) load of females by area and season. Mites were counted inspecting the whole body surface of the lizards

Anyway, as year-area interaction was not significant, differences between areas were stable over years. Not differences were found among sectors (GLZM, $F_{1,\,292}$= 1.72, P = 0.19). But the interaction season-area was statistically significant (GLZM, $F_{1,\,287}$= 12.57, P < 0.001). Thus, not all the sectors vary among seasons in the same way and, moreover, differences between sectors changed throughout the season. Parasite load decreased in summer in undisturbed area (GLZM, $F_{1,\,96}$= 61.11, P = 7.30x10^{-12}) but not in visitors area (GLZM, $F_{1,\,196}$= 2.47, P = 0.12). While in spring no differences between sectors appeared (GLZM, $F_{1,\,176}$= 2.09, P = 0.15; Table 1), in summer differences were found (GLZM, $F_{1,\,116}$= 16.43, P = 9.20x10^{-5}; Table 1).

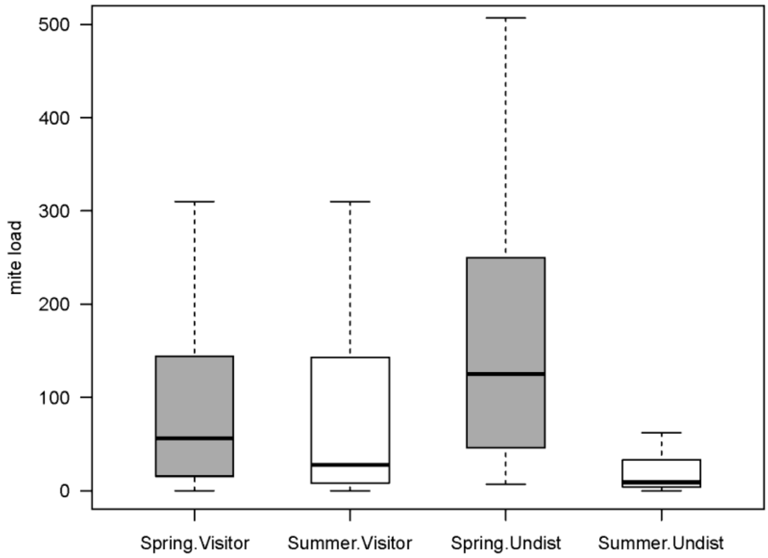


Figure 1. Boxplot of mite loads of males in both areas for spring and summer

For adult females, results were very similar. Three-way interaction and the interaction area-year were not significant (all P > 0.05) and were removed from the model. We obtained significant differences between seasons (GLZM, $F_{1,\,180}$= 32.26, P = 1.55x10^{-8}) showing summer fewer parasite load, and years ($F_{3,\,177}$= 13.01, P = 1.06x10^{-7}). Differences were due also to lower parasite load in 2007 ($\bar{x}$±se = 10.95±3.25) respect 2008 ($\bar{x}$±se = 88.02±14.99) (Z = 3.72, P < 0.001), 2009 ($\bar{x}$±se = 84.07±12.45) (Z = 3.48, P = 0.002) and 2010 ($\bar{x}$±se = 88.83±14.49) (Z = 4.14, P < 0.001). Interaction season-year was significant (GLZM, $F_{3,\,173}$= 5.43, P= 0.001), due to no differences between seasons in 2007 ($F_{1,\,17}$= 0.99, P = 0.33), probably due to small sample size. Moreover, as it happens with adult males, not differences were found among sectors (GLZM, $F_{1,\,181}$=

0.49, P = 0.48). But the interaction season-area was statistically significant (GLZM, $F_{1, 176}$= 17.04, P = 5.69x10^{-5}). Again in females, parasite load decreased in summer in undisturbed area (GLZM, $F_{1, 62}$= 63.64, P = 4.33x10^{-11}) but not in visitors area (GLZM, $F_{1, 117}$= 3.40, P = 0.07). While spring showed no differences between sectors (GLZM, $F_{1, 103}$= 2.73, P = 0.10; Table 2), in summer, differences were found (GLZM, $F_{1, 76}$= 15.67, P < 0.001; Table 2).

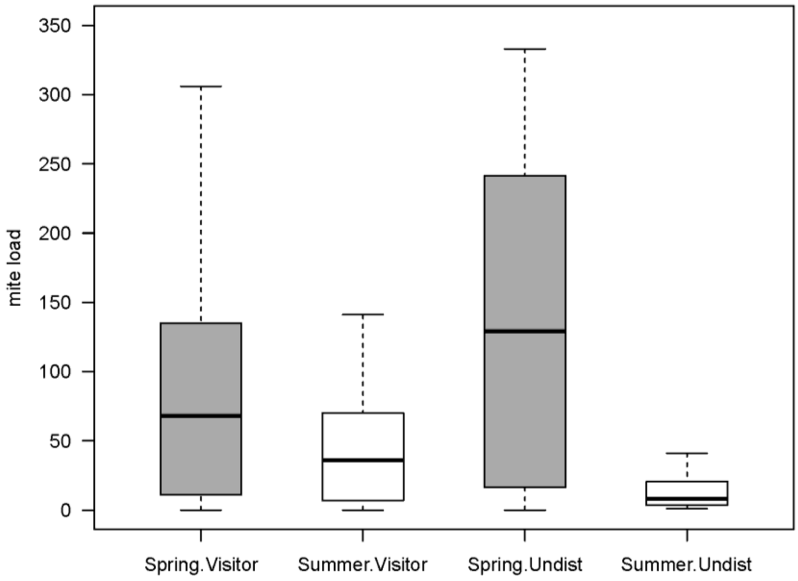


Figure 2. Boxplot of mite loads of females in both areas for spring and summer

In juvenile's model, differences appeared between seasons (spring: $\bar{x}$±se = 63.09±14.11; summer: $\bar{x}$±se =14.05±5.09; GLZM, $F_{1, 39}$= 34.97, P = 1.00x10^{-6}), areas (visitors area: $\bar{x}$±se = 57.52±13.13; undisturbed area: $\bar{x}$±se =33.00±11.15; GLZM, $F_{1, 40}$= 4.87, P = 0.03) and years (GLZM, $F_{2, 37}$= 6.28, P = 0.005). Post hoc test only show differences between 2007 ($\bar{x}$±se = 8.71±5.63) and 2010 ($\bar{x}$±se = 62.91±23.30) (Z = 2.65, P= 0.02). The only significant interaction was season-year (GLZM, $F_{2, 35}$= 4.22, P= 0.02), due, again, to no differences between seasons in 2007 ($F_{1, 5}$= 2.88, P= 0.15), probably due to small sample size.

Because ectoparasite load could be related with host body size, we check for differences in SVL between areas. In spring, adult males from visitors area were significantly larger (GLZM, $F_{1, 176}$= 11.43, P < 0.001), but also in summer (GLZM, $F_{1, 115}$= 22.11, P < 0.001). In females no differences were found (spring: GLZM, $F_{1, 105}$= 0.06, P = 0.81; summer: GLZM, $F_{1, 77}$= 1.41, P = 0.24).

Lizard's body sizes were unrelated with prevalence during spring (adult males: GLZM, χ^2= 1.84, df = 1, P = 0.17; adult females: GLZM, χ^2= 0.73, df = 1, P = 0.40), as well as during summer (adult males: GLZM, χ^2= 1.71, df = 1, P = 0.19; adult females: GLZM, χ^2= 0.10, df = 1, P = 0.75). Parasite load was not related with body size during spring in adult males, (GLZM, $F_{1,\ 177}$= 0.07, P = 0.79) or adult females (GLZM, $F_{1,\ 103}$= 0.36, P = 0.55). While during summer, we found a higher parasite load in larger lizards (adult males, GLZM, $F_{1,\ 115}$= 19.36, P = 2.43x10^{-5}; adult females: GLZM, $F_{1,\ 76}$= 14.04, P < 0.0005). However, analysing summer results separately for each sector, only larger lizards from visitors area showed a higher parasite load (in visitors area, adult males, GLZM, $F_{1,\ 64}$= 16.04, P = 0.0002; adult females: GLZM, $F_{1,\ 44}$= 6.13, P = 0.02; in undisturbed area: adult males, GLZM, $F_{1,\ 49}$= 3.98, P = 0.52; adult females, GLZM, $F_{1,\ 30}$= 0.35, P = 0.56).

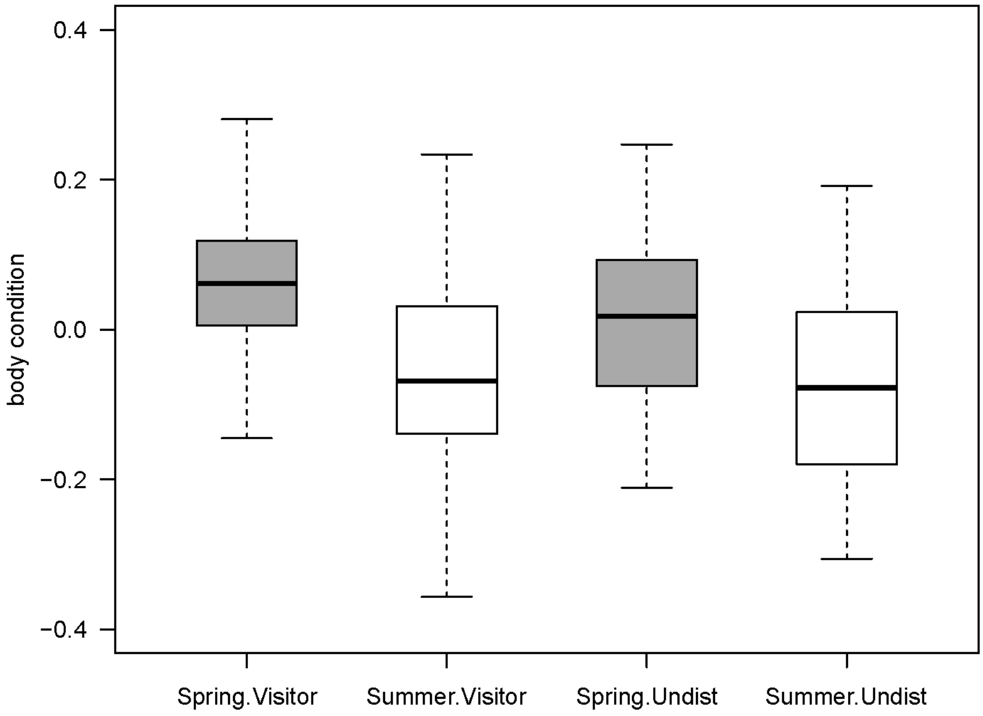


Figure 3. Boxplot of body condition of males in both areas for spring and summer

We also examined changes in body condition for each sector and season. In adult lizards, body condition strongly decreased in summer in visitors area (adult males: GLZM, $F_{1,\ 191}$= 55.55, P = 3.08x10^{-12}; adult females: GLZM, $F_{1,\ 113}$= 38.00, P = 1.12x10^{-8}) and, in a lesser extent in undisturbed area (adult males: GLZM, $F_{1,\ 95}$= 7.91, P = 0.006; adult females: GLZM, $F_{1,\ 62}$= 10.48, P = 0.002). In the same direction, for adult males differences between sectors appeared in spring (GLZM, $F_{1, 171}$= 8.88, P = 0.003), showing better condition visitors area individuals, but these differences disappeared in summer (GLZM, $F_{1, 115}$= 0.007, P = 0.93). Similar tendency were found for females. While in spring body condition was clearly similar (GLZM, $F_{1, 101}$= 0.05, P= 0.82), in summer, females' condition from undisturbed area was marginally better (GLZM, $F_{1, 74}$= 3.32, P = 0.07).

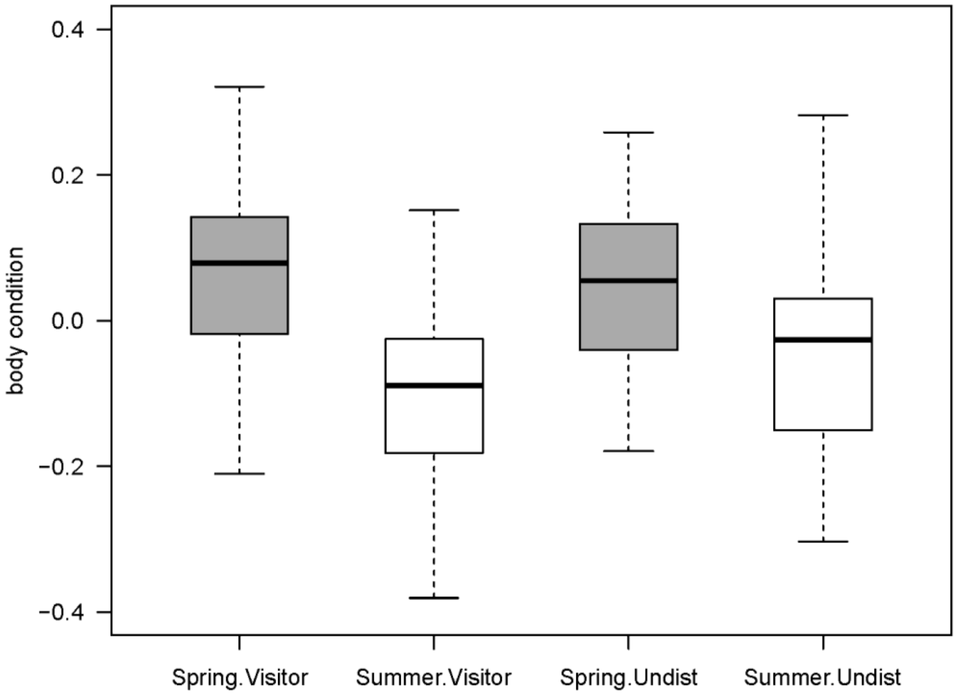


Figure 4. Boxplot of body condition of males in both areas for spring and summer

We were unable to find any relation between body condition and prevalence of infestation (ANCOVA: adult males: $F_{1, 287}$= 1.32, P = 0.25; adult females: $F_{1, 174}$= 0.39, P = 0.54). In addition, we did not find differences between prevalence and body condition, as a categorical variable ('healthy' and 'unhealthy' lizards) during spring (Fisher's test: adult males, P = 0.56; adult females, P = 0.49) nor during summer (Fisher's test: adult males, P = 0.71; adult females, P = 0.53). Nevertheless, for the whole island, we found a negative correlation between parasite load and body condition during spring (Spearman´s rank correlation test: adult males, S = 1102902, P = 0.002; adult females, S = 233047.8, P = 0.004), but not during summer (Spearman´s rank correlation test: adult males, S = 249095.8, P = 0.4745; adult females, S = 65630.69, P = 0.38).

4. Discussion

This is the first study on ectoparasites of the Balearic lizard. Prevalence of the Balearic lizard from Aire Island reached almost 97% of individuals, one of the highest value even recorded (Table 3). Only an insular population of *Gallotia galloti eisentrauti* infected by *Ophionyssus galloticolus* in Tenerife Island (Canary Islands, Spain) reached similar values (Bannert et al., 2000). Regarding parasite load, the average intensity was up to 88.63±5.3 mites per individual. These results are also higher than those found in other lacertid populations. For example, *P. melisellensis* showed a parasite burden lower than 1.63 (Huyghe et al., 2009). In *P. muralis,* mite load ranged from 0 to 16 (Amo et al., 2006) and the median of ectoparasite load found in *Sceloporus occidentalis*, was also very low (median of ticks = 16; median of mites = 3; Schall et al., 2000). Even on insular population values are lower; García-Ramírez et al., (2005) found an average intensity of 6.25 in *G. atlantica* from Alegranza islet (Canary Islands). The lack of predation and competitors allow high lizard densities in several Mediterranean islets.

Specie	origin	Prevalence (%)	Authors
Podarcis lilfordi	Insular	97%	Present study
Gallotia galloti eisentrauti	Insular	100 %	Bannert et al., 2000
Gallotia atlantica	Insular	53.3%	Bannert et al., 2000
Podarcis muralis	Continental	29.7%	Amo et al., 2006
Lacerta agilis	Continental	10%	Gwiazdowicz and Filip, 2009
Podarcis muralis	Continental	2 individuals	Strijbosch, 1980
Sceloporus occidentalis	Continental	48 %	Schall et al., 2000

Table 3. Prevalence of infestation by ectoparasites in different lacertid lizards species

In Aire Island the density reaches over 4,000 individuals per hectare (Pérez-Mellado et al., 2008) and density is considered a good predictor of the degree of infestation by parasites (Svahn, 1974). Even if the Balearic lizard lacks social organization the high density could promote a higher rate of contacts and interactions among individuals and, consequently, higher probabilities of infection (but see Godfrey et al., 2008 for a lack of relation between host's density and prevalence). Alternatively, the high parasite rates observed in Aire Island could be related with the bottleneck effect and the degree of inbreeding, intrinsic to the origin of many insular populations. That is, the loss of genetic variability was proposed as one of the causes that can erode the resistance to parasitism (Hamilton et al., 1990). Particularly, in populations where individuals are closely related, the expected low level of genetic variability would facilitate a parasite found lower genetic resistance in the host (Shykoff and Schmid-Hempel, 1991).

Probably due to the high proportion of infected individuals, we did not found general differences in prevalence between sectors or age-sex categories. However, we found differences between adults and juveniles and between adult males and females in parasite infestation. Sex differences in parasite load are frequent (see review in Klein, 2004). Immunological divergences between sexes have been mainly proposed to be responsible of these differences, but also genetic and behavioural differences may explain some variability in response to infection (Klein, 2004). Furthermore, in Aire Island, males show higher mobility (Pérez-Mellado et al., 2007) and interact more frequently with conspecifics, probably leading to an increase in exposure to parasite's vectors from infected conspecifics (Klein, 2004). In addition, adults are more prone to be infected as they monopolize the best foraging and basking places where probably became repeatedly re-infected (Amo et al., 2005), while juveniles are normally located at less favourable areas. In fact, on Aire Island, the proportion of different age classes is significantly different at different areas of the islet (Pérez-Mellado et al., 2007; Garrido and Pérez-Mellado, 2013). In prevalence rates, only for adult males we found differences between seasons. The rate of infected males was higher in spring, probably because of the immunosuppressive effects of testosterone during the breeding season (Klein, 2004).

We found no significant differences in prevalence, neither among sectors nor along the four years of study. The stability of prevalence for long periods of time was also detected in other species of reptiles (Smallridge and Bull, 2000; Salkled and Schwarzkopf, 2005). The absence of differences between years would reflect the stability of parasite-host interaction in the system under study (Amo et al., 2005). We suggest that because of the high lizard density, differences in prevalence values (near 100%), between years are difficult to emerge.

Parasite load decreased in summer for all age-sex categories. We agree with Amo et al. (2005) when suggest that infections occur early in the breeding season, when lizards are more active. It is also when many resources are invested in reproductive effort (Salvador et al., 1996). After that period, resources previously invested in courtship and reproduction could be allocated to fight against parasites. Alternatively, the maximum activity period for *P. lilfordi* takes place in April and May (Pérez-Mellado, 1998). Thus, contacts with conspecifics and subsequent reinfections are more probable during spring. However, trends found in parasite load for each sector were different. Thus, in spring no differences were observed between areas, but in summer undisturbed area individuals showed fewer mites. From another point of view, in visitors' area ectoparasite load was maintained across seasons while in undisturbed area decreased substantially in summer. It might assume that differences in parasitism are due to differences in body size. However, males from visitors' area were larger, but in both seasons and no differences in females' SVL were found between areas. Thus, the observed differences in ectoparasite load between areas could not be due to differences in lizards' body size from those areas. Moreover, we were unable to find a general relationship between parasitism and body size. Surprisingly, this relation only appeared during summer for males and females and only at visitors' area. Amo et al. (2006) also found a lack of relation between parasite load and SVL during breeding season and a significant relation during summer at the area with a higher human impact. As they proposed, in less disrupted areas larger lizards obtained enough resources to have a good defence against parasites, while in visitors' area didn´t, as they expend more resources to perform escape responses.

The impact caused by humans on natural populations is currently in the spotlight. Typically, the disturbance has been associated with changes in the behaviour of animals, which even has been taken as an index of disturbance effects (Carney and Sydeman, 1999). Lizards perceive humans as potential predators, adapting their escape behaviour with an increasing avoidance response (see for example Amo et al., 2006, 2007). Thus, even if parasite load strongly decreased in undisturbed area in both sexes, at visitors' area it was maintained. Lizards inhabiting areas with high tourism levels presumably perform more often escape responses. The costs derived from these behaviours and the time lost for other activities, as foraging, can reduce body condition of lizards (Amo et al., 2006, 2007). For example, individuals of *P. muralis* and *P. algirus* exposed to a high predation pressure suffered a loss of body mass (Martín and López, 1999; Pérez-Tris et al., 2004).

Our results support this hypothesis, the loss of body condition is stronger for both sexes in the area of greater human impact, and could be reflected in other fitness-related components, as parasite load. As a consequence of the loss of body condition, the ability to invest in defence against parasites could also decrease (Cooper et al., 1985; Smallridge and Bull, 2000), as we found in lizards from visitors area. Alternatively, Navarro et al. (2004) showed that over perceived risk of predation, individuals tend to reallocate available resources. Thus, the immune system receives a reduced priority because its elevated costs. Under experimental conditions, the individuals exposed to predators reduced their ability to cope with parasitism (Navarro et al., 2004). Anyway, it appears that in Aire Island, human disturbance can cause a break in the parasite-host equilibrium of lizard population. Body condition supports this idea, as it follows different trends between both areas, similar to those obtained for parasitism intensity, body condition decrease in between seasons was stronger in visitors' area.

Additionally, we did not found a direct correlation between prevalence and body condition or with the categorical variables of body condition (healthy and unhealthy, see Material and Methods section). For parasite load, only during spring a negative correlation appeared. Thus, during spring, healthier individuals loaded fewer mites. In Mediterranean islets, trophic availability is frequently limited (Pérez-Mellado, 1989; Pérez-Mellado and Corti, 1993) and the climate is characterized by strong seasonal fluctuations (Fuentes, 1984), with significant variations in food resources. During summer, the drought is particularly strong at Aire islet and very few arthropods are active (unpub. results). Consequently, individuals could not have enough available resources to maintain an optimal body condition against parasite attack. Probably, because lizards had a better body condition in spring, they can afford the costs of defence against parasites.

Mediterranean islands, as Aire, have isolated lizard populations on relatively simple environments. Thus, historically, parasite-host interactions had probably suffered less disruption than adjacent continental areas. Individual lizards showed values of parasitism, both prevalence and intensity, which are among the highest found to date.

These values are explained by the ecological and life history characteristics of insular population of the Balearic lizard (very high density values, low predation pressure and the scarcity of resources).

Moreover, our results suggest how the appearance of human disruption may break this parasite-host equilibrium. In our case, the impact of visitors affects the natural dynamic of parasite-host system. Because humans apparently are perceived as potential predators, lizards would perform more frequently escape responses, altering their natural behaviour. Throughout the summer, when drought is particularly strong at Mediterranean islands and resources are scarce, these islands receive the major bulk of tourists. The higher parasite load and stronger decrease of body condition observed in visitor's area during summer could be a competitive disadvantage for individuals from that area, compromising the fitness and survival probabilities of lizards. This could be of particular interest as it happens in especially sensitive ecosystems: small island populations.

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Capítulo 5

En un entorno insular, la velocidad de las lagartijas se ve afectada por parásitos sanguíneos, pero no por ectoparásitos

En un entorno insular, la velocidad de las lagartijas se ve afectada por parásitos sanguíneos, pero no por ectoparásitos

Resumen

Los parásitos son esenciales para comprender la dinámica poblacional y la ecología de los hospedadores. Por ello, se ha propuesto a los parásitos como una de las fuerzas evolutivas más importantes. En numerosas islas del Mediterráneo, poco se sabe acerca de los parásitos que infectan a las lagartijas del género *Podarcis*. Los estudios realizados sobre ectoparásitos que infectan a las lagartijas de este género se centran principalmente en estudiar el impacto que tienen sobre los rasgos vitales de los hospedadores. Sin embargo, el conocimiento de los niveles naturales de infestación y prevalencia parasitaria es un primer paso necesario para entender cómo un parásito interactúa con sus hospedadores. En este trabajo estudiamos los factores que afectan el ectoparasitismo en una lagartija endémica, *Podarcis lilfordi* (Günther, 1874), en la isla del Aire (Menorca, Islas Baleares, España). Centramos nuestra atención en la influencia de las perturbaciones humanas sobre el parasitismo. Ambas, la prevalencia y la intensidad de la infección se encuentran entre las mayores que se ha encontrado en las poblaciones de lacértidos mediterráneos. La reducida variabilidad genética y/o la alta densidad propia de las islas se han propuesto para explicar tales valores de intensidad y prevalencia. La intensidad de la infección fue mayor en adultos, probablemente debido a que monopolizan los mejores lugares donde las posibilidades de re-infección son mayores. Como en muchas otras poblaciones, entre los adultos, los machos mostraron mayor carga parasitaria. Diferencias inmunológicas, genéticas y de comportamiento se proponen para explicar esta variabilidad. Además, las lagartijas reconocen a los humanos como depredadores, realizando conductas de defensa, que son costosos en términos fisiológicos. Por lo tanto, la perturbación humana también podría afectar a otros rasgos vitales, como la defensa contra los parásitos. Durante el verano, cuando el turismo es particularmente intenso, los machos y hembras adultos que habitan las zonas visitadas de la isla mostraron una mayor carga de ectoparásitos y una disminución más

marcada de la condición corporal en comparación con los individuos de áreas no visitadas. Proponemos que los costes derivados de realizar conductas de escape, como respuesta a la presencia humana, son responsables de las diferencias observadas. En consecuencia, la presión humana afectaría el equilibrio parásito-hospedador y la condición corporal de los hospedadores, disminuyendo así su estado físico y sus probabilidades de supervivencia.

Sprint speed of lizards is affected by blood parasites, but not ectoparasites, in an insular environment

Abstract

Parasites can have detrimental effects on locomotor performance of their hosts. In this study, the effect of parasitism on burst speed of the Balearic lizard, *Podarcis lilfordi,* at its population of Aire Island (Balearic Islands, Spain), was examined studying haemogregarines and mites. According to the minimal adequate GLM model obtained, the relative length of hind legs did not influence locomotor performance of lizards. Concerning parasites, mites were retained as a factor by GLM minimal model, but their effect on burst speed was not significant. Co-infection by both parasites had also no effect on running speed of hosts. However, the intensity of infection by blood parasites showed a significant influence on burst speed. Body condition had also a significant influence. Lizards with a lower blood parasite load and better body condition showed a faster burst speed. We compare our results with those from other lizard species living in different habitats, discussing the role of insular conditions, with a low predation pressure and high lizard densities.

Keywords: Burst speed; Blood parasites; Ectoparasites; Body condition; Islands; Predation pressure; Balearic lizard; *Podarcis lilfordi*.

Introduction

Locomotor capabilities, such as maximal sprint speed or burst speed, are good indicators of performance abilities (Robson and Miles 2000). In addition, there is a clear link of locomotor performance and individual’s fitness, through its effect on foraging efficiency (Huey and Pianka 1981; Magnusson et al. 1985), escape from predators (Shine 1980; Bauwens and Thoen 1981; Webb 1986), and agonistic interactions among conspecifics (Trivers 1976; Pough and Andrews 1985; Robson and Miles 2000). Nevertheless, there are few experiments looking for the effect of parasitism on locomotor capabilities. Only few studies of reptiles were able to find a clear correlation between parasite load and burst speed. Oppliger et al. (1996) found a significant relation of haemogregarines and burst speed in *Zootoca vivipara* and Main and Bull (2000), described a similar relation in juveniles of *Tiliqua rugosa* and ectoparasites. In both cases, burst speed decreased with higher parasite loads. According to Schall et al. (1982), because aerobic capacity is reduced in lizards infected with malaria, lizard activity should be reduced. Schall et al. (1982) were unable to find an effect of parasitism on burst speed but found a clear effect of parasitism on running stamina.

The Balearic lizard (*Podarcis lilfordi)* is an endemic lacertid lizard from Balearic Islands (Spain). In Aire Island, low predation pressure, high lizard density and scarcity of food resources promoted an increased number of physical contacts and agonistic interactions among conespecifics (Garrido and Pérez-Mellado in press, 2013), also allowing a higher chances of parasite transfers (Garrido and Pérez-Mellado in press, 2013). In Aire Island, rates of parasitism by haemogregarines and mites are among the highest found in Mediterranean lizards (Garrido and Pérez-Mellado in press, 2013). Because this population is exposed to a combination of different parasites, it is critical to understand how each parasite affect host's performance, as well as the interaction between them. (Jolles et al. 2008; Harries et al. 2001, Graham et al. 2005).

In this work we study the impact that haemogregarines and mites have on locomotor performance on the Balearic lizard in Aire Island, where. If predators are preying upon most parasitized individuals (Temple 1987; Navarro et al. 2004; Genovart et al. 2010), those individuals would be eliminated from the population by predators, obscuring the relation between parasites and burst speed that, finally, would be analyzed with remaining survivors. In Aire Island, predation pressure is very low (Cooper and Pérez-Mellado 2012) and, consequently, heavily infested individuals probably are not excluded by predators (Temple 1987; Navarro et al. 2004; Genovart et al. 2010).

We predict that most parasitized individuals will show a lower sprint speed. We also predict a higher detrimental effect of haemogregarines than mites on sprint speed, as it was shown for other aspects of lizard physiology (Oppliger et al. 1996; Sorci et al. 1996; Oppliger and Clobert 1997).

Material and methods

P. lilfordi is a medium-sized lacertid lizard (snout-vent length, SVL, up to 81 mm. in males and 75 mm. in females; Pérez-Mellado 1998). The study was conducted during June 2011 in Aire Island. Lizard density can be very high, over 4,000 individuals per hectare (Pérez-Mellado et al. 2008).

The term haemogregarine refers to coccidial blood parasites of the family Haemogregarinidae, containing the genera *Hepatozoon*, *Haemogregarina* and *Karyolysus* (Telford 2008). Roca and Galdón (2010) suggested that blood parasites from lacertid lizards of the Iberian Peninsula and Canary Islands can be ranged within genera *Hepatozoon* or *Hemolivia*.

Mites of the genus *Ophionyssus* (Acari; Mesostigmata; Macronyssidae) are mainly parasites of Squamata. This genus includes 15 species, 13 of them confined to lizards of various genera (Fain and Bannert 2000). Lacertid lizards are parasitized by six species of *Ophionyssus*, two of them, *O. Galloticolus* and *O. setosus*, parasites of the genus *Gallotia* from Canary Islands (Bannert et al. 2000). In the case of the genus *Podarcis* from Spain or the rest of the Mediterranean basin, we do not know any taxonomic study of their ectoparasites. Nevertheless, *Ophyonissus* is present in many other lizard species from Iberian Peninsula (Bannert et al. 2000; Moraza et al. 2009; Majlathova 2010) and in other *Podarcis* species from Europe (Strijbosch 1980). The ectoparasites of this study were tentatively assigned to this genus.

Lizards were collected by noosing on sunny days, between 8.00 and 12.00 GMT, when lizards where more active. We only studied adult males. We obtained body temperatures of individuals immediately after performance trials. For each individual, we recorded its snout-vent length (SVL), body weight and right and left hind limb lengths (HLL). Relative sizes of hind limbs (rHLL) were calculated from the residuals of the regression of log-transformed average of left and right HLLs versus log transformed SVL.

Trials were done in the field just after capture. Locomotor performance trials were carried out on a racetrack with cork substrate. Two marks separated by two meters defined the start and the end of the run (see a full description of the apparatus in Cooper et al. 2004). Two observers timed with digital stopwatches the passage through these two marks. We classified the quality of each run as 'good' or 'poor' (van Berkum and Tsuji 1987; Husak 2006). Poor runs (runs with pauses or reversals) were discarded. We included three good runs for each individual. One problem with measuring sprint speed on a racetrack is the difficulty to correct for differences among individuals in their motivation to run (Sorci et al. 1995). Thus, after three poor runs, a lizard was discarded. For each individual, we used in analyses its fastest run.

Body condition was estimated from the residuals of the regression of log transformed body weight versus log transformed SVL. We also counted the number of ectoparasites observed on the body surface immediately after capture. To obtain blood samples, we made a slight longitudinal cut in the dorsal base of the tail with a sterile scalpel. With the blood drop we obtained a blood smear *in situ*. Blood smears were placed on microscopic slides and air dried in the field. In the lab, slides were fixed with absolute methanol for 10 minutes and then stained with modified Giemsa for 20 minutes. Samples were analyzed using an optical microscope at 400X. Blood parasites were haemogregarines (see above). The intensity of parasitism was estimated on a total of 2,000 counted cells per sample. Specimens were always released at the site of capture.

The statistical analyses were carried out in R environment (ver. 2.12.1, R Development Core Team 2010). We analyzed the effect of haemogregarine load, mite load and body condition on burst speed by using general linear models (GLM, Crawley 2007). As hindlimb length could influences burst speed (Bauwens et al. 1995 and references therein), we include the relative sizes of hind limbs (see above) in the model. Interaction terms of rHLL with the rest of variables revealed whether the influence parasitism and condition over burst speed varied in relation to rHLL.

We started with a saturated model, considering effects of all independent variables as well as all interactions between them. To determine the minimum adequate model (Crawley 2007) we use a backward stepwise logistic regression made on the basis of deletion tests (Crawley 2007). Non significant factors and interactions were removed from the GLM models until we obtained a minimal adequate model, taking into account at each step the minimal value of the Akaike's Information Criterion

(AIC). Post-hoc comparisons were made using the R multcomp package (Hothorn et al. 2009). Effect sizes reported are partial eta-squared (η_p^2) and may be interpreted similarly as R^2 (Cohen 1988). As R^2, η_p^2 is a dimensionless measure of effect size independent of the degrees of freedom used in the analyses (Tabachnick and Fidell 2001).

Results

The measures of burst speed by both observers were highly correlated, indicating a high repeatability (Spearman's rank correlation test, R_s= 0.64, n = 49, P = 1.55×10^{-6}). Thus, we obtained the mean of the measurements of the two observers for each trial. Body temperatures of experimental lizards ranged between 31.30 and 38.30 ºC (mean ± SE = 35.74 ± 0.45). During trials, we did not detect any significant influence of body temperature in the maximum speed of lizards (Spearman's rank correlation test, R_s= -0.21, n = 30, P = 0.15). HLL averages ranged between 32.36 – 40.36 mm (mean ± SE = 36.52±0.27 mm).

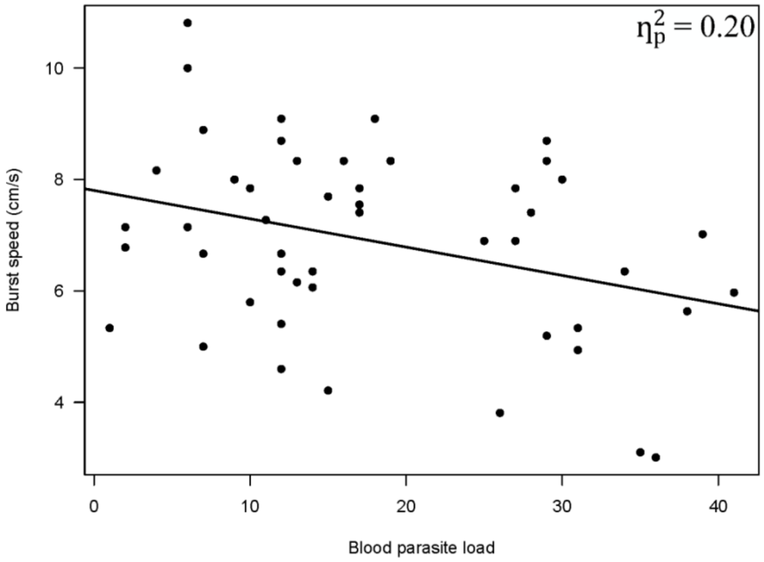

Figure 1. Relationship between burst speed and blood parasite load in the Balearic lizard, *P. lilfordi*. η_p^2: Cohen's partial eta-squared.

In the GLM model, none of the interaction terms was significant (all P> 0.05) and consequently, all of them were removed. The GLM minimal adequate model was significant (R^2 = 0.30, $F_{4, 40}$ = 4.17, P = 0.006). We found no influence of rHLL over burst speed of lizards ($F_{1, 40} = 2.41$, $P = 0.13$). Ectoparasite load was retained in the model, but its effect remained non significant ($F_{1, 40} = 0.93$, $P = 0.34$). Intensity of infection by haemogregarines showed a clear influence ($F_{1, 40} = 6.01$, $P = 0.02$; $\eta_p^2 = 0.15$; Fig. 1). Finally, body condition was also significantly related with burst speed ($F_{1, 40} = 7.34$, $P = 0.01$; $\eta_p^2 = 0.18$; Fig. 2). Thus, burst speed was higher for lizards with a lower blood parasite load and a higher body condition.

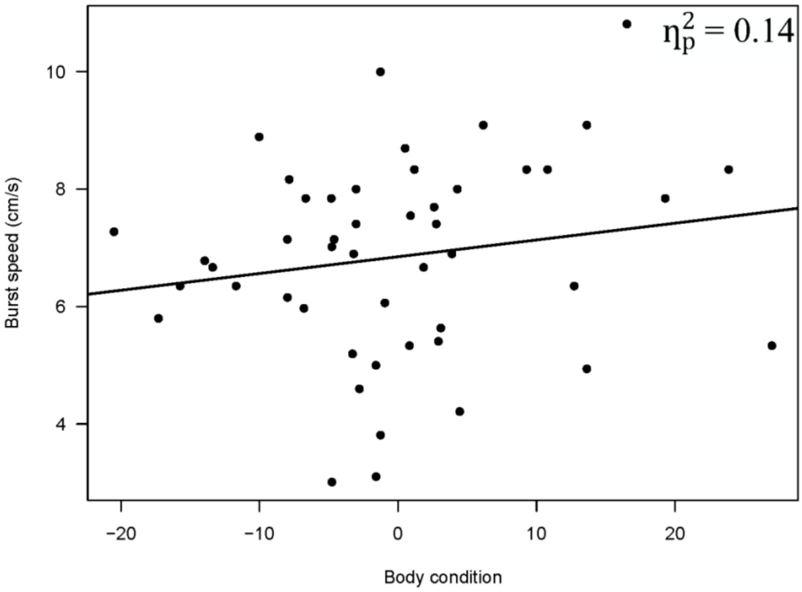

Figure 2. Relationship between burst speed and body condition in the Balearic lizard, *P. lilfordi*. $\boldsymbol{\eta_p^2}$: Cohen's partial eta-squared

Discussion

The predictions of biomechanical models and the results of studies for some lacertid lizards, showed a positive relationship between the relative length of hind limbs and burst speed (Bauwens et al. 1995) and morphology had a high explanatory power in multispecies comparisons (Pounds 1988; Bauwens et al. 1995; Bonine and Garland 1999; Van Damme and Vanhooydonck 2001). But, at intraspecific level, some studies showed the weakness of this relation (Garland 1985; Van Damme et al. 1997; Macrini and Irschick 1998). In our case, we were unable to find any direct relation between burst speed and the relative hind limb length.

According to our predictions, we found a strong negative relationship between blood parasite load and running speed. Haemogregarines that infected lizards may reduce their haemoglobin concentrations, lowering their capacity for oxygen transport to muscle tissue (Oppliger et al. 1996), involved in several aspects of lizard physiology and behaviour, such as foraging efficiency (Caudell et al. 2002). Our results confirm that individuals who run faster showed a better body condition, being also less parasitized by haemogregarines. In the same population, Garrido and Pérez-Mellado (in press) found a negative correlation between haemogregarines intensity and body condition of individuals from a large sample of lizards studied from 2007 to 2010. But, only from observations of a natural population, a correlation between parasitism and fitness does not necessarily imply causation (Main and Bull 2000). For other reasons than parasite load, some individuals may have a different behaviour or fitness than the rest of the population, and can be more susceptible to parasites. Consequently, these individuals can have reduced locomotor capabilities.

Negative effects of blood parasites over burst speed of lizards were found by Oppliger et al. (1996). Why other studies were unable to find such correlation between parasite load and locomotor performance? Predators can usually capture most parasitized individuals (Temple 1987). For Oppliger et al. (1996), this fact implies that most parasitized lizards may be more vulnerable to predation as their mobility was reduced. Therefore, most parasitized lizards are eliminated from the population by predators, and the relation between parasites and burst speed can be obscured. In Aire Island predation risk over *P. lilfordi* is very low and we can avoid this factor.

Our results showed no influence of ectoparasites load on running speed. In laboratory trials, *Tiliqua rugosa* lizards infested by mites runned slower and had a lower endurance (Main and Bull 2000). With the exception of this experiment, no correlation of locomotor performance and ectoparasites was found in reptiles, at least in natural populations. Dunlap and Mathies (1993) found harmful effects of mites only when lizards were co-infected with other parasites as malaria. We did not find an influence on maximal running speed of the interaction of both parasites. Hence, co-infection do not increase the impairment of locomotor performances of lizards from Aire Island.

Blood parasites seem to have several consequences, as a lower maximal sprint speed (Oppliger et al. 1996), a lower resting metabolic rate (Oppliger et al. 1996), an increased reproductive effort (Sorci et al. 1996) and a decreased ability to regrow the tail (Oppliger and Clobert 1997). At least in lizards from Aire Island, blood parasites have a greater negative effect on the fitness of individuals than mites, in a system where predation pressure could be discarded as a disturbing factor to interpret the results.

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Capítulo 6

Relaciones complejas entre la carga parasitaria, los comportamiento específicos masculinos y el comportamiento de escape

Relaciones complejas entre la carga parasitaria, los comportamientos específicos masculinos y el comportamiento de escape en una lagartija insular

Resumen

Los modelos de escape economicistas predicen las decisiones de escape que toman las presas cuando un depredador se acerca. La distancia de inicio de la huida (la distancia entre el depredador y la presa cuando esta inicia comienza a huir, FID en sus siglas en inglés) y la distancia huida (DF, en sus siglas en inglés) son las principales variables utilizadas para medir las respuestas de escape. Según la teoría óptima de escape, FID aumenta a medida que el coste de no huir aumenta, disminuye cuando el coste de huir debido a la pérdida de oportunidades aumenta, y aumenta a medida que aumenta la aptitud de la presa. Algunos factores, como el parasitismo, pueden afectar a más de uno de estos predictores de la FID. Las presas parasitadas pueden tener una menor aptitud inicial, la capacidad de escape deteriorada y / o una disminución de la capacidad de forrajeo para evitar la depredación, reduciendo aún más el coste de huir. Por ejemplo, si los parásitos disminuyen la condición corporal, la aptitud de la presa se reduce y la capacidad de escape puede ser afectada. Por lo tanto, la influencia global del parasitismo sobre la FID es difícil de predecir. Hemos examinado las relaciones entre las decisiones de escape y la carga parasitaria, el tamaño corporal y el estado de condición sobre la FID y la DF en la lagartija balear (*Podarcis lilfordi*).

Las lagartijas con una mayor carga de hemogregarinas tenían una FID mayor y una DF más corta. La FID estaba negativamente relacionada con la condición corporal en los individuos con cargas más altas de ácaros. No está claro si las diferencias observadas se deben a los efectos de los parásitos, ya que las diferencias de comportamiento entre machos que tienen diferentes cargas parasitarias podrían explicar, al menos en parte, las correlaciones entre los parásitos y el comportamiento de escape. Un conocimiento detallado de la ecología y comportamiento de las presas es necesario para evaluar el

aparente efecto del parasitismo en los estudios de campo no experimentales. Se discuten los hallazgos en relación a las predicciones de la teoría óptima de escape.

Complex relationships among parasite load, male-specific behaviors and escape behavior in an insular lizard

Abstract

Economic escape models predict escape decisions by prey being approached by predators. Flight initiation distance (FID, the predator-prey distance when the prey begins to flee), and distance fled (DF) are major variables used to measured escape responses. In optimal escape theory, FID increases as cost of not fleeing increases, decreases as cost of fleeing due to lost opportunities increases, and increases as the prey's fitness increases. Some factors, including parasitism, may affect more than one these predictors of FID. Parasitized prey may have lower initial fitness, impaired escape ability and/or reduced foraging ability to avoid predation, further reducing opportunity cost of fleeing. For example, if parasites decrease body condition, prey fitness is reduced and escape ability may be impaired. Hence, the overall influence of parasitism on FID is difficult to predict. We examined relationships between escape decisions and parasite load, body size and body condition on FID and DF in the Balearic lizard (*Podarcis lilfordi*). Lizards showing higher haemogregarines load had longer FID and shorter DF. FID was negatively related to body condition among individuals with high mite loads. It is uncertain whether the observed differences are caused by effects of parasites, because eco-behavioral differences among males having different parasite loads may account at least in part for the correlations between parasites and escape behavior. Detailed knowledge of a prey's ecology and behavior is necessary to evaluate apparent effect of parasitism in non experimental field studies. We discuss the findings discussed in relations to predictions of Optimal Escape Theory.

Keywords: flight initiation distance; distance fled; escape behavior; body condition; parasitism; lizard.

1. Introduction

To recognize and avoid predators is an essential activity of animals because a mistake may lead to death. Consequently, there is strong selection on escape behavior (Ydenberg and Dill 1986). Flight initiation distance (= FID), the distance separating an approaching predator from prey when escape begins, is the primary escape variable used to assess processes associated with escape decisions by prey. Distance fled (DF) is another important indicator of assessed risk of not fleeing and cost of fleeing. Several models have been suggested to explain decisions made by prey based on costs of not fleeing (predation risk) and of fleeing (due to loss of opportunities). In the first economic model (Ydenberg and Dill 1986), prey begin to flee when a predator reaches the distance where cost of not fleeing and cost of fleeing are equal. Because cost of not fleeing increases and cost of fleeing decreases as predator-prey distance decreases, cost of not fleeing exceeds cost of not fleeing at all distances closer than the predicted FID.

According to optimal escape theory (OET; Cooper and Frederick 2007, 2010), FID is optimized rather than maximized or limited to the case of equal costs of not fleeing and of fleeing. OET predicts escape decisions based on effects of predation risk, cost of escaping, and current fitness (Cooper and Frederick 2007). By including prey's initial fitness, OET incorporates Clark's asset protection principle (1994), which states that individuals having greater residual reproductive value should be more risk-averse (Clark 1994; Roff 2002). In the context of escape, prey should have longer FID to protect their expected lifetime fitness. Individuals with low expectations should be relatively risk-prone because they have little to lose. Recently, Cooper and Frederick (2010) extended optimal escape theory by introducing predator lethality. Optimal FID increases as predator lethality increases, matching intuitive expectations that prey should not flee when contact entails no fitness loss but should be increasingly wary as expected fitness loss on contact increases. Hence, inclusion of lethality as a predation risk factor improves the ability of optimal escape theory to predict escape decisions. In this same work, Cooper and Frederick (2010) discussed multiple potential effects of some factors, such as autotomy of body parts and parasitism, on escape decisions.

Parasitism has multiple potential effects on escape decisions as described by the model of Cooper and Frederick (2010). For parasitized, injured or diseased organisms, lower initial fitness favors decreased FID (Clark 1994; Cooper and Frederick 2010).

However, reduced ability of parasitized individuals to obtain benefits reduces opportunity cost of fleeing and impairment of escape ability increase risk of not fleeing. Unless escape tactics change for parasitized individuals, these effects favor increased FID (Cooper and Frederick 2010). Hence, the influence of parasitism on FID is difficult to predict (Cooper and Frederick 2010). The objective of the present study is to assess how parasitism affects the perceived risk of predation as reflected by FID.

Parasites can reduce host fecundity (Schall 1983; Møller 1993) and reproductive success (Schall 1996; Oppliger et al. 1997), and can affect sexual selection of hosts (Hamilton and Zuk 1982; Møller et al. 1999). Parasitism is an important cause of host mortality (Schall 1983, 1996; but see Eisen 2001), reducing life expectancy (e.g. Sorci and Clobert 1995; Møller 1997) and residual reproductive value of hosts. This may reduce risk aversiveness by hosts. In accord with life-history theory (Clark 1994; Roff 2002), optimal escape theory predicts that this aspect of parasitism favors shorter FID in highly parasitized prey due to their lower initial fitness.

Parasitism may have another major effect, reduction of escape ability that counters the effect on initial fitness. Several studies have shown that parasitized hosts suffer greater predation risk (Temple 1987; Navarro et al. 2004; Møller and Nielsen 2007; Genovart et al. 2010). Møller and Nielsen (2007) suggested that individuals with high intensities of infection have a disproportionately larger risk of predation. The metabolic efficiency of infected lizards is impaired (Schall et al. 1982). Impaired locomotor performance reduces escape ability (Shine 1980; Bauwens and Thoen 1981; Webb 1986). Impairment of running speed and possibly endurance in hosts suggests that parasitized individuals may flee shorter distances than unparasitized individuals. In lizards running speed decreases as loads of blood parasite (Oppligeret al. 1996) and ectoparasites increase (Main and Bull2000). Because the escape ability of highly parasitized prey is reduced, their perception of risk is predicted to be greater than that of parasite-free or lightly parasitized individuals unless the parasites also affect cognitive processes related to escape. Consequently, if risk assessment is unimpaired, parasitism's effect on escape ability is predicted to lead to longer FID.

The overall effect of parasitism on FID is difficult to predict (Cooper and Frederick 2010). The balance between effects of factors that lead to an increase and decrease in FID determines the predicted overall effect of parasitism on optimal flight initiation distance. FID may be shorter if the effect of lower fitness is stronger than the effect of lower speed; it may be longer if the greater predation risk due to lower speed predominates (Cooper and Frederick 2010).In several studies on escape behavior, individuals in better condition (i.e. lizards with intact tails or higher body condition) had longer FIDs (Stankowich and Blumstein2005), which is consistent with a predominant effect of initial fitness. Because individuals with lower condition run more slowly, lower body condition predicts longer FID; the reduced fitness associated with poorer body condition predicts shorter FID, as observed. Therefore, asset protection appears to have outweighed the greater risk associated with reduced running speed in these cases. These findings suggest that worsened body condition may have a stronger effect on FID via reduction of initial fitness than through greater risk imposed by reduction of running speed. Nevertheless, because parasitism may have additional effects, including reduction in opportunity cost of fleeing, we made no prediction about its effect on FID.

We examined relationships between parasites and escape decisions of hosts in an insular population of the Balearic lizard, *Podarcis lilfordi*. On Aire Island (an islet off Menorca, Balearic Islands, Spain) lizards are infected by blood-borne microparasites (haemogregarines) and macroparasites (mites) (Garrido and Pérez-Mellado 2012, 2013). We examined effects of parasite load and body condition on FID and DF.

2. Material and methods

Podarcis lilfordi is a medium-sized lacertid lizard (maximum adult male snout-vent length = SVL = 80 mm; Barbadillo et al. 1999) endemic to the Balearic Islands (Pérez-Mellado 1998). The field work was done in June 2011 on Aire, a small islet having a surface area of 34 hectares. Aire's vegetation is very influenced by strong winds, high salinity and long periods of drought. Low Mediterranean shrubs and a variety of herbaceous species sparsely cover the island (Pérez-Mellado et al. 2007). Lizard densities can be very high, over 4,000 individuals per hectare (Pérez-Mellado et al. 2008). Aire has a Mediterranean mesothermic climate characterized by mild winters and hot dry summers.

Escape behavior trials were conducted on warm, sunny days from 0700 to 1200 h GMT when lizards were fully active. We recorded FID with a metric rule to the nearest 0.01 m. Because escape behavior often differs among age and sex groups (Stankowich and Blumstein 2005), we tested only adult males. After each trial, the lizard was captured by noosing. For each individual we recorded SVL, tail length, hind leg length (HLL) and body mass. At the site of capture, we counted ectoparasites, using a 5× monocle to inspect the whole body surface. To obtain blood samples, we made a slight longitudinal cut in the dorsal and proximal side of the tail with a sterile scalpel. We smeared a drop of blood on a slide *in situ* and air dried it in the field. After blood sampling, all lizards were released at the site of capture. In the laboratory, slides were fixed with absolute methanol for 10 min and then stained with modified Giemsa for 20 min. Samples were analyzed using an optical microscope at 400×. The only blood parasites identified were haemogregarines (Garrido and Pérez-Mellado 2012, 2013). The intensity of parasitism was estimated from 2,000 counted cells per sample. Prevalence was estimated as the percentage of infected individuals. To estimate body condition and relative HLL, we used un-standardized residuals from linear regressions of log-transformed body mass and HLL on log-transformed SVL, respectively.

The statistical analyses were carried out in R ver. 2.12.1 (R Development Core Team 2010). The assumption of homogeneity of variance was examined using Fligner's tests and normality was assessed using Shapiro-Wilk's tests. Data that did not meet the assumptions were logarithmically or square-root transformed. Normality and homogeneity of variances of the transformed were verified prior to analysis. In addition to measured variables noted above, we included a categorical variable for hour of day. Because body size influences FID in some lizards (Bauwens et al. 1995; Cooper 2011) and can affect parasite load (Amo et al. 2004, 2005; but see Garrido and Pérez-Mellado 2012 for the same population), we included SVL as an independent variable in the model. Interaction terms of SVL with the rest of variables were included in the regression model to reveal whether the influence parasitism and condition on FID varied in relation to SVL.

We used general linear models (GLM, Crawley 2007) to assess the significance of effects of lizard characteristics (SVL, body condition and/or haemogregarine and mite loads, the temporal variables and relative HLL) on log-transformed FID and log-transformed DF. We started with a saturated model, considering effects of all independent variables as well as all of their interactions. To determine the minimum adequate model (Crawley 2007), we applied a backward stepwise procedure with stepping criteria of 0.05 for entry and 0.1 for removal (Crawley 2007). Non significant factors and interactions were removed from GLM models until we obtained a minimal adequate model, taking into account at each step the minimal value of the Akaike's Information Criterion (AIC). Effect sizes reported are partial eta-squared (η_p^2) which may be interpreted similarly to R^2 (Cohen1988). As R^2, η_p^2 is a dimensionless measure of effect size having range 0 -1 that is independent of the degrees of freedom used in the analyses (Tabachnick and Fidell 2001).

3. Results

Preliminary analyses

Neither GMT (Fligner test: $\chi^2 = 28.94$, df = 27, $P = 0.36$; GLM, $F_{1,\,28} = 1.79$, $P = 0.19$), nor hour ($\chi^2 = 6.10$, df = 4, $P = 0.19$; one-way ANOVA, $F_{4,\,25} = 0.84$, $P = 0.51$) were significantly related to FID. The variables that might affect running speed were not significantly associated with FID (relative HLL -GLM, $F_{1,\,21} = 1.25$, $P = 0.28$; SVL - GLM $F_{1,\,28} = 1.67\text{x}10^{-5}$, $P = 1$).

Results were similar for DF. Neither GMT time (Fligner test: $\chi^2 = 28.71$, df = 27, $P = 0.38$; GLM, $F_{1,\,28} = 0.47$, $P = 0.50$) nor hour of day (Fligner test: $\chi^2 = 2.17$, df = 4, $P = 0.36$; one-way ANOVA, $F_{4,\,25} = 1.15$, $P = 0.36$) were significantly related to DF. The effect of relative HLL on DF was not significant (GLM, $F_{1,\,21} = 1.61$, $P = 0.22$). However, DF increased significantly as SVL increased (GLM, $F_{1,\,28} = 4.56$, $P = 0.04$). Thus, we included SVL in the GLM model for DF.

FID model

Neither mite load ($F_{1,\,24} = 1.89$, $P = 0.18$) nor body condition ($F_{1,\,24} = 2.32$, $P = 0.14$) were significantly related to FID; these variables were not included in the final model. However, the interaction of mite load and body condition was significant and was included in the final model. Because none of the other interaction terms were significant (all $P > 0.05$), they were removed from the model (Table 1).

	Df	Sum Sq	Mean Sq	F	Pr(>F)	Slope	$\boldsymbol{\eta_p^2}$
Haemogregarines	1	0.54	0.54	5.23	0.031*	0.055	0.17
Mites:condition	1	0.55	0.55	5.24	0.030*	-0.0016	0.17
Residuals	26	2.73	0.11				

Table 1. FID's minimal adequate model. η_p^2 : Cohen's partial eta-squared.

SVL and FID were not significantly related ($F_{1,\ 22} = 0.04$, $P = 0.85$). The minimal adequate GLM model was significant ($R^2 = 0.29$, $F_{2,\ 26} = 5.24$, $P = 0.01$). Haemogregarine load was positively correlated with FID ($F_{1,\ 26} = 5.23$, $P = 0.03$; Figure 1), with a moderate effect size ($\eta_p^2 = 0.17$); FID increased with higher intensity of infection by blood parasites. Finally, the interaction of mite load and body condition with FID was significant ($F_{1,\ 26} = 5.53$, $P = 0.03$) although its effect size ($\eta_p^2 = 0.17$) was

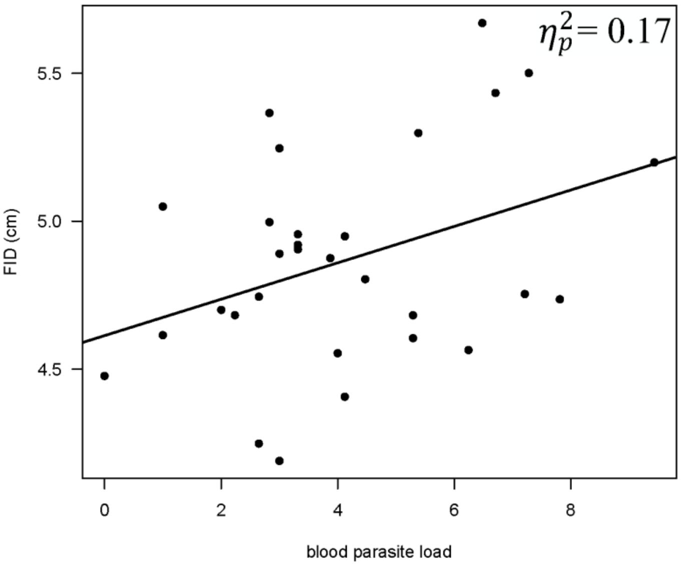

Figure 1. Relationship between FID and blood parasite load in *P. lilfordi*. η_p^2: Cohen's partial eta-squared.

small. FID was negatively correlated with body condition among heavily infested lizards, but was not significantly correlated with body condition in lightly infested lizards.

Because the interaction of body condition and mite load was significant, we investigated the relationship between body condition and FID taking into account the mean value of squared transformed mite load of all individuals ($\bar{x}$±SE = 7.47±0.77). Individuals having values of square-root transformed ectoparasite load above the mean were considered to be heavily infected'; individuals with values below the mean were considered lightly infected. Among lightly infected lizards, body condition was not correlated with FID (GLM, $F_{1,\,11}$ = 0.052, P = 0.82; slope: -0.003; η_p^2 = 0.004; Figure 2). Among highly infected individuals, FID was negatively correlated with body condition (GLM, $F_{1,\,14}$ = 5.18, P = 0.04; slope: -0.02; η_p^2 = 0.33; Figure 3). In an analysis using untransformed mite load, for which the distribution deviated only slightly from normality, lizards were again grouped as heavily or lightly infected based on the mean mite load ($\bar{x}$±SE = 72.62±11.18). We obtained identical results because the same individuals were heavily and lightly infested for the two groups using the raw and transformed data.

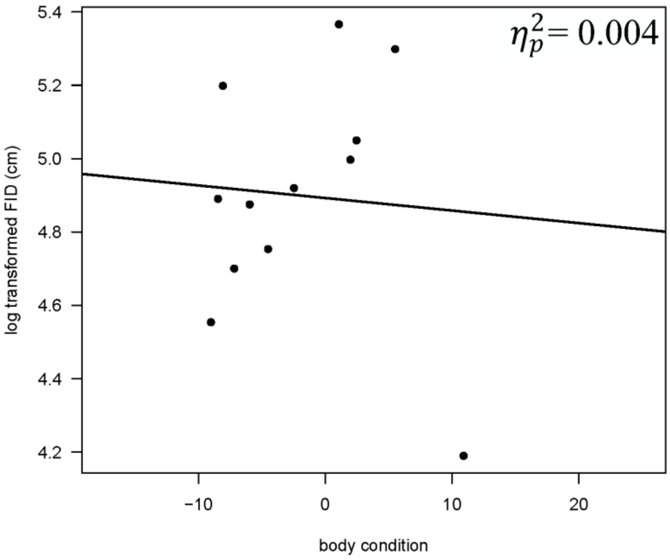

Figure 2. Relationship between FID and body condition among poorly infected individuals in *P. lilfordi*. η_p^2: Cohen's partial eta-squared.

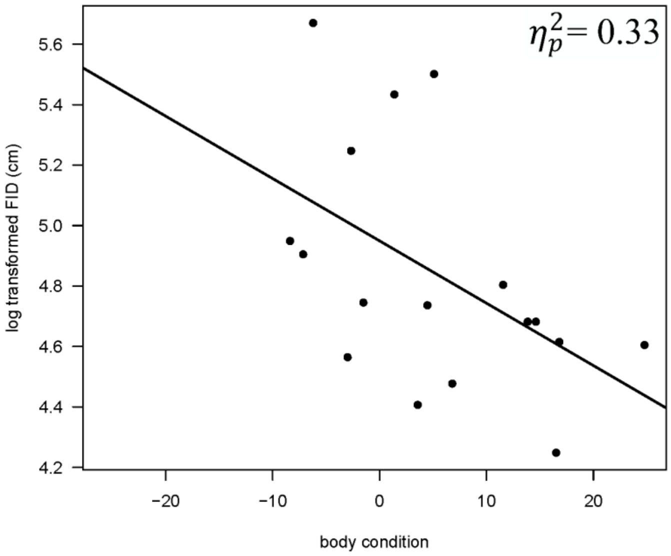

Figure 3. Relationship between FID and body condition among highly infected individuals in *P. lilfordi*. η_p^2: Cohen's partial eta-squared.

DF model

The best model for DF (R^2 = 0.30, $F_{2,\ 27}$ = 5.75, P = 0. 01) included only SVL ($F_{1,\ 27}$ = 5.64, P = 0.03; η_p^2 = 0.17) and haemogregarine load ($F_{1,\ 27}$ = 5.87, P = 0.02; η_p^2 = 0.18; Table 2). DF increased as SVL increased, but decreased as blood parasite load increased (Figure 4).

	Df	**Sum Sq**	**Mean Sq**	**F**	**Pr(>F)**	**Slope**	η_p^2
Haemogregarines	1	0.62	0.54	5.87	0.031*	-0.068	0.18
Body size	1	0.60	0.55	5.64	0.030*	0.040	0.17
Residuals	27	2.87	0.11				

Table 2. DF's minimal adequate model. η_p^2 : Cohen's partial eta-squared.

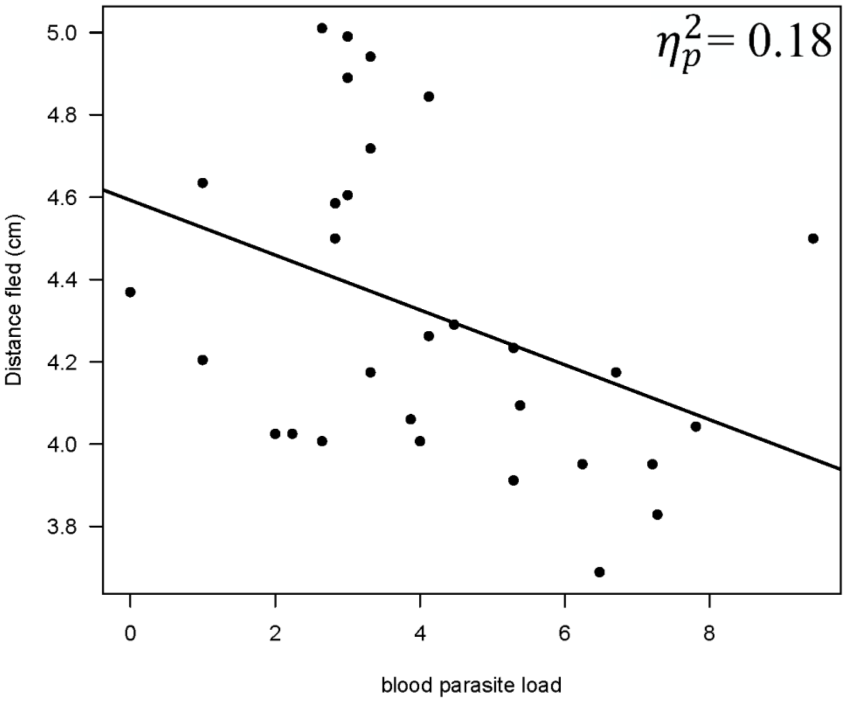

Figure 4. Relationship between DF and blood parasite load in *P. lilfordi*. η_p^2: Cohen's partial eta-squared.

4. Discussion

Relationship to previous reports

Both types of parasites affected FID by *P. lilfordi*, but only haemogregarines affected DF. Haemogregarine load was related to the escape variables in opposite ways: FID increased as haemogregarine load increased, but DF decreased as haemogregarine load increased. Body length was unrelated to FID, but DF increased as SVL increased. The significant interaction between body condition and ectoparasite load on FID is particularly interesting. In heavily infested lizards, body condition was negatively correlated with FID, but in lightly infested lizards body condition and FID were not significantly related.

Previous studies of lizards demonstrated that for blood parasites, but not ectoparasites, infected individuals tended to have reduced escape ability due to lowered metabolic efficiency (Atkinson and Van Riper 1991; Chen et al. 2001). Blood-borne parasites have more harmful effect on the physiology of hosts than mites or ticks (Schall et al. 1982; Oppliger et al. 1996; Garrido and Pérez-Mellado 2012 and references therein).

Our findings for lizards lightly infested by ectoparasitic mites agree with those of previous findings, but the negative correlation between FID and body condition among the heavily infested lizards is a novel finding. Our results suggest that only above a certain degree of infestation do ectoparasites affect the relationship between body condition and FID. Probably, this threshold mite load value indicates the maximum load that hosts can tolerate without actually causing significant impairment of escape ability. If so, body condition of lizards affects escape responses only when individuals have to deal with high ectoparasite loads.

For the same lizard and mite species at the same site as the present study, Hawlena et al. (2009) found a weak positive correlation between body condition and FID. In light of our findings, regarding the interaction between mite load and body condition, the correlation was presumably low because parasite loads differed among the individuals they sampled. Our findings that HLL and SVL did not affect FID also agree with those of Hawlena et al. (2009). However, because both studies focused on adults, SVL might affect FID if juveniles are included.

Our finding of a negative relationship between FID and body condition among lizards heavily infested with mites agrees with that of a previous study of the wood pigeon, *Columba palumbus* (Kenward 1978). However, the relationship in wood pigeons was not stated to be restricted in heavily parasitized individuals.

To our knowledge, this is the first study to report a positive correlation between FID and parasite load, in this case for haemogregarines, at the individual level. Several studies have shown that FID is affected by tail autotomy positively, negatively, or not at all (Stankowich and Blumstein 2005; Cooper 2007; Cooper and Wilson 2008). In the lizard *Sceloporus virgatus* running speed is reduced (Cooper et al. 2009) and FID is increased following tail loss (Cooper 2007; Cooper and Wilson 2008). Sprint speed in adult male *P. lilfordi* decreases as haemogregarine load increases and as body condition worsens (Garrido and Pérez-Mellado in review).

A final difference between the two escape variables is that FID was unrelated to SVL, but DF increased as SVL increased. In previous lizard studies, FID increased with body size in 10 species (Shallenberger 1970; Martín and López 1995, 2003; Whiting et al. 2003; Carretero et al. 2006; Brecko et al. 2008; Cooper 2011), and decreased from hatchlings to adults in another (Berger et al. 2007). Because all of these studies included hatchlings and/or juveniles as well as adults, the range of SVL among adult males in our study was likely too small to reveal any covariation of SVL and FID.

In four earlier studies of the relationships between body size and DF in lizards, the relationships were variable: DF increased as body size increases in one species (Martín and López 1995), decrease in another (Whiting et al. 2003), and was unrelated to body size in two others (Martín and López 2003). The increase in DF as SVL increases in adult male *P. lilfordi* agrees with the relationship found in a study of another lacertid species in which the entire size range from hatchlings to adults was examined (Martín and López 1995).

Causes of variation are less well understood for DF than for FID but DF is strongly affected by distance to refuge in some prey (Stankowich and Blumstein 2005) and may vary among age/size groups (Stankowich and Blumstein 2005). The lack of relationship between SVL and FID and positive correlation between SVL and DF in *P. lilfordi* is unique. The increase in DF as SVL increases might simply indicate that larger lizards ran farther while expending equal effort to flee. Alternatively, the relationship suggests that larger individuals representing a greater energetic benefit to predators might be at greater risk of being pursued or more likely to be pursued more vigorously once they begin to flee. Larger adults might also be farther from effective refuges.

Fit between effects of parasites and escape theory

Haemogregarines. As detailed in the introduction, optimal escape theory (Cooper and Frederick 2007, 2010) predicts that FID increases as risk of predation assessed by the prey increases, decreases as cost of fleeing increases, and increases as the prey's fitness (residual reproductive value) increases. For haemogregarines, the increase in FID as parasite load increases is consistent with assessment by the prey that risk of being captured is greater, opportunity cost of fleeing is lower, or the prey's initial fitness is greater at higher parasite loads. Effects of the blood parasites on costs of not fleeing and of fleeing might be mediated by decrease of running speed or other aspects of escape ability and lower feeding rates among heavily parasitized individuals, respectively.

Because running speed decreases as haemogregarine load increases (Garrido and Pérez-Mellado in review), predation risk at a given predator-prey distance increases with load. Therefore, the observed increase in FID with haemogregarine load corresponds to the prediction by escape theory that FID increases as cost of not fleeing (predation risk) increases. Larger, more heavily infected males have been better able than other lizards to obtain food associated with available resources (Garrido and Pérez-Mellado 2013), which would lower the cost of missing a feeding opportunity to flee. Furthermore, although dominant males defend dead horse arum plants while flowers are blooming (Pérez-Mellado et al. 2007; Garrido and Pérez-Mellado 2013), we have no evidence that they defend the fruits that were present later in the year during our study. Therefore, the increase in FID as haemogregarine load increases might be attributable to the greater initial fitness and reduced running speed of large males having high haemogregarine loads, but not to greater cost of fleeing.

Haemogregarine infections can be fatal in laboratory conditions (Schall 1996), but effects of lower loads observed in our study on mortality and health are unknown. Assuming that fitness is reduced as haemogregarine load increases to high levels, the effects of the greater initial fitness and greater assessed predation risk appear to have outweighed any reduction in fitness due to the observed haemogregarine loads in *P. lilfordi*. Either the haemogregarines loads prevailing in our population of lizards having a limited, but adequate, food supply and exposed to low levels of predation (Pérez-Mellado and Corti 1993; Cooper et al. 2009b; Cooper and Pérez-Mellado 2012) do not reduce fitness substantially. Alternatively, enhanced thermoregulatory and feeding opportunities of large males might have counteracted any negative effects of the parasites. That body condition was unrelated to either FID or DF in relation to haemogregarine load further suggests that haemogregarines may not greatly affect fitness in the population studied.

This lack of effect of body condition on FID occurred despite slower running speed of lizards in worse body condition (Garrido and Pérez-Mellado in review). Large males have high initial fitness and may have high haemogregarine loads acquired via high activity, aggressive behavior and possibly enhanced by immunosuppression due to aggression that causes greater susceptibility to infection (Garrido and Pérez-Mellado 2013). These findings suggest that the high initial fitness of large males, not greater predation risk, is the primary factor responsible for the increase in FID as haemogregarine load increases.

Haemogregarine load also affected the distance fled by lizards, but DF decreased as parasite load increased. If assessed risk were the major factor affecting DF by parasitized lizards, DF would be predicted to increase as parasite load increases, as did FID. If initial fitness were the main factor affecting DF, DF would be predicted to increase with haemogregarine load because larger males with higher loads have better body condition and higher fitness. Our results, therefore, were opposite to those predicted by escape theory for effects of predation risk and initial fitness. Therefore, for the costs considered by escape theory to account for effects of haemogregarine load on DF, cost of fleeing must increase as load increases. We do not know why load might increase cost of fleeing. Alternative hypotheses are greater reliance on refuge use by heavily parasitized individuals, which might flee into or near refuges before stopping, and impaired endurance. Because DF decreased as haemogregarine load increased, factors affecting the decision to begin fleeing and how far to flee must be affected differently by haemogregarines or associated ecological or behavioral factors.

The difference for the two variables, FID and DF, could indicate that risk assessment mechanisms differ for FID and DF in parasitized lizards. Parasites can drastically alter host behavior, including antipredatory behavior (e.g., Moore 2002; Liebersat et al. 2009; e.g., rabies). Another possibility consistent with previous finding for lizards having impaired escape ability (Cooper et al. 1990) is that lizards more heavily parasitized by haemogregarines might stay closer to refuge, thereby shortening FID. This is unlikely because, although *P. lilfordi* sometime use refuges, typically they remain in sight after fleeing. It is also possible, but very unlikely, that endurance is reduced sufficiently for DF, which is short, to be shortened in heavily infected lizards.

Mites. Effects of mite infestation on escape behavior differed dramatically from those of haemogregarines in that FID was negatively correlated with body condition only among individuals heavily infested by mites and mite load did not affect DF. In heavily infested individuals, the decrease in FID as body condition improved is inconsistent with a predominant effect of mites on asset protection. Because FID in this group of lizards increased as body condition deteriorated, the findings are consistent with the predictions of optimal escape theory for prey that assess greater predation risk or have lower opportunity cost of fleeing when poor body condition reduces escape ability.

Animal prey are scarce during warm summer months, but lizards in some parts of Aire then eat nectar of several flowering plants (Pérez-Mellado and Corti 1993). If the total food supply is adequate, cost of fleeing is low. Even if food is scarce, fleeing when a feeding opportunity is not immediately present has very low cost. Even if worsening body condition impairs ability to obtain food, the cost of fleeing cannot be reduced much because it is already low in the absence of a potentially very profitable feeding opportunity while a predator approaches. However, if the ability of prey in low body condition to capture prey is impaired, cost of fleeing is reduced to some extent, favoring longer FID. This is the opposite of our finding, suggesting that the effect of body condition on FID in lizards heavily infested by mites is not mediated by an effect on cost of fleeing. Assessment of greater risk of being captured by lizards having poor body condition and heavy mite load is the most likely explanation for the observed relationship.

Taken together the lack of relationship between FID and body condition at low mite loads and the negative relationship between FID and body condition at high mite loads suggest that there may be a threshold level of mite infestation below which body condition and FID are unrelated. Above the threshold load, FID increases as body condition declines. Lizards heavily infested with mites appear to perceive predation risk to be greater when body condition is poorer. We do not know the mechanism underlying the joint effect of mite load and body condition on FID. A study of joint effects of mite load and body condition on sprint speed could be informative, but heavy mite load might affect some combination of activity, foraging ability, social status, and long-term fitness in ways that affect escape decisions in relation to body condition.

Conclusions

Loads of both haemogregarine blood parasites and ectoparasitic mites are related to escape decisions in *P. lilfordi*. However, because our study was correlational rather than experimental, the relationships that we detected do not necessarily indicate that parasite loads caused modification of escape decisions. In the case of haemogregarines, ecological and behavioral factors that influence both FID and parasite load account for the correlation between haemogregarine load and FID. In the absence of other influences, FID is expected to increase as body condition increases because initial fitness increases as body condition increases. Males that aggressively defend resources from conspecifics have high body condition. Their aggression exposes them to blood of conspecifics. They also have high activity levels that may lead to increased contact with mites that are vectors of haemogregarines. Therefore, aggression and high activity can account for the higher haemogregarine load among males in good body condition. Males with high parasite loads presumably have longer FID because they are protecting the asset (Clark, 1994) of higher fitness associated good condition. In contrast, reduction of fitness in heavily parasitized prey would predict decreasing FID as load increases to levels that would reduce fitness substantially. In our study haemogregarine loads were too low to have substantially reduced fitness.

For mites, the relationship to escape behavior is not a direct one, but an effect on the relationship between FID and body condition restricted to males with high mite loads. It is uncertain whether mite load affected escape decisions in relation to body condition or the relationship between mite load and escape was a consequence of ecological and behavioral differences among heavily and lightly infested males. The differences in relationships between escape decisions and parasite loads between haemogregarines and mites suggest that different eco-behavioral factors would be required to account for the relationships or that mite loads *per se* may affect escape decisions. To differentiate between these two possibilities, further research is needed on factors leading to increasing mite loads and effects of mites on body condition and other factors that might affect escape decisions. Investigation of ecological differences between males that differ in mite load could be illuminating.

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Capítulo 7

La prueba de destrucción bacteriana revela diferentes estrategias inmunológicas en lacértidos

La prueba de destrucción bacteriana revela diferentes estrategias inmunológicas en lacértidos

Resumen

El enfoque clásico de la inmunología se basa en la resistencia parasitaria por la cual los animales tratan de prevenir la infección o reducir el crecimiento del parásito. La tolerancia es otro mecanismo de defensa que puede evitar la disminución de la aptitud biológica del huésped sin una reducción de la infección parasitaria. En las plantas, ambas estrategias pueden coexistir en la misma población. La eco-inmunología se centra en la descripción y explicación de la variación natural de las funciones inmunes en organismos en libertad, de acuerdo con los factores bióticos y abióticos. Hemos utilizado una prueba bactericida (BK en sus siglas en inglés) para probar la capacidad que tienen las lagartijas en estado salvaje para mantener una respuesta inmune. Las características de esta técnica encajan adecuadamente con el objetivo de conocer el rendimiento del sistema inmune en las poblaciones naturales. En el presente estudio, evaluamos la respuesta inmune de los individuos en relación con la carga parasitaria y condición corporal. Los resultados mostraron que la respuesta inmune se correlaciona con la carga de ectoparásitos, pero no con la de hemogregarinas. No se encontró tampoco correlación con condición corporal. Sin embargo, la interacción entre la carga de parásito sanguíneos y la condición sí fue significativa. El uso de métodos estadísticos específicos para la interpretación de la interacción entre las variables continuas reveló dos diferentes estrategias en relación con la defensa inmunitaria en una población natural de *Podarcis lilfordi*. Entre los individuos poco infectados apareció una correlación positiva entre la condición corporal y la capacidad de destrucción bacteriana de plasma. Esta relación desaparece en individuos que estaban altamente infectados, lo que sugiere que algunas lagartijas no dan prioridad a la inversión en la función inmune. Desde un punto de vista eco-inmunológico, las características de esta población insular de lacértidos les permite escoger entre dos diferentes mecanismos de defensa: algunas lagartijas pueden priorizar el sistema inmune, mientras que otras asignarían esa energía

a otros requisitos. Es decir, algunas pueden optar por la resistencia, mientras que otras optan por la tolerancia.

Bacterial killing reveals different immunological strategies in lacertid lizards

Abstract

Classic immunology relies on parasite resistance by which animals prevent infection or reduce parasite growth. Tolerance is another defence mechanism that can avoid the decrease in host’s fitness without reducing parasite infection. In plants both strategies can co-exists in the same population. Eco-immunology focuses in the description and explanation of natural variation of immune functions in free-living organisms, according to biotic and abiotic factors. Bacterial killing (BK) assay was used to test the ability to maintain an immune response in free-living lizards. BK characteristics fit adequately with our purpose to comprehend performance of immune system in wild populations. Here, we assess immune response of individual lizards in relation to their parasite load and body condition. Results showed that immune response is correlated with ectoparasite, but not with haemogregarine, load. No correlation was found with body condition. The interaction between haemogregarine load and condition was significant. Specific statistical methods for interpreting interaction between continuous variables revealed two different strategies in relation with immune defence in a wild population of *Podarcis lilfordi*. Lightly infected individuals showed a positive correlation between body condition and bactericidal capability of plasma. This relation disappears among heavily infected lizards, suggesting that some lizards do not prioritize the investment in immune function. From an eco-immunological viewpoint, the characteristics of insular population allow individual lizards to choose between two different defence mechanisms: some lizards may prioritize immune system, while other lizards would allocate energy to other requirements. Some may opt for resistance while others for tolerance.

Keywords: bacterial killing assay; parasites; body condition; immune response; immunological strategies; islands.

1. Introduction

The fitness of an organism is critically dependent on its immune system that provides an efficient protection against parasites and pathogens (Schulenburg et al. 2009). Typically, the study of the immunological function has been context-independent (Martin et al. 2011) and the knowledge of the mechanisms of the immune system in free-living animals is still scarce (Sheldon and Verhulst 1996; Schmid-Hempel 2003). Likewise, it is becoming increasingly evident that immune response plays a significant role in an organism's physiological, biochemical and behavioural responses to its environment and thus have the potential to shape the evolution of life-history strategies. Ecological immunology (or eco-immunology) field emerges as a response to these demands. The aims of eco-immunology are to understand immune function in an evolutionary context, explaining natural variation in immune functions (Sheldon and Verhulst 1996). Specifically, why and how biotic and abiotic factors contribute to variation in immunity of free-living organisms.

Resistance, defined as a series of defence mechanisms by which animals prevent infection or reduce parasite growth, have obvious fitness benefits by reducing the fitness losses attributed to infection, but it is not the unique defence mechanism (Schulenburg et al. 2009). Tolerance is also a kind of defence that can reduce or alleviate the reduction in fitness owing to parasite infection, but without reducing parasite infection or growth. Tolerance minimizes the consequences of damage caused by parasites, instead of minimizing the burden of parasites (Baucom and De Roode 2011). Although both defence mechanisms serve the same purpose for the host (the retention of fitness) they may have dramatically different consequences for the ecology and evolution of hosts and parasites, since resistance directly reduces parasite fitness, whereas tolerance does not (Baucom and De Roode 2011). In addition, several studies have showed the existence of a trade-off between tolerance and resistance in plants, that can be also present in animals (Baucom and De Roode 2011 and references therein). Because that trade-off, both strategies may be represented within a population (Baucom and De Roode 2011).

In this work, we study immune defence strategies adopted by individuals of an insular population of lizards. In addition, we examine parasite load and body condition in relation to immune response of *Podarcis lilfordi* in Aire Island (Balearic Islands, Spain). Assuming eco-immunological premises (Sheldon and Verhulst 1996), immune function cannot be understood if we leave aside environmental characteristics under which a population lives. Aire Island, as other insular ecosystems of the Mediterranean basin, is characterized by a limited number of terrestrial vertebrates and a low predation pressure (Pérez-Mellado et al. 2007; Cooper and Pérez-Mellado 2012). Consequently, it is usual to find high densities at these populations (Pérez-Mellado et al. 2008). In Aire Island, high lizard density promotes an increased number of physical contacts and agonistic interactions among conespecifics (Garrido and Pérez-Mellado in press, 2013), allowing a higher probability of parasite transfers (Garrido and Pérez-Mellado in press and 2013). Otherwise, it is well known that to maintain an immune response is costly (Sheldon and Verhulst 1996). The scarcity of resources is almost the norm at Mediterranean Islands (Pérez-Mellado and Corti 1993) and, when resources are limited, allocation of energy to immune defences may be modulated by the need to spend energy in other functions such as growth, reproduction, and maintenance (Nelson and Demas 1996).

Classic immunology approach relies on resistance and argues that differences in parasitic infection reveals differences in immune response, and expects to find a positive relation between immune response and body condition and/or a negative relation with parasite burden (Cooper et al. 1985; Sorci, 1995; Smallridge and Bull 2000; Madsen et al. 2005). However, the main premise of ecological immunology is that host defences are costly, and should be employed or evolve only when costs are acceptable and are outweighed by benefits (Baucom and De Roode 2011). If ecological demands are great, or if fitness can be maximized via growth or reproduction instead survival infection, immune defences may be lowered, altered, or outright compromised (Martin et al. 2011). Hence, we do not expect to find just a classical immune strategy, because some individuals may opt for tolerance and not for resistance since the costs of mount an immune response would not compensate. This immunological effort would be particularly profitless in insular populations as Aire Island where the high risk of parasitic infection is continuous and predation pressure is low. Particularly if we take into account that one of the major cost to lack an immune response is death, because predators would capture the sickest or most parasitized individuals (Temple 1987; Navarro et al. 2004; Genovart et al. 2010).

Aire Island provides a suitable site to explore a range of individuals that differ in their parasite loads. Because of low predation pressure, the possibility that heavily infested individuals were excluded from the dataset killed by predators (Temple 1987; Navarro et al. 2004; Genovart et al. 2010) is minimal. Moreover, in Aire Island, rates of parasitism by haemogregarines and mites are among the highest ever found in Mediterranean lizards (Garrido and Pérez-Mellado in press and 2013). Thus, we expected to find both immunological strategies to be represented in *P. lilfordi* population on Aire Island.

2. Material and methods

2.1 Area and species under study

P. lilfordi is a medium-sized lacertid lizard (snout-vent length, SVL, up to 81 mm. in males and 75 mm. in females, Pérez-Mellado, 1998). The study was conducted between 16th and 23th, June 2011 in Aire Island, a small islet off Menorca. It is a typical Mediterranean coastal islet, with vegetation highly influenced by its environmental characteristics, strong winds, high salinity and long drought periods. As in other Mediterranean islands, food resources are scarce (Pérez-Mellado and Corti 1993). Lizard densities can be very high, over 4000 individuals per hectare (Pérez-Mellado et al. 2008).

Lizards were collected by noosing on sunny days, between 8.00 and 12.00 GMT, when lizards were more active. We only studied adult males. For each individual, we recorded snout-vent length (SVL) and body weight. Body condition was estimated from the residuals of the regression of log-transformed body weight on log-transformed SVL. Ectoparasites were counted at the capture place with a 5X monocle, inspecting the whole body surface of the lizards. To obtain blood samples we made a slight longitudinal cut in the dorsal and proximal side of the tail with a sterile scalpel immediately upon recapture. Blood samples for immune analysis (see below) were collected in a heparinized microcapillary tube. Then, we obtained a blood smear to estimate blood parasite load. Blood smears were placed on microscope slides and air dried in the field. In the lab, slides were fixed with absolute methanol for 10 minutes and then stained with modified Giemsa for 20 minutes. Samples were analyzed using an optical microscope at 400X. The only blood parasites identified were haemogregarines (see above). The intensity of parasitism was estimated on a total of 2,000 counted cells per sample. Prevalence was estimated as the percentage of infected individuals at each of the two areas under study. Specimens were always released at the site of capture.

Blood samples for immune response assays were stored with ice until centrifuged at 3600 rpm for 12 minutes and plasma separated within eight hours of collection. We acquired approximately 30 µl. of plasma from each lizard and kept plasma frozen at –20°C until immune assays were conducted.

2.2 Plasma bacterial killing capacity

Based on the review of available methods to quantify the immune response (see Demas et al. 2011), we assessed immunity response of individuals by quantifying the bacterial killing (BK) capacity of blood plasma. Because this test evaluates the ability to remove an actual pathogen, it provides a functionally relevant evaluation of host immune function. In addition, this technique determines the immune function without inducing an external response by injecting relatively artificial antigens or mitogens (Demas et al. 2011). Moreover, it provides a more functionally relevant assessment of host immune function in comparison with assays of isolated immune components and it is preferable to monitoring techniques (e.g. leukocyte counts, lymphoid tissue masses), which can vary quantitatively (Demas et al. 2011). For various reasons, the interpretation of these monitoring techniques, as measures of immunocompetence, is somewhat problematic (Norris and Evans 2000; Demas et al. 2011). Thus, BK fits adequately with our purpose to know the performance of the immune system in wild populations.

Bacterial killing assay is used to measure a functional response by the animal's innate immune system against a relevant pathogen, *Escherichia coli* (French et al. 2010; Ruiz et al. 2011 and references therein). The bacteria killing assay is emerging as a valuable tool to measure overall intrinsic immune investment across a variety of organisms (Chester et al. 2010; Matson, Tieleman and Klasing 2006; Zimmerman et al. 2010). We determined immune response following a protocol similar to that developed by Ruiz et al. (2010, 2011). We prepared a bacterial stock solution by diluting 2x10^{-6} times a saturated concentration of *E. coli* in a CO_2-independent media with 2.34 mg of L-glutamine (Sigma-Aldrich, St Louis, MO). Pilot trials (unpub. results) showed that this concentration was suitable to get quantifiable positive controls (avoiding bacterial background lawn) but with enough colonies to estimate killing capabilities of lizard plasma. Then, we diluted plasma samples in 200 µl. of the working bacterial solution. These plasma/bacteria cocktails were incubated for 30 min at 37 °C. Subsequently, we plated each sample on agar plates including two positive controls (of only bacteria) and a negative control (without bacteria). Plates were incubated overnight at 37 °C to allow colony growth. We then counted the number of colonies on each plate and compared them with the positive control plate in order to calculate the percentage of bacteria killed by each lizard plasma sample [percentage of killed bacteria= 100 – (average # of colonies on sample plate/average # of colonies of positive control plates)*100]. No colonies were formed on negative control plates. Petri plates in which we detected contamination by other bacterial colonies were discarded. Thus, we calculate the *ex vivo* bacterial killing capability of collected plasma exposed to *E. coli*.

2.3 Statistical analyses

The statistical analyses were carried out in R environment (ver. 2.12.1, R Development Core Team 2010). We analyzed the relation of haemogregarines load, mites load and body condition with innate immunity of lizards by using general linear models (GLM) (Crawley 2007). Haemogregarines and mite loads were log transformed to reach normality assumptions. Following Crawley (2007), factors and interactions without significance were removed from the GLM model until we obtained a minimal adequate model. Effect sizes reported are partial eta-squared (η_p^2) and may be interpreted similarly as R^2 (Cohen 1988). As R^2, η_p^2 is a dimensionless measure of effect size independent of the degrees of freedom used in the analyses (Tabachnick and Fidell 2001).

Interaction terms between continuous variables were interpreted following Aiken and West (1991) and Cohen et al. (2003) method. Specific statistical techniques were used to interpret results in order to obtain a proper understanding of real effects of variables under study. In the multiple regression models, with several continuous variables, it is usually considered that the effects of the predictors are additive. Even if additivity is appropriate for many situations, there are times when it does not apply (Dallal 2001). Sometimes, the purpose of a study is to formally test whether additivity holds. Perhaps the way a response variable varies with one predictor depends on another predictor. One way to investigate this is by including an interaction term in the model (Dallal 2001). Interaction between continuous variables in multiple regression model were analyzed using Aiken and West (1991) and Cohen et al. (2003) method, which provide detailed information about the influence of body condition on the response variable (immune response). The purpose of the analysis is to determine the values of one continuous predictor in which another continuous predictor shows an effect over response variable (Aiken and West 1991; Cohen et al. 2003).

3. Results

Except from the interaction between haemogregarines load and body condition, none of the interaction terms was significant (all P > 0.05) and they were removed from the model (Table 1). Therefore, the relation of each variable in the model was independent from the relation of the remaining variables. Blood parasite load was unrelated with immune response ($F_{1,\ 16} = 0.18$, P = 0.68), but it was a significant interaction with body condition. The GLM minimal adequate model was significant (Adjusted R^2 = 0.61, $F_{3,\ 17}$ = 9.00, P < 0.001).

	Df	Sum Sq	Mean Sq	F	Pr(>F)	Slope	η_p^2
Mites	1	96.69	96.69	20.94	<0.001*	-1.45	0.55
Condition	1	2.47	2.47	0.54	0.47	21.09	0.03
Hemogregarines:condition	1	25.53	25.53	5.53	0.03*	-7.98	0.25
Residuals	17	78.49	4.62				

Table 1. BK's minimal adequate model. Just intensity of ectoparasites and the interaction between haemogregarines load and body condition were significant and retained in the model. η_p^2: Cohen's partial eta-squared

Ectoparasite load was negatively correlated with innate immune system ($F_{1,\,17}$ = 20.94, P < 0.001; slope = -1.45; Fig. 1). Mite load diminished as bacterial killing capabilities of plasma increased. Finally, Body condition was retained in the model but effects remained no statistical significant by themselves ($F_{1,\,17}$ = 0.54, P = 0.47; slope = 21.09). The interaction of log transformed values of haemogregarines load and body condition was significantly related with the immune response of hosts ($F_{1,\,17}$ = 5.53, P = 0.03; slope = -7.98). In addition, no relation was found between condition and haemogregarine load (GLM, Adjusted R^2 = 0.04, $F_{1,\,19}$ = 1.93; P = 0.18).

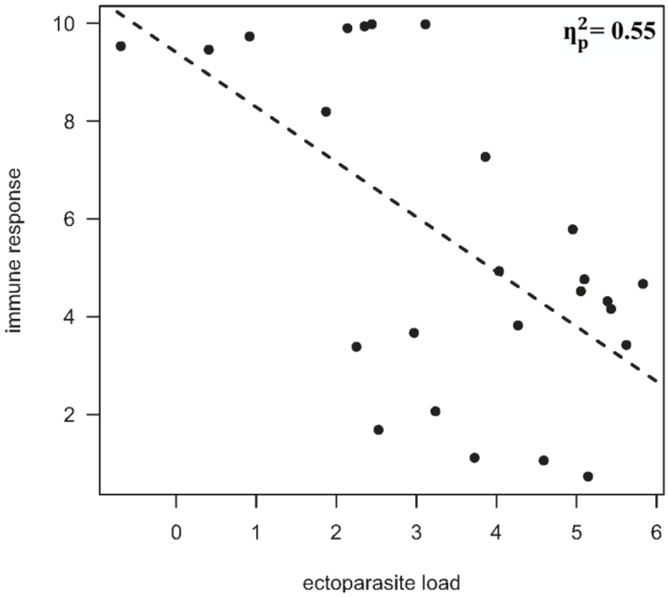

Figure 1. Relationship between bacterial killing capability of plasma and ectoparasite load in the Balearic lizard, *P. lilfordi*. η_p^2: Cohen's partial eta-squared

Because it was involved in an interaction term of the GLM model, given a constant value of ectoparasite load, the relation of body condition and immune response was different for different values of hemogregarine load (Dallal 2001; Sweet and Martin 2011). We analysed interaction between continuous variables (condition and blood parasite load) following Aiken and West (1991) and Cohen et al. (2003). To generate simple regression equations recommended by these authors, we choose three different values of haemogregarines load (HL): the median, as being the value that divides the sample into two equal groups, one standard error over the median and one standard error below the median. We referred them as medium (HL_M), high (HL_H) and low (HL_L)

haemogregarines load; as median was 12 and SE was 10, these values were HL_M= 12; HL_H= 22; HL_L= 2. Since we divided sample size according to median value, we can consider individuals having more than 12 haemogregarines/ 2000 cells to be ‘highly infected’, being ‘lightly infected’ lizards with less of 12 haemogregarines/ 2000 cells.

	Simple slope	**Standard error**	**t-test**	**df**	**P-value**
HL_H	b_H= -3.57	s_H= 4.93	t = -3.57 / 4.93 = -0.72	16	P = 0.24
HL_M	b_M= 1.22	s_M= 4.11	t = 1.22/ 4.11 = 0.30	16	P = 0.39
HL_L	b_L= 15.58	s_L= 6.85	t = 15.58/ 6.85 = 2.27	16	P = 0.02*

Table 2. Computation of t-test for slopes of simple regression equations (see Aiken and West, 1991)

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After the generation of simple regression equations for HL_M, HL_H and HL_L, we computed t-tests for the slopes (Table 2 and Fig. 2). Thus, t-tests revealed that just for HL_L simple regression, slope was statistically different from zero. Non significant results were obtained for HL_M or HL_H.

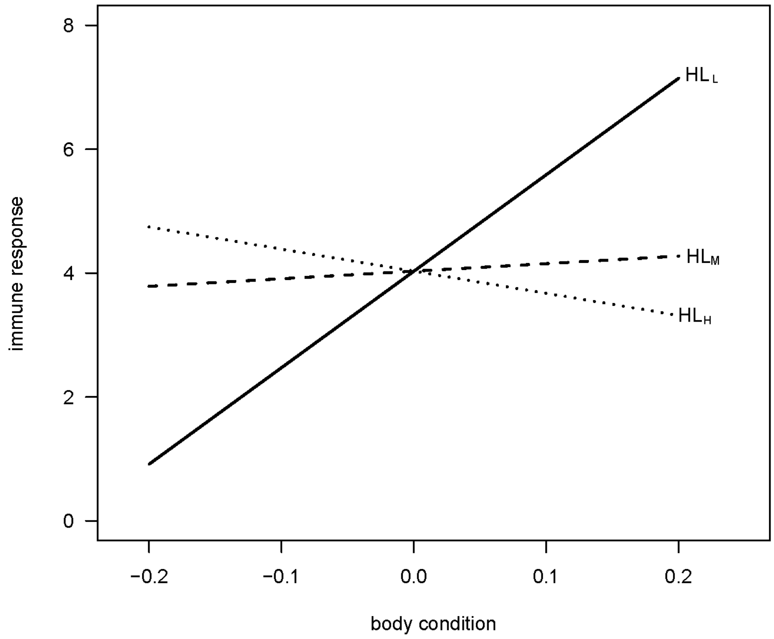

Figure 2. Regression of immune response on body condition at three different levels of blood parasite load (HL_H, HL_M and HL_L, see Aiken and West, 1991 and Cohen et al., 2003).

Hence, we can conclude, that body condition was positively correlated with immune response only in ‘lightly infected’ individuals. No relation appeared for highly infected lizards. Furthermore, body condition was similar between lightly and highly infected individuals (Fligner test: $\chi^2 = 0.18$, df = 1, P = 0.67; one-way ANOVA, $F_{1,\ 19} = 0.11$, P = 0.74).

4. Discussion

According to the eco-immunological approach (Sheldon and Verhulst 1996), we did not expect to find a direct correlation between parasite load and the immune response, because, taking into account the environmental conditions of insular populations, lizards should opt for tolerance instead of resistance strategy. Surprisingly, as classical immunology predicts (Cooper et al. 1985; Sorci, 1995; Smallridge and Bull 2000; Madsen et al. 2005), we found a negative correlation between immune response and ectoparasite load. Several works have documented this relation previously (Cooper et al. 1985; Sorci 1995; Smallridge and Bull 2000; Madsen et al. 2005). However, this approach does not explain what is observed for the remaining variables. We found that immune response was uncorrelated with condition and blood parasite load, but it was a significant interaction of both variables. We suggest that immunosuppressive effect of ectoparasite saliva would be responsible for the direct correlation between immune response and ectoparasite load (Moore 2002; Ribeiro et al. 1985, 1990; Titus and Ribeiro 1990) and not a host’s decision regarding investment in its immune system, as proposed by eco-immunology. Moreover, if our results would be consistent with the classical view, the immune response should also be linked with the intensity of haemogregarine load.

The most interesting result of our study is the influence of the blood parasite load on body condition and immune response. The significant interaction term revealed that the relationship between body condition and immune response varies according to the parasite load. Applying Aiken and West (1991) and Cohen et al. (2003) methods, we found that in lightly infected lizards, body condition was positively correlated with immune response, while in highly infected lizards, this correlation was absent. In a manipulative experiment, Ruiz et al. (2010) found a context-dependent response of testosterone on the relation between food intake (related to condition) and bacteria killing capability. Food supplementation increased immune response and plasma levels. Furthermore, testosterone treatment enhanced immune response in food supplemented animals (Ruiz et al. 2010). Our results showed that lizards in better condition had a better immune response, but only among lightly parasitized individuals. Moreover, body condition is similar between lightly and heavily infested lizards.

Alternatively, because resource availability and pathogens fluctuate over time and space (Nelson et al. 2002) and immune defence is costly (Martin et al. 2008 and references therein), animals must adaptively allocate resources among physiological systems to maximize fitness (Stearns 1992; Ricklefs and Wikelski 2002). We suggest that this could result in different strategies regarding immune system: some individuals may prioritize the immune system against other physiological requirements, while other individuals first satisfy other demands. That is, individuals adopt different defence mechanisms, some may opt for resistance while others do for tolerance (Baucom and De Roode 2011).

Lizards opting for resistance prioritize immune system and should mount a better antiparasite response. Those individuals showed an intensity haemogregarine load lower than the median value and we refer to them as lightly infected individuals. Among them, we expected to find a positive relation between body condition and immune response. Immune response is condition-dependent in animals in a better health or having a greater endogenous energy/resource reserve, typically maintaining better immune responses than those in poor body condition (Lifjeld et al. 2002). Hence, those with better body condition showed a stronger immune response and a lower parasite load. Several authors (e.g. Cooper et al. 1985; Smallridge and Bull 2000), argued that the loss of body condition could means a reduction in resources for defence against parasitic infection, and would reduces the ability of lizards to develop an immune response to infection. In a population of *P. muralis* from Spain, Amo et al. (2007) found that lizards with poorer body condition had lower cell mediated immune responses and a higher blood parasite load.

However, as to mount a good immune defence is costly and host resources are limited, there is often a trade-off between the immune response and other demands, such as growth or reproduction (Sheldon and Verhulst 1996; Møller et al. 1999). Some individuals would opt to not prioritize the immune system. As an alternative strategy, they prefer to satisfy other physiological requirements first, and adopt tolerance as defence mechanism. Lizards prone to this strategy, would present a higher parasite burden than those that invest in defence system. And among them, body condition is not related with immune response.

The strategy of not prioritize the immune system can only be followed by populations where the negative effects are acceptable. That is, where such strategy is effective in terms of fitness. The more severe injury in fitness of this strategy is the increased risk of predation. Predators mainly capture sickest or most parasitized individuals (Navarro et al. 2004; Genovart et al. 2010). Thus, individuals opting for this strategy would be removed from the population more easily and the strategy would not be detectable by researchers. For a population with low predation pressure, as in the case of lizards from Aire Island (Cooper and Pérez-Mellado 2012), this would not happen, and both strategies could co-exist. With a low predation pressure, both, high or low parasitized individuals are not habitually preyed. Accepting a tolerance defence mechanism, energy saved could be invested in other life-history traits, increasing individual's fitness.

The coexistence of different strategies is not new, it is even common in many other components of life-history traits of individuals within a population. For example, there are consistent individual differences in many behavioural traits like boldness, exploration, sociability or aggressiveness (Rodríguez-Prieto et al. 2011), foraging tactics (Trillmich and Trillmich, 1986) or mating and reproductive strategies (Gross, 1996). The ecological and evolutionary consequences of such immunological variation must be considered. Plants would be either resistant or tolerant, but not at the same time (Weinig 2003 and references therein). Because of costs derived from both strategies, plants may opt for one or another strategy depending on different environmental factors, so can create temporally or spatially fluctuating selection on resistance and tolerance, modifying the relative costs and benefits of resistance and tolerance (Weinig 2003 and references therein). Analogously, animals could change their strategy according to changes in biotic and abiotic conditions, physiological requirements or other components of their life-history. In any case, these results must be interpreted with caution, since the combined influence of the parasite load and body condition on the immune response has not been thoroughly studied.

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Capítulo 8

Evaluación de los factores que influyen en la asimetría fluctuante en cuatro poblaciones insulares de lagartos

Evaluación de los factores que influyen en la asimetría fluctuante en cuatro poblaciones insulares de lagartos

Resumen

En los animales, la estabilidad en el desarrollo se evalúa frecuentemente mediante el nivel de asimetría fluctuante (FA en sus siglas en inglés). Varios factores de estrés ambiental y genético pueden aumentar la inestabilidad en el desarrollo en una población. Sin embargo, el uso de FA como medida de la inestabilidad en el desarrollo y su relación con otras medidas de estrés genético y ambiental, siguen siendo controvertidos. Estudiamos FA en la lagartija balear, *Podarcis lilfordi*. Se examinaron cuatro poblaciones de *P. lilfordi* que habitan diferentes islas costeras de Menorca (Islas Baleares, España). Estas cuatro poblaciones muestran diferencias ecológicas y genéticas claras que nos ha permitido estudiar los posibles factores que influyen en la FA. Analizamos las siguientes predicciones: (1) FA aumenta en las islas más pequeñas, (2) FA aumenta en islas con una densidad mayor lagartija, (3) FA aumenta en islas con lagartijas más parasitados y (4) las poblaciones con mayor endogamia niveles serían más susceptibles al estrés ambiental. Nuestros resultados apoyarían parcialmente la cuarta predicción de que existe una FA mayor en la población con menor variabilidad genética y, en consecuencia, con un nivel de endogamia superior. Probablemente, otros factores ambientales modulan esta relación. No hemos podido confirmar las tres primeras predicciones mencionadas.

Assessing factors involved in fluctuating asymmetry of four insular populations of lizards

Abstract

In animals, developmental stability is frequently assessed by level of fluctuating asymmetry (FA). Several environmental and genetic stress factors can increase developmental instability in a population. Anyhow, the use of FA as a measure of developmental instability, and its relationship with other measures of genetic and environmental stress, remain controversial. We studied FA in the Balearic lizard, *Podarcis lilfordi*. We examined four populations of *P. lilfordi* inhabiting different coastal islets of Menorca (Balearic Islands, Spain). These four populations show clear ecological and genetic differences that allowed us to study factors potentially involved in the level of FA. We examine the following predictions: (1) FA will increase on smaller islands; (2) FA will increase on islands with a higher lizard density; (3) FA will increase on islands with more parasitized lizards and (4) Populations with higher inbreeding levels would be more susceptible to environmental stress. Our results would partially support the fourth prediction of a higher FA in population with lower genetic variability and, consequently, a higher inbreeding level. Probably, other environmental factors modulate this relationship. Thus, we were unable to confirm the first three predictions above mentioned.

Keywords: developmental stability; island; genetic variability; environmental stress; parasites; *Podarcis lilfordi*.

Introduction

Developmental stability is viewed as an individual’s ability to buffer its development against random perturbations (Van Dongen and Lens, 2000). Frequently, it is assessed by the level of fluctuating asymmetry (FA), which corresponds to the small and random differences between the right and left sides of an otherwise bilaterally symmetrical character (Van Valen, 1962). Several environmental and genetic stresses can increase developmental instability. FA was defined as a population parameter, but nowadays, it is also used as an indicator of individual´s quality (Møller and Swaddle, 1997).

This study tries to approach the subject of FA employing the Balearic lizard, *Podarcis lilfordi*, as a model. We examine four populations of *P. lilfordi* inhabiting different coastal islets around Menorca (Balearic Islands, Spain). Although ectotherms are generally considered to be good monitors of environmental stress (Leary and Allendorf, 1989), there are few studies in developmental stability of reptiles. In addition, lizards have many metric (as limbs or head dimensions) and meristic (as scales, femoral pores or subdigital lamellae) paired characteristics relatively ease to measure (Soulé, 1967) and they are excellent models for ecological or eco-morphological studies (Pianka, 1986; Losos, 2009). At population level, small islands around Menorca are ideal scenarios to study the effects of genetic and ecological factors over development stability, because they host isolated lizard populations on relatively simple ecosystems. Also, developmental instability measured as FA is, presumably, more evident in small island populations because may endure more environmental (MacArthur and Wilson, 1967) and genetic stress (Young, Boyle and Brown, 1996; Frankham, 1998; Crawford et al., 2001).

In the absence of human intervention, gene flow between islands is limited or absent. Crnobrnja-Isailović, Aleksić and Bejaković (2005) argued that conditions of isolated populations as bottleneck effect, long-term strong selection, low effective population size or crowding effects, may lead to high inbreeding levels, periods of intense directional selection and extreme homozygosity. Consequently, the balance of genome is disrupted and the ability of the organism to buffer its developmental pathways against the production of random error can be reduced. Hence, theory suggests a positive correlation between FA and levels of inbreeding as it increases homozygosis (Møller and Swaddle, 1997).

In addition, small populations may also withstand more environmental stress through, for example, competition for limited resources and/or co-existence with predator species (MacArthur and Wilson, 1967; Crnobrnja-Isailović, Aleksić and

Bejaković, 2005). Due to increased genetic stress, small populations may be more susceptible to environmental stress. Inbred populations may suffer greatly changes in the environment that not inbred populations perceive as not stressful, indicating that the genetic and environmental stress can interact (Kristensen et al., 2006). Such interactions can lead to the synergy between both stresses, causing that environmental conditions, normally benign, may be detrimental (Kristensen et al., 2006). To assess the impact of competitor or predator species, we deliberately chose populations where we have four combinations of presence or absence of rats and gulls. These two species are often perceived as significant agents of change in the alteration of many ecological communities, particularly in islands (Pérez-Mellado, 2002; Oro and Martínez-Abraín, 2007).

Among non-genetic factors, parasitism and population density have been the most widely studied factors in relation to FA. Møller and Swaddle (1997) and Møller and Manning (2003) presented large evidences from a range of vertebrate taxa and conclude that population density could results in higher levels of FA, presumably generated by a lower resource availability at higher densities and density-dependent competition. However, other authors were not able to found this relationship between population density and FA (Wiener and Rago, 1987) or totally (Kruuk et al., 2003).

Parasitism and FA are supposedly linked through poor environmental conditions that can increase developmental instability. So do their measures, as fluctuating asymmetry. Poor environmental conditions are also known to reduce the efficiency of the immune system (Møller and Swaddle, 1997). In theory, among populations, those exposed to more stressful conditions, should show higher levels of parasitic load and FA. Møller (1996, 2006) reviews the literature suggesting a positive relationship between parasitism and increased levels of asymmetry, although the link between both variables is diverse. Møller (1996, 2006) also warns that this correlation does not imply causation and that the damage caused in the host is related to parasite´s virulence. Anyhow, several studies did not find that relation (Bergstrom and Reimchen, 2005 and references therein).

In lizards, isolation of insular populations has been shown to increase inbreeding and genetic drift (Madsen, Stille and Shine, 1996; Madsen et al., 2000) and, with small island size, it has been associated with an increase in FA (Soulé, 1967; Sarrè and Dearn, 1991; Sarrè, 1996; Vervust et al., 2008; Băncilă et al., 2010). However, no increase of FA was also found in small islands populations of *Podarcis muralis* (Crnobrnja-Isailović, Aleksić and Bejaković, 2005). At individual level, some studies linked asymmetry of hatchlings with temperature during egg incubation (Ji, Qiu, and Diong, 2002; Qualls and Andrews, 1999), physiological performances (Martín and López, 2001; López and Martín, 2002; Vervust, 2008; but see also Warner and Shine, 2006) and mating and reproductive success (Martín and López, 2000 and 2006; but see Lailvaux and Irschick, 2006).

The objective of our work is to study the influence of genetic and non-genetic (density, competitor species and parasitism) factors, as related with the FA levels of individuals and populations. We predict that FA will increase: (1) on smaller islands; (2) on islands with a higher lizard density; (3) on islands with more parasitized lizards. We also expect that populations with higher inbreeding levels would be more susceptible to environmental stress. That is, the impact of competitor species should increase with inbreeding levels.

Material and methods

Species under study

The Balearic lizard, *Podarcis lilfordi* (Günther, 1874) (Squamata, Lacertidae), is a medium-sized lizard endemic to coastal islets of Mallorca, Menorca and the Cabrera archipelago (Balearic Islands). Lizards were collected by noosing. For each individual, were recorded age, sex, SVL, tail length and body weight. Body condition was estimated from the residuals of the regression of log-transformed body weight versus log-transformed SVL.

We quantified prevalence and intensity of infection of two different parasites, mites and haemogregarines. Both kinds of parasites were previously studied (Garrido and Pérez-Mellado, 2012 and 2013). Mites of *P. lilfordi* were provisionally assigned to the genus *Ophionyssus*, while blood parasites were ranged within the haemogregarines group (Garrido and Pérez-Mellado, 2012, 2013). Ectoparasites were counted at the site of capture with a 5x monocle, inspecting the whole body surface of the lizards. To estimate blood parasitism we obtained blood samples making a slight longitudinal cut in the dorsal and proximal side of the tail with a sterile scalpel. With the blood drop, we obtained a blood smear *in situ*. Specimens were always released at the site of capture. Blood smears were placed on microscopic slides and air dried in the field. In the lab, slides were fixed with absolute methanol for 10 minutes and then stained with modified Giemsa for 20 minutes. Samples were analyzed using an optical microscope at 400X. The only blood parasites identified were haemogregarines. The intensity of haemogregarines was estimated on a total of 2,000 counted cells per sample. Prevalence was estimated as the percentage of infected individuals at each of the two areas under study.

Balearic lizards were studied in four different islands: Aire, Colom, Binicodrell and Tosqueta. Being relatively simple ecosystems allow us to control the ecological variables that differ between populations. We chose deliberately these four populations knowing the ecological and genetic differences between them (Pérez-Mellado et al., 2008, see **Table 1**) that allowed us to study the factors potentially involved in the level of FA.

Population	NPS	Pi	K	S	Rats	Seagulls	D	B	density±SE	Biom
Aire	1	0.000200	0.50	29.80	−	+	6.10	5.58	4098.60±586.60	32.54
Colom	4	0.00095	2.07	51.14	+	+	7.62	9.38	1615.50±294.93	11.02
Binicodrell	0	0.00	0.0	0.29	−	−	0.57	3.84	1180.50±264.45	7.59
Tosqueta	0	0.00	0	0.29	+	−	0.57	4.91	1703.00±501.41	11.29

Table 1. Genetic data and environmental factors of each population under study: NPS: total number of polymorphic sites; Pi: nucleotide diversity; k: average number of nucleotide differences; S: island area (in hectares); Rats: presence (+) or absence (−).

FA estimation

We measured one metric, hindlimb length (HLL), and 6 meristic traits from the left and right sides: Femoralia (FEM), the number of femoral pores at the hind leg, lamellae (LAM), the number of subdigital lamellae of the fourth toe, the number of supraocular scales (SPO), the number of supralabial scales (SPL), the number of infralabial scales (INL) and the number of submaxilary scales (SMX). To verify that the traits met the requirements for FA (see below), we took three repeated measured of them in a sample of 30 lizards.

For metric and meristic traits where the absolute differences of right minus left (|R - L|) sides are large (4-5 units), Palmer (1994) and Palmer and Strobeck (1986) described a test to establish whether the between-sides variation is significantly larger than the measurement error (ME). The test is a two-way ANOVA (sides x individuals), and should be conducted routinely as a part of any study of FA (Palmer and Strobeck, 1986). This procedure tests for the significance of all between-sides variation relative to measurement error, including antisymmetry (AS) and FA. One advantage of factorial ANOVA procedure is that the significance of directional asymmetry (DA) can be tested at the same time as that of FA relative to measurement error. For meristic traits, where the difference between sides is small (1-2 units) and where a low level of scoring error may exist, ANOVA technique does not work properly. Palmer (1994 and pers. comm.) recommended a likelihood ratio or G-test (Sokal and Rohlf, 1995). If the test is statistically significant, it is still needed to confirm that the mean of the differences between both sides of the character (|(R - L)/n|) is greater than mean of the difference between all measures (|Mx - My|/n), where ‘n’ is the number of replicate counts. In these cases, DA must be estimated with a one-sample t-test of the mean (R-L), because departures from normality can mask DA (Palmer, 1994).

Once we tested for DA and ME, tests for the presence of AS should also be conducted in studies of FA. Typically, AS shows a platykurtic or bimodal frequency distribution in a given population, while DA presents skewness (Møller and Swaddle, 1997). To check for kurtosis and skewness, we applied the Anscombe-Glynn and D'Agostino tests, respectively (Zar, 2010). Finally, Palmer and Strobeck (1986) suggested looking for dependence of FA to body size, correcting for this dependence if necessary. Palmer (1994) pointed out that body size may reflect some aspect of body condition. Thus, controlling for trait size may partially control for body condition and render asymmetry estimates that are condition-independent. Accordingly, we chose to avoid the correction for size dependence, because body size could be correlated with factors under study and that correction can mask their association with FA (Palmer and Strobeck, 1986; Băncilă et al., 2010).

For our purpose, we used a composite FA index (CFA), as the analysis of composite indexes is more powerful than analyses based on single traits (Møller, 2006; see discussion in Leung, Forbes and Houle, 2000). In addition, analyses that combine information across traits should prove to be more reliable detectors of stress (Leary and Allendorf, 1989; Watson and Thornhill, 1994). Therefore, after each trait were analysed for FA, they were combined per individual in the composite CFA2 (Leung, Forbes and Houle, 2000).

Statistical analysis

First, we searched for differences in the level of asymmetry between islands. To avoid spurious differences that are really caused by sex differences, we introduce this factor in a two-way ANOVA together with the 'island' factor. CF2 data were Box-Cox transformed to get homoscedasticity. Then, to test the correlation between parasitism and FA at population level, we ran Pearson's correlation tests between averages FA and the averages of haemogregarines and mites loads of each island. Distributions of population averages of FA were normal, as distribution of average parasites loads (see 'Results'). If FA levels differed between populations, we searched for correlations between asymmetry and prevalence or intensity of parasitism, independently for each island. In all cases, as CFA2 had homogeneous variances (see 'Results'), relations with variables were analyzed using general linear models (GLM) with Poisson distribution (Grafen and Hails, 2002; Crawley, 2007).

The statistical analyses were carried out in R environment (ver. 2.12.1, R Development Core Team, 2010). We started with a saturated model, considering effects of all independent variables as well as all interactions between them. To determine the minimum adequate model (Crawley, 2007), we use a backward stepwise logistic regression made on the basis of deletion tests (Crawley, 2007). Non significant factors and interactions were removed from the models until we obtained a minimal adequate model, taking into account AIC (Akaike's Information Criterion) at each step. Post-hoc comparisons were made using multcomp package (Hothorn, Bretz and Westfall, 2009). Effect sizes reported are partial eta-squared (η_p^2) that can be interpreted similarly as R^2

(Cohen, 1988). As R^2, η_p^2 is a dimensionless measure of effect size, independent of the degrees of freedom used in the analyses (Tabachnick and Fidell, 2000).

Results

Assessing FA

All meristic traits show an average difference between sides less than 1 (**Table 2**). Thus, we applied Palmer's recommended G-test to look if ME was bigger than FA (**Table 2**). G-test was significant and the mean |(R - L)/2| is greater than mean |M1 - M2| for all traits, except for SPO and SMX where we couldn't apply G-test as there were no variation in the repeated measures, so, the repeatability of measures was 100% (**Table 2**). For these traits, we obviously considered that variation between sides will not be due to ME. Moreover, in all meristic traits, normality was tested to discard DA (see above), we obtained that distributions were normal for all meristic traits (**Table 3**). For HLL (metric trait), two-way ANOVA recommended by Palmer showed that DA is not present and that variation due to non directional asymmetry was higher than variation due to ME (**Table 4**).

	FEM	**LAM**	**SPO**	**SPL**	**INL**	**SMX**
$\bar{x}$±SE of|R - L|	0.75±0.04	0.83±0.05	0.07±0.01	0.12±0.02	0.19±0.02	0.03±0.01
Palmer's G-test	$G_5 = 34.89$ $P < 0.001$	$G_9 = 6.54$ $P < 0.001$		$G_2 = 7.72$ $P < 0.05$	$G_1 = 4.95$ $P < 0.05$	
$\bar{x}$|M1- M2|	0.03	0.49		0.01	0	
$\bar{x}$|(R - L)/2|	0.074	1		0.14	0.07	
FA>ME	yes	yes	yes	Yes	yes	yes

Table 2. Results of G-tests and the protocol (see Palmer, 1994) to estimate FA relative to ME in meristic traits whose average different between sides is low.

	FEM	**LAM**	**SPO**	**SPL**	**INL**	**SMX**
Fisher's	$t_{511} = -0.35$	$t_{348} = -1.01$	$t_{521} = -0.93$	$t_{518} = -0.12$	$t_{522} = 0.19$	$t_{522} = 0.89$
t-test	$P = 0.73$	$P = 0.31$	$P = 0.35$	$P = 0.91$	$P = 0.83$	$P = 0.37$

Table 3. One sample t-test: testing for a departure of the mean of (R - L) from an expected mean of zero. This method allowed discarding the presence of DA in the different characters.

	Df	**Sum Sq**	**Mean Sq**	***F***	***P***
side	1	0.00	0.001	0.0085	0.9265
Individual	25	1090.96	43.638	274.8742	< 0.001*
Side:Individual	25	28.37	1.135	7.1472	< 0.001*
Residuals	104	16.51	0.159		

Non DA (FA and AS)				DA			Between replicate variation (MS$_m$)
Trait	**MS**	**df**	***F***	**MS**	**df**	***F***	
HLL	1.135	25,104	7.14*	0.001	1,25	0.0008^{NS}	0.159

Table 4. Two-way mixed model ANOVA: testing the significance of between-side individual interaction (non DA), between-side variation (DA) and between-replicates variation (ME) in *Podarcis lilfordi*. Asterisk (*) indicates significant factors; NS indicates no significant factors.

Visual inspection of data plots revealed no evidence for clustering of vectors of shape asymmetry that can suggest the presence of AS. Although, significant levels of skewness for the distribution of right minus left differences were found for SMX, but not for the remaining traits (**Table 5**). All traits showed a distribution significantly leptokurtic (**Table 5**). Since there were no platykurtosis or bimodality, we eliminate the possibility of AS acting as a confounding factor in our tests for FA.

To sum up, significant FA was detected for all characters measured in *P. lilfordi*, except for the skewness found in SMX, probably due to the relevance in the test of just two asymmetrical individual for this trait and as a consequence of the little variation of this trait. We decided to discard SMX in further analyses.

Trait	Skewness	*P*	kurtosis	*P*
LMP	0.69	0.48	3.81	< 0.001*
FEM	0.1813	0.26	4.080	< 0.001*
LAM	-0.0022	0.99	4.099	< 0.001*
SPO	-2.31	0.02	11.54	< 0.001*
SPL	0.19	0.24	9.97	< 0.001*
INL	1.59	0.11	7.37	< 0.001*
SMX	2.44	< 0.001*	14.69	< 0.001*

Table 5. Skewness (D'Agostino test) and kurtosis (Anscombe-Glynn test) estimates for each measured trait. Asterisk (*) indicates significant factors.

FA between populations

Two-way ANOVA showed no significance for the interaction term sex:island ($F_{3,\ 609}$ = 1.15, *P* = 0.33), that was excluded from the model. Males exhibited more asymmetrical FA values (mean ± SE = 6.01 ± 0.34; *n* = 249) than females (mean ± SE = 4.68 ± 0.28; *n* = 368), but we did not find statistical differences between sexes ($F_{1,\ 612}$ = 2.49, *P* = 0.11), and the effect size was very small (η_p^2 = 0.004).

	Binicodrell	**Aire**	**Colom**
Aire	*t* = 2.68 *P* = 0.04 *		
Colom	*t* = -1.80 *P* = 0.26	*t* = 1.31 *P* = 0.54	
Tosqueta	*t* = 4.05 *P* < 0.001 *	*t* = 7.52 *P* < 0.001 *	*t* = 6.63 *P* < 0.001 *

Table 6. Tukey's post-hoc test for differences in CF2 between populations. Asterisk (*) indicates significant differences.

Between islands, strong differences were found ($F_{3,\ 612}$ = 21.14, *P* < 0.001), but with a moderate effect size (η_p^2 = 0.094). Tukey's post hoc (**Table 6**) revealed that Tosqueta (mean ± SE = 12.68 ± 1.07, range = 23.89), showed statistically highest rates of asymmetry than Binicodrell (mean ± SE = 8.15 ± 1.47, range = 60.52), Colom (mean ± SE = 5.91 ± 0.38, range = 27.89) and Aire (mean ± SE = 5.14 ± 0.26, range = 25.10). Binicodrell individuals presented differences with Aire. But surprisingly, despite showing higher FA absolute values, Binicodrell not showed differences with Colom. Lizards from Colom were not more asymmetric than those from Aire.

Apparently, at population level, parasitism is not a good indicator of developmental stress measured as FA. No relation appeared between population levels of FA (Shapiro-Wilk normality test, $W = 0.88$, $P = 0.36$) with average haemogregarines load ($W = 0.93$, $P = 0.60$; Pearson's correlation, $t_2 = 1.32$, $P = 0.32$, $cor = 0.68$), nor with mites intensity ($W = 0.92$, $P = 0.57$; Pearson's correlation, $t_2 = 1.34$, $P = 0.31$, $cor = 0.69$).

Discussion

We used CFA2 to test some hypotheses elaborated respect asymmetry FA. First, no found any differences between sexes in the levels of FA. In contrast to some previous studies where males were more asymmetric than females (Møller and Swaddle, 1997), here, we reported no differences between sexes, despite the differences in absolute values between both groups. According to the assumptions of FA theory, we can conclude that both sexes are exposed to similar levels of stress.

FA levels were significantly different among all island populations, with the exception of Aire-Colom comparison, with similar values of asymmetry. Despite we expected to find higher FA levels in smaller islands due to increased levels of inbreeding, there was a lack of difference between Binicodrell and Colom, probably due to their unequal sample size and the wide range of FA values in Binicodrell. Density has been pointed out as severely affects developmental stability, increasing FA (see, for example, Møller and Swaddle, 1997; Møller and Manning, 2003), and very few studies have questioned this relationship (e.g. Kruuk et al., 2003). In our study, this relationship is not as obvious as the theory shows. Even, we could say that the opposite happens and that density, therefore, was not the key factor leading developmental stability Aire Island showed much higher density but the lowest FA values along with Colom. And among the other islands, with similar population densities, FA differed strongly (**Table 1**).

Immune system and parasitism also could be linked with FA in poor environmental conditions (Møller and Swaddle, 1997). But in our case, parasitism is not a good predictor of the degree of asymmetry of a population. The degree of parasitism present in a population depends, not only on immune system efficiency, but also depends on the likelihood of becoming infected with the parasite. These probabilities vary according to different ecological and environmental factors (Biaggini, Berti and Corti, 2009). Even depends on the behaviour of individuals within a population (Garrido and Pérez-Mellado, 2012 and 2013, and references therein). In this sense, Binicodrell population provides a good example because no ectoparasites were found, despite its FA levels.

From our viewpoint, genetic stress is a more reliable indicator of the degree of asymmetry. Theory predicts that small populations may endure more genetic stress than large populations due to erosion of genetic variation (Young, Boyle and Brown, 1996; Frankham, 1998; Crawford et al., 2001). Also, this loss of genetic variation and deeper inbreeding depression can cause a fitness decline (Crnokrak and Roff, 1999; Frankham, 2005; Allendorf and Luikart, 2007). Genetic variability was significantly higher in larger populations (**Table 1**). Hence, Tosqueta and Binicodrell showed the lowest genetic variability and the highest levels of FA of four populations under study. Among the two less asymmetrical populations, Colom and Aire, no differences in CFA2 were found, but Colom showed a higher genetic variability. Despite this exception, our results are consistent with other studies, which prove the influence of genetic variability on FA levels. We propose that genetic variability affects stability when it is below a given level of inbreeding. Above that level, as it is the case of Aire and Colom, there is no relationship between FA and homozygosis.

According with our results, genetic stress could be good predictor of the degree of asymmetry of populations, although it may not be the only one. There may be any environmental factor generator of stress that impairs the effective development of individuals. Presumably, insular populations must also withstand great environmental stress, due to cohabite with the mentioned competitor-predator species, the ship rat and yellow-legged seagull. *L. michahellis* is present in Aire and Colom (**Table 1**), both presented the lowest CF2 value. Presumably, gulls are not responsible for differences in the development between islands. Our results are in line with some recent papers in which it is argued that their negatively impact on the population trends of most species is not so clear (e.g. Oro and Martínez-Abraín, 2007).

The ship rat is an alien species, arrived to Balearic Islands with human occupation. Invasive species have reportedly altered endemic populations as predate over them, but also because they compete for resources (Case and Bolger, 1991). Particularly, rodents have often responsible of the most devastating effects on invaded habitats (Pérez-Mellado, 2002). In the case of Tosqueta and Binicodrell, there are no differences in genetic variability, neither in density or biotic capacity (**Table 1**). In fact, the main difference between Tosqueta and Binicodrell could be the presence of *R. rattus* in Tosqueta and its absence in Binicodrell. Reptiles show a high vulnerability to rodent presence (Towns, Atkinson and Daugherty, 2006). As we expected, results derived from environmental stress were in accordance with Kristensen et al. (2006). They showed that inbred populations may suffer greatly certain changes in the environment that inbred populations perceive as not stressful, indicating that the genetic and environmental stress can interact (Kristensen et al., 2006). Thus, it seems that Tosqueta individuals could be more sensitive to the presence of rats that Colom ones, probably because Colom lizards had higher levels of heterozygosis (Pérez-Mellado et al., 2008).

In summary, although the genetic variability is considered the best predictor of the degree of asymmetry of populations, other stressors may modulate this effect, in our case perhaps the presence of rats. If parasites are a cause of asymmetry, it is possible that the average intensity of parasite load in these populations do not affect the development of individuals. Here we studied two kinds of parasites with different virulence (see Material and Methods) and four populations with very different intensities of infection. As Ward, Thomas and Poulin (1998) propose if FA could be an indicator of susceptibility to parasitism, we should find correlation between FA and parasitism, unless: (1) highly-asymmetrical individuals could experience high mortality rates and consequently masked the relation; (2) we may expect no relationship between FA and parasitism among adults, as not reflecting what happened earlier during development.

From our viewpoint, the only conclusion is that FA and parasitism are not related at population level in *P. lilfordi*. But, according with Ward, Thomas and Poulin (1998), perhaps the relationship between parasitism and FA is not as general as suggested by other authors (Møller, 1996; Polak, 1997; Møller, 2006). Palmer (2002) considers that FA is a poor indicator of the quality of individual development, as there may be random deviations from symmetry. In addition, Floate and Fox (2000) showed that increased stress reduced population fitness but did not affect FA and Rasmuson (2002), reviewing the relationships between developmental noise and fitness indices, notes that a comprehensive theory of the causes of developmental instability still is absent. Moreover, Hendrickx, Maelfait and Lens (2003) observed no relationship between fluctuating asymmetry and fitness under stressful conditions and Kruuk et al. (2003) did not reveal any relationship, at individual level, between the levels of FA and fitness.

There has been a recent debate over the importance of publication biases in our understanding of ecological processes (Møller and Jennions, 2001 and references therein). Fanelli (2012a, b) argued that papers with negative results are disappearing from most disciplines, as in all Animal and plant sciences. It is even more serious when dealing with relatively recent issues. It is the case of FA, where statistical methodology is being updated continuously. Ward, Thomas and Poulin (1998) warn "that any interpretation based solely on the proportion of studies reporting a positive association may be misleading".

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Capítulo 9

Discusión

Discusión

El estudio de los distintos indicadores del estado de las poblaciones de *Podarcis lilfordi* evidencia, en un primer momento, la influencia que sobre ellos tienen las características propias de las islas. Estas singularidades, que hemos ido citando a lo largo de todo el trabajo, como el grado de aislamiento de las poblaciones, las altas densidades de población, la baja presión de depredación o la ausencia de competidores, repercuten sobre los estimadores de tal modo, que los resultados observados no serían fácilmente explicables fuera del contexto insular.

Es habitual encontrar altas densidades de lagartijas en casi la totalidad de las poblaciones mediterráneas. Dentro de la cuenca mediterránea, las Islas Baleares constituyen una de los regiones en las que los lagartijas endémicas alcanzan densidades más elevadas (Salvador, 1986; Pérez-Mellado, 1989, 1998), pudiendo llegar en la Isla del Aire hasta los 4000 individuos/hectárea (Pérez-Mellado et al., 2008). Estos valores son señalados como el factor determinante en las altas tasas de prevalencia e intensidad parasitarias halladas en la Isla del Aire para ambos parásitos, una de las más elevadas encontradas hasta ahora en lacértidos mediterráneos (Capítulos 2 y 4). A pesar de que la lagartija balear carece de una organización social definida, la exposición a los ectoparásitos de conespecíficos infectados aumenta con la densidad. A su vez, los ácaros como hospedadores intermedios de hemogregarinas, son el factor crítico de la elevada prevalencia de parásitos sanguíneos (Svahn, 1974).

Dentro de la propia isla del Aire, los valores de densidad varían entre distintas áreas (Pérez-Mellado et al., 2007). Está demostrado que los animales no se distribuyen al azar, ya que algunos lugares son mejores que otros en términos de disponibilidad de recursos teniendo igualmente en cuenta los riesgos y los costes de adquisición de esos recursos (Zug et al., 2001). En las pequeñas islas del Mediterráneo, la escasez de alimentos y la imprevisibilidad en la disponibilidad de recursos es un patrón habitual

(Pérez-Mellado y Corti, 1993). En estas circunstancias, algunos recursos se vuelven extremadamente importantes durante algunos períodos del año, como ocurre con la rapa mosquera, *Dracunculus muscivorus* (Araceae). Además, Hews (1993) sugirió que los recursos alimenticios influyen sobre la abundancia de los reptiles y que tanto el comportamiento social como la distribución espacial en respuesta a la abundancia de alimentos son diferentes entre ambos sexos. En este estudio, hemos visto como la floración durante la primavera de *D. muscivorus* en la zona central del Aire provoca una distribución desigual de los individuos de la población (Capítulo 3). En esta zona, hallamos una mayor abundancia de machos, siendo éstos además de mayor tamaño que en las dos áreas con densidades más bajas de rapa mosquera. Asimismo, la prevalencia de infestación parasitaria era mayor en dicha zona. Por otra parte, aparecieron diferencias en la intensidad de infección. Ésta fue mayor en los individuos de la zona central como consecuencia de una mayor concentración de individuos dominantes y agresivos, y de un aumento de la probabilidad de encuentros agonísticos, lo que incrementaría las posibilidades de reinfección.

Hay que destacar, que tanto la alta densidad existente como el elevado número de contactos sociales, ambos responsables de los resultados comentados hasta ahora, son en gran medida consecuencia de la ausencia de competidores y de la baja presión de depredación a la que está sometida dicha población. Los depredadores capturan habitualmente presas enfermas y/o altamente parasitadas (Temple, 1987; Navarro et al., 2004; Genovart et al, 2010). Esto no ocurre probablemente en la isla del Aire, debido a la baja presión de depredación, lo que facilita que encontremos individuos con tan elevadas tasas de infestación parasitaria, al no ser eliminados de la población (Capítulo 2 y 4). Esta particular situación en la isla del Aire nos sitúa en una posición ideal para estudiar los posibles efectos deletéreos del parasitismo. Así, hemos detectado que la velocidad de carrera está estrechamente relacionada con la intensidad de parásitos sanguíneos (Capítulo 5) y con la condición que, a su vez, se halla negativamente relacionada con la carga de hemogregarinas (Capítulo 2). Para Oppliger et al. (1996), este hecho implica que los individuos más parasitados pueden ser más vulnerables a la depredación, ya que su movilidad se ve reducida. Puede ser, por lo tanto, que las lagartijas más parasitadas sean eliminadas de la población por los depredadores, y, por lo tanto, este hecho puede oscurecer la relación entre el parasitismo y velocidad de carrera. Debido a ello, muchos estudios con poblaciones continentales y/o mayor presión de depredación no encontraron esta relación. En el Aire, la depredación sobre *P. lilfordi* es probablemente baja, en comparación con una zona continental y se puede evitar este factor al estudiar el efecto directo de los parásitos.

La ausencia de depredadores también posibilitaría la existencia de dos estrategias diferenciadas respecto a la inversión en el sistema inmune. Los resultados sugieren que algunos individuos pueden priorizar la inversión en el sistema inmune frente a otras necesidades fisiológicas, mientras que otros satisfacen primero las demandas de otras necesidades (Capítulo 7). La estrategia de no priorizar el sistema inmune sólo podría aparecer en poblaciones en las que los efectos negativos de la misma pueden ser aceptados, es decir, cuando esta estrategia es efectiva en términos de aptitud biológica (Capítulo7). El mayor perjuicio para los individuos que adoptan esta estrategia sería el aumento del riesgo de depredación, que, como ya hemos dicho, es mayor en individuos más parasitados y enfermos (ver más arriba). Si así fuera, los individuos que optan por dicha estrategia se eliminarían de la población con más facilidad y la misma no sería detectable por los investigadores. En la Isla del Aire, debido a la baja presión de depredación, esta estrategia sería perfectamente asumible. Es más, la energía ahorrada se podría invertir en otros rasgos vitales. También hay que tener en cuenta que una estrategia inmune, centrada en la eliminación sistemática de los parásitos, podría no ser efectiva cuando las probabilidades de reinfección son continuas y muy elevadas. Sería necesario llevar a cabo en el futuro experimentos adicionales para confirmar están hipótesis.

El aumento de presión humana durante el verano es otro factor habitual en determinados islotes del Mediterráneo. En general, la mayor presencia de visitantes durante esa época puede considerarse una característica propia de dichas poblaciones. Las lagartijas perciben a los seres humanos como potenciales depredadores (Amo, López y Martín, 2006), provocándose una serie de respuestas antidepredadoras. En consecuencia, en algunas zonas de la Isla del Aire, la percepción que tienen las lagartijas de ser capturadas es elevada, aunque la depredación real sea escasa. Los comportamientos de escape son costosos en términos fisiológicos, causando efectos negativos como la disminución de la condición corporal (Martín y López, 1999; Pérez-

Tris et al, 2004), lo que podría reflejarse en otros componentes relacionados con la eficacia biológica, como la carga parasitaria. Durante la temporada estival, cuando la sequía y la escasez de recursos son más pronunciadas, algunas poblaciones insulares de *P. lilfordi*, como la del Aire, reciben el mayor flujo de visitas de todo el año. Durante esa misma estación, las lagartijas de la zona más visitada de la isla mostraron una mayor carga de ectoparásitos y una mayor disminución de la condición corporal en comparación con los individuos de áreas del islote no visitadas por turistas (Capítulo 4). Proponemos que los costes de las respuestas de escape motivadas por los visitantes humanos son responsables de las diferencias observadas.

Además, hemos estudiado los efectos del parasitismo sobre la conducta de escape de los individuos (Capítulo 6). Según la teoría óptima de escape (Cooper and Frederick 2007, 2010), el parasitismo podría afectar a más de uno de los predictores de la FID. Por tanto, sus efectos son difícilmente predecibles. En este estudio hemos observado que las lagartijas con una mayor carga de hemogregarinas tenían una FID mayor, pero también una DF (distancia a la que se huye) más corta. Además, la FID estaba negativamente relacionada con la condición corporal entre los individuos con cargas más altas de ácaros. Los hallazgos se discuten en relación a las predicciones de la teoría óptima de escape (Capítulo 6).Así, aunque las cargas de ambos parásitos están relacionadas con las decisiones de escape en *P. lilfordi*, debido a que nuestro estudio es correlacional y no experimental, estas relaciones no indican necesariamente que las cargas parasitarias modifican las decisiones de escape. Las diferencias de comportamiento entre machos que tienen diferentes cargas parasitarias podrían explicar, al menos en parte, las correlaciones entre los parásitos y el comportamiento de escape. Un conocimiento detallado de la ecología y comportamiento de las presas es necesario para evaluar el aparente efecto de parasitismo en los estudios de campo no experimentales.

Por otra parte, al ser finitos los recursos de los que dispone un individuo, el aumento del coste energético derivado de las respuestas de escape podría afectar a otros requerimientos vitales. Algunos autores, como Navarro et al. (2004), mostraron que ante la percepción del riesgo de depredación, los individuos tienden a redistribuir los recursos disponibles. Así, el sistema inmune recibe una prioridad reducida porque sus costes son elevados. En este estudio se estimó la respuesta inmune innata de los individuos mediante un ensayo inmunológico bactericida (*bacterial killing assay*) (Capítulo 7). En este caso, hemos hallado una fuerte influencia negativa del ectoparasitismo sobre la respuesta inmune (Capítulo 7). Dado que el ectoparasitismo aumenta con la presencia humana (Capítulo 4), podríamos argumentar que el impacto humano afecta indirectamente al sistema inmune de *P. lilfordi*, a través del aumento en la intensidad de la carga parasitaria externa. En resumen, la presión humana afectaría tanto a la condición corporal de las lagartijas como a su carga parasitaria, ya través de esta también a su capacidad de montar una buena respuesta inmune, disminuyendo sus probabilidades de supervivencia. Esto puede ser de especial interés, ya que ocurre en una pequeña población insular de una lagartija endémica.

Teniendo en cuenta los datos de la literatura y los resultados obtenidos en este estudio (Capítulos 2, 4, 5, 6 y 7), se puede concluir que, al menos en la lagartija Balear, los efectos deletéreos de los distintos parásitos sobre diferentes componentes de la aptitud biológica o sobre sus rasgos vitales, son notablemente diversos. Mientras que las hemogregarinas alteran capacidades locomotoras como la velocidad de carrera (Capítulo 5) o la distancia de huída (Capítulo 6), los ectoparásitos parecen disminuir la capacidad de la respuesta inmune (Capítulo 7). Además, ambos tipos de parásitos están relacionados con una peor condición corporal (Capítulo 2 y 4). Así, los resultados parecen indicar que los efectos nocivos están más vinculados a su campo de acción que a la potencialidad nociva del parásito, sin tener que concluir que uno sea más perjudicial que el otro. Debería prestarse una mayor atención a los efectos perjudiciales de los parásitos, en todos los ámbitos, desde el estudio de la fisiología o el comportamiento de los hospedadores, hasta la elaboración de planes de conservación. Especialmente si tenemos en cuenta que algunas actividades humanas, tales como el ecoturismo, están relacionadas con la carga parasitaria mayor de los lagartos que habitan en estas áreas (Amo et al., 2006).

Sin embargo, las razones argüidas hasta aquí para explicar la variación existente en los indicadores a nivel intrapoblacional, como los patrones de parasitismo o el estado de condición, no funcionan cuando comparamos poblaciones diferentes, probablemente debido a las singularidades ecológicas de cada una de ellas. Por ejemplo, los valores de densidad no explicarían por sí solos los niveles de parasitismo descritos y las diferencias entre cuatro poblaciones estudiadas, Aire, Colom, Tosqueta y Binicodrell. La población de Tosqueta presenta la mayor tasa de intensidad, tanto de ecto- como de endoparásitos (Capítulo 8), y también uno de los menores niveles de variabilidad genética. Shykoff y Schmid-Hempel(1991) sostienen que en las poblaciones donde los individuos están estrechamente emparentados, el bajo nivel de variabilidad genética facilitaría la infestación de un parásito, ya que éste se encuentra menor resistencia genética en la población. Se ha propuesto que el stress genético puede tener un alto poder explicativo al comparar los niveles de parasitación de distintas poblaciones (Hamilton et al., 1990). Sin embargo, el caso del islote de Binicodrell no concuerda con esta hipótesis. En dicha población existen niveles de homocigosis y densidades similares a los de Tosqueta, pero en Binicodrell observamos una ausencia total de ectoparásitos y la más baja tasa de infestación por hemogregarinas. Este resultado complica la construcción de una hipótesis explicativa sobre las tasas parasitarias. Probablemente, sea necesario tener en cuenta tanto las particularidades genéticas como las condiciones ecológicas, e incluso históricas de cada población para poder comprender las relaciones entre parásito y hospedador de la misma.

Algo similar ocurre con la asimetría fluctuante (Capítulo 8), que es, probablemente, el indicador menos fiable de los cuatro usados en este estudio (Floate y Fox, 2000; Rasmuson, 2002; Hendrickx et al., 2003; Kruuk et al., 2003). Las diferencias en los valores de asimetría hallados no pueden explicarse tan solo en base a un patrón, sino como una combinación de varios factores presentes en una población. En Menorca, la variabilidad genética se muestra como el mejor predictor del grado de asimetría de las poblaciones, aunque modulado por determinados factores de stress ambiental, como la presencia de la rata negra, *Rattus rattus*. Esta especie se encuentra en las islas de Colom y Tosqueta. Sin embargo, su presencia parece tener más impacto sobre los niveles de asimetría de ésta última, que muestra además valores más bajos de variabilidad genética. Hay evidencias de que un mayor estrés genético provoca una mayor susceptibilidad a los factores de estrés ambiental. Las poblaciones más consanguíneas pueden sufrir de modo intenso determinados cambios en el medio ambiente que otras poblaciones perciben como no estresantes, lo que indica que el estrés genético y el ambiental pueden interactuar (Kristensen et al., 2006).

Siguiendo las hipótesis propuestas por otros autores (Møller, 1996; Møller y Swaddle, 1997), hemos tratado de establecer una relación entre la asimetría fluctuante y otros indicadores, pero no hemos hallado evidencias claras que permitan demostrar una relación entre las medidas de parasitismo o la condición corporal con los niveles de asimetría. Esta ausencia de correlación con las hipótesis de Møller (1996) y Møller y Swaddle (1997) no es nueva (Ward et al., 1998; Floate y Fox, 2000; Rasmuson, 2002; Hendrickx et al., 2003; Kruuk et al., 2003). Existe un vivo debate sobre la importancia que tienen ciertos sesgos en los resultados publicados sobre nuestra comprensión de los procesos ecológicos (Møller y Swaddle, 1997; Møller y Jennions de 2001 y sus referencias; Møller, 2006). Puede ser éste el caso de la asimetría fluctuante, cuya metodología estadística está actualizándose continuamente (e.g. Leung et al., 2002) y que, a día de hoy, no cuenta aún con amplio soporte experimental. Fanelli (2012a, b) señala que los documentos con resultados negativos están desapareciendo de la mayoría de las disciplinas, y que esto es especialmente grave cuando se trata de cuestiones relativamente recientes y no profundamente consolidadas, como puede ocurrir con el estudio de la estabilidad en el desarrollo. De hecho, Ward et al. (1998) advirtieron de que "cualquier interpretación basada únicamente en estudios que informan de una asociación positiva entre variables puede ser engañoso".

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Capítulo 10

Conclusiones

Conclusiones

1. Las tasas de intensidad y prevalencia de infestación por hemogregarinas y ectoparásitos halladas en la población insular de *P. lilfordi* en Aire están entre las más elevadas conocidas hasta el momento para poblaciones de lacértidos.

2. En general, las tasas de infestación son mayores en machos adultos que en hembras o juveniles. Probablemente, las diferencias inmunológicas, genéticas y de comportamiento entre grupos de edad y sexo sean responsables de esta variabilidad.

3. La prevalencia de infestación por parásitos sanguíneos y ectoparásitosse mantiene estable a lo largo de los años del período de estudio, sugiriendo un equilibrio parásito-hospedador relativamente invariante en la población investigada.

4. Las intensidades de infestación, sin embargo, varían entre estaciones. La carga de parásitos sanguíneos aumenta en verano, cuando la disponibilidad de recursos y la condición corporal disminuyen, mientras que la carga de ectoparásitos es mayor en primavera, probablemente debido a que durante el verano las condiciones ambientales son más adversas para los ácaros.

5. La presión humana en la isla del Aire puede provocar una perturbación en el equilibrio natural entre parásito y hospedador. En la zona donde se concentran la mayoría de los visitantes, la carga de ectoparásitos no disminuye en verano, pero sí se observa una fuerte disminución de la condición corporal.

6. Debido a la escasez y la imprevisibilidad en la disponibilidad de alimentos propios de las pequeñas islas del Mediterráneo, algunos recursos efímeros se

vuelven extremadamente importantes durante algunos períodos del año. En estas circunstancias, la floración durante la primavera de *D. muscivorus* en la zona central del Aire y la habilidad para luchar y poder tener así acceso a ella puede influir en los patrones de parasitismo de los individuos.

7. A nivel individual, la tasa de infestación por hemogregarinas está negativamente relacionada con la condición corporal de los individuos. Dicho efecto no se observa en el caso de los parásitos externos.

8. La carga de parásitos sanguíneos tiene una influencia negativa sobre la velocidad de carrera de los machos adultos de la lagartija balear. Este resultado y el anterior reflejan el impacto negativo de las hemogregarinas sobre la fisiología y los rasgos vitales del hospedador.

9. Tanto los ectoparásitos, como los parásitos sanguíneos tienen una influencia directa sobre la conducta de huída de los individuos. Los individuos menos parasitados por hemogregarinas muestran una menor precaución frente al riesgo de depredación, permitiendo un mayor acercamiento de los depredadores. Sin embargo, este riesgo se compensa, ya que huyen más lejos que los individuos más parasitados, probablemente debido a las limitaciones fisiológicas derivadas del parasitismo.

10. Los ectoparásitos influyen indirectamente sobre la distancia de huida, potenciando en ciertos casos el efecto de la condición corporal sobre el riesgo de depredación percibido por las lagartijas. De este modo, sólo entre los individuos más cargados de ácaros, la distancia de huida y la condición corporal están inversamente relacionadas.

11. Hemos estimado la capacidad de respuesta inmune de las lagartijas de la Isla del Aire mediante un ensayo bactericida. Probablemente debido a que la saliva de los parásitos hematófagos tiene compuestos inmunosupresivos, la intensidad de infección por ectoparásitos tiene un efecto negativo sobre la capacidad de respuesta inmune.

12. La influencia conjunta de la condición corporal y la carga parasitaria sanguínea indica que, entre los individuos de la Isla del Aire, parecen existir dos estrategias inmunológicas diferenciadas. Algunos individuos parecen priorizar el sistema inmune, mientras que otros asignarían los limitados recursos energéticos a otros requisitos. Es decir, unas lagartijas pueden optar por la resistencia frente a los parásitos, mientras que otras optan por la tolerancia.

13. No hemos hallado ninguna relación de la asimetría fluctuante con el resto de indicadores estudiados. A nivel poblacional, no parece haber relación entre las tasas de parasitismo, interno o externo y los valores de asimetría fluctuante.

14. Los valores de asimetría hallados en las distintas poblaciones no pueden explicarse como consecuencia de un único factor, sino como el resultado de una combinación de varios factores. En nuestro estudio de cuatro poblaciones, la variabilidad genética se muestra como el mejor predictor del grado de asimetría de las poblaciones, aunque probablemente modulado por agentes causantes de stress ambiental.

15. De los cuatro indicadores estudiados, la asimetría fluctuante parece el menos fiable.

16. El grado de infestación parasitaria externa e interna, la condición corporal y la respuesta inmune pueden considerarse como indicadores fiables del estado de salud de las poblaciones estudiadas.