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BIOGEOGRAPHY OF BLOOD PARASITES IN A MODEL AVIAN HOST  
WITH DIVERSE MIGRATORY STRATEGIES

THE BLACKCAP *SYLVIA ATRICAPILLA*

(BIOGEOGRAFÍA DE LOS PARÁSITOS SANGUÍNEOS EN UN  
HOSPEDADOR AVIAR MODELO CON DIVERSAS ESTRATEGIAS  
MIGRATORIAS

LA CURRUCA CAPIROTADA (*SYLVIA ATRICAPILLA*)

TESIS DOCTORAL DE:  
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# Biogeography of blood parasites in a model avian host with diverse migratory strategies: the blackcap *Sylvia atricapilla*

(Biogeografía de los parásitos sanguíneos en un hospedador aviar modelo con diversas estrategias migratorias: la curruca capirotada *Sylvia atricapilla*)



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**INDEX**

|   |            |
|---|------------|
| <b>Abstract</b>   | <b>7</b>   |
| <i>Resumen</i>  | <b>13</b>  |
| <b>Chapter I: General section</b>                                 |            |
| <b>General introduction</b>                                       |            |
| 1. A World of parasites   | <b>21</b>  |
| 2. Avian haemosporidians as model parasites                       | <b>24</b>  |
| 3. Blackcaps as model hosts                                       | <b>29</b>  |
| <b>Objectives</b>   | <b>33</b>  |
| <b>General material and methods</b>                               |            |
| 1. Sampling localities  | <b>37</b>  |
| 2. Bird capture and sampling techniques                           | <b>40</b>  |
| 3. Laboratory procedures  | <b>41</b>  |
| <b>General results and discussion</b>                             |            |
| 1. Parasite diversity and environmental variation                 | <b>45</b>  |
| 2. Current and future predictions of parasite influence           | <b>46</b>  |
| 3. Parasite diversity and transmission strategies                 | <b>49</b>  |
| 4. The particularities of island parasites                        | <b>50</b>  |
| 5. Migration and parasite diversity                               | <b>53</b>  |
| 6. Parasite vectors   | <b>55</b>  |
| 7. Guidelines for future research                                 | <b>56</b>  |
| <b>Concluding remarks</b>   | <b>58</b>  |
| <br>  |            |
| <b>Chapter II: Ecological determinants of parasite diversity</b>  | <b>63</b>  |
| <b>Chapter III: Parasite range shifts and global warming</b>      | <b>75</b>  |
| <b>Chapter IV: Seasonal transmission in avian blood parasites</b> | <b>91</b>  |
| <b>Chapter V: Blackcap haemosporidians in the Macaronesia</b>     | <b>105</b> |
| <br>  |            |
| <b>Acknowledgements/Agradecimientos</b>                           | <b>119</b> |
| <b>Supporting information</b>                                     | <b>125</b> |
| <b>References</b>   | <b>137</b> |





## ABSTRACT

### *Theoretical framework*

Parasitism is a symbiotic relationship in which one organism lives at the expense of the other (the parasite and the host, respectively) affecting its fitness (survival and reproductive success) by causing host mortality, deterioration of body condition or reduced fertility. Parasitism is the most common lifestyle on Earth, so that the ubiquity of the action of pathogens and parasites is such that they can in all justice be considered as one of the major forces driving evolution... but how 'ubiquitous' is actually the action of parasites? There are several factors which promote inequalities in the distribution of parasite diversity and impact; and knowing them is crucial to foresee and prevent the negative effects that emergent diseases more and more represent in a World under a process of global change:

First of all, parasite distribution is under the influence of global patterns of climate and landscape variation, which are major determinants of the distribution of biodiversity. Furthermore, most of the World is under the clear influence of seasonal climatic factors, making the habitat suitability for parasites vary in the same place during the year. Knowing how climate and habitat affect parasite diversity is critical to choose the appropriate variables when making models to predict parasite influence, both currently and in the future (assuming different scenarios of climate change and alterations in landscape features). It is important as well, to the same end, to understand how parasites adjust their life cycles to seasonal variation.

In addition to global environmental trends, and depending on their transmission ability, some parasitic groups might see limited their access to remote regions, namely oceanic islands. Several island biocenoses have evolved freed from the parasitic pressures that suffer their mainland counterparts, acting as natural laboratories in which to examine which were the features that allowed the few successful parasites to colonise them; and hence determine which parasites having those features are to be considered as potentially hazardous given their invasive potential.

Animal dispersal and migration, acting both at the host(s) and the vector(s) levels, is a further key aspect to consider as a driver of parasite diversity, constituting an opportunity to extend over large areas and to reach more potentially susceptible hosts. From the host point of view, the avoidance of areas subjected to strong parasitic pressure is a major factor prompting and maintaining migration among diverse animal groups. Both sides make essential to combine the study of animal migration and parasite diversity.

### *Model system*

The present thesis analyses the biogeographical patterns of parasitism, including the study of broad effects of climate and habitat and the peculiarities of insular scenarios, both currently and in a context of global change. It also integrates the effect of host migration as a determinant of parasite distribution and diversity, as well as the idiosyncrasy associated to vector-borne parasitism, given that many parasites rely on vector species to complete their life cycles and to get dispersed. To this end, the study

model conformed by avian haemosporidians (genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon*; order Haemospororida), blackcaps (*Sylvia atricapilla*, order Passeriformes), and the Iberian Peninsula and the Macaronesian archipelagos was chosen. Avian haemosporidians, which are easy to detect and study by using molecular methods, are a group of vector-borne parasites extraordinarily diversified in blackcaps, a model host which in turn displays several different migratory behaviours. The archipelagos of Madeira and the Canary Islands offer the setting in which to test a number of aspects of parasite island biogeography, and the wide range of environmental conditions of the Iberian Peninsula allow to analyse their effects over parasite diversity.

### *Objectives and results*

I. Understanding how environmental variation influences the distribution of parasite diversity is critical if we are to anticipate disease emergence risks associated with global change. However, choosing the relevant variables for modelling current and future parasite distributions may be difficult: candidate predictors are many, and they seldom are statistically independent. This problem often leads to simplistic models of current and projected future parasite distributions, with climatic variables prioritized over potentially important landscape features or host population attributes. We studied avian haemosporidians in 37 Iberian blackcap populations, using Partial Least Squares regression to assess the relative importance of a wide array of putative determinants of variation in the diversity of these parasites, including climate, landscape features and host population migration. Both prevalence and richness of parasites were predominantly related to climate (an effect which was primarily, but not exclusively driven by variation in temperature), but landscape (notably topographic) features also contributed to explain variation in parasite diversity. Our results show that parasite distribution models, which are usually based on climatic variables alone, improve by including other types of predictors. Remarkably, different models emerged for each parasite genus, although all parasites were studied in the same host species and share many aspects of their life cycles; so that closely related parasites may show different relationships with the same environmental influences (both in magnitude and direction). Thus a model used to develop one parasite distribution can probably not be applied identically even to the most similar host-parasite systems.

II. Although there is an ever-growing number of works dealing with human and livestock pathogen models, we lack specific models to forecast disease risks of most wildlife parasites; both currently and in the future under a global change scenario. We used MaxEnt to model present-time distribution of areas of high impact of haemosporidian parasites on Iberian blackcap populations. Our results show that the three parasite genera considered show contrasting geographic patterns of variation in prevalence and richness, in agreement with their ecological constraints. We also assessed how the areas of high influence of these parasites are expected to change by the end of the XXI century, assuming several scenarios of climate change. Regardless of the global warming scenario considered, we show that both *Haemoproteus* and *Leucocytozoon* will lose areas of high richness and prevalence in the future, whereas *Plasmodium* is expected to expand its range of high influence. The comparisons among different climate change scenarios show nevertheless that future parasite impacts are prone to be more dependent of locally restricted environmental configurations (instead of following broad-scale trends) as the higher the predicted temperature rise. The structure of local parasite assemblages is expected to change across the blackcap's Iberian range,

modifying the interaction arena in which parasite virulence evolves; with unknown effects for their hosts.

III. In temperate regions, many vector-borne parasites maximize their transmission prospects by adjusting themselves to the seasonal dynamics that condition the cycles of host susceptibility and vector availability. Nevertheless, if in some regions environmental circumstances are convenient enough during the whole year, parasites could benefit from a switch to an unseasonal transmission strategy. We performed a Bayesian estimation of how different transmission strategies (summer transmission, extended summer transmission and year round transmission) have evolved throughout the phylogeny of *Haemoproteus parabelopolskyi*, a much diversified avian blood parasite which infects blackcaps as well as two other host species with contrasting migratory activity. Our results indicate that, coming from an ancestor which was a summer transmitted parasite of blackcaps, year round transmission and host switches arose recently several independent times. However, year-round transmitted parasites did not seem to diversify as much as seasonally transmitted parasites; suggesting that, although such strategy may be ecologically successful at present-time, seasonal transmission may be more stable over evolutionary time. Switches from seasonal to unseasonal transmission strategies could pose a problem if they promote the spread of noxious parasite lineages, so that a deeper knowledge of the transmission strategies of other parasites in temperate areas is compulsory.

IV. The study of parasite biogeography on islands is important for our understanding of both the processes involved in the evolution of parasite assemblages worldwide, and the ecology and conservation of insular communities. The Macaronesian archipelagos offer a particular scenario in which to study the haemosporidian parasites of blackcaps, which are recent colonizers there. We tested how several processes involved in parasite colonization and community assembly have shaped its parasite community prior to its permanent isolation on islands. The prevalence of parasites in the island populations of blackcaps was lower than in mainland blackcap populations and parasite richness decreased with increasing island distance to the continent. None of the parasites observed on the islands were blackcap specific. Some of the observed parasites appear to have switched from blackcaps to other Macaronesian host species, while others were of Afrotropical origin and were acquired after blackcaps colonised the islands. Macaronesian blackcaps do not face the strong parasite load encountered by their mainland counterparts despite the fact that blackcap migration from the continent may directly transport mainland blackcap parasites to the islands. These results support the idea that normal mainland host-parasite associations are compromised on islands, and that parasite island syndromes (low richness, frequent host-switching, and reduced specialization) evolve already at early stages of the insular colonization process.

V. The interactions at different scales between host migration and parasites are considered throughout the thesis. First its role as a factor promoting or hampering current parasite diversity at a population level is assessed. We detected a slight significance of the effect of migration over *Leucocytozoon* diversity (which was greater in sedentary blackcaps) and a lack of effect over *Plasmodium* and *Haemoproteus* diversity, which allow us to tentatively exclude host migratory behaviour as a factor to take into account while making predictive parasite modelling; an interesting tip given that whether migratory behaviour will evolve and change among geographic areas or not is difficult to foretell. Secondly, we also took advantage of the coexistence during

the wintering season in different habitat types of migrant and sedentary blackcaps to determine which variable (bird's origin or habitat) is more important (if any) on determining parasite transmission out of the breeding season. We detected that bird origin rather than habitat type is the primary factor shaping the distribution of parasites during winter among blackcaps. Finally, we analysed to what extent an annual influx of migrating blackcaps coming from the continent (with their parasitic load) is noticeable among insular blackcaps, detecting very slight effects which do not impede the insular parasite community to diverge on its own.

### *Conclusions*

I. The ability to forecast the evolution of parasite diversity is critical in a changing World even-threatened by emergent diseases. Conveniently, we demonstrate that climatic variables are reliable predictors of present-time haemosporidian diversity among Iberian blackcap populations. We reveal that the inclusion of topographic features helps to improve the power of predictive models, whereas other variables difficult to include such as land uses or aspects of host biology like migratory behaviour are not that important. These facts, which may be common to other similar host-parasite systems, confirm us on the trustworthy nature of predictive parasite models based upon these variables. We also promote the use of PLS regression, a statistical tool rarely used in ecological studies, to assess the relative importance of candidate variables as an essential prior step before developing accurate predictive models.

II. Although broad-scale parasitic surveys of wildlife can be unaffordable in terms of logistics or economic costs, we show that even a discrete population sample size is enough to make meaningful predictions about how parasite diversity is currently structured and how it might be in the future. We foresee geographic shifts of areas of strong parasite influence; highlighting how such changes are likely to vary among parasite types and which implications such differences may have on the impact of parasitism on host populations.

III. We also warn about how future parasite pressures can noticeably vary according to different scenarios of climate change. As a rule, the models of climate change which are constructed assuming a more environmentally friendly outlook, predicts a climate effect over parasite diversity more homogeneous throughout the current range of parasite influence. This in turn could favour future adaptation of blackcaps as well as other hosts to the novel climatic conditions by lessening the stochasticity associated to parasite pressure; making thus of it a more desirable situation.

IV. Even if our models of parasite diversity are insightful, they suffer from the lack of relevant information about hosts and vectors, which could contribute to produce even better predictive models. There is therefore a need of basic research on the field of wildlife diseases and disease vectors, as these are key components to make meaningful predictions which allow foreseeing and preventing the effect of emerging diseases.

V. The reconstruction of the evolution of seasonal transmission stages indicates that, although these changes do not show a long term survival, they can arise frequently and have a temporary great ecological success. Switches on transmission seasonality could promote the spread of host generalist parasite haplotypes, and the arbitrariness of these switches claims in favour of parasite monitoring surveys addressed to analyse several

host species across both seasons and years, in order to determine how widespread different transmission strategies are and to detect any possible hazard that, in a context of fast and unpredictable global change, might represent a risk for biodiversity.

VI. Macaronesian blackcaps do not face the strong parasite pressure encountered by their mainland counterparts, despite the fact that blackcap migration from the continent may directly transport mainland blackcap parasites to the islands. These results support the idea that parasite island syndromes (low richness, frequent host-switching, and reduced specialization) evolve even before insular host populations become completely isolated from their mainland counterparts.

VII. The reduced parasite burden in the Macaronesian populations of blackcaps and other species raises a cautionary word on the possible hazardous effects that the introduction of novel pathogens might have on such unique island bird communities.



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## RESUMEN

### *Marco teórico*

El parasitismo es un tipo de relación simbiótica en la que uno de los organismos vive a expensas del otro (el parásito y el hospedador, respectivamente), mermando su eficacia biológica (supervivencia y éxito reproductivo) al aumentar la mortalidad del hospedador, afectar a su condición física o reducir su fertilidad. El parasitismo es el estilo de vida más común entre los seres vivos, llegando a ser tal la ubicuidad de la acción de parásitos y otros patógenos que se les puede considerar justamente como una de las principales fuerzas evolutivas... pero ¿cómo de ubicua es en verdad la acción de los parásitos?. El hecho es que existen varios factores que determinan desigualdades en la distribución de la diversidad de los parásitos y, consecuentemente, de su impacto. Conocer estos factores es crucial para poder prever y prevenir los efectos negativos de las enfermedades emergentes, tanto más preocupantes cuanto más intenso es el proceso de cambio global en que estamos inmersos.

En primer lugar, la distribución de los parásitos depende de los patrones globales de variación del clima y la estructura de los hábitats, que son los principales factores que determinan la distribución de la biodiversidad. A mayores, en gran parte del mundo el clima se ve claramente afectado por variaciones estacionales, haciendo que la idoneidad del medio para los parásitos cambie en un mismo lugar a lo largo del año. Cuando se quieren elaborar modelos predictivos del posible impacto de los parásitos, tanto en el presente como en el futuro (asumiendo distintos escenarios de cambio climático o de variaciones en el uso del suelo), conocer el efecto preciso del clima y la estructura del hábitat sobre la diversidad de los mismos es vital a la hora de escoger las variables adecuadas. Con el mismo fin, es igualmente importante entender cómo los parásitos ajustan sus ciclos vitales a las variaciones estacionales.

En función de su propia capacidad de dispersión, y además de estar sujetos a factores medioambientales globales, algunos grupos de parásitos pueden no aparecer representados en regiones remotas, en particular en islas oceánicas. Muchas biocenosis insulares han evolucionado así ajenas a la presión que los parásitos ejercen en las regiones continentales sobre comunidades equivalentes; constituyendo laboratorios naturales en los que examinar qué características poseen los pocos parásitos exitosos que han conseguido establecerse, y así determinar que parásitos, gracias a poseer esas mismas propiedades, deben ser considerados como especialmente peligrosos debido a su potencial como agentes invasores.

El fenómeno de la dispersión y las migraciones de los distintos grupos animales, actuando tanto a nivel de los hospedadores de los parásitos como de sus posibles vectores, es otro aspecto clave a tener en cuenta como promotor de la diversidad de parásitos, al determinar la existencia de oportunidades para colonizar áreas mayores y de entrar en contacto con nuevos hospedadores potencialmente susceptibles. Desde el punto de vista del hospedador, por otra parte, intentar evitar las áreas en las que la presión de los parásitos es mayor puede ser uno de los factores que promuevan la aparición y el mantenimiento de los movimientos migratorios. Ambas caras de la misma moneda hacen esencial combinar el estudio de las migraciones animales y de la diversidad de los parásitos.



*Modelo de estudio*

Esta tesis analiza los patrones biogeográficos de la diversidad de parásitos, incluyendo tanto el efecto a gran escala del clima y la estructura del hábitat como las peculiaridades de los escenarios insulares; tanto en el presente como en el futuro, en un contexto de cambio global. Además integra el efecto de la migración de los hospedadores como uno más de los factores determinando la distribución y la diversidad de los parásitos; así como las peculiaridades asociadas a la transmisión indirecta de los parásitos a través de vectores, que es una característica común a muchos parásitos. Para tal fin, se ha escogido como modelo de estudio el tándem formado por la curruca capirotada (*Sylvia atricapilla*, orden Passeriformes) como hospedador y sus parásitos hemosporidios de los géneros *Plasmodium*, *Haemoproteus* y *Leucocytozoon*; además de la península Ibérica y los archipiélagos de Canarias y Madeira como zonas de estudio. Los hemosporidios son un grupo de parásitos transmitidos por vectores fácil de detectar y estudiar usando técnicas moleculares, que además está ampliamente diversificado dentro de la curruca capirotada. Ésta a su vez añade al modelo multitud de estrategias migratorias distintas. Asimismo, los archipiélagos considerados son el marco ideal para testar varios aspectos de biogeografía insular de los parásitos; y la península Ibérica presenta un rango de variación ambiental lo suficientemente extenso como para examinar cómo afecta a la diversidad de parásitos.

*Objetivos y resultados obtenidos*

I. Si pretendemos anticiparnos al riesgo inherente a las enfermedades emergentes en un contexto de cambio global, es de vital importancia entender cómo la variación ambiental influye en la diversidad de parásitos. Pero escoger las variables más relevantes a la hora de realizar modelos de distribución de parásitos tanto en el presente como en el futuro puede ser complicado: las variables candidatas son muchas, y a menudo están correlacionadas entre sí, dificultando los cálculos estadísticos. Esto hace que con frecuencia los modelos de predicción de impacto de los parásitos sean simples en exceso, dando prioridad al uso de variables climáticas frente a otras de estructura del hábitat o de atributos del hospedador que pudieran ser igualmente importantes. Nosotros estudiamos la diversidad de parásitos hemosporidios de 37 poblaciones peninsulares de curruca capirotada; y analizamos la importancia relativa que como determinantes de dicha diversidad tienen multitud de variables (tanto climáticas como de estructura del hábitat, además del comportamiento migratorio del hospedador) mediante regresión de mínimos cuadrados parciales (*PLS regression*). Tanto la riqueza como la prevalencia de los parásitos estudiados estuvo predominantemente ligada al clima (sobre todo -aunque no exclusivamente- a variables relacionadas con la temperatura), pero las variables de estructura del hábitat (sobre todo las topográficas) también contribuyeron a explicar en buena medida las variaciones en la diversidad de parásitos. Nuestros resultados indican que los modelos de distribución de parásitos, que habitualmente se basan sólo en el uso de variables climáticas, mejoran cuando se incluyen en los modelos variables predictivas adicionales. Es importante destacar a mayores que, aunque los parásitos estudiados infectan al mismo hospedador y poseen ciclos vitales muy similares, el efecto de las variables ambientales cambió mucho entre géneros; tanto en magnitud como en sentido. De esta manera debe extremarse la precaución a la hora de extrapolar modelos, pues éstos pueden no ser equivalentes incluso entre los sistemas parásito-hospedador más próximos.

II. Aunque el número de trabajos que ofrecen modelos predictivos de riesgo para parásitos que afectan al hombre o al ganado (tanto en el presente como en escenarios futuros de cambio global no deja de aumentar, este tipo de conocimiento es todavía deficitario para muchos patógenos de la fauna silvestre. Nosotros utilizamos MaxEnt para elaborar modelos predictivos de qué poblaciones ibéricas de curruca capirotada están actualmente bajo un mayor efecto de los hemosporidios; demostrando que los tres géneros de parásitos considerados presentan patrones geográficos distintos entre sí y consistentes con sus diferentes requerimientos ecológicos. También analizamos cómo se espera que haya variado la distribución de esas áreas sometidas a un gran impacto de los parásitos a finales del siglo XXI, asumiendo distintos escenarios de cambio climático. Independientemente del escenario considerado, se prevé una situación en la que disminuirán las áreas sometidas a una gran influencia de tanto *Haemoproteus* como *Leucocytozoon*; mientras que aumentarán aquellas bajo una influencia alta de *Plasmodium*. Sí se espera sin embargo que en el futuro las diferencias entre zonas relativas al impacto de los parásitos dependa más de factores microclimáticas locales (en oposición a tendencias climáticas a gran escala) cuanto mayor sea el aumento de temperatura predicho. Se espera que la estructura de las comunidades de parásitos cambie en las distintas poblaciones ibéricas de curruca, modificando el escenario de interacciones que determina la evolución de la virulencia, con un resultado incierto para sus hospedadores.

III. En las regiones templadas, muchos parásitos transmitidos por vectores maximizan sus perspectivas de ser transmitidos ajustando sus ciclos vitales a las dinámicas estacionales que condicionan la susceptibilidad de sus hospedadores y la disponibilidad de vectores. Sin embargo, hay zonas dentro de esas mismas regiones en las que el ambiente es lo suficientemente propicio como para permitir que haya una transmisión de parásitos continua a lo largo del año, beneficiando a los parásitos capaces de cambiar desde una estrategia de transmisión estacional a una continua. Nosotros realizamos una estimación bayesiana de cómo tres estrategias de transmisión estacional (transmisión limitada al verano, extendida más allá del verano hasta el invierno, y continua a lo largo de todo el año) han podido ir evolucionando a lo largo de la filogenia de *Haemoproteus parabelopolskyi*, un parásito hemosporidio con una diversidad de linajes notable, que infecta a la curruca capirotada y otras dos especies con diversos comportamientos migratorios. Nuestros resultados indican que, a partir de un ancestro propio de la curruca capirotada y de transmisión limitada al verano, los cambios de hospedador y de estrategia de transmisión surgieron recientemente en varias ocasiones independientes. Sin embargo los parásitos transmitidos a lo largo de todo el año no parecen diversificarse tanto como los transmitidos sólo durante el verano, sugiriendo que aunque la estrategia de transmisión no estacional puede ser actualmente ecológicamente favorable, la transmisión limitada al verano puede ser más estable a largo plazo. Los cambios entre estrategias de transmisión estacional y no estacional pueden ser preocupantes si favorecen la propagación de linajes de parásitos particularmente perjudiciales, por lo que es imprescindible contar con un conocimiento más profundo de las estrategias de transmisión de otros parásitos en zonas templadas.

IV. El estudio de la biogeografía de parásitos en islas es muy relevante, tanto para entender los procesos implicados en la evolución de las comunidades de parásitos a escala global, como la ecología y la conservación de los ecosistemas insulares. Los archipiélagos macaronésicos representan un escenario particular en el que estudiar los

parásitos hemosporidios de las currucas capirotadas, que son colonizadores recientes en la zona. Nosotros estudiamos cómo diversos procesos implicados en la colonización de los parásitos han podido determinar la comunidad parasitaria de las currucas isleñas, que no están todavía totalmente aisladas. La prevalencia de la comunidad de parásitos de las poblaciones isleñas de currucas fue mucho menor que la de las poblaciones de currucas peninsulares, y la riqueza de parásitos decreció con el aumento de la distancia de las islas al continente. Ninguno de los parásitos encontrados en las islas era específico de las currucas. Algunos de los parásitos detectados parecen haber colonizado otras especies a partir de las currucas; y al contrario, otros parásitos de origen afrotropical han colonizado esta especie con posterioridad a su llegada a las islas. Las currucas macaronésicas no se ven enfrentadas al gran impacto parasitario que afrontan sus equivalentes continentales. Estos resultados apoyan la idea de que las relaciones parásito-hospedador normales se ven afectadas en las islas; así como que los típicos síndromes insulares referentes a los parásitos (baja riqueza, frecuentes cambios de hospedador y poca especialización) pueden aparecer incluso en estadios tempranos del proceso de colonización insular.

V. La interacción a distintas escalas entre los parásitos y la migración de los hospedadores se analiza a lo largo de la tesis. Primero, comprobamos su efecto como factor promoviendo o limitando la diversidad de parásitos a escala poblacional. Detectamos un ligero efecto significativo sobre la diversidad de *Leucocytozoon* (que fue mayor en poblaciones sedentarias) y una falta de efecto sobre *Plasmodium* o *Haemoproteus*, lo que nos permite sugerir que es un factor que puede ser excluido de los modelos predictivos de parásitos, algo interesante en sí mismo debido a lo complicado de predecir la evolución futura del comportamiento migratorio de los hospedadores. En segundo lugar, también aprovechamos el periodo en que currucas migradoras y sedentarias conviven durante el periodo de invernada para determinar qué variable tiene mayor importancia, si el origen de las aves o el hábitat de invernada, a la hora de determinar la transmisión de parásitos fuera de la época de cría. Determinamos que, aunque el efecto es muy ligero, el origen de las aves tiene mayor importancia a la hora de definir la distribución invernal de los parásitos entre individuos. Finalmente, analizamos hasta qué punto la llegada anual de currucas continentales migradoras (y de sus parásitos) puede estar promoviendo de forma continua la llegada de nuevos parásitos a las islas macaronésicas, detectando un efecto muy ligero que no impide la evolución independiente de las comunidades insulares de parásitos.

### *Conclusiones*

I. En un mundo en cambio bajo la amenaza constante de las enfermedades emergentes, la capacidad de predecir la evolución de la diversidad de parásitos es vital. Demostramos que las variables climáticas son adecuadas para predecir la diversidad actual de hemosporidios en las poblaciones ibéricas de currucas capirotadas; y además que incluir variables topográficas en los análisis ayuda a mejorar el poder de los modelos predictivos, mientras que otras variables tales como los distintos usos del suelo, o aspectos de la biología de los hospedadores como el comportamiento migratorio, pueden obviarse por no ser tan determinantes. Estos detalles, que probablemente sean compartidos por otros sistemas de parásitos y hospedadores, nos reafirman en la fiabilidad de los modelos predictivos elaborados en base a esas variables. Además, defendemos el uso de *PLSR*, una técnica estadística muy poco utilizada en estudios ecológicos, como herramienta conveniente a la hora de establecer

la importancia relativa de diferentes variables, que es un paso previo esencial a la elaboración de modelos predictivos precisos.

II. Aunque los muestreos parasitológicos a gran escala de fauna salvaje puedan ser difíciles de realizar por cuestiones logísticas o económicas, demostramos que incluso muestreando un número limitado de poblaciones se pueden realizar predicciones significativas acerca de cómo se estructura la diversidad de los parásitos a día de hoy y de cómo puede estar distribuida en el futuro. Prevedemos que se puedan dar cambios de las áreas sometidas a gran influencia de los parásitos hoy en día, destacando cómo es probable que esos cambios varíen entre parásitos y qué implicaciones pueden tener a la hora de afectar a sus hospedadores.

III. También advertimos de que los cambios futuros en el impacto de los parásitos puede variar notablemente según sea el escenario previsto de cambio climático. Como norma, los escenarios que asumen puntos de vista más benévolos con el medio ambiente predicen efectos del clima sobre los parásitos más homogéneos. Esto podría favorecer la futura adaptación tanto de las currucas como de otros hospedadores a las nuevas condiciones climáticas, a base de disminuir la aleatoriedad asociada a la influencia de los parásitos; haciendo así de estos escenarios una opción más deseable.

IV. Aunque nuestros modelos de predicción de diversidad de parásitos son reveladores, adolecen de la falta de información pertinente acerca de los vectores, que podría contribuir a la construcción de mejores modelos. Es preciso pues que se sille llevando a cabo investigación fundamental en el campo de las enfermedades de la fauna salvaje y de sus vectores, ya que son piezas clave a la hora de hacer predicciones que nos permitan prever y prevenir el efecto de las enfermedades emergentes.

V. La reconstrucción de la evolución de las distintas estrategias de transmisión estacional indica que, aunque los cambios acaecidos no presenten una pervivencia larga en términos evolutivos, pueden surgir con frecuencia y tener un alto éxito momentáneo. Los cambios en las estrategias de transmisión podrían pues promover la expansión de linajes parasitarios generalistas; de modo que la imprevisibilidad de estos cambios es un punto a favor del desarrollo de programas de monitorización de parásitos que incluyan varias especies de hospedadores, años y estaciones del año; para determinar cómo de extendidas están las distintas estrategias de transmisión y detectar cualquier posible factor de amenaza que, en un contexto de cambio global rápido e imprevisible, pueda representar un riesgo para la biodiversidad.

VI. Las currucas macaronésicas no están sometidas a la gran presión parasitaria que sufren sus equivalentes continentales, a pesar de que cada año éstas al migrar transporten parásitos a las islas. Esto apoya la idea de que los síndromes insulares referentes a los parásitos (baja riqueza, frecuentes cambios de hospedador y poca especialización) pueden aparecer ya al inicio del proceso de colonización insular.

VII. La reducida carga parasitaria de las poblaciones macaronésicas de currucas, que podría ser compartida por otras especies, constituye una llamada de atención acerca de los posibles efectos nocivos que la introducción de nuevos agentes patógenos podría tener en esas comunidades insulares únicas.



## CHAPTER I: GENERAL SECTION



In the extreme SW of Spain the woods of evergreen oak species, like the Algerian oak in the picture, are home to a population of sedentary birds which have been occupying that region since before the Quaternary Glaciations; and which gave origin to the migratory blackcap populations of Western Europe after the retreat of the ice. All along this process, blackcaps have evolved developing the widest range of migratory behaviours known for a single bird species.



## GENERAL INTRODUCTION

### A World of parasites

Every time we look around, fascinated by the astonishing diversity of the natural world; no matter how keen the eye, we generally fail to see most of the creatures that are actually present around us. And this is not just a matter of scale, of some organisms being too small to be considered in a brief glimpse, but a matter of mind; while considering species diversity we usually overlook more than half of the living species: parasites. Parasitism is a symbiotic relationship in which one organism lives at the expense of the other (the parasite and the host, respectively) affecting its fitness (survival and reproductive success) by causing host mortality, deterioration of body condition or reduced fertility; all these negative effects on host fitness are collectively known as parasite virulence (Clayton & Moore, 1997). Parasitism is the most common lifestyle on Earth (Combes, 2005): even if we only consider macroparasites (parasites with indirect transmission, typically animals and protozoa; “microparasites” referring to bacteria or viruses) they are still estimated to represent on average about 40% of the species of their respective groups (Dobson, 2008); so that all living beings (parasites themselves as well) are susceptible of being parasitized at any point of their life cycles (Combes, 2005).

Early wisdom stated that parasitism was a transient situation

headed toward either mutualism or parasite extinction, until some seminal works (Anderson & May, 1979; May & Anderson, 1979; Price, 1980) highlighted that the natural condition of the host-parasite interactions is instead that of a coevolutionary continuum: the parasite tries to maximize its reproductive output at the expense of the host's resources; whereas the host attempts to minimize the damage caused by the parasites, either by mounting a stronger immune defence which reduces parasite load (resistance) or by physiologically minimising the suffered damage without actually altering parasite load (tolerance). Since then, the number of publications dealing with the influence of parasites over the most diverse aspects of their hosts' lives is overwhelmingly large: parasitism has been shown to be involved in the apparition and maintenance of sexual reproduction (Hamilton *et al.*, 1990), sexual selection (Hamilton & Zuk, 1982), genetic diversity (Decaestecker *et al.*, 2007), life history traits (Møller, 1997), community structure (Mouritsen & Poulin, 2005), reproductive success (Gustafsson *et al.*, 1994) or predation risk (Lafferty & Morris, 1996); to say but a few examples. In short, the ubiquity of the action of pathogens and parasites is such that they can in all justice be considered as one of the major forces driving evolution (Clayton & Moore, 1997; Combes, 2005).

This late affirmation brings a question attached: how ‘ubiquitous’ is



actually the action of parasites? Parasites are not alien to the general trends that, imposed by global patterns of climate and landscape variation, are major determinants of the distribution of biodiversity (Pearson & Dawson, 2003; Foley *et al.*, 2005). Besides, most of the World is under the clear influence of seasonal climatic factors, making the habitat suitability for parasites vary in the same place during the year (Altizer *et al.*, 2006). These determinants, acting on the parasites themselves or on their hosts, make self-evident that the different parasites will not be as diverse and abundant in all places on Earth (or during all times of the year); creating asymmetries on the parasite influence over a given host species. Knowing which factors are under these differences is crucial to understand how host-parasite relationships have evolved throughout space and time.

In addition to global environmental trends, and depending on their transmission ability, some parasitic groups might see limited their access to remote regions, such as isolated islands. Islands have been the focus of intense biogeographical research because of the opportunities they offer to understand the processes involved in species' range expansion and the evolutionary consequences of population isolation (Whittaker & Fernández-Palacios, 2007). Although the study of parasite biogeography is still in its infancy, a number of papers have examined the patterns of island colonization (Ishtiaq *et al.*, 2010; Cornuault *et al.*, 2012), host-parasite coevolution across different islands (Fallon *et al.*, 2003), or the development of island syndromes by parasites (changes in life-history, behaviour, or physiology after island colonization; Nieberding *et al.*, 2006). The island biogeography of parasites shed light as well over the patterns of disease introduction into new areas:

several studies have addressed what enables a parasite to be a successful invader, or instead why some are lost during host range expansion (MacLeod *et al.*, 2010; Ewen *et al.* 2012). The implications of this research are many; for instance, the introduction of exotic parasites offers dramatic examples of parasite-driven extinction in insular faunas (Wikelski *et al.*, 2004; Atkinson & LaPointe, 2009).

In relation to this late topic, a further aspect of animal life with which parasites are also intimately linked and that dramatically determines their distribution patterns is the migration of their host species (Altizer *et al.*, 2011). The development of migratory movements, a behavioural feature widespread across animals of many different taxa, is promoted by seasonal changes in resource availability (Dingle & Drake, 2007). In this context, from the host point of view, the avoidance of areas subjected to strong parasitic pressure (which can be viewed as a way of preserve the acquired resources) is a major factor prompting and maintaining migration among diverse animal groups, such as insects (Bartel *et al.*, 2011), fishes (Poulin *et al.*, 2012), birds (Piersma, 1997) or mammals (Folstad *et al.*, 1991). Parasites play also a role acting as a filter which culls, during the resource-demanding migration periods, the most disease-susceptible individuals of a given population (Van Gils *et al.*, 2007; Bartel *et al.*, 2011). Besides, parasites can determine the geographical configuration of the routes followed by migrating animals (Møller *et al.*, 2011; Møller & Szép, 2011), as well as the habitats selected during migration (Mendes *et al.*, 2005). Finally, as a result of the aforementioned issues, migratory movements have an effect over parasite infection risk: given that seasonal movements diversify the range of

habitats and regions that migratory species visit year round, it is commonplace that they suffer from a more diverse parasite community (Peterson *et al.*, 2008; Altizer *et al.*, 2011; Jenkins *et al.*, 2012; Pérez-Rodríguez *et al.*, 2013). In consequence, migratory animals tend to have a more robust immune system than their resident counterparts (Møller & Erritzøe, 1998). From the parasite point of view in turn, host migration represents an opportunity to extend over large areas, reaching more potentially susceptible hosts (Reed *et al.*, 2003; Olsen *et al.*, 2006; Breurec *et al.*, 2011; Vollmer *et al.*, 2011), although this is very dependent on the existence of the suitable transmission conditions at both ends of the migratory route (Hellgren *et al.*, 2007a).

Animal migration is thus capable of promoting the spread of parasites, but in a somehow evolutionary bearable and predictable way. In contrast, other factors enhancing parasite activity are not so predictable: since the Age of Discoveries and especially from the dawn of the Industrial Revolution onwards, human activity has been progressively acquiring the potential of profoundly influencing and modifying the natural world. The process of global change we are witnessing, which include worldwide habitat modifications and climate change (Vitousek, 1994), has a lot to do with current patterns of distribution and transmission of many parasites (Patz *et al.*, 2000; Lafferty, 2009; Mostowy & Engelstadter, 2011; Morgan *et al.*, 2012). Emerging infectious diseases (EID), interpreted *sensu* Daszak *et al.* (2000) as those that, either caused by a novel or a previously known pathogen, unexpectedly affect new host species and/or expand over new geographical areas, are one of the major factors threatening human health and jeopardizing biodiversity

conservation at a global scale (Daszak *et al.* 2000; Jones *et al.*, 2008; Smith *et al.*, 2009; Thompson *et al.*, 2010): the general global warming trend is promoting the spread in temperate areas of parasites previously limited to tropical regions (Peterson, 2009; González *et al.*, 2010), and large-scale habitat alterations prompt the emergence and maintenance of zoonoses and other diseases among humans and wildlife altogether (Harrus & Baneth, 2005; Woolhouse & Gowtage-Sequeria, 2005). These links of parasite risk with climate and habitat modifications, altogether with the progressively better accuracy of the predictive models of global change, are behind the growing interest of developing models to predict which areas are likely to suffer from high parasite impacts both currently and in the future (Thomson *et al.*, 2006; Brooker & Clements, 2008; Lafferty, 2009; González *et al.*, 2010; Loiseau *et al.*, 2013).

Another of the major biodiversity challenges associated to global change is the threat that human-mediated (either deliberate or accidental) introductions of species outside their natural ranges can pose (Mack *et al.*, 2000): parasites can behave as invasive species, with devastating effects among immunologically naïve native host reported thorough the World after a novel pathogen establish itself in a new area; especially on oceanic islands (Daszak *et al.*, 2003; Wikelski *et al.*, 2004; Atkinson & LaPointe, 2009; Tompkins & Jakob-Hoff, 2011). Furthermore, alien species can also prosper if in the introduction areas they are freed of the burden imposed by native parasites in their original ranges, or if the parasites they might carry among them are more pathogenic to the possible competitor native species in the

novel regions (Strauss *et al.*, 2012). Additionally, once an EID gains strength, naturally migrating animals can enhance its transmission through vast areas, multiplying its negative effects (Reed *et al.*, 2003; Olsen *et al.*, 2006; Lawson *et al.*, 2011; Fuller *et al.*, 2012); hence the interest of study the combination of host migratory behaviour and the effect of global change over disease dynamics at the same time.

The depicted situation of parasitic risk unpredictability under global change becomes even more complicated when we consider that a third actor is usually pushing its way into the play, as many parasites rely on vector species to complete their life cycles and to get dispersed. Although vector availability and diversity is certainly a limiting factor of vector-borne diseases (Cumming & Guégan, 2006; Gager *et al.*, 2008), there are several reasons explaining why vector transmission, namely that associated to blood-feeding arthropods, gives parasites a clear benefit over other ways of transmission: first, vector's piercing bite allows the parasite to successfully skip the infection-detering barrier that host's skin represents. In addition, vectors can act as very efficient parasite dispersing mechanisms, especially in the case of flying insects. And depending on vector specificity, specific parasites can make sure they will be transmitted only to the right host species (Black, 2003). Vectors own environmental needs make vector-borne parasites dependent on habitat modifications that could facilitate or impede vector's diversity or abundance, and on climatic constrains controlling their life cycle and activity regimes. These features make vector-borne parasites especially liable to be influenced in a context of climate change (Kovats *et al.*, 2001;

Tabachnick, 2010; Colwell *et al.*, 2011), and also good candidates for modelling their distributions in accordance with environmental variables; providing that the adequate predictors are correctly identified and accurate datasets available (Thomson *et al.*, 2006; Peterson, 2009; González *et al.*, 2010; Costa & Peterson, 2012).

The general outline exposed here conveys the convenience of jointly analysing the biogeographical patterns of parasitism both currently and in the future in a context of global change, the effect of host migration as a determinant of parasite distribution and diversity, and the peculiarities associated to vector-borne parasitism. For technical convenience, it is easier to restrict a research study to a single model organism or a group of related organisms. So, is there any parasite group, homogeneous in terms of biological features, which allows the combined study of all these questions? Given the outwardly endless diversity of parasitic forms of life, the answer is, quite unsurprisingly, "yes".

### **Avian haemosporidians as model parasites**

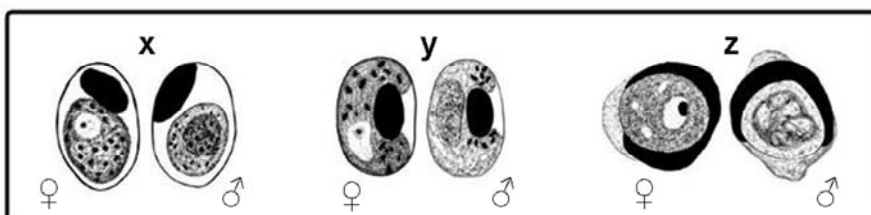
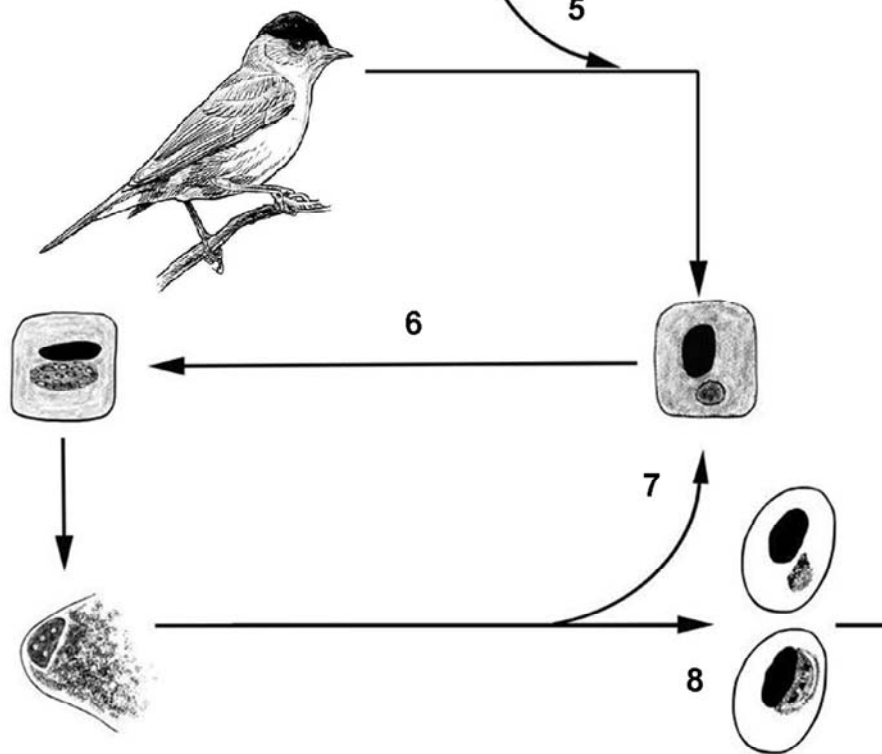
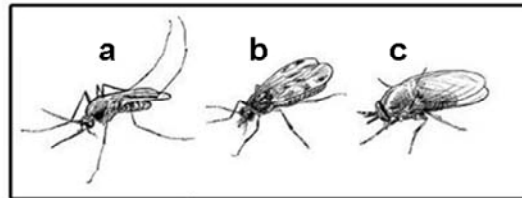
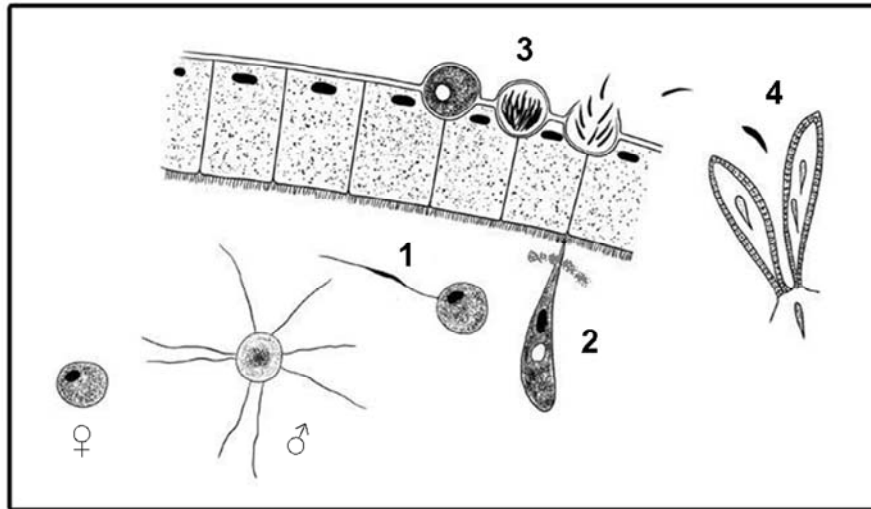
Avian haemosporidians (phylum Apicomplexa, order Haemospororida), a group of vector-borne protozoan parasites including bird-infecting *Plasmodium spp.* and the species of the related genera *Haemoproteus* and *Leucocytozoon*; possess a wide display of features that make them very appealing as model parasites. This was already discovered long time ago: avian haemosporidians were first described by a Russian physician, Danilewsky, in 1884; and before the onset of the studies with rodent and primate parasites, chicken *Plasmodium* was crucial to unravel many aspects of the life cycle of

human malaria, including its mosquito-borne transmission (Valkiūnas, 2005). Haemosporidians considered in the present thesis belong to three different families: *Plasmodium* to Plasmodiidae, *Haemoproteus* to Haemoproteidae and *Leucocytozoon* to Leucocytozoidae, but all of them share the basic aspects of their life cycles (Figure 1; next page). Although in recent years the phylogenetic relationships among them have been subjected to much debate (Perkins & Schall, 2002; Pérez-Tris *et al.*, 2005; Martinsen *et al.*, 2006; Martinsen *et al.*, 2008), the most recent view tends to consider *Haemoproteus* and *Leucocytozoon* as sister genera, forming altogether a clade within *Plasmodium* and sister to *Plasmodium* species that infect birds and lizards. All these parasites would in turn be a sister taxa of mammal-infecting *Plasmodium* parasites; rendering thus *Plasmodium* as traditionally considered a paraphyletic group (Outlaw & Ricklefs, 2011). The last in-depth taxonomic revision of the group included 205 described morphospecies (that is parasites recognizable by their microscopic appearance; Valkiūnas, 2005), but since then a number of other species has been described (see for instance Valkiūnas *et al.*, 2007; Valkiūnas *et al.*, 2009; Križanauskienė *et al.*, 2010, Valkiūnas *et al.*, 2010 or Iezhova *et al.*, 2011).

Once a bird has been bitten by an infected vector and the sporozoites are established among its internal tissues, there is a stage of latency (a period during which parasites reproduce asexually); followed by the acute phase of the infection, which takes place when parasites invade the circulating blood. During this period the infected bird suffers the most negative impacts, derived from the anaemia linked to the destruction of red blood cells. Once the acute phase of the infection is over, there remains a “baseline” level of

circulating infected red blood cells, which represent the chronic phase of the infection. Parasites may even disappear from circulating blood while remaining dormant in the internal tissues of the bird; and relapse and show up again in the circulating blood in due course (Valkiūnas, 2005). This seasonal relapse, which seems to be mediated by day length (Valkiūnas *et al.*, 2004), is a typical feature of infections in temperate areas: they refrain from circulating blood during winter and relapse in spring, coincident with the reproductive season of their birds' hosts (and hence the appearance of immunologically naïve young birds) and the re-emergence of their insect vectors. In tropical regions in contrast infections are usually present in blood year round (Valkiūnas, 2005). The pathogenicity of the different parasites considered is liable to a great deal of variation, depending on the species of both the parasite and the host and on the intrinsic features of the infected bird; but as a rule *Plasmodium* parasites are the most harmful of the three, followed by *Leucocytozoon* and then *Haemoproteus* infections (Valkiūnas, 2005).

The traditional study of avian haemosporidians, consisting on the examination of morphological characteristics of parasites in stained blood smears obtained from infected birds, was greatly boosted with the development of several PCR-based molecular techniques that rendered possible the performance of large-scale parasite surveys (Bensch *et al.*, 2000; Perkins, 2000; Ricklefs & Fallon, 2002). The molecular techniques developed by the team of Staffan Bensch (Lund University, Sweden; Bensch *et al.*, 2000; Hellgren *et al.*, 2004; Waldenström *et al.*, 2004) in particular, which amplify a 479 bp fragment of the cytochrome *b* gene of



**Figure 1.** Parasite gametocytes present in the blood of an infected bird enter the body of an insect vector while feeding, and within its gut gametocytes produce gametes and the parasites undergo sexual reproduction (1). After fertilization, the resulting ookinete penetrates the peritrophic membrane of the insect (2) and develops as an oocyst attached to the gut wall (3). The oocyst matures and eventually sporogony takes place; sporozoites are then released into the insect's hemolymph and migrate to the insect's salivary glands (4). When an infected blood-sucking insect bites a bird, parasite's sporozoites, which are the infective form for the bird, are released within its salivary fluids (5). Inside the bird, sporozoites invade internal tissues and reproduce asexually, producing merozoites (6) which can in turn infect the internal tissues again, repeating this cycle several times (7). Eventually merozoites penetrate red blood cells (8) and mature as gametocytes\* to start the cycle again (Valkiūnas, 2005). All the considered parasites use dipteran insects as vectors, but the precise group differs among parasite genera: avian *Plasmodium* is vectored by several genera of Culicidae mosquitoes (a), *Haemoproteus* (subgenus *Parahaemoproteus*; the one considered in the present work) by *Culicoides* spp. biting midges (family Ceratopogonidae, b) and *Leucocytozoon* by many species of Simuliidae blackflies (c).

\* Blackcap haemosporidian examples: x. *Plasmodium relictum*. y. *Haemoproteus parabelopolskyi*. z. *Leucocytozoon majoris*.

the three considered parasite genera, are now widely used. This has rendered possible the marshalling of a large on-line repository of haemosporidian cytochrome *b* gene sequences: MalAvi (Bensch *et al.*, 2009), allowing the performance of analyses that encompass a great diversity of parasites from all the World infecting a large number of hosts. Molecular techniques unveiled as well a previously unknown cryptic diversity of avian haemosporidian parasites, showing that several parasite morphospecies were actually composed of independent genetic lineages that behave as “good” species (Bensch *et al.*, 2004). Genetic analyses have also showed that multiple infections (those comprising more than one parasite genotype) are readily identifiable and can be very common in wild birds (Pérez-Tris & Bensch, 2005a, Martínez *et al.*, 2009). PCR analyses are as a rule more sensitive than microscopic surveys, so that they also allow to better estimate parasite prevalence (Pérez-Tris & Bensch, 2005a); nevertheless, an expert survey of blood smears can be comparatively as good (Garamszegi,

2010). There is also a good match between morphospecies and genetically related parasite haplotypes (Hellgren *et al.*, 2007b), and microscopic examination is especially useful at revealing the existence of hidden multiple infections (because if several parasites are represented in a sample, but in unbalanced proportions, PCR analyses usually detect only the most abundant; Pérez-Tris & Bensch, 2005a; Valkiūnas *et al.*, 2006); so that the traditional microscopic study of avian haemosporidians is still highly informative (Valkiūnas, 2011).

The easiness introduced by molecular detection techniques on parasitic surveys have certainly favoured the actual appreciation of avian haemosporidians as study subjects (Bensch *et al.*, 2009), but this is by no means the only reason. So which other characteristics of avian haemosporidians make them so appealing on the study of host-parasite interactions?

- a. First of all, avian haemosporidians are extraordinarily abundant, appearing all over the World except for the Polar Regions (probably due to the absence of insect vectors) and infecting almost all the birds groups studied up to date (Valkiūnas, 2005); although in some groups such as seabirds they are comparatively very scarce (Quillfeldt *et al.*, 2011). This renders possible the performance of wide-scale studies of parasite diversity and its effects, as well as the study of parasite colonization processes of isolated areas such as archipelagos (Ishtiaq *et al.*, 2010; Ricklefs *et al.*, 2011; Cornuault *et al.*, 2012).
- b. Depending on the type of parasites, host diversity is also very variable. Some parasites such as *Plasmodium relictum* haplotypes P-SGS1 or P-GRW4 infect hosts of very diverse phylogenetic and geographic ascriptions (Bensch *et al.*, 2009), whereas others are apparently very host specific (Reuiller *et al.*, 2006; Pérez-Tris *et al.*, 2007). In general, *Plasmodium* parasites seem to have wider host ranges than *Leucocytozoon* or *Haemoproteus* ones; although there is a great deal of variation (Hellgren *et al.*, 2009). This variation in host ranges makes possible to study patterns of host-parasite coevolution and to compare the relative virulence of generalist versus specialist parasites. Vector specificity has been in contrast by far less studied, so that although there seems to be also a lot of variation in terms of vector range, more studies are needed before trends could be defined (Hellgren *et al.*, 2008; Kimura *et al.*, 2010; Njabo *et al.*, 2010; Martínez-de la Puente *et al.*, 2011; Kim & Tsuda, 2012; Santiago-Alarcón *et al.*, 2012).
- c. Although the classical view was that avian haemosporidian infections were essentially harmless, this has been shown not to be the case (Valkiūnas, 2005): avian haemosporidian infections entail an evident, measurable cost for their hosts in terms of individual survival and reproductive output (Merino *et al.*, 2000; Marzal *et al.*, 2005; Knowles *et al.*, 2010; Ashgar *et al.*, 2011), so that they can drive the evolution of many hosts' traits (Bonneaud *et al.*, 2006; Møller & Nielsen, 2007; Arriero & Møller, 2008; Garamszegi & Møller, 2012; Westerdahl *et al.*, 2012). All these effects make of them interesting study subjects in the evolutionary ecology field; including the examination of the effects of multiple infections (Marzal *et al.*, 2008; Palinauskas *et al.*, 2011).
- d. At a broader scale, their effect over hosts' health status cannot be neglected. Although infrequently, avian haemosporidians are responsible of mortality episodes in poultry industries or avian collections (Valkiūnas, 2005). But these parasites are notably infamous by their role in the extinction of many native Hawaiian birds, where they were accidentally introduced (LaPointe *et al.*, 2012); so that the study of avian haemosporidians is also timely from the conservation biology point of view.
- e. Given their wide distribution and detrimental effects, avian haemosporidians must be prime targets on the study of the interactions between parasitism and global change, although few studies have covered this topic. Avian haemosporidians are expected to spread in a context of global warming, both at a wide scale (Møller, 2010; Garamszegi, 2011; Fuller *et al.*, 2012; Loiseau *et al.*,

2013) and locally through altitudinal shifts (Freed *et al.*, 2005; Zamora-Vilchis *et al.*, 2012); and their diversity is also dependent of broad-scale habitat changes (Chasar *et al.*, 2009; Sehgal, 2010). They are also intimately linked to events of species introductions, with novel parasites taking over the new available hosts (Ewen *et al.*, 2012; LaPointe *et al.*, 2012) or with the introduced bird species taking advantage of a reduced parasite pressure (Marzal *et al.*, 2011; Ventim *et al.*, 2012).

- f. Given their broad host spectrum, avian haemosporidians are a superb model for the study of host migration and parasite risk, as many of their hosts are migratory species. Following the general trend, migratory species usually sustain a greater parasite diversity than the sedentary ones (Figuerola & Green, 2000; Waldenström *et al.*, 2002; Jenkins *et al.*, 2012; Pérez-Rodríguez *et al.*, 2013). This fact however does not seem to facilitate the exchange of parasites among biogeographical areas, which seems to be rare in evolutionary time given that many parasites are either transmitted only during the breeding season or in the wintering areas (Hellgren *et al.*, 2007a). The diverse host range of different parasites have delivered contrasting results while trying to specifically link particular parasites and migratory routes (Fallon *et al.*, 2006; Svensson *et al.*, 2007).
- g. Finally, and although the use of avian haemosporidians as laboratory model parasites for the study of human malaria has long been abandoned (Valkiūnas, 2005), given that avian haemosporidians are free of the socioeconomic and sanitary factors that mask and make difficult

modelling the natural spread of human or livestock diseases, they are still very useful as models of wide scale disease risk prediction, for malaria as well as for other vector-borne diseases (Garamszegi, 2011).

The reasons supporting the choice of avian haemosporidians as model parasites for the present thesis are now clear, but we lack still the choice of a model bird host. The ideal host should harbour a diverse community of avian haemosporidians, so that research would benefit from the advantages of this model parasite group. It should also be broadly distributed, in order that the effect of diverse environmental factors (both climate and habitat) over parasite diversity could be checked. And it should ideally exhibit a varied display of migratory strategies, so as to study the effect of different host migration strategies over parasites in the absence of the confounding factors linked to between species comparisons... So, does that ideal host exist? Surprisingly enough the answer is, again, “yes”.

### **Blackcaps as model hosts**

The blackcap *Sylvia atricapilla* (order Passeriformes, family Sylviidae) is a small (bill-tail length 13-15 cm, weight 14-20 g), sexually dimorphic bird (Figure 2). It is specially suited as a model species for the purposes of the present work for a number of reasons:

- a. The first and most important one is that blackcaps harbour by far and large the greatest diversity of avian haemosporidians known in a single host (Pérez-Tris *et al.*, 2007; Križanauskienė *et al.*, 2010;





**Figure 2.** A pair of blackcaps *Sylvia atricapilla*, the model host used in this Thesis. Males (bird on the left) and females (bird on the right) can be readily distinguished by the colour of the crown (black and rufous respectively); juvenile birds of both sexes before their first moult instead look alike, resembling adult females, and can only be sexed by molecular means. This picture was taken in the extreme south of Spain, where sedentary (male blackcap) and migratory birds (female blackcap) coexist during the wintering season. Both classes of blackcaps can be distinguished by their morphological measures, given that sedentary birds have comparatively shorter and rounder wings and longer tails than the migratory ones. *Picture credit: Javier Pérez-Tris*

Santiago-Alarcón *et al.*, 2011), which comprises no less than 50 different parasite haplotypes of the three considered genera (MalAvi database, accessed February 2013; Bensch *et al.*, 2009). Parasites of two morphospecies of the genus *Haemoproteus* in particular: *H. pallidulus* and above all *H. parabelopolskyi*; have undergone a tight process of differentiation within blackcaps, developing a wide diversity that blackcaps do not share with other hosts except for a couple of closely related *Sylvia* species; so that blackcaps are very convenient to study many aspects of host-parasite relationships in a system somehow closed and

independent of the presence of other host species. Besides, and although parasite prevalence shows a great deal of variation among populations (Pérez-Tris & Bensch, 2005b), it is usually high in blackcaps; allowing to examine the causes and consequences of variation in parasite influence.

b. Blackcap distribution is restricted to the Western Palaearctic (including the Macaronesian archipelagos) and adjacent parts of Asia, but throughout its distribution range it is generally a widespread and abundant species (Shirihai *et al.*, 2001), making it easy to capture a reasonable sample of birds per

locality. Blackcaps usually thrive in areas such as open woods, riparian woods, mature hedges, orchards or gardens; with light tree cover and dense bush undergrowth, not refusing human vicinity. Areas with this physiognomy are widespread across its distribution range, and therefore blackcaps can be found in regions that otherwise present contrasting climatic conditions and diverse wide-scale habitat configurations, allowing to test how parasite diversity reacts faced to different environmental conditions.

- c. Although as much as five blackcap subspecies have been described, the variation among them is small and largely clinal, making it almost impossible to assign individual birds to a given subspecies (Shirihai *et al.*, 2001). This is a reflection of the slight among-populations genetic differentiation of this species (Pérez-Tris *et al.*, 2004; Dietzen *et al.*, 2008); a fact which helps to reduce the uncontrolled variance that could be due to genetic effects while comparing parasite pressures among blackcap populations.
- d. And last but not least, the blackcap is renowned for displaying the widest range of migratory strategies known in a single bird species, which has converted it in a preferred model for the study of the evolution of bird migration (Berthold, 2001). The meridian situated roughly at 10° E marks a migratory divide: blackcaps west to this line migrate with a SW direction and with a SE direction east to it (Møller *et al.*, 2011). As a general rule, the northerly the population the further south they migrate, so that Scandinavian and Russian blackcaps usually migrate to tropical Africa; whereas blackcaps from Central Europe spend the winter in the Mediterranean region, where native

blackcaps are short-distance migrants or even sedentary (Shirihai *et al.*, 2001). There are blackcap populations in which individuals show different migratory behaviours (Berthold & Querner, 1982), and a whole population with a totally different migration direction can arise within another very rapidly (Berthold *et al.*, 1992). This broad array of migratory behaviours makes possible to compare how migration can influence the parasite community of a given host population.

All the actors of the present thesis, avian haemosporidians and the blackcap, have already been introduced; but it is still necessary to select an appropriate stage: one which allows making the most of the convenient characteristics of the model system... and such scenery also exists: the Iberian Peninsula. Located in the extreme SW of Europe and spanning over 582,000 Km<sup>2</sup>, the Iberian Peninsula covers, in a tractable area, a great deal of environmental variation. This is derived from the fact that it is under the influence of two biogeographical regions (the Euro-Siberian and the Mediterranean), and its highly mountainous landscape promotes the presence of many climatic gradients (Ninyerola *et al.*, 2005). Blackcaps breed commonly all over the Iberian Peninsula, although they become scarcer towards the more arid regions of the south and east extremes (Carbonell, 2003). Most of the Iberian blackcap populations are composed of short-distance migrants that spend the wintering season, altogether with blackcaps from elsewhere further north in Western Europe, in the Iberian Mediterranean lowlands. There they co-occur with local breeders, which are sedentary (Pérez-Tris & Tellería, 2002).

Migratory and sedentary blackcaps can safely be told apart from one another by their morphology: in relation to body size, migrant birds have longer and more pointed wings and a shorter tail (Figure 2). These differences make possible to use discriminant functions to distinguish between both types of birds when captured side by side (Pérez-Tris *et al.*, 1999; De la Hera *et al.*, 2007); a distinction further supported by stable isotopes analysis (De la Hera *et al.*, 2012).

Apart from the Iberian Peninsula, blackcap populations living on the Macaronesian archipelagos of the Canary Islands and Madeira are also subject of the present thesis. Both island groups of volcanic origin lie near the

Saharan African coast and share many biotic traits with the Mediterranean region (Whittaker & Fernández-Palacios, 2007; Fernández-Palacios *et al.*, 2011; Illera *et al.* 2012). Blackcaps occupying those islands are sedentary, but originated at the end of the Last Glacial Maximum from migratory individuals of European origin (Pérez-Tris *et al.*, 2004). Additionally, a number of migrating blackcaps of continental origin still spend the wintering season on the islands, coexisting with local birds. Island blackcaps offer thus a rare opportunity to study how an island parasite community starts its divergent evolution, before host populations become completely isolated from their mainland counterparts.

## OBJECTIVES

The general objective of this thesis is to study how vector-borne parasite diversity is biogeographically structured depending on regional environmental variation and host migratory behaviour. Given their many convenient traits, avian haemosporidians infecting blackcaps are used as model parasites; and their diversity studied throughout the annual cycle in a number of selected localities. The specific guidelines of this thesis are:

- a. In a context of global change, only a deep knowledge of whether and how different environmental factors influence current host-parasite interactions, as well as how parasite-environment relationships may vary among parasite types, will allow us to foresee future parasite impacts and their potential effects on biodiversity. Hence **Chapter II** examines how environmental variation (decomposed into several climatic and habitat variables) determine parasite richness and prevalence during the host breeding season. Its main goal is to assess whether the relationships between environmental or host population attributes and parasite diversity are similar or vary among the three parasite genera considered. It also tests if climate-based parasite distribution models could be improved by considering variables such as landscape and host population attributes. Once it is thus stated which variables are the ones with the stronger influence on parasite diversity; **Chapter III** makes use of this knowledge to elaborate specific predictions of how the areas with the greatest parasite diversity are structured in the Iberian Peninsula.
- b. The information about how climatic variables determine where it is fair to expect a high parasitic pressure over blackcap populations is also employed on **Chapter III** to forecast, using several models of climate change, how these areas are likely to be distributed by the end of the XXI century. A particular relevance is given to the study of whether the three parasite genera considered will follow the same general trends or not; and to examine the relationship between current and future parasite influence predictions, aiming to determine if the expected changes are likely to be the same in the whole study region or to vary locally instead. This is crucial to know if changes in parasite pressure will affect their host populations in the same way, or if asymmetries in relation to the current situation are likely to arise, reshuffling the present map of interactions between host and their different parasites (and their likely consequences for host populations).
- c. Seasonal variation is also considered in this thesis, and how different seasonal transmission strategies have arisen throughout parasite phylogeny is studied in **Chapter IV**, using the diversified parasite morphospecies *Haemoproteus parabelopolskyi* as a particular model. The influence of host switches as a factor promoting parasite diversification is also addressed.

- d. The study of parasite biogeography on islands is important for our understanding of both the processes involved in the evolution of parasite assemblages worldwide, and the ecology and conservation of insular communities. The Macaronesian archipelagos offer a particular scenario in which to study the haemosporidian parasites of blackcaps, which are recent colonizers there. **Chapter V** test how different hypotheses relating to the processes involved in parasite colonization and community assembly have shaped the parasite community of Macaronesian blackcaps prior to its permanent isolation on islands.
- e. The interactions at different scales between host migration and parasites are considered throughout the thesis. In **Chapter II** its role as a factor promoting or hampering current parasite diversity at a population level is assessed. **Chapter IV** takes advantage of the coexistence during the wintering season in different habitat types of migrant and sedentary blackcaps to determine which variable (bird's origin or habitat) is more important (if any) in determining parasite transmission out of the breeding season. Finally, **Chapter V** analyses to what extent an annual influx of migrating blackcaps coming from the continent (with their parasitic load) allows the insular parasite community to diverge on its own.



Bird ringing is a technique well regulated in most European countries, which has allowed researchers to marshal important knowledge about all aspects of bird's lives. Blackcaps, being common all over the continent, are among the most captured and ringed species.



## GENERAL MATERIAL AND METHODS

### Sampling localities

#### A. Breeding season: the Iberian Peninsula

The localities in which blackcaps were sampled during the breeding season were selected trying to cover the whole distribution of the species in the Iberian Peninsula (Carbonell, 2003), including populations potentially migratory and sedentary and a broad range of environmental variation in terms of altitude, climate and habitat physiognomy. During four years (2008-2011) a total of 37 localities (Figure 3) was sampled at the end of the blackcap breeding season (mid-July to early August, to avoid a temporal coincidence with the onset of autumn migration of blackcaps and thus capturing migrant birds from elsewhere), yielding a total of 882 blackcaps (mean  $n = 24$  birds per locality).

To perform the environmental characterization of each locality, a set of climatic and habitat variables was obtained (downloaded as Geographic Information System -GIS- layers) from the Spanish Laboratory of Informatics Biogeography\*: mean, maximum and minimum monthly temperatures, monthly rainfall, 19 bioclimatic variables\*\*, GETOPO30 topographic features, CORINE 2000 Land Cover land uses and mean monthly NDVI index (see in Figure 4 an example of how the main climatic factors are distributed in the Iberian Peninsula). Monthly measures were combined to obtain both annual and breeding season

(March to June) means. An area of 10 Km<sup>2</sup> surrounding each location (as obtained on the spot with a GPS device) was defined as the geographical working unit. All GIS analyses were done using ESRI® ArcMap™ 9.3.

#### B. Wintering season: Campo de Gibraltar

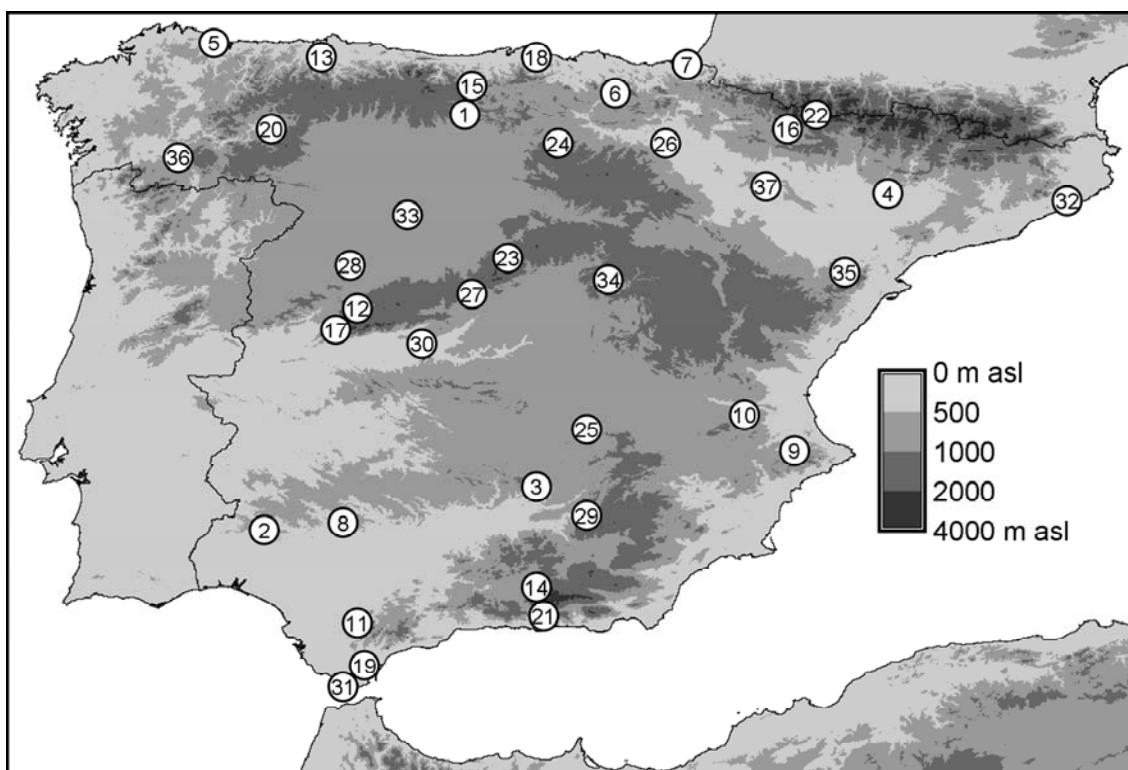
Wintering blackcaps were captured in the Campo de Gibraltar region (extreme south of Spain, Cádiz province; 36° 01' N, 5° 36' W) on a monthly basis, from September 2006 to March 2007. The landscape is composed of low hills (100 to 300 m asl) covered by forests (thereafter “woodlands”) of evergreen oaks (mostly cork oak *Quercus suber* and Algerian oak *Quercus canariensis*) with a dense understory of fruiting shrubs and vines. As a result of human clearance for cattle stockbreeding, most lowland areas consist on open meadows with scattered, dense shrubby patches (thereafter “shrubland”) of mastic (*Pistacia lentiscus*) and wild olive trees (*Olea europaea*) which offer to birds an abundant supply of berries. As stated in Chapter III, sedentary blackcaps occur only on woodlands, whereas migratory blackcaps occupy both woodlands and shrubland. Accordingly, blackcaps up to a total of 571 birds were captured in both habitats types at four different locations: two in each, belonging to the municipalities of Tarifa and Los Barrios (close to points 19 and 31 in Figure 3).

\* Natural Sciences National Museum <http://www2.mncn.csic.es/LBI/Presentacion.htm>

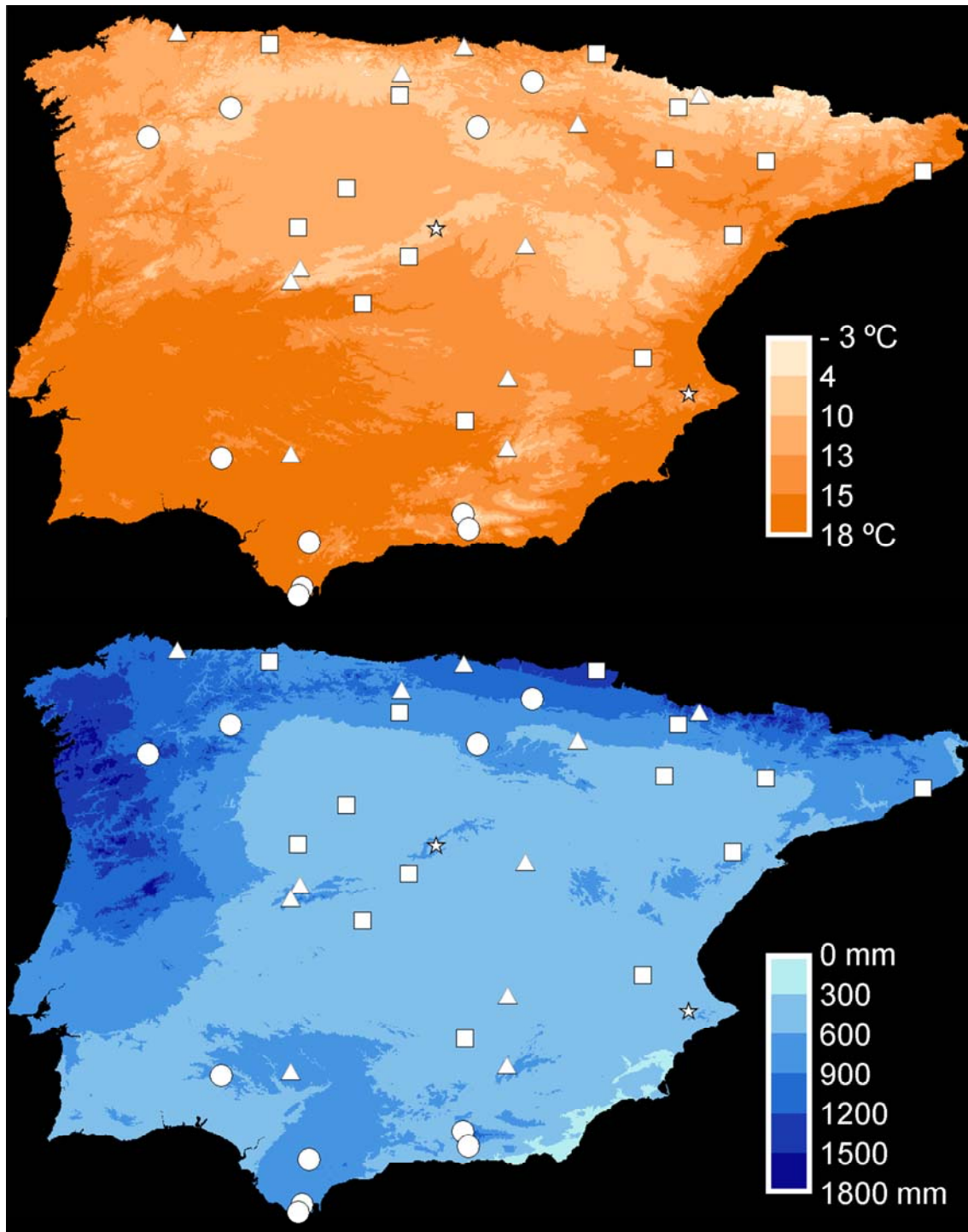
\*\* WorldClim <http://www.worldclim.org/bioclim>



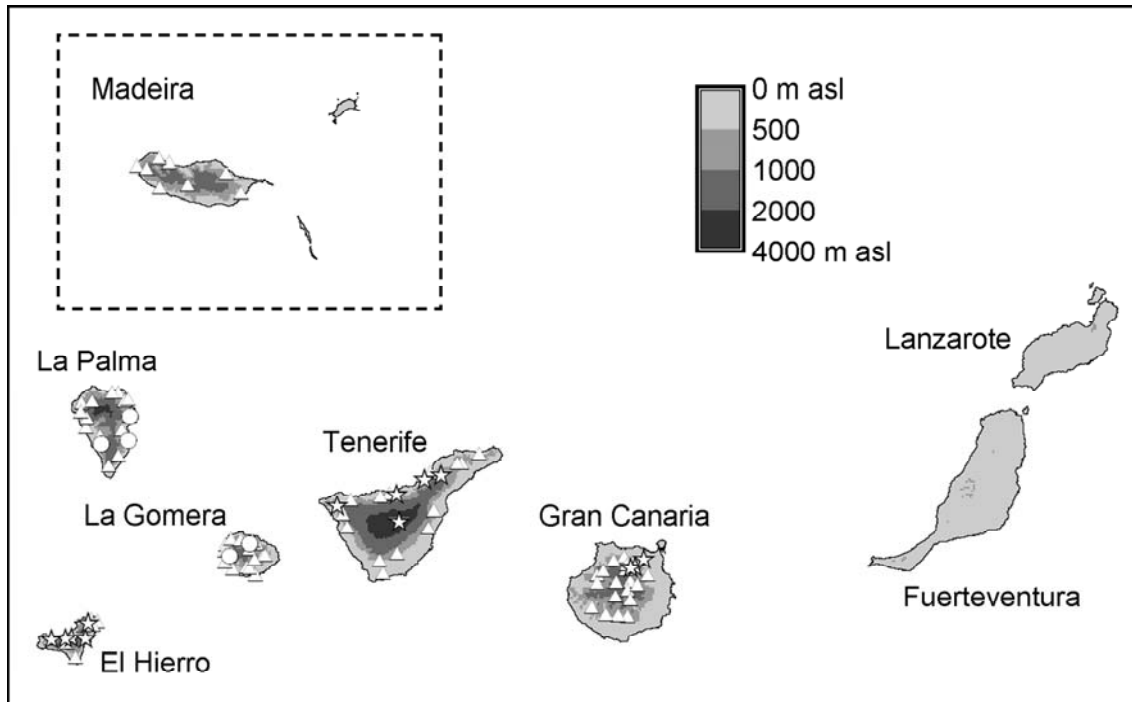
**Figure 3.** Geographic location of the 37 sampling localities in the Iberian Peninsula, with relief. Locality names are the municipalities where sampling took place; the name of the Spanish provinces to which those municipalities belong is stated in parentheses.



| ID | Locality name                           | ID | Locality name                       |
|----|---|----|-------------------------------------|
| 1  | Aguilar de Campoo (Palencia)            | 20 | Molinaseca (León)                   |
| 2  | Alájar (Huelva)                         | 21 | Pampaneira (Granada)                |
| 3  | Aldequemada (Jaén)                      | 22 | Panticosa (Huesca)                  |
| 4  | Alfarràs (Lérida)                       | 23 | Pinilla del Valle (Madrid)          |
| 5  | Barreiros (Lugo)                        | 24 | Pradoluengo (Burgos)                |
| 6  | Barrundia (Álava)                       | 25 | Ruidera (Ciudad Real)               |
| 7  | Bera (Navarra)                          | 26 | San Adrián (Navarra)                |
| 8  | Cazalla de la Sierra (Sevilla)          | 27 | San Lorenzo de El Escorial (Madrid) |
| 9  | Cocentaina (Alicante)                   | 28 | Santa Marta de Tormes (Salamanca)   |
| 10 | Cofrentes (Valencia)                    | 29 | Santiago-Pontones (Jaén)            |
| 11 | El Bosque (Cádiz)                       | 30 | Talavera de la Reina (Toledo)       |
| 12 | Gilbuena (Ávila)                        | 31 | Tarifa (Cádiz)                      |
| 13 | Grado (Oviedo)                          | 32 | Tordera (Barcelona)                 |
| 14 | Güéjar-Sierra (Granada)                 | 33 | Tordesillas (Valladolid)            |
| 15 | Hermandad de Campoo de Suso (Santander) | 34 | Trillo (Guadalajara)                |
| 16 | Jaca (Huesca)                           | 35 | Valderrobres (Teruel)               |
| 17 | Jerte (Cáceres)                         | 36 | Vilar de Barrio (Orense)            |
| 18 | Limpias (Santander)                     | 37 | Zuera (Zaragoza)                    |
| 19 | Los Barrios (Cádiz)                     |    |                                     |



**Figure 4.** Mean annual values of temperature and rainfall in the Iberian Peninsula. The different symbols indicating the sampling localities specify the sampling year (circle: 2008; square: 2009; star: 2010; triangle: 2011).



**Figure 5.** Sampling localities on the Macaronesian archipelagos of Madeira and the Canary Islands. The different symbols indicating the sampling localities specify the sampling year (star: 2003; circle: 2004; triangle: 2007).

### *C. Island populations: Madeira and the Canary Islands*

Blackcaps were sampled throughout the species' range on the Canary Islands and on Madeira during the breeding seasons (May to July) of 2003, 2004 and 2007: all islands were visited twice except for Madeira (Figure 5). Given the patchy distribution of blackcaps in the Macaronesia as a result of the dominant aridity, individuals were captured at between 10 - 23 sites on each island, numbering in total 380 birds.

### **Bird capture and sampling techniques**

Depending on local blackcap abundance and access facilities, in each locality a variable number of mist-nets were set, and tape-lures were used to increase the number of. Shortly after capture, birds were individualised with a metal ring; and aged and sexed by plumage details (Svensson, 2009). The

length of the eighth primary (feathers numbered from the body to the wing tip), tail length and distance between the wing tip and the tip of the first and ninth primary feathers to the nearest half mm were measured using appropriate rulers. These measures were used to classify each blackcap population as being migratory or sedentary, according to the discriminant function developed by Pérez-Tris *et al.* (1999). This method classifies Iberian blackcaps as belonging to migratory or sedentary populations with a success rate of around 90% (De la Hera *et al.*, 2007). Populations were assumed to be migratory if the majority of their individuals scored migratory-like morphology, and sedentary otherwise. A blood sample of each bird (~ 200 µl) was collected from the jugular vein and stored refrigerated in absolute ethanol for molecular analyses.

### Laboratory procedures

Total DNA was extracted from blood samples following a standard ammonium acetate protocol. After quantification, extracted DNA was diluted to a working concentration of 25 ng/μl. To test for sample quality and suitability for PCR all samples were previously tested using a standard sexing protocol (Griffiths *et al.*, 1998); this also allowed determining the sex of juvenile birds, which do not express the plumage sexual traits until their first moult. Samples were screened for positive parasite infections following the nested PCR protocols of either Hellgren *et al.* (2004) (valid for *Haemoproteus*, *Plasmodium* and *Leucocytozoon*) or Waldenström *et al.* (2004) (which amplifies only *Haemoproteus* and *Plasmodium*). Both protocols amplify the same 479 bp fragment of the parasite cytochrome *b* gene. PCR results were checked in 2% agarose gels stained with ethidium bromide or GelRed™ (Biotium, USA) under UV light, looking for bands of the appropriate size. Negative samples were rerun to look for false negatives. A positive control (using DNA template from an infected bird) and eight negative controls (using distilled water instead of template DNA) were included in every 96-well PCR batch to test for reaction quality and possible PCR contamination (no negative control produced positive result).

All positive samples were sequenced on an ABI Prism 3730 capillary robot (Applied Biosystems, USA) using the primers HaemF (for *Plasmodium* and *Haemoproteus*) or HaemFL (for *Leucocytozoon*). Sequences were edited manually using BioEdit 7.0.5.3 and cytochrome *b* gene haplotypes, defined by a sequence difference of at least one base in the amplified fragment, were identified using the Nucleotide BLAST

application of GenBank\*. Mixed infections were recognized by the presence of double peaks on the electropherograms (Pérez-Tris & Bensch, 2005a) and if possible the identities of the parasites involved were assessed by comparing the double peak patterns with previously known sequences of parasite haplotypes infecting blackcaps. Those cytochrome *b* haplotypes found which were not previously described were confirmed by repeating the PCR and then sequencing from both ends with primers HaemF and HaemR2 or HaemFL and HaemR2L. New sequences were given a name according to MalAvi nomenclature (Bensch *et al.*, 2009) and deposited in GenBank.

\* <http://blast.ncbi.nlm.nih.gov/Blast.cgi>





Blackcaps' taste for berries, altogether with their bold and inquisitive nature, makes of it a bird common in gardens, parks and orchards all along Europe. The vast olive grooves grown in the southern European peninsulas hold great figures of wintering blackcaps, making up for the maquis plenty of fruiting shrubs that once covered the same regions where blackcaps used to winter. In the last decades, among the central European birds traditionally heading towards the SW during winter, a whole new population of blackcaps have arisen which migrates following a NW direction, to spend the winter in southern England feeding on garden berry-carrying bushes and birdfeeders.

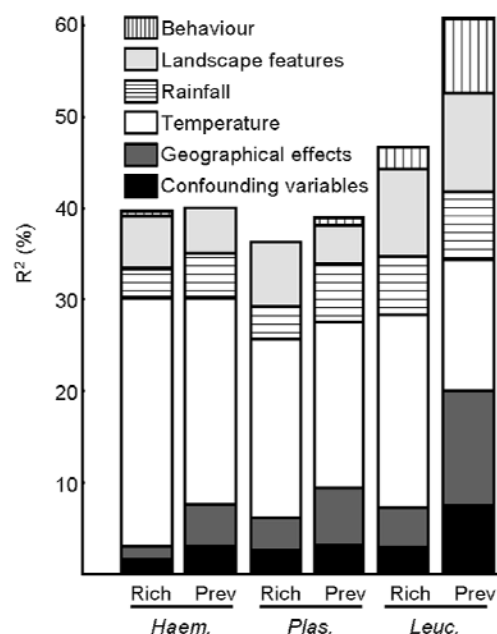


## GENERAL RESULTS AND DISCUSSION

## Parasite diversity and environmental variation

All blackcap populations screened for parasites in the Iberian Peninsula hosted at least one haemosporidian haplotype, although both parasite richness and prevalence showed a great deal of variation among localities, providing with the foundations to assess which environmental features had the greatest influence on parasite diversity. In broad numbers, 65.3% of the blackcaps screened were infected by at least one parasite haplotype; and a grand total of 24 haplotypes were found.

We analyzed the relationship between parasite richness and prevalence and the considered environmental variables using Partial Least Square (PLS) regression (Carrascal *et al.*, 2009). Our PLS analyses were able to significantly explain a diverse amount of variance of the response variable (Figure 6), but the comparison of our models with alternative PLS regressions built with climatic variables alone showed that in all cases adding non-climatic variables significantly improved climate-based models. Interestingly, the variation among genera in the amount of variance explained by PLS models can be linked to differences in their degree of environmental specialization; which may be mediated either by constraints on the parasites themselves or by their vectors (Loiseau *et al.*, 2011a). In fact, *Plasmodium* parasites are often host generalists transmitted by a wide array of ornithophilic mosquitoes (Gager *et al.*, 2008; Kimura *et al.*, 2010; Njabo *et*



**Figure 6.** Decomposition of  $R^2$  values (expressed as percentage of variance explained) of PLS regression models into partial contributions of different types of predictors. The figure shows the results of models built for variation in haplotype richness and prevalence of the three parasite genera. Climatic variables (temperature and rainfall) accounted for the greater percentage of explained variance, but they had contrasting effects according to parasite genera: whereas both *Haemoproteus* and *Leucocytozoon* favoured year-round colder and rainier locations, *Plasmodium* in turn was found to thrive in warmer, drier sites. The effect of landscape features was mainly related to altitude and slope, while broad scale land uses had only a marginal effect. Finally, *Leucocytozoon* was the only parasite genus showing a significant relationship with host migratory behaviour; both its richness and prevalence were higher in sedentary blackcap populations.

*al.*, 2010); whereas *Haemoproteus* and *Leucocytozoon* establish more specific associations with both their avian hosts



and their insect vectors (Valkiūnas & Iezhova, 2004; Hellgren *et al.*, 2008; Martínez-de la Puente *et al.*, 2011). This variable degree of habitat specialization among parasites may greatly influence the performance of predictive models, which usually work better for specialist species (Evangelista *et al.*, 2008).

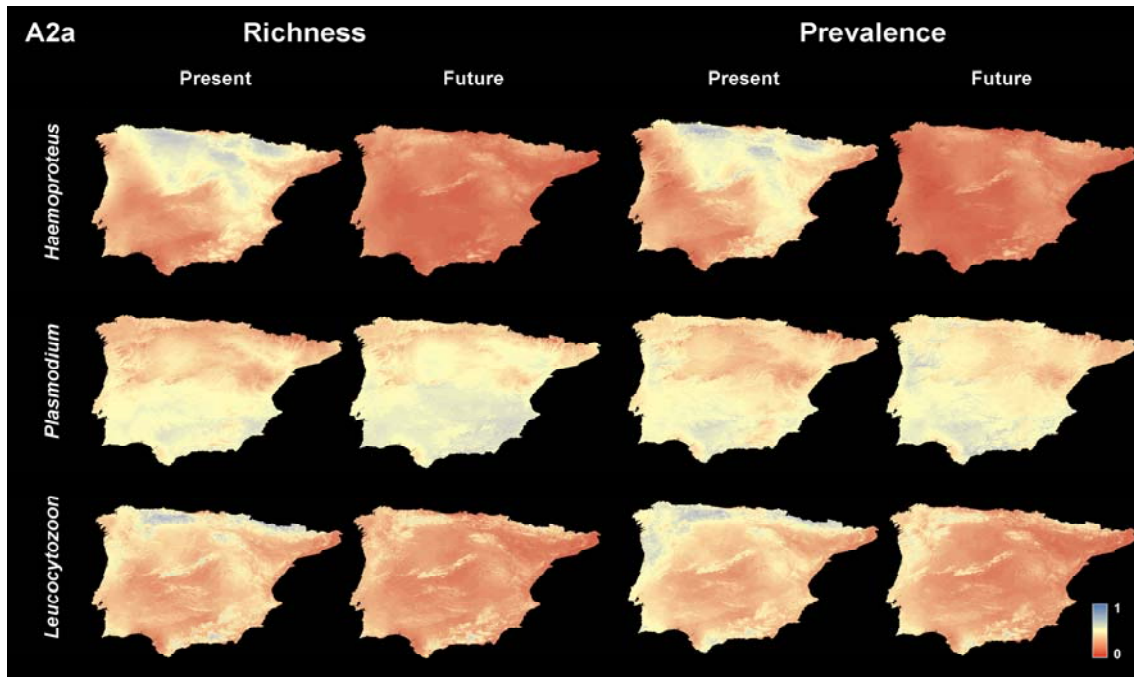
Climatic predictors accounted for the greatest proportion of explained variance of all response variables. This is interesting in its own right: given the current concern posed by climate change, better climate forecasting models are developed everyday; so that if parasite distributions maintain a noticeable link with climatic features, parasite predictive models are also candidate to a continuous improvement. Regarding landscape features, it is worth noting the importance of both altitude and slope for explaining geographic variation in parasite diversity; most likely acting as a proxy for other unconsidered landscape or biotic variables (for instance the linkage of *Leucocytozoon* to areas with steep slopes probably reflects the dependence of their insect vectors on running water). The importance of topographic variables for parasite diversity modelling detected in this thesis adds to recent literature advocating the incorporation of these variables in ecological modelling (Hof *et al.*, 2012). Broad patterns of land uses, in turn, did not play a prominent role as predictor variables.

Predictions about how parasite diversity is currently distributed are largely dependent on the identification of the right variables. Although a great effort is being devoted to create accurate climatic models, land uses are subject to swift changes (Foley *et al.*, 2005) and their integration as variables in predictive distribution models is difficult (Pearson *et al.*, 2004).

Nevertheless we have shown that in our study system variation in parasite diversity is related up to a great degree to climatic and topographic features such as altitude or slope (which are virtually static variables), so that predictions of host-parasite interactions are likely to be realistic. In this sense, PLS regression demonstrated to be a very useful tool, allowing us to test at one time several variables that are often discarded in modelling analyses for the sake of statistical simplicity.

### **Current and future predictions of parasite impact**

MaxEnt, a widely used Species Distribution Modelling tool (Phillips *et al.*, 2006), was employed to elaborate predictions on the distribution of the areas of the Iberian Peninsula likely to hold high values of parasite richness and prevalence. Predictions were made both for the present time and for the future, considering three different scenarios of climate change which differ in their degree of predicted climate disturbance. The direction of the relationships between parasite diversity and climatic and topographic features observed in the PLS analyses (Chapter II) suggested two major trends of parasite distribution in the Iberian Peninsula which were backed up by MaxEnt modelling outputs (Figure 7): on the one hand, *Plasmodium* richness and prevalence are expected to be higher in the warmest Iberian areas (near the coastlines and in the Mediterranean lowlands located towards the south). On the other hand, both *Haemoproteus* and *Leucocytozoon* would thrive in colder locations, either in the north of the study region or in the southern mountain ranges. In sum, both groups of parasites were strongly influenced by opposite trends. Consistent with present-time influence



**Figure 7.** Predicted current and future distribution of the probability (ranging between 0 -red- and 1 -blue-) of finding areas with high richness and prevalence of the three considered parasite genera in the Iberian Peninsula. According to current conditions, the areas with the highest *Haemoproteus* richness and prevalence are located in the northern half and the southern mountain ranges of the Iberian Peninsula. *Leucocytozoon* follows the same trends, but with a tighter link to the mountainous areas. In turn, *Plasmodium* high prevalence and richness are likely to occur throughout the whole Iberian Peninsula except for highest latitudes and elevations. For the predicted climatic values by the end of the XXI century, clear differences among parasite genera arise: both *Haemoproteus* and *Leucocytozoon* areas of high influence are predicted to shift northwards and upwards in mountain ranges, whereas *Plasmodium* areas of high influence expand over the entire Iberian Peninsula. Future projections depicted are those of the A2b emission scenario of climate change, which of the three scenarios considered in Chapter III is the one that represents a medium temperature increase; but predictions were equivalent among scenarios.

of environmental features over parasite diversity, a process of climate warming would likely derive in a future situation in which *Plasmodium* would increase its range at the expense of both *Haemoproteus* and *Leucocytozoon*, which would lose areas of high influence in the Iberian Peninsula; a picture reflected again on MaxEnt models (Figure 7).

An analysis of the shape of the relationship between current and future conditions for the same localities revealed further sources of variation among parasite genera and scenarios of climate change (Table 3, Figure 16). The shape of these relationships

suggested that the regions under high parasite impacts of *Haemoproteus*, and to a certain degree *Leucocytozoon*, will collapse except where currently the greater probabilities of sustaining high levels of parasite influence are held. In turn and within a general trend of increased probabilities of high parasite influences, the areas best currently suited to favour *Plasmodium* are those in which the probability is less likely to rise; probably because of climatic threshold limitations operating after the maximum has been exceeded. The analysis of the comparison of these relationships among scenarios also revealed that, as more extreme climatic conditions are expected, there would be

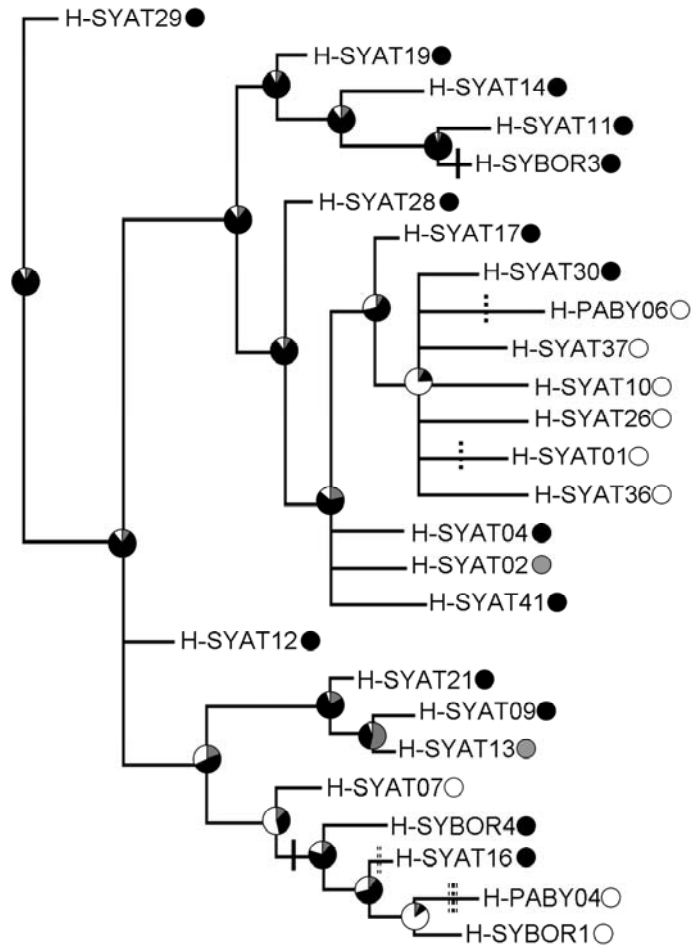
a stronger effect of local microclimatic factors as determinants of parasite predictions; in opposition to a generalised effect of broader climatic conditions expected under milder climatic predictions.

According to our results, and independently of the particular predictions linked to specific scenarios of climate change, the general trend for the end of the century is one of simplified parasite component communities among Iberian blackcap populations, driven by reduced prevalence and richness of *Haemoproteus* and *Leucocytozoon*. These are currently the most prevalent and lineage rich haemosporidian parasite genera in blackcaps in the Iberian peninsula, but they might in the future be dependent on “parasite refuges”, determined by particularly favourable microclimatic conditions, to sustain areas of high influence; which in addition are likely to be few and patchily distributed. On the other hand, *Plasmodium* is expected to experience strong and broadly homogeneous rises of future high influence.

The spatial configuration imposed by environmental conditions to the areas of high parasite pressure means that birds living in different Iberian regions currently face fundamentally different parasite communities. This implies that for a blackcap dispersal potentially comes at a cost of encountering parasite environments different from those that it was adapted to; with an associated fitness cost (Møller & Szép, 2011). Our models agree in foreseeing a situation in which parasite influence is not expected to vary in the same way for all three parasite genera, so that in the future the predicted situation entails a generalized reorganization of local parasite component communities. Furthermore,

we showed that under more stressful future climatic conditions, the differences among localities, even if close among them, could show a great deal of variation in terms of parasite environments; as a result of the discussed local microclimatic influences. Blackcap dispersal could then be limited in the future by the randomness of parasite pressure, acting eventually as a further factor limiting the ability of the species to cope with the future environmental conditions.

Furthermore, the reshuffling of the current interaction scenario anticipates a changing role of parasites on host population regulation (Dobson *et al.*, 2008): parasite diversity can drive the evolution of resistance mechanisms (Westerdahl *et al.*, 2005; Bonneaud *et al.*, 2006; Loiseau *et al.*, 2011b), so that a simplification of parasite communities may induce a relaxation of the host resistance mechanisms (Goüy de Bellocq *et al.*, 2008). This could in turn limit the ability of host populations to deal with novel, emerging pathogens favoured by global change. Additionally, in-host competition among different parasite strains promotes virulence in haemosporidian parasites (Råberg *et al.*, 2006; Wargo *et al.*, 2007), but it is unclear to what extent the expected change in structure of parasite communities is likely to affect within-host parasite assemblages: the combined retreat of *Haemoproteus* and *Leucocytozoon* should ease competition among parasites, which somewhat predicts lower virulence in the future (De Roode *et al.*, 2003). However, an expansion of *Plasmodium*, the most virulent of all three parasite genera (Valkiūnas, 2005), predicts instead an increase in virulence of future parasite component communities; an effect that may be exacerbated by in-host competition among more virulent parasites, associated with the increase of



**Figure 8.** Bayesian reconstruction of ancestral states of seasonal transmission strategy and host species of parasites belonging to the *Haemoproteus parabelopolskyi* clade assigning a current character state to all the parasite lineages. Current transmission states and estimated posterior probabilities for the transmission state of the Most Recent Common Ancestor are identified by colour (Black: summer transmission. Grey: extended transmission. White: year round transmission. The shaded percentage of each circle indicates the probability of the MRCA showing that trait). Changes of host are distinguished by vertical bars (Solid line: from *Sylvia atricapilla* to *S. borin*. Dotted line: from *S. atricapilla* to *S. abyssinica*. Double dotted line: from *S. borin* to *S. atricapilla*. Triple dotted line: from *S. borin* to *S. abyssinica*). The MRCA of the whole group is estimated to be a summer transmitted (posterior probability of 0.84) blackcap-infecting parasite (posterior probability of 0.93).

*Plasmodium* haplotype richness and prevalence.

**Parasite diversity and transmission strategies**

We performed an extensive literature search, as well as a parasitic screening of several hundred blackcaps captured throughout a whole wintering season in the extreme south of Spain (September

to March), to retrieve the current states of transmission seasonality of parasite lineages belonging to the morphospecies *H. parabelopolskyi*. We could detect the existence, besides the division between year round and summer transmitted lineages established by Pérez-Tris & Bensch (2005b), of hidden variations in the year round transmission strategy: remarkably a distinction between “year round transmission *sensu stricto*” (lack of

noticeably variations in prevalence throughout the year) and “extended summer transmission” (parasites with high prevalences in autumn that drop in winter and quickly recover in early spring). A Bayesian reconstruction of the ancestral state of host species and parasite transmission seasonality suggested with a high posterior probability that the *H. parabelopolskyi* parasite clade originated in blackcaps from a summer transmitted common ancestor (Figure 8). Both changes in the seasonality of parasite transmission and host switches seem to be as a rule of recent origin in the evolutionary history of the group.

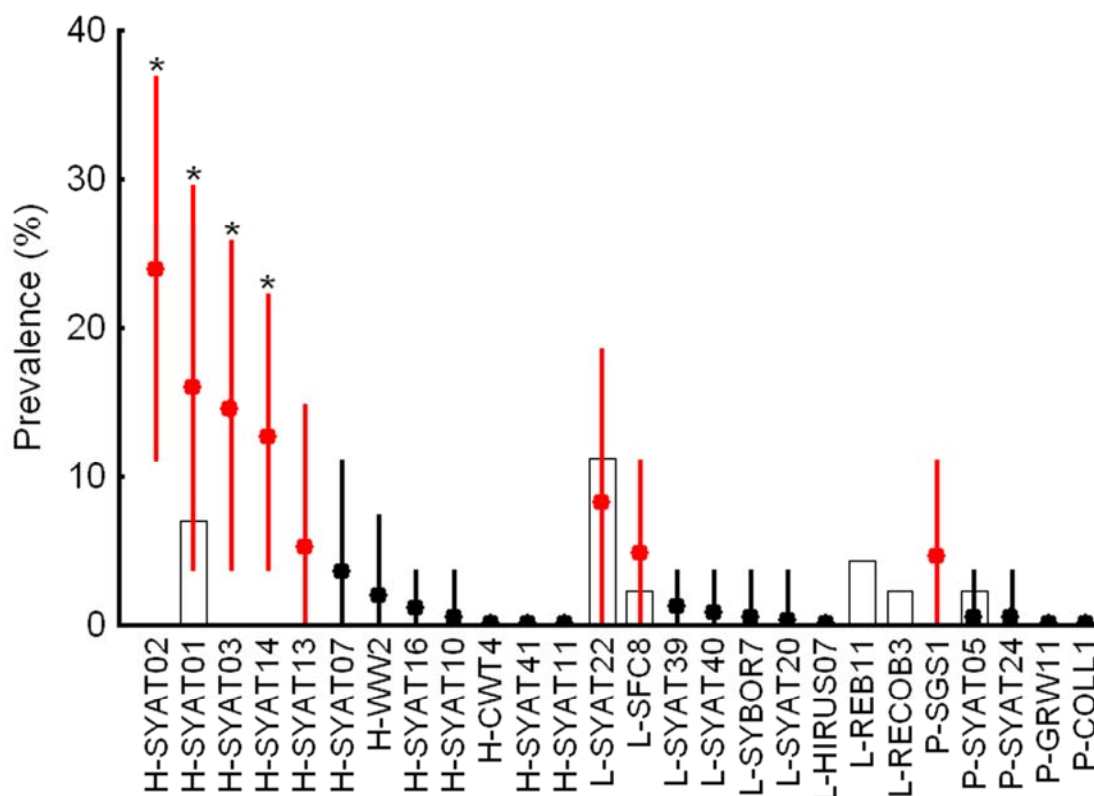
Why all the events of switching from summer transmission to other strategies appear as recent phenomena in the evolutionary history of the parasite group, if they otherwise seem to be advantageous in terms of parasite spread (Pérez-Tris & Bensch, 2005b)? An intrinsic genetic limitation does not seem to be the most likely explanation, given that these changes are not rare in the parasite phylogeny; so natural selection acting on the long term against enlarged transmission periods may be a more probable cause: the longer a parasite is affecting its host out of the breeding season, when the challenge posed by the immune system is smaller; the more chances it has of being targeted by it. Parasites could then risk to be eliminated by the immune system of the host or reverse instead to a summer transmission strategy. This situation seems to be generalized among parasites in temperate areas, given that in spite of the advantages of year round transmission, most of them seem to stick to seasonal transmission (Hellgren *et al.*, 2007a).

Host switches arise also as recent events scattered throughout the *H. parabelopolskyi* phylogeny.

Interestingly, in our study case host switches accompany several steps of parasitic lineage diversification. Diversification throughout recurrent host switches is a process common to many host-parasite systems (Schluter, 2000; de Vienne, 2012) and widespread among avian haemosporidians (Ricklefs & Fallon, 2002, Ricklefs *et al.*, 2004; Jenkins *et al.*, 2012); but when a given lineage starts an in-host diversification process, its opportunities of switching to additional hosts seem to become highly compromised (Pérez-Tris *et al.*, 2007; Jenkins *et al.*, 2012), as in our study case. The coevolutionary arms race between host and parasites, which leads to high rates of in-host diversification, probably comes at the cost of losing the capacity of infecting other host species.

### **The particularities of island parasites**

The haemosporidian parasite community of the examined Macaronesian blackcaps was very different from that of their peninsular counterparts (Chapter II) in terms of haplotype richness, haplotype composition and prevalence. In all, only 27 out of 380 blackcaps (7.1%) were found to be infected by six parasite haplotypes: four of them commonly observed among mainland blackcaps and two not previously found infecting this species (Figure 9). Parasite prevalence varied greatly among years and among islands. The structuring of the insular parasite assemblage was highly divergent as well, with *Leucocytozoon* dominating in terms of prevalence and haplotype richness, whereas in mainland Europe *Haemoproteus* is the predominant haemosporidian genus in blackcaps (Križanauskienė *et al.*, 2010; Santiago-Alarcón *et al.*, 2011; Chapter II). Besides, although more than a half of



**Figure 9.** Comparison between actual island parasite richness and prevalence (open bars) and mainland parasite diversity. A simulated population of 100,000 infected blackcaps, in which each parasite haplotype occurred with the same frequency as observed across all Iberian blackcap populations (Chapter II), was created. From this simulated population, 10,000 randomly selected groups of 27 parasite infections (the actual number of parasite infections found on islands) were created and used to calculate the expected island parasite prevalence of each mainland parasite (dots represent means, with 95% CI). Based on individual probabilities of occurrence ( $P_0$ ) obtained from these simulations, four parasites were predicted to occur on islands (marked with stars); and another eight showed  $P_0 > 0.69$  (in red), which is the average probability of occurrence computed for the four mainland parasites that were found on islands. L-REB11 and L-RECOB3 were only found on islands. The initial of haplotype names stand for parasite genera (H: *Haemoproteus*, P: *Plasmodium*, L: *Leucocytozoon*).

the parasites found in the Iberian Peninsula are blackcap-specific, none of them were present among the six insular parasites, which were all host generalist.

Parasite richness was independent of the size of blackcaps' range on each island; something to be expected given their lack of specificity, as generalist parasites can rely on other host species to thrive. However, there was a negative correlation between distance from the continent and parasite richness, which could result from various, not mutually exclusive processes: (1) sequential founder

population bottlenecks in the parasites associated with the blackcaps (or other European host species) having first colonized the easternmost islands then spread westwards across islands, (2) recurrent parasite colonization favoured by seasonal migration of blackcaps from Europe (which concentrate on the easternmost islands; Shirihaï *et al.*, 2001), or (3) generalist parasites being transported from Africa and spreading westwards across the archipelago according to a stepping-stone pattern of island colonization. Whatever the mechanisms, our results support the basic principle of island biogeography

that the balance between colonization and extinction favours greater richness on islands that are located near the putative source of colonizers (MacArthur & Wilson, 1967).

Apart from being less prevalent, parasites demonstrated apparent temporal instability in blackcap island populations, an observation which is at odds with previous studies showing temporal consistency in the structure of haemosporidian assemblages (Pérez-Tris & Bensch, 2005b; Durrant *et al.*, 2008; Spurgin *et al.*, 2012; but see Bensch & Åkesson, 2003). Low and unstable parasite prevalence might indicate that parasites on the blackcaps on the islands are more susceptible to extinction. This idea is supported by the composition of the parasite assemblage (comprising non-specific parasites capable of exploiting multiple hosts), which may benefit from the exploitation of alternative host species in years when particular hosts (blackcaps in our case) may for some reason be less available. Decreasing richness of parasites with increasing island distance to the continent (a putatively uninterrupted source of parasite colonisation) may be further evidence of frequent parasite extinction on islands, with parasite communities being re-seeded from the continent.

The different parasite haplotypes present on the islands offer attractive insights of which characteristics a successful island-colonizer parasites must present. The only *Haemoproteus* parasite we found on insular blackcaps, *H. parabelopolskyi* H-SYAT01, is the only parasite of the *H. parabelopolskyi* group which is not host specific (it can infect the Afrotropical species *Sylvia abyssinica* as well; Chapter III); therefore it is possible that H-SYAT01 might have benefited from its ability to thrive in hosts other than the blackcap,

or from being transmitted by vectors of African origin, to be able to thrive in the Canary Islands, where other *Haemoproteus* haplotypes as frequent as itself in the continent are not present. It is also worth pointing out that H-SYAT01 was only found in Gran Canaria, the insular blackcap population closest to the continent; providing additional support for the idea that migrating blackcaps may permanently enhance parasite diversity on easternmost islands. The *Leucocytozoon* haplotype L-SYAT22, which behaves as a blackcap-specialist in Europe, does infect the Berthelot's pipits in the Macaronesia as well (Spurgin *et al.*, 2012); a possible evidence of ecological fitting (Agosta & Klemens, 2008). Conversely, both L-REB11 and L-RECOB3 are *Leucocytozoon* haplotypes of putative tropical African adscription. These parasites had the opportunity in the Macaronesia to access host species of Palaearctic origin, e.g. blackcaps, as evidenced on our present study; representing clear cases of local parasite acquisition by a host species that lost other parasites after range expansion (Marzal *et al.*, 2011).

The broad picture uncovered in our study is that Macaronesian blackcaps sustain a totally different parasite community from that of their mainland counterparts. This can be partially interpreted as the likely outcome of two processes known as 'missing the boat' (if parasites failed to arrive with its host to the new area) or 'drowning on arrival' (if they did arrive but failed to become established; MacLeod *et al.*, 2010) during island colonization. However, blackcaps have not only lost their usual continental parasites after island colonization, they have also gained new parasites from those that were present in insular populations of other species.

### Migration and parasite diversity

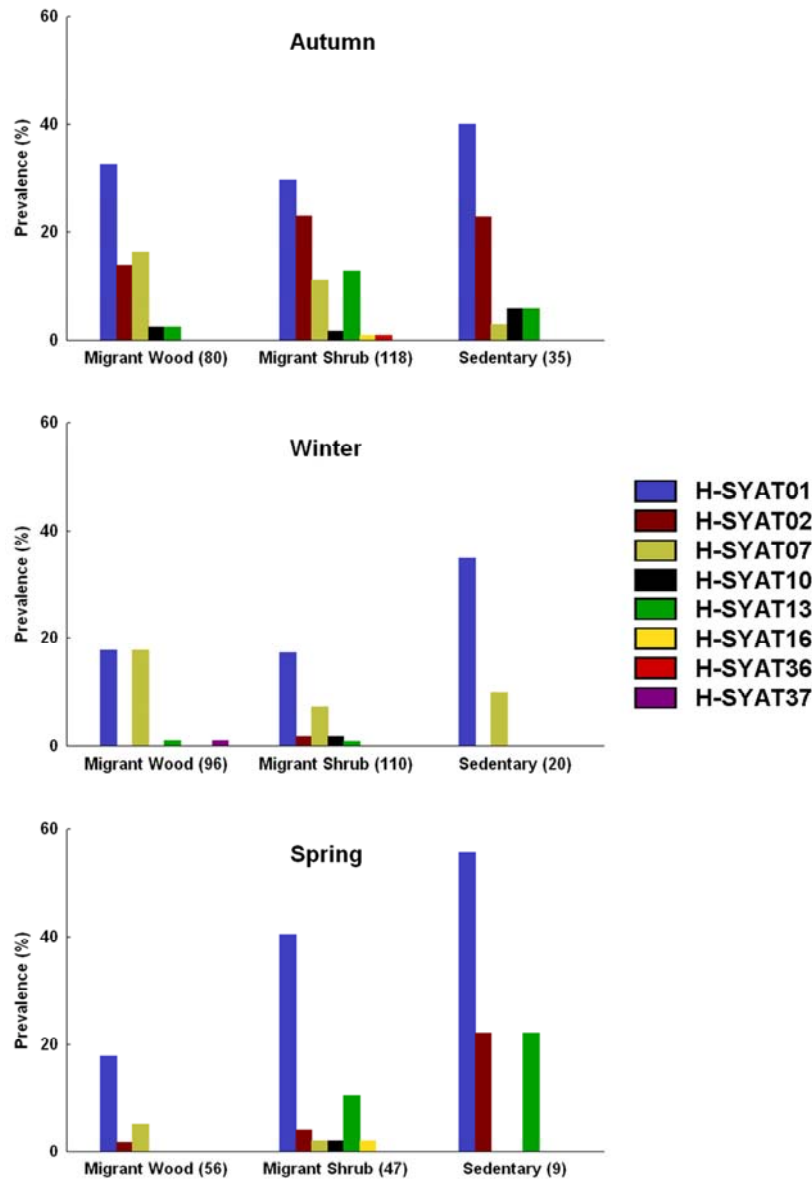
The wide display of different migratory behaviours that even geographically close blackcap populations show, and the easiness to identify them attending to birds' morphology; helped to consider the relationship between host migratory behaviour and parasite diversity at different stages of this thesis. In broad terms, our study of parasite diversity in the Iberian Peninsula during the breeding season failed to find significant effects of migration on it except for the case of *Leucocytozoon* parasites (Figure 6). Remarkably, we observed that both *Leucocytozoon* richness and prevalence were higher on sedentary blackcap populations; a fact at odds with previous findings reporting higher *Leucocytozoon* diversity on migratory birds in an interspecific comparison (Jenkins *et al.*, 2012). Nevertheless, the slight significance of the effect of migration over *Leucocytozoon* diversity and its effect over *Plasmodium* and *Haemoproteus* allow us to tentatively exclude host migratory behaviour as a factor to take into account while making predictive parasite modelling; an interesting tip given that whether migratory behaviour will evolve and change among geographic areas or not is difficult to foretell (Pulido & Berthold, 2010).

Parasite screening of both sedentary and migratory birds coexisting together during the wintering season shed some light over the processes regulating haemosporidian gametocyte production suppression at the end of the breeding season: frequent summer-transmitted parasites, such as *H. parabelopolskyi* haplotypes H-SYAT14 or H-SYAT16, were absent or almost absent among the birds sampled from September to March, regardless that the climatic conditions of early

autumn or spring would remain highly favourable for parasite transmission. Given that this happened among migratory and sedentary birds as well, a day-length mediated suppression of gametocyte production seems to be the most likely mechanism (as for the spring relapse; Valkiūnas *et al.*, 2004); independent thus of weather changes or physiological adjustments on the birds' organism related to migration activity.

As long as blackcaps structure themselves during the wintering season between habitats according to their migratory behaviour (sedentary blackcaps remain in the woodlands where they live year round; whereas migrants occupy woodlands and shrublands as well; Chapter IV), an opportunity arises to test if host origin and/or habitat have an effect on parasite transmission during the wintering period. After dividing wintering birds in three groups (migrant blackcaps in woodlands, migrant blackcaps in shrublands and sedentary blackcaps - only in woodlands-), the variations in prevalence registered pointed towards a stronger effect of origin versus habitat on the differences in prevalence (Figure 10). The lack of third order associations among any given parasite x group x season, implying a homogenization of the parasite communities between groups along the wintering period; suggest that parasite transmission is actually relatively limited on a yearly basis. Shrublands seem to harbour less parasitized birds, but although these differences are usually attributed to unequally favoured transmission among habitats (Chasar *et al.*, 2009; Yohannes *et al.*, 2009), they could arise as well if blackcaps with different parasite loads make their habitat choice driven by factors such as dominance or geographical origin for instance (Pérez-Tris & Tellería, 2002).





**Figure 10.** Seasonal variation in prevalence (in %) of *Haemoproteus parabelopolskyi* lineages among the three studied seasons and the three blackcap groups considered. The number of sampled birds per group is stated between parentheses. A log-linear analysis showed that the prevalence of the four most prevalent lineages (H-SYAT01, H-SYAT02, H-SYAT07 and H-SYAT13) varied among seasons and groups; but whereas the temporal patterns were clearly understandable, differences among groups per parasite haplotypes could not be assigned a biological meaning.

As stated before, our study showed that in the Canary Islands parasite richness is clearly structured as a function of the distance to the continent, pointing towards the yearly influx of migrating European birds with their parasite loads as a possible factor promoting parasite diversity in these islands. Nevertheless, several particularities of insular blackcaps' parasite communities could be detected

despite this influx; so that our results suggest the idea that the typical characteristics of insular parasite assemblages (low lineage richness, frequent host-switching, or reduced specialization of host-parasite relationships; Fallon *et al.*, 2005) may evolve before insular host populations become completely isolated from their mainland counterparts.

### Parasite vectors

Although the specific study of parasite vectors was not one of the objectives of this thesis, their influence stands out clear throughout it; and there are several characteristics of the insect vectors which are crucial to understand many of the results exposed here. For instance, the link of haemosporidian parasites with environmental features depicted on Chapter II could be mediated either by constraints on the parasites themselves or by their vectors: not only by their degree of host specialization, as stated before; but also by their own habitat requirements: whereas the mosquitoes which transmit *Plasmodium* (Fam. Culicidae) can breed on a wide variety of shallow pools, the vectors of the other two parasite genera are more habitat-specialists: *Haemoproteus* vectors (*Culicoides* biting midges, Fam. Ceratopogonidae) rely on constant ground humidity; and *Leucocytozoon* vectors (black flies, Fam. Simuliidae) require running, unpolluted water for breeding (Santiago-Alarcón *et al.*, 2012).

The predicted changes in the areas of high influence of haemosporidian parasites may well be interpreted thus as a likely consequence of the different ecological requirements of their vectors. *Culicoides* midges are generally considered to be among the organisms that will be favoured by climate change (Wilson & Mellor, 2008; Acevedo *et al.*, 2010), but more specific studies show that not all *Culicoides* species thrive in the same environmental conditions, and so they are not expected to react in the same way faced with climate change (Calvete *et al.*, 2008). According to our predictions this is probably the case for the *Culicoides impunctatus* species group, which is the vector of *H. parabelopolskyi* (Valkiūnas & Iezhova,

2004), the species that makes the bulk of *Haemoproteus* diversity in blackcaps. Likewise, previous studies indicate that Simuliidae blackflies are likely to benefit from the expected environmental shifting in southern Europe towards more seasonal, torrential streams with warmer waters (Bonada *et al.*, 2007; Feio *et al.*, 2010); but again our predictions suggest that the projected situation could be above the suitability threshold for those vectors in many areas of the Iberian Peninsula. *Plasmodium* vectors in turn do favour warm locations and have very plastic breeding site requirements (Santiago-Alarcón *et al.*, 2012); so that their future abundance doesn't seem to be a problem limiting *Plasmodium* expansion. This situation is even more probable given that all *Plasmodium* haplotypes detected in blackcaps in our survey are both host and vector generalists, so that they could easily skip local extinctions of a given vector or blackcap population and keep expanding their ranges (Woolhouse *et al.*, 2001).

Vector availability is capital for haemosporidian transmission, so that an obvious candidate to explain the low richness and altered prevalence patterns observed on islands could be a reduced availability of appropriate vectors. This may be particularly relevant to the many blackcap-specific *Haemoproteus* haplotypes known from continental populations which are lacking on the Macaronesia. Given the vector-specificity of *Haemoproteus* parasites (Martínez-de la Puente *et al.*, 2011), the apparent inability of blackcap-specific parasites to thrive on islands could be associated with their specific vectors having failed to colonize Macaronesian archipelagos. If the midge species that have colonised the islands do not target blackcaps, or if they are not able to transmit blackcap-specific parasites, this

would explain the absence of these parasites from the islands. Nevertheless, a thorough study of the Macaronesian vector-bird-parasite interactions must be performed before we could state with certitude the role of vectors in driving the distribution of haemosporidians in insular blackcaps.

### **Guidelines for future research**

Although we demonstrate that climatic and topographic variables are reliable predictors of present-time haemosporidian diversity, we are aware that our predictions are to be taken with caution, as our models by no means explain all the variance in parasite richness or prevalence. The inclusion of biotic interactions helps to ameliorate SDM (Araújo & Luoto, 2007), so that the incorporation of vector information into parasite modelling is a crucial step to improve our predictions. Sadly, competent vectors and their current distributions are virtually unknown for most haemosporidian parasites of birds, as the studies on the subject are still scarce (Imura *et al.*, 2010; Njabo *et al.*, 2010; Martínez-de la Puente *et al.*, 2011; Santiago-Alarcón *et al.*, 2012), which makes modelling vector distributions unaffordable for the time being. Basic research on vector biology, that nowadays is almost limited to the vectors of those parasites of human or livestock sanitary importance, is a critical basis to develop better parasite predictive models.

To complete our understanding of the processes regulating parasite transmission throughout the year, it would be interesting to know the precise nature of the mechanisms regulating parasite retreat from the peripheral blood for all the considered transmission strategies. Future studies considering several wintering periods

could for instance look for relationships between specific environmental conditions and variations in prevalence of the different parasite haplotypes. If any climatic variable happens to have a detectable effect on the average winter prevalence of a given parasite, then the ongoing process of climate change could modify parasite abundances by affecting parasite transmission on the wintering grounds; and not only on the breeding grounds, where studies addressing this issue usually insist (Møller, 2010; Garamszegi, 2011; Loiseau *et al.*, 2013). Other host species should be studied as well, to know if the depicted set of different transmission strategies is exclusive of blackcap haemosporidians, or if on the contrary is widespread across the parasites infecting other birds in temperate regions.

The apparent temporal instability of insular blackcaps' parasite communities is a further issue to explore: why parasite prevalence changes so much between years on islands, and whether parasites persist in other host species during periods of low prevalence in blackcaps; remain questions for future research. The parasite communities of other Macaronesian bird species should be examined as well: if the impoverishment of parasite community detected in blackcaps is a general situation, then the arrival of alien pathogens (perhaps not from the temperate region, but from the tropical one) could become a threat for biodiversity conservation.

In short, given the importance that global change has been shown in this thesis to have as a factor rearranging parasite diversity, a claim must be made in favour of parasite monitoring surveys dealing with several host species across both seasons and

years; in order to both improve our current knowledge of parasite diversity and how it is structured, and to detect any possible emerging danger at its primary stages (Fuller et al. 2012). The natural role of the Iberian Peninsula as

an entrance of tropical pathogens into Europe invites to intensify this kind of surveys here; as well as to start them in the north of Africa, which is currently a completely unknown territory in terms of avian haemosporidian research.

## CONCLUDING REMARKS

1. The ability to forecast the evolution of parasite diversity is critical in a changing World even-threatened by emergent diseases. Conveniently, we demonstrate that climatic variables are reliable predictors of present-time haemosporidian diversity among Iberian blackcap populations. We reveal that the inclusion of topographic features helps to improve the power of predictive models, whereas other variables difficult to include such as land uses or aspects of host biology like migratory behaviour are not that important. These facts, which may be common to other similar host-parasite systems, confirm us on the trustworthy nature of predictive parasite models based upon these variables. We also promote the use of PLS regression, a statistical tool rarely used in ecological studies, to assess the relative importance of candidate variables as an essential prior step before developing accurate predictive models.

2. Although broad-scale parasitic surveys of wildlife can be unaffordable in terms of logistics or economic costs, we show that even a discrete population sample size is enough to make meaningful predictions about how parasite diversity is currently structured and how it might be in the future. We foresee geographic shifts of areas of strong parasite influence; highlighting how such changes are likely to vary among parasite types and which implications such differences may have on the impact of parasitism on host populations.

3. We also warn about how future parasite pressures can noticeably vary according to different scenarios of climate change. As a rule, the models of

climate change which are constructed assuming a more environmentally friendly outlook, predicts a climate effect over parasite diversity more homogeneous throughout the current range of parasite influence. This in turn could favour future adaptation of blackcaps as well as other hosts to the novel climatic conditions by lessening the stochasticity associated to parasite pressure; making thus of it a more desirable situation.

4. Even if our models of parasite diversity are insightful, they suffer from the lack of relevant information about hosts and vectors, which could contribute to produce even better predictive models. There is therefore a need of basic research on the field of wildlife diseases and disease vectors, as these are key components to make meaningful predictions which allow foreseeing and preventing the effect of emerging diseases.

5. The reconstruction of the evolution of seasonal transmission stages indicates that, although these changes do not show a long term survival, they can arise frequently and have a temporary great ecological success. Switches on transmission seasonality could promote the spread of host generalist parasite haplotypes, and the arbitrariness of these switches claims in favour of parasite monitoring surveys addressed to analyse several host species across both seasons and years, in order to determine how widespread different transmission strategies are and to detect any possible hazard that, in a context of fast and unpredictable global change, might represent a risk for biodiversity.

6. Macaronesian blackcaps do not face the strong parasite pressure encountered by their mainland counterparts, despite the fact that blackcap migration from the continent may directly transport mainland blackcap parasites to the islands. These results support the idea that parasite island syndromes (low richness, frequent host-switching, and reduced specialization) evolve even before insular host populations become completely isolated from their mainland counterparts.

7. The reduced parasite burden in the Macaronesian populations of blackcaps and other species raises a cautionary word on the possible hazardous effects that the introduction of novel pathogens might have on such unique island bird communities.





In temperate European regions blackcaps occupy during the breeding season a vast range of deciduous woodland areas, where fruiting trees and bushes such as the buckthorn, the privet or the wild cherry (in the picture) supplement their insect-based diet. But the subsequent depletion of cover and food during autumn forces them to abandon these regions in search of most suitable wintering areas.



This chapter is based upon the manuscript: **Pérez-Rodríguez, A.**, Fernández-González, S., De la Hera, I., Pérez-Tris, J. (2013) Finding the appropriate variables to model the distribution of vector-borne parasites with different environmental preferences: climate is not enough. *Global Change Biology*. In press. doi: 10.1111/gcb.12226

## CHAPTER II: ECOLOGICAL DETERMINANTS OF PARASITE DIVERSITY

*Abstract:* Understanding how environmental variation influences the distribution of parasite diversity is critical if we are to anticipate disease emergence risks associated with global change. However, choosing the relevant variables for modelling current and future parasite distributions may be difficult: candidate predictors are many, and they seldom are statistically independent. This problem often leads to simplistic models of current and projected future parasite distributions, with climatic variables prioritized over potentially important landscape features or host population attributes. We studied avian blood parasites of the genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon* (which are viewed as potential emergent pathogens) in 37 Iberian blackcap *Sylvia atricapilla* populations. We used Partial Least Squares regression to assess the relative importance of a wide array of putative determinants of variation in the diversity of these parasites, including climate, landscape features and host population migration. Both prevalence and richness of parasites were predominantly related to climate (an effect which was primarily, but not exclusively driven by variation in temperature), but landscape features and host migration also explained variation in parasite diversity. Remarkably, different models emerged for each parasite genus, although all parasites were studied in the same host species. Our results show that parasite distribution models, which are usually based on climatic variables alone, improve by including other types of predictors. Moreover, closely related parasites may show different relationships to the same environmental influences (both in magnitude and direction). Thus a model used to develop one parasite distribution can probably not be applied identically even to the most similar host-parasite systems.

*Keywords:* Avian haemosporidians, Blackcap *Sylvia atricapilla*, Environmental constrains, *Haemoproteus*, Host migration, *Leucocytozoon*, *Plasmodium*, Partial Least Squares regression.

### Introduction

Global patterns of climate and landscape variation are major determinants of the distribution of biodiversity (Pearson & Dawson, 2003; Foley *et al.*, 2005), a tenet which is also true for parasites. Given that parasitic diseases are both a major ecological factor affecting wildlife (Combes, 2005)

and a public health concern (Daszak *et al.*, 2000), unravelling the environmental correlates of parasite diversity, transmission success and virulence has deserved considerable attention (Patz *et al.*, 2000; Altizer *et al.*, 2006; Lafferty, 2009; Mostowj & Engelständer, 2011). Furthermore, with increasing global change (widespread climate change and large-scale man-

made habitat modifications; Vitousek, 1994; Sala *et al.*, 2000), understanding current parasite distributions and their environmental determinants becomes urgent, in order to anticipate future risks of disease spread and emergence (Peterson, 2008; Fuller *et al.*, 2012).

The relationships between parasites and the environment are difficult to comprehend because parasites depend on their hosts to complete their life cycles. Thus, it is hard to determine whether a given environmental feature determines the probability of the occurrence of a parasite directly or indirectly through their hosts (Hance *et al.*, 2007; Cardon *et al.*, 2011). Moreover, many parasites are transmitted among hosts by vectors (usually ectothermic, seasonally-active invertebrates), and this further complicates the ways in which parasites may be affected by climatic and landscape features (Dobson & Carper, 1992; Patz *et al.*, 2000; Gage *et al.*, 2008; Loiseau *et al.*, 2011a). However, most parasite distribution models have so far relied on climatic variables alone, largely neglecting the potential role that landscape features or variation in host population attributes could play as determinants of parasite diversity. But the question remains whether parasite distributions may be adequately modelled based on climate variables alone, or if the models may significantly improve by including landscape variables or host population attributes as predictors (Lafferty 2009; Hof *et al.*, 2012). Answering this question will improve our understanding of parasite distributions, as well as our predictions regarding their future configuration under different global change scenarios.

Avian haemosporidians (including species of *Plasmodium*, *Haemoproteus* and *Leucocytozoon*, order Haemospororida; Valkiūnas,

2005) are a closely related group of vector-borne parasites (Outlaw & Ricklefs, 2011). They are harmful to their hosts (Merino *et al.*, 2000; Marzal *et al.*, 2008; Knowles *et al.*, 2010), and are infamous for the conservation problems caused by their introduction in naïve host populations isolated on oceanic archipelagos (Atkinson & van Riper III, 1991; Freed *et al.*, 2005; Howe *et al.*, 2012). They are easy to study in wild bird populations, and have become models for research on host-parasite relationships and their evolutionary and ecological implications (Bensch *et al.*, 2009). But in spite of their importance, there is a general lack of knowledge of the match between ecological features and diversity of avian haemosporidians. Few studies have developed predictive models for these parasites, most of which focused on *Plasmodium* (Sehgal *et al.*, 2010; Loiseau *et al.*, 2012; 2013). Consequently, knowledge of the environmental requirements of parasites and their vectors is poorly known in comparison with that of human malaria parasites (Carter *et al.*, 2000; Drakeley *et al.*, 2006; Murdock *et al.*, 2012). Nevertheless, it is anticipated that avian haemosporidians will probably expand their ranges with global warming (Garamszegi, 2011; Zamora-Vilchis *et al.*, 2012, Loiseau *et al.*, 2013). Broad-scale habitat modifications are also responsible for changes in their richness and prevalence, although studies on the subject are scarce (Chasar *et al.*, 2009; Sehgal, 2010).

Together with environmental features, an important determinant of the diversity of avian parasites may be the migratory behaviour of their host populations. Migratory birds may be exposed to different parasite faunas throughout the year, as they regularly visit multiple sites, potentially separated by long distance, by moving between

breeding and nonbreeding grounds. As a consequence, migratory birds are expected to harbour more diverse parasite communities than their sedentary relatives. This has been extensively documented in birds and their pathogens (Peterson *et al.*, 2008; Altizer *et al.*, 2011), including all avian haemosporidian genera (Figuerola & Green, 2000; Waldenström *et al.*, 2002; Jenkins *et al.*, 2012); but to our best knowledge host migration is still to be implemented as a predictor variable in parasite diversity modelling.

We studied which environmental factors best explain variation in haemosporidian parasite richness and prevalence among Iberian populations of blackcaps (*Sylvia atricapilla*). The blackcap is an excellent model species for our purposes because it harbours a diverse community of haemosporidians, many of which are strict blackcap-specific parasites which often attain high prevalences that vary among geographic areas (Pérez-Tris & Bensch 2005b; Pérez-Tris *et al.*, 2007; Križanauskienė *et al.*, 2010). By studying parasite richness and prevalence in a single host species, we overcome the problem of differential parasite detection associated with heterogeneous sample size among host species (Dimitrov *et al.*, 2010; Loiseau *et al.*, 2011a). Also conveniently, the Iberian Peninsula shows marked topographic and environmental heterogeneity at a reduced geographic scale, and blackcaps span most of the range of Iberian environmental conditions (Carbonell, 2003). In addition, Iberian blackcaps include both migratory and sedentary populations (Tellería *et al.*, 2001).

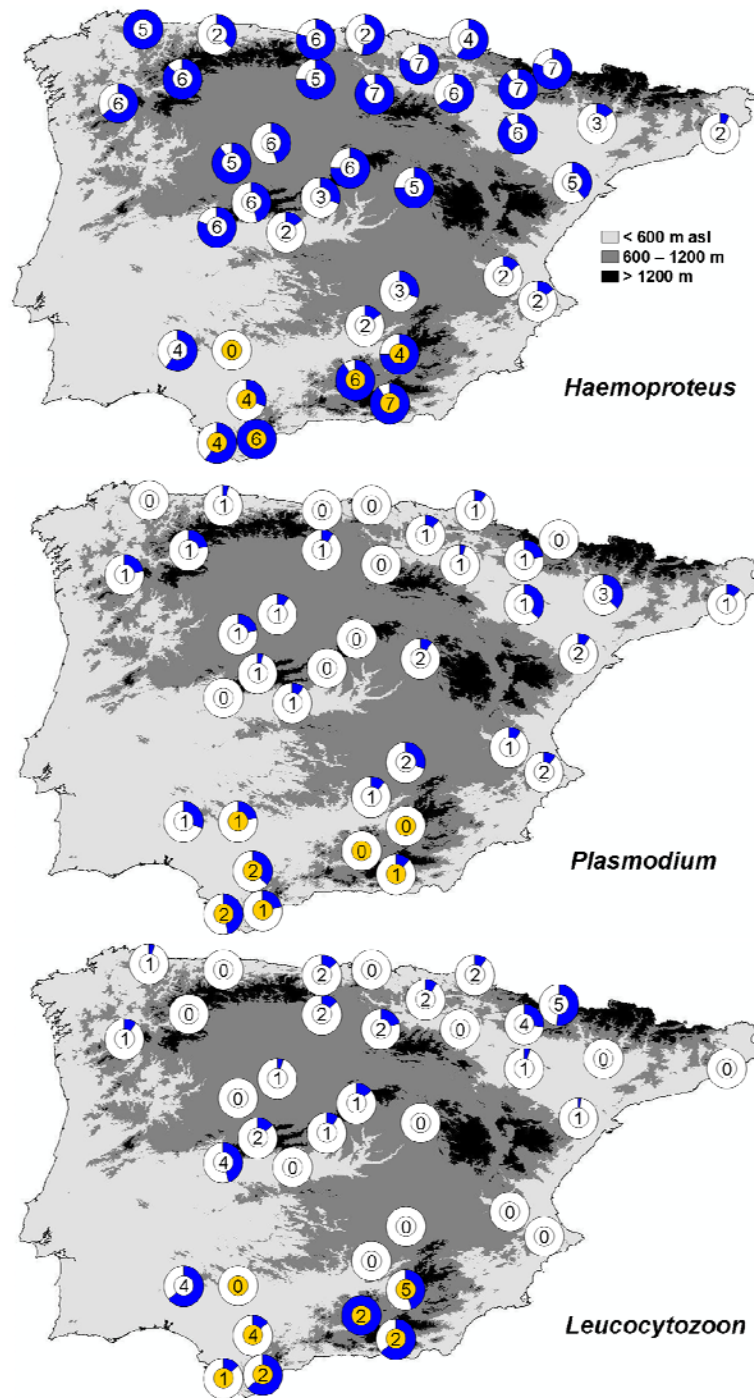
Our goal is to assess whether the relationships between environmental or host population attributes and parasite prevalence and richness are similar or

vary among the three parasite genera (the latter possibility may be expected because their vectors have different environmental needs and each parasite type may face different physiological constraints). Given the need for solid conceptual and empirical foundations for developing models of parasite distribution under different global change scenarios, we also set out to test if climate-based parasite distribution models may be improved by considering variables such as landscape and host population attributes (Lafferty 2009; Hof *et al.*, 2012). In a context of global change, only a deep knowledge of whether and how different environmental factors may affect host-parasite interactions, including how parasite-environment relationships may vary among parasite types, will allow us to foresee future parasite impacts and their potential effects on biodiversity.

## Material and Methods

### *Statistical analyses*

To meet statistical requirements, all variables were log- (numerical data) or arcsin-transformed (proportion data). We used Partial Least Squares regression (PLS) to test for the effects of different variables on richness and prevalence of each parasite genus. PLS is an extension of the multiple regression analysis explicitly designed to deal with databases in which the predictors are numerous (especially if there are more predictors than study cases) and potentially highly collinear (Carrascal *et al.*, 2009). PLS regression gathers the predictors in one or several multivariate factors according to the underlying relationships among the original variables. It considers both the dependent variable and the predictors at the same time and forces them to maximize the explained variance of the former. If there is more than one



**Figure 11.** Haplotype richness and prevalence per parasite genus. The degree of filling of the outer circle represents parasite prevalence (%). The number in the inner circle indicates the number of different haplotypes detected. The colour of inner circles distinguishes between sedentary (shaded) and migratory (white) blackcap *S. atricapilla* populations.

significant factor, they are orthogonal and account for successive lower proportions of the original variance of the dependent variable. The factor or factors extracted can be interpreted as

environmental gradients defined by a particular ordering of the predictors (Carrascal *et al.*, 2009). In each factor, every predictor is given a weight that represents its particular importance on

defining the factor, regardless of its collinearity with the other predictors. Therefore, PLS allows analysis of the effect of variables that, despite being highly correlated, can explain different aspects of the variation in the response variable which would be dismissed by other methods.

Although rarely used in ecological studies, PLS is viewed to perform equally or better than other regression methods that handle highly collinear data, such as stepwise multiple regression, Principal Components regression, or model fitting techniques that apply Maximum Likelihood or Bayesian theory (Helland, 2001; Carrascal *et al.*, 2009). PLS is preferred above other methods when the number of predictors is high for the available sample size and error variance is large (Garthwaite, 1994; Carrascal *et al.*, 2009). Importantly, PLS regression also allows a first assessment of the relative importance of each of a large set of predictors to be made, which is helpful for selecting the appropriate variables (or their surrogates instead) before designing more specific predictive models.

To perform the PLS analyses we used the NIPALS algorithm with seven-fold cross-validation implemented in STATISTICA 7.0 (StatSoft, 2004). The NIPALS algorithm evaluates the significance of each factor assessing not only the proportion of the original variance explained by it, but also its predictive power through cross-validation. The relative contribution of each predictor to the derived factors and its significance can be assessed by the square of its predictor weight: given that the sum of all the square predictor weights equals 100% of the explained variance, every variable with a square weight greater than  $1/k$  (where  $k$  is the number of predictor variables)

significantly contributes to the final design of the factor. Conversely, the general  $R^2$  of each factor can be decomposed among predictors, as they contribute to it proportionally to their square predictor weights. In order to test whether adding non-climatic predictors improved climate-based models, we conducted PLS analyses on 100 databases obtained by bootstrap (with replacement) of the original data. We derived significance values for the difference between the models that included all variables and climate-only models from pairwise comparisons of  $R^2$  values obtained using bootstrapped data.

We are aware that the effects of climatic or habitat variables do not need to be linear, but the inclusion of the quadratic terms of these variables in preliminary analyses did not qualitatively affect our results. In models including linear and quadratic terms together, quadratic terms were given similar or lower weights compared to the corresponding linear terms. Total variance explained often remained unchanged or, in some instances, even decreased (~2%) when quadratic terms were added in the models. Therefore, we decided to analyse our data using linear terms alone.

## Results

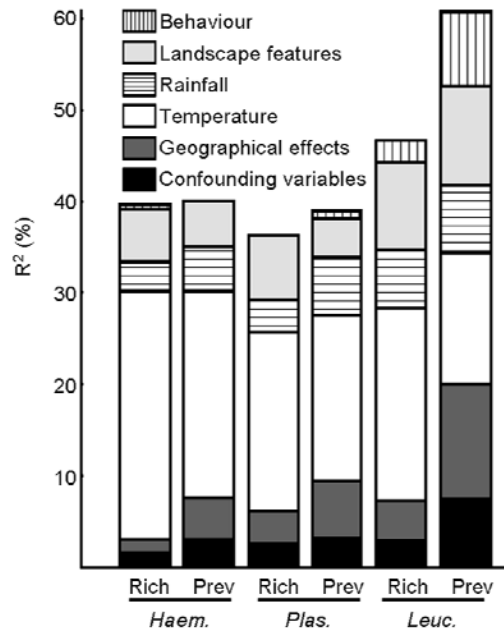
### *Parasite screening*

In all, 65.3% of the blackcaps screened were infected by at least one of the three parasite genera, but parasite prevalence and richness greatly varied among localities (Fig. 11, Table S1). *Haemoproteus* appeared in all but one of the localities, with an average prevalence of 58.2%, followed by *Plasmodium* (27 localities, 13.3% prevalence) and then *Leucocytozoon* (23

CHAPTER II

**Table 1.** Partial Least Squares regression weights of each predictor variable on the factors (columns) retained in the models obtained for each dependent variable (haplotype richness and prevalence of each parasite genus). In all cases, only one factor was retained in the final model. For the sake of clarity, independent variables have been grouped into different categories (whose joint contributions are shown in Fig. 12), and the variables that did not significantly contribute to explain variance in any of the dependent variables have been omitted (see the complete structure of factors in Table S4). R<sup>2</sup> values of each model are indicated at the bottom of the table.

| Variable   | <i>Haemoproteus</i> |             | <i>Plasmodium</i> |             | <i>Leucocytozoon</i> |             |
|--|---------------------|-------------|-------------------|-------------|----------------------|-------------|
|  | Richness            | Prevalence  | Richness          | Prevalence  | Richness             | Prevalence  |
| <b>Confounding variables</b>                             |                     |             |                   |             |                      |             |
| Proportion of adults                                     |                     |             |                   |             |                      | 0.151       |
| Sampling year: 2008                                      | 0.157               | 0.184       | -0.172            | -0.197      | -0.144               | 0.254       |
| Sampling year: 2009                                      |                     | -0.170      |                   |             |                      | -0.187      |
| Sampling year: 2010                                      |                     |             |                   |             | 0.166                |             |
| <b>Geographical effects</b>                              |                     |             |                   |             |                      |             |
| Latitude (Y)   |                     |             |                   | -0.160      |                      | -0.197      |
| X*Y  |                     |             |                   | 0.200       | 0.160                |             |
| Y <sup>2</sup>   |                     | 0.148       |                   |             | 0.151                | 0.237       |
| X*Y <sup>2</sup>   |                     |             |                   | -0.147      |                      |             |
| X <sup>3</sup>   |                     | -0.185      |                   |             |                      |             |
| Y <sup>3</sup>   |                     |             |                   | -0.197      |                      | -0.233      |
| <b>Temperature</b>                                       |                     |             |                   |             |                      |             |
| Annual mean temperature                                  | -0.275              | -0.221      | 0.232             | 0.221       | -0.212               |             |
| Maximum annual mean temperature                          | -0.274              | -0.238      | 0.246             | 0.233       | -0.211               |             |
| Minimum annual mean temperature                          | -0.253              | -0.188      | 0.207             | 0.199       | -0.200               | -0.151      |
| Breeding mean temperature                                | -0.265              | -0.226      | 0.247             | 0.233       | -0.232               | -0.165      |
| Maximum breeding mean temperature                        | -0.262              | -0.247      | 0.266             | 0.251       | -0.235               |             |
| Minimum breeding mean temperature                        | -0.245              | -0.194      | 0.227             | 0.217       | -0.207               | -0.165      |
| Minimum temperature of the coldest month                 |                     | 0.169       |                   |             |                      |             |
| Temperature annual range                                 |                     | -0.147      |                   |             |                      |             |
| Temperature mean diurnal range                           | -0.200              | -0.231      | 0.182             | 0.169       | -0.145               |             |
| Isothermality (mean diurnal range/annual range)          | -0.219              |             | 0.161             | 0.162       | -0.177               | -0.153      |
| Mean temperature of the wettest quarter                  | -0.205              | -0.200      | 0.204             | 0.144       | -0.208               | -0.211      |
| Mean temperature of the warmest quarter                  | -0.271              | -0.271      | 0.230             | 0.208       | -0.209               |             |
| Mean temperature of the coldest quarter                  | -0.237              | -0.148      | 0.189             | 0.184       | -0.174               |             |
| <b>Rainfall</b>  |                     |             |                   |             |                      |             |
| Annual rainfall  |                     | 0.143       |                   |             | 0.165                |             |
| Breeding rainfall  |                     | 0.148       |                   |             | 0.199                |             |
| Rainfall of the driest month                             |                     |             | -0.155            | -0.219      |                      |             |
| Rainfall seasonality (coefficient of variation)          | -0.154              |             |                   | 0.174       |                      |             |
| Rainfall of the wettest quarter                          |                     |             |                   |             | 0.144                |             |
| Rainfall of the driest quarter                           |                     |             |                   | -0.197      |                      |             |
| Rainfall of the warmest quarter                          |                     |             |                   | -0.182      |                      |             |
| Rainfall of the coldest quarter                          |                     | 0.158       |                   |             | 0.187                | 0.176       |
| <b>Landscape features</b>                                |                     |             |                   |             |                      |             |
| Altitude   | 0.158               |             |                   |             | 0.185                | 0.169       |
| Slope  |                     |             | -0.228            | -0.175      | 0.272                | 0.309       |
| Percentage of broadleaf forest                           |                     |             | -0.185            |             | 0.181                |             |
| Percentage of wooded croplands                           | -0.269              | -0.264      |                   |             |                      |             |
| Percentage of arable land                                |                     |             | 0.153             |             |                      |             |
| Percentage of urban areas                                |                     |             |                   |             | -0.202               | -0.171      |
| <b>Behaviour</b>   |                     |             |                   |             |                      |             |
| Migration: Sedentarism*                                  |                     |             |                   |             | 0.151                | 0.259       |
| <b>R<sup>2</sup> of the model (% variance explained)</b> | <b>39.5</b>         | <b>40.0</b> | <b>36.1</b>       | <b>38.8</b> | <b>46.5</b>          | <b>60.6</b> |



**Figure 12.** Decomposition of  $R^2$  values (expressed as percentage of variance explained) of PLS regression models into partial contributions of different types of predictors. The figure shows the results of models built for variation in haplotype richness (Rich) and prevalence (Prev) of parasites of the three genera (Haem.: *Haemoproteus*; Plas.: *Plasmodium*; Leuc.: *Leucocytozoon*).

localities, 17.0% prevalence). Although we failed to determine haplotype identity in a number of parasite infections, we found a total of 24 parasite haplotypes (Fig. 11, Table S2). Unidentified parasite haplotypes were evenly distributed across localities, so they do not affect our estimates of parasite richness per locality. Neither parasite richness nor prevalence of any of the three genera showed any significant relationship with locality sampling size (all  $p > 0.05$ ), and a 1000-fold bootstrap calculation with replacement of mean parasite richness and prevalence per locality revealed no significant differences with the actual dataset (all correlations between field and bootstrapped values were highly significant  $p < 0.001$ ).

### PLS analyses

The comparison of our PLS models with alternative models built with climatic variables alone showed that in all cases adding non-climatic variables significantly improved climate-based models (Table S3). PLS analyses always yielded only a single significant multivariate factor for each dependent variable. In general, both richness and prevalence followed the same trends for each parasite genus (Table 1, Fig. 12, and Table S4).

Temperature-related variables showed a strong negative relationship with *Haemoproteus* richness. Rainfall was not noticeably associated with *Haemoproteus* richness, which was instead negatively correlated with the abundance of wooded croplands. *Haemoproteus* prevalence followed nearly the same trends.

*Plasmodium* in turn was found to thrive in warmer sites: its richness and prevalence increased with temperature, towards the south and near the coastal regions of the Iberian Peninsula. Rainfall variables were also conspicuously associated with *Plasmodium* prevalence, which rose up when rainfall was concentrated out of the warmest months. In terms of habitat features, *Plasmodium* favoured also flat, open areas.

*Leucocytozoon* was the only parasite genus showing a significant relationship with host migratory behaviour; both richness and prevalence were higher in sedentary blackcap populations. Landscape characteristics, noticeably terrain steepness and the presence of areas with a high percentage of broadleaf forests and few human settlements, were related with greater *Leucocytozoon* diversity, as well as year-round cold and rainy locations,



which were located both in the north of the Iberian Peninsula and in the southern mountain ranges.

## DISCUSSION

Our results reveal the types of environmental variables that best explain the geographical variation in prevalence and richness of vector-borne parasites in Iberian blackcaps. PLS regression analyses were able to detect major gradients of environmental configurations, as defined by particular combinations of values of the predictor variables, which adequately explained variation in parasite diversity. Geographic variation in temperature and precipitation, but also in factors other than climate, is associated with variation in prevalence or haplotype richness of all parasite genera analysed. However, *Haemoproteus*, *Plasmodium* and *Leucocytozoon*, while phylogenetically closely related, do not respond in the same way to environmental variation.

The amount of variance in parasite richness and prevalence explained by PLS analyses ranged between 36-61%, and it was greatest for *Leucocytozoon*, then for *Haemoproteus* and finally for *Plasmodium*. Variation among genera in the amount of variance explained by PLS models can be related to differences in their degree of environmental specialization. This has a great influence on the performance of predictive models, which is usually higher for specialist species (Evangelista *et al.*, 2008). Environmental specialization could be mediated either by constraints on the parasites themselves or by their vectors (Loiseau *et al.*, 2011a); *Plasmodium* parasites often are host generalists transmitted by a wide array of ornithophilic culicine mosquitoes (Gager *et al.*, 2008; Kimura *et al.*, 2010;

Njabo *et al.*, 2010), whereas *Haemoproteus* and *Leucocytozoon* establish more specific associations with both their avian hosts and their insect vectors (Valkiūnas & Iezhova, 2004; Hellgren *et al.*, 2008; Martínez-de la Puente *et al.*, 2011). The vectors of these two genera are also known to be more habitat-specialists: *Haemoproteus* vectors (*Culicoides* biting midges, Fam. Ceratopogonidae) rely on constant ground humidity, and *Leucocytozoon* vectors (black flies, Fam. Simuliidae) require running, unpolluted water for breeding (Santiago-Alarcón *et al.*, 2012).

Climatic predictors accounted for the greatest proportion of explained variance of all response variables, determining two major trends of parasite distribution in the Iberian Peninsula. On the one hand, *Plasmodium* richness and prevalence increased in the warmest Iberian areas (the Mediterranean lowlands located towards the south and near coastlines). On the other hand, both *Haemoproteus* and *Leucocytozoon* were found to thrive in cooler locations, either in the north of the study region or in the southern mountain ranges. In sum, both groups of parasites were strongly influenced by the opposite trends. Thus birds living in different Iberian regions face fundamentally different parasite communities. Regarding landscape features, it is worth noting the importance of both altitude and slope for explaining geographic variation in parasite diversity. This is especially true for *Leucocytozoon*, though linkage to areas with steep slopes probably reflects the dependence of their insect vectors on running water. We found topographic variables important for parasite diversity modelling, which adds to recent literature advocating of the incorporation of these variables in ecological modelling (Hof *et al.*, 2012).

Land use, by contrast, did not play a prominent role as a predictor variable. Nevertheless, the negative effect of the percentage of urban areas on both prevalence and richness of *Leucocytozoon* can be interpreted as further evidence that haemosporidian prevalence decreases in disturbed habitats, as described for other host-parasite systems (Bonneaud *et al.*, 2009; Sehgal, 2010). Finally, sedentary blackcap populations scored higher richness and prevalence for *Leucocytozoon*. This result suggests that sedentary blackcaps face more complex parasite communities, an observation which is opposed to our initial expectations (Jenkins *et al.*, 2012) and deserves further research.

A putative problem linked to our geographically restricted sampling is that this excluded from our analyses part of parasites' geographical ranges (all three parasite genera have been found in blackcaps outside the Iberian Peninsula; Bensch *et al.*, 2009), and thus capture a fraction of the environmental range in which each parasite can thrive. As a consequence, the conclusions drawn from our analyses might only apply to the Iberian Peninsula, especially in relation to climatic effects (Beale *et al.*, 2008; Jiménez-Valverde *et al.*, 2009; Peterson *et al.*, 2009). Nevertheless, the localities sampled in our study span a wide range of climatic variation, with mean annual temperature ranging between 7.2 °C and 17.3 °C or mean annual rainfall ranging between 366.1 mm and 1361.9 mm. In addition, we found a strong climatic effect on parasite diversity. Therefore, even if the above effects could become more marked by including more sampling localities, we believe that their relative magnitudes or directions would not vary greatly.

The effect of climatic variables on parasite diversity is not always easy to interpret without the concurrence of other variables (Thomson *et al.*, 2005), hence the convenience of multivariate analyses such as PLS regression. The lack of statistical restrictions on the number of examined predictors allowed us to test several variables in a single analysis that, although potentially meaningful from a biological point of view, are often discarded in predictive modelling analyses for the sake of statistical simplicity. Forecasting parasite activity is largely constrained by the availability of accurate predictive models which correctly describe how parasite distributions are expected to change in the future. Although great effort has been devoted to creating predictive climatic models, the future evolution of habitats is far from clear (Stanton *et al.*, 2011), let alone whether migratory behaviour will evolve and change among geographic areas (Pulido & Berthold, 2010). Even so, we have shown that variation in parasite diversity is mainly related to climatic or virtually static variables (such as altitude or slope), so future predictions of host-parasite interactions are likely relevant. For instance, according to the general trends shown in this paper, a process of climate warming is likely to lead the current scenario of host-parasite interactions to one in which *Plasmodium* may increase its range at the expense of both *Haemoproteus* and *Leucocytozoon*, which are likely to lose areas of high diversity in the Iberian Peninsula. This brings a word of caution as well, because there is always a risk of applying the assumptions derived from particular host-parasite systems to others simply because they include related species. A deep knowledge of parasite ecology is vital before making such extrapolations; given that closely related parasites, as we show here, can follow different environmental trends.

Anticipating the relationship between parasite diversity and the emergence of infectious diseases in a changing world is urgent (Fuller *et al.*, 2012). Conveniently, we demonstrate that environmental variables are reliable predictors of present-time parasite diversity among Iberian blackcap populations, a circumstance which may be general to other host-parasite systems, even if the shape of parasite-environment relationships is likely to change among parasite groups. Species distribution modelling using environmental variables is a helpful tool

for developing robust predictions about how parasite diversity may change over time or across geographical areas, but this is particularly true providing that the appropriate variables are used. To this end, assessing the relative importance of candidate variables may prove an essential prior step before developing predictive models. Better models, based on an appropriate selection of predictor variables, will help us to avoid being caught off guard by the consequences of global change on the natural systems.



In the Mediterranean regions subjected to an intense summer drought, blackcaps occur where the presence of water courses sustain overgrowth riparian woods, most noticeably those including dense bramble thickets. If in the future the climate is to become harsher, the preservation of these habitats would become more and more crucial in order to preserve blackcaps as well as many other species.

This chapter is based upon the manuscript: **Pérez-Rodríguez, A.**, De la Hera, I., Fernández-González, S., Pérez-Tris, J. Global warming will reshuffle the areas of high influence of three genera of avian haemosporidians. *In preparation*

## CHAPTER III: PARASITE RANGE SHIFTS AND GLOBAL WARMING

*Abstract:* The impact of infectious diseases on wild populations is directly linked to the local diversity and abundance of parasites. In a geographic context, populations living in areas where parasites are most diverse and prevalent should be under stronger parasite-mediated selection, and dispersal into these populations from less affected areas should involve fitness costs. We used MaxEnt to model the distribution of areas of high impact of *Plasmodium*, *Haemoproteus* and *Leucocytozoon* parasites among Iberian blackcap (*Sylvia atricapilla*) populations, both at present and by the end of the XXI century, assuming three future scenarios that foresee light, medium and severe changes in climate. We specifically tested (1) whether parasites are expected to undergo range shifts that modify the degree of exposure to parasites of host populations, and (2) if differences among localities in the probability of high parasite impact will remain the same or will change in the future, thereby altering the relative impact of parasites in a geographic context. Our models show that both *Haemoproteus* and *Leucocytozoon* will lose areas of high richness and prevalence in the future, whereas *Plasmodium* is expected to expand its range of high impact. In addition, among-population differences in the expected impact of parasites will decrease in the future, most clearly for *Haemoproteus* (currently the most common parasite genus in blackcaps). Future parasite impacts are expected to become more dependent on local environmental configurations than on broad-scale trends when our models assumed future scenarios of major change in climatic conditions. Regardless of the global warming scenario considered, our predictions foresee an altered geographic mosaic of blackcap-parasite relationships, which will modify the interaction arena in which the impact of parasites on host populations evolves.

*Keywords:* Avian haemosporidians, Blackcap *Sylvia atricapilla*, Global warming, *Haemoproteus*, *Leucocytozoon*, MaxEnt, *Plasmodium*, Species Distribution Modelling

### Introduction

Among the many factors influencing the population dynamics of species, the impact of parasites is not to be neglected: a growing body of scientific literature shows how parasites affect host population attributes as varied as life history traits (Møller, 1997), reproductive success (Gustafsson *et al.*,

1994), genetic diversity (Decaestecker *et al.*, 2007) or predation risk (Lafferty & Morris, 1996). However, parasite impacts are unevenly distributed throughout host species' ranges. In principle, the impact of parasites is expected to be stronger on host populations that face more diverse parasite communities, or where more virulent parasites are abundant (Petney

& Andrews, 1998; Pedersen & Fenton, 2007; Smith *et al.*, 2009). These features of the parasitic landscape may depend on many interacting factors, including environmental constraints that may differently affect the performance of each kind of parasites (Hechinger & Lafferty, 2005; Crossan *et al.*, 2007; Dobson *et al.*, 2008; Mostowj & Engelstädter, 2011). The link of parasite ranges with environmental features has prompted a growing interest in using Species Distribution Modelling tools (SDM; Guisan & Zimmermann, 2000) to predict parasite occurrence in space and time. But whereas many studies have assessed which areas are likely to harbour a given parasite or group of parasites (occurrence; Patz *et al.*, 2000; Lafferty, 2009), the number of studies forecasting actual parasite impacts (abundance) is in comparison quite reduced (Thomson *et al.*, 2006; González *et al.*, 2010; Loiseau *et al.*, 2013). If we are to anticipate potential disease emergence risks, we are urged to improve our ability to predict where parasite impacts are likely to be stronger, both currently and in the future (Fuller *et al.*, 2012).

Parasite impacts may be viewed to evolve in a geographic mosaic of host-parasite interactions (Thompson, 2005), in which host populations living in areas where parasites are most diverse and prevalent should be under stronger parasite-driven selection, and individual hosts that disperse into these areas from less parasite-affected areas may incur fitness costs (Møller & Szep, 2011). From this perspective, it is important to know whether different types of parasites affecting one host species will likely undergo parallel or dissimilar shifts in the distribution of their impact among host populations. If the different elements of the parasite community shift their distributions in similar ways, overall prevalence or

richness of parasites would increase or decrease among localities due to climate change, but such changes would not modify the relative impact of different parasite types among localities. However, if each parasite underwent distinct shifts in the geographic distribution of its impacts, climate change would modify the assembly of component parasite communities (the subsets of parasite diversity that occur in each locality where the host is found; Poulin, 2007), thereby altering the geographic mosaic of host-parasite interactions.

Vector-borne parasites are particularly dependent on environmental variables, because both the life cycle and activity regimes of invertebrate vectors are tightly constrained by climate (Kovats *et al.*, 2001; Cumming & Guégan, 2006; Gager *et al.*, 2008). This makes vector-borne parasites good candidates to shift their distribution with climate change (Thomson *et al.*, 2006; Peterson, 2009; González *et al.*, 2010; Costa & Peterson, 2012), a relevant fact given that these include the causal agents of notorious diseases such as malaria, filariasis or trypanosomiasis (Tabacknick, 2010). But although disease impacts (including those caused by vector-borne parasites) are major threats to biodiversity, we still lack specific models to forecast disease risks of most wildlife parasites (Smith *et al.*, 2009). From this perspective, avian haemosporidian parasites (protozoans of the genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon*) are convenient for modelling disease impacts (Fuller *et al.*, 2012). These parasites have a negative effect on their hosts' fitness (Marzal *et al.*, 2008; Knowles *et al.*, 2010; Palinauskas *et al.*, 2011), and consequently may drive the evolution of many hosts' traits (Møller & Nielsen, 2007; Arriero & Møller, 2008;

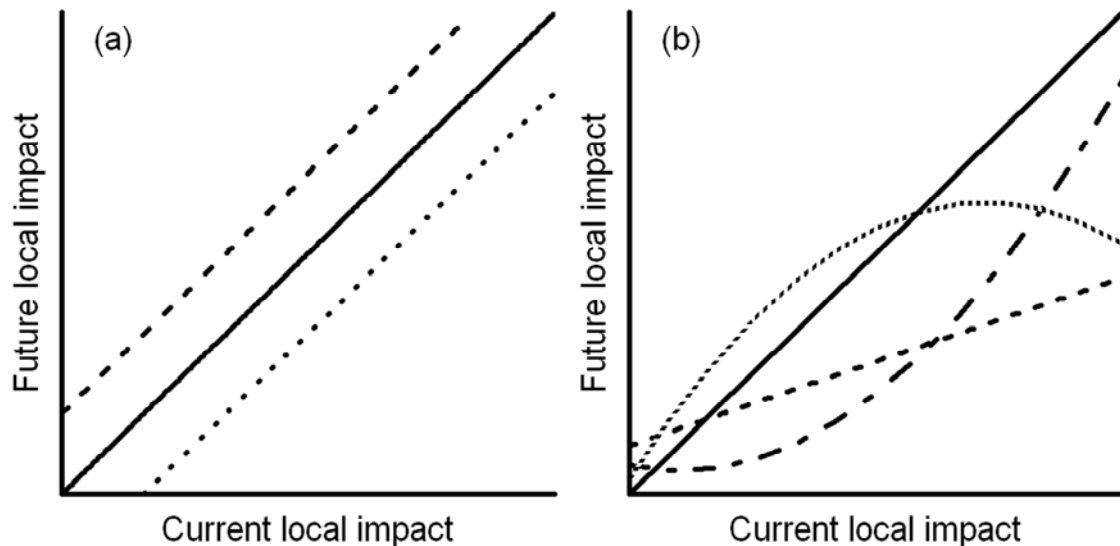
Garamszegi & Møller, 2012; Westerdahl *et al.*, 2012). Moreover, haemosporidian parasites (especially *Plasmodium*) are infamous for their capacity to establish in areas where they are accidentally introduced (Ewen *et al.*, 2012) and the conservation problems they may cause to naïve host species that become affected (LaPointe *et al.*, 2012). Despite the interest of these parasites, there are few predictive models of their distribution (Sehgal *et al.*, 2010; Loiseau *et al.*, 2012; 2013), and these have seldom explicitly attempted to predict where different parasite types are expected to exert their strongest impact on host populations, or how the geographic distribution of relative parasite impacts is likely to change in the future.

We used a SDM approach to analyse the current and future geographic distribution of prevalence and richness of *Plasmodium*, *Haemoproteus* and *Leucocytozoon* among Iberian populations of a passerine bird species, the blackcap *Sylvia atricapilla*. Our goal was to model where in the Iberian Peninsula blackcaps are likely to face high impacts of each of these parasites, and to predict how the foreseen climate change towards the end of the century may modify the spatial configuration of such impacts. Iberian blackcaps make an excellent model to study diversity of avian blood parasites because of various reasons. Firstly, blackcaps are host of a highly diverse assemblage of haemosporidian parasites (Pérez-Tris *et al.* 2007), which varies in richness and prevalence among the wide range of Iberian habitats occupied by the species (Chapter II). This variation is primarily explained by climatic and orographic variables, but it is largely independent of other environmental influences (such as land uses or host population attributes; Chapter II), which are more

difficult to implement in SMD (Franklin, 2010). Therefore, it is possible to predict how blackcap parasite communities are likely to change in the future relying on the realistic assumption that preferences of each parasite genus in relation to climatic and orographic influences will remain the same as observed today. Secondly, modelling the distribution of parasites of a single host species removes any bias derived from the different composition of host communities sampled in different localities, or from variable sampling effort among host species. Thirdly, climate change takes place at the global scale, but its effects are heterogeneous in space (Walther *et al.*, 2002), so a geographically restricted scenario (such as the Iberian Peninsula) is desirable for the study of its fine-grained expected impacts, which may not be correctly captured by models based on data from locations distantly located and climatically too contrasted. Finally, the Iberian Peninsula is considered to be a natural entrance of tropical pathogens into Europe (e.g., Sáinz-Elipe *et al.*, 2010), so it is very important to have explicit models that deal with expected parasite geographic range shifts in this region in the decades to come.

Adding interest to our study system, the environmental preferences of haemosporidian parasites found in Iberian blackcaps vary among genera (Chapter II), what may render the general patterns of expansion or retreat of this group of parasites under climate change more complex than usually viewed. In principle, the fact that parasites of the genera *Haemoproteus* and *Leucocytozoon* favour fresh environments, while *Plasmodium* parasites favour warm areas, predict different trends of range shift among the three genera in a context of global warming (Chapter II). However, the





**Figure 13.** Example models of the variation between current and future expectations of high parasite impact. The solid line represents a scenario in which parasite impact has the same probability to be high today and in the future. The other lines indicate possible ways in which future values may differ from present-time ones. In (a), parasite impact increases (dashed line) or decreases (dotted line) equally in all localities. In (b) future parasite impact varies in different ways, with different consequences regarding the distribution of parasite impacts among future populations, as compared to the current situation.

magnitude of the change in the expected impact of each parasite genus might differ under different climate change scenarios, a possibility which remains to be investigated. More importantly, the fact that different parasite genera may undergo dissimilar distribution shifts in the future may affect the structure of parasite component communities. This may modify the pattern of variation in parasite impact among host populations, thereby reshuffling the geographic mosaic of host-parasite interactions as we observe it today.

We set out to investigate (1) whether *Haemoproteus*, *Plasmodium* and *Leucocytozoon* are expected to gain or lose areas of high impact among Iberian blackcap populations, (2) how such predictions change under different climate change scenarios, and (3) how future differences among populations in the predicted impact of each parasite genus compares with present-time predicted impacts. A way to explore the

latter issue is to consider the shape of the relationship between current and future predictions regarding local parasite impacts (Figure 13). For a given parasite, a linear relationship with slope equal to one would support a homogeneous pattern of variation between current and future predictions of parasite impact across the blackcap's Iberian range. Such pattern would indicate that the parasite is expected to either gain or loss areas of high impact in the future (the direction and magnitude of the change would be indicated by the intercept of the regression line; Figure 13a); however, this range shift would not modify relative differences in parasite impact among host populations. On the contrary, non-linear relationships, or regression slopes different from one, would suggest alternative scenarios, in which among-population differences in future parasite impact are expected to change along the range of current impacts. For example, if the slope of the

relationship between current and future model predictions of parasite impact is lower than one, then relative differences in parasite impact among localities are expected to lessen in the future, thereby decreasing the variance in fitness among host populations attributed to heterogeneously distributed parasite impacts. However, among-population inequalities are expected to increase in the future if the slope of the relationship is higher than one. Moreover, the relationship between current and future conditions may not be linear, so that the slope of the relationship may change along the range of variation of present-time parasite impacts (Figure 13b).

In turn, some parasites may increase local impacts at the expense of others (Pedersen & Fenton, 2007), and such changes may be more accentuated in some regions than in others. In addition, future changes in the composition of parasite assemblages may either exacerbate or ease parasite pressures upon local bird populations depending on the relative virulence of the parasites that are locally favoured (for instance, *Plasmodium* is generally viewed as the most harmful for the host of the three parasite genera studied here; Valkiūnas, 2005). Which of the alternative scenarios depicted above for the geographic layout of future host-multiparasite interactions is the most realistic one, remains an essential open question in our understanding of disease risks associated with climate change.

## Material and Methods

### *Parasite distribution models*

Given that our main interest was to model quantitative attributes of the distribution of parasites (differences in parasite richness and prevalence), we first analysed our data using Random Forest (Breiman, 2001), a machine

learning technique that allows the handling of continuous response variables. Random Forest has been shown to perform as a superior SDM tool in comparison with other techniques (Sehgal *et al.*, 2010; Bisrat *et al.*, 2012; Tôrres *et al.*, 2012; Loiseau *et al.*, 2013). However, the explanatory power of the obtained models was very low, arguably because of the difficulty to make accurate quantitative predictions of parasite prevalence or richness (see a detailed account of methods and results of these analyses in the Appendix S1). Consequently, we decided to sacrifice quantitative data to favour better prediction, for which we used MaxEnt, release 3.3.3 (Phillips *et al.*, 2006; Elith *et al.*, 2011). MaxEnt is a SDM software based on a maximum-entropy density approach: it takes as input a set of layers of environmental variables together with a set of geo-referenced localities with presence of a given organism. According to the characteristics of the places in which that organism occurs, the program produces a predictive model of its geographical range, which consists of a map of the studied region in which suitability for the presence of the assessed organism ranges from 0 to 1. Providing that the appropriate data layers are available, it can also elaborate predictions of suitability over different geographical areas or under climatic conditions different of those of the present time. MaxEnt has been used for predicting the current and future potential ranges of dipteran vectors (Foley *et al.*, 2010; Fischer *et al.*, 2011) and vector-borne diseases (Perkins *et al.*, 2007; Sehgal *et al.*, 2010; Machado-Machado, 2012).

Previous research allowed us to determine which among a large set of environmental variables (including climate, orography, land uses and host population attributes) best explain

geographic variation in prevalence and richness of haemosporidian parasites among Iberian blackcap populations (Chapter II). Based on this knowledge, we selected nine variables to construct MaxEnt models: altitude, steepness of terrain slope, and the bioclimatic variables bio01 (annual mean temperature), bio02 (temperature annual range), bio03 (temperature mean diurnal range), bio05 (isothermality), bio14 (rainfall of the driest month), bio15 (rainfall seasonality) and bio16 (rainfall of the wettest annual quarter). Given that MaxEnt uses only unique-event data (typically the presence of the species whose distribution is being modelled), we converted our quantitative measures of haplotype richness and prevalence of parasites into qualitative data. To do that, for each parasite genus we classified all localities as sites in which haplotype richness or prevalence scored values above (1) or below (0) the median of all localities. Thus, for each genus localities scoring 1 could be viewed as sites of high parasite impact, where haplotype richness or prevalence values are above present-time median values in the Iberian Peninsula; those localities are the ones we used for MaxEnt analyses. Therefore, what we actually modelled is the range of environmental configurations suitable for the occurrence of diverse and/or abundant parasite communities. We preferred the median rather than the mean as a reference for high or low local parasite impact because the former was a more central value in the distribution of prevalence and richness values. Nevertheless, we conducted our analyses using the mean as the reference, and our results remained qualitatively unchanged.

Future climatic conditions under the CGCM2 emission scenarios for the 2080-2100 period were downloaded

from the GCM Downscaled Data Portal (Ramírez & Jarvis, 2008). We considered three different scenarios for future climate conditions: A1b, A2a and B2a. A-group scenarios consider a future in which environmental protection is subordinated to economical growth, whereas B-group scenarios consider the opposite situation. Inside each group, family 1 scenarios put a stronger focus on global policies and family 2 in local ones. Every family is divided then in particular scenarios according to more specific settings. None of the scenarios take into account the adoption of specific measures to correct the causes or mitigate the effects of climate change. The different scenarios are described in detail in the Special Report of Emission Scenarios of the IPCC ([http://www.grida.no/publications/other/ipcc\\_sr](http://www.grida.no/publications/other/ipcc_sr)). With our scenario selection we tried thus to cover a wide array of forecasted conditions, including light (B2a), medium (A2a) and severe (A1b) change in future climate conditions.

Final MaxEnt outputs are the average of 100 bootstrap analyses for each dependent variable. In every run, a random 75% of the included localities was used as training data to construct the model and the remaining 25% was used as test data to assess the predictive power of the model. Model performance was assessed using the area under the receiver operator characteristic curve (AUC), which can be seen as the probability that the model assigns a higher occurrence probability to a randomly selected locality with presence of the analysed response variable than to a randomly selected absence point.

#### *Current–future conditions comparisons*

MaxEnt outputs were exported as GIS layers and then the predicted values of

the same set of 1000 random geographic locations were taken from each predictive map (present and future predictions, for the three scenarios of climate change considered). To examine variation between present and future conditions and the shape of this variation (i.e., whether the impact of all the three parasite genera varies in the same direction or not), the values of present and future conditions were compared with a repeated measures ANOVA. The relationships at a local scale between current and future predictions were examined as well using regression models, in which future predictions were the dependent variable and present predictions the independent variable. We tested for both linear and non-linear (quadratic) relationships, and conducted separate analyses for parasite richness and prevalence under the three scenarios of climate change. To assess which type of relationship (linear or non-linear) best fitted to the data, we compared the absolute residual values of each model by means of repeated-measures ANOVA. When a linear relationship was supported by the data, we tested for deviation of the regression slope from  $b = 1$ . All statistical analyses were performed using STATISTICA 7.0 (Statsoft, 2004).

## Results

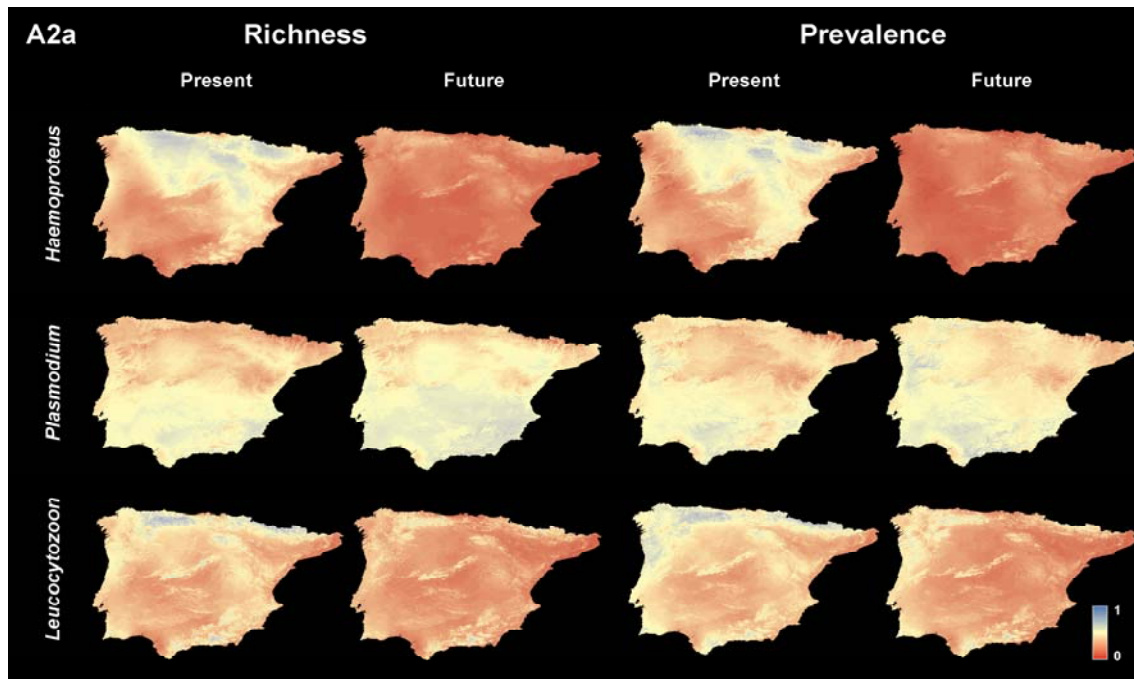
All MaxEnt predictive modelling outputs achieved good prediction scores (Table 2). According to present-time conditions, the areas with the highest *Haemoproteus* richness and prevalence are located in the northern half and the southern mountain ranges of the Iberian Peninsula. *Leucocytozoon* follows the same trends, but with a tighter link to the mountainous areas. In turn, *Plasmodium* high prevalence and richness are likely to occur throughout the whole Iberian Peninsula except for

highest latitudes and elevations. For the predicted climatic values by the end of the XXI century, clear differences among parasite genera arise: both *Haemoproteus* and *Leucocytozoon* areas of high impact are predicted to shift northwards and upwards in mountain ranges, whereas *Plasmodium* areas of high impact expand over the entire Iberian Peninsula (Figure 14; see Figure 3 for a depiction of the main Iberian mountain ranges).

**Table 2.** Evaluation of MaxEnt model performance based on the area under the ROC curve (AUC; average values of 100 replicates). The figures between parentheses indicate the number of localities out of the initial 37 that scored values of parasite richness or prevalence above the average, and were thus employed to construct each model. AUC-values range from 0 to 1 (perfect discriminative model); values of AUC above 0.7 can be considered good; values over 0.9 excellent (Phillips *et al.*, 2006).

| AUC scores    |            | Training sample | Test sample |
|---------------|------------|-----------------|-------------|
| <i>Haem.</i>  | Rich. (16) | 0.845           | 0.777       |
|               | Prev. (18) | 0.857           | 0.801       |
| <i>Plasm.</i> | Rich.(7)   | 0.838           | 0.738       |
|               | Prev. (18) | 0.817           | 0.746       |
| <i>Leuc.</i>  | Rich. (15) | 0.864           | 0.795       |
|               | Prev. (18) | 0.846           | 0.774       |

The repeated measures ANOVA analyses (Table S5) showed that the differences in the expected occurrence of areas of high local parasite impact between present and future conditions were highly significant for all parasite genera (Figure 15). We found a general rise on the expectation of the presence of areas with high impact of *Plasmodium*, and conversely a decrease on the predicted areas of high impact of *Haemoproteus* and *Leucocytozoon*. The choice of modelling scenario affected significantly such trends: under scenarios A1b and A2a (which foresee

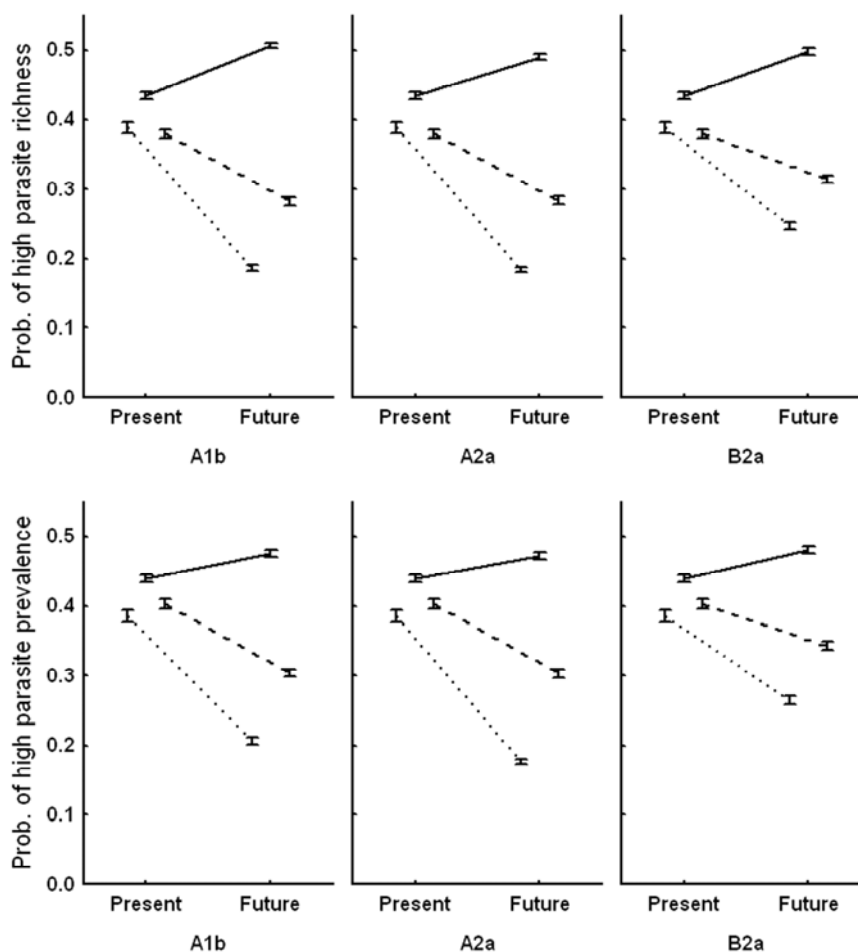


**Figure 14.** Present and future distribution of the probability of areas with high richness and prevalence of *Haemoproteus*, *Plasmodium* and *Leucocytozoon* in Iberian blackcaps. The colour range varies between red (0) and blue (1). Future distribution of probability is projected according to a scenario for future climate assuming medium changes (A2a emission scenario). Similar figures showing the results obtained assuming light and severe scenarios are shown online in Figures S1 and S2.

severe to medium future changes in climate conditions), the expected loss of areas of high impact was more pronounced for *Leucocytozoon* and, most remarkably, for *Haemoproteus*; as compared to a scenario of comparatively light climate changes (B2a). However, the gain of areas of high impact by *Plasmodium* parasites was similar under the three climate change scenarios considered.

In general, current and future expectations of high impact were highly correlated across Iberian localities for the three parasite genera, regardless of the variable used to measure impact (parasite richness or prevalence) or the climate change scenario that was assumed. However, the exact trends changed among parasite genera: whereas the shape of the relationships fitted to a linear pattern for *Plasmodium* richness and prevalence under all

climate change scenarios (Table 3, Figure 16), the relationships better fitted to non-linear patterns for *Haemoproteus* and *Leucocytozoon* (for which future predictions of high impact stayed low along much of the range of current predictions of high impact; Table 3, Figure 16). Nevertheless, these patterns changed among climate change scenarios: the more tempered the expected effects of climate change, the more linear the relationship between current and future expectations of high impact of *Haemoproteus* and *Leucocytozoon*. In fact, non-linear trends were specially marked for these two genera under the most severe scenario (A1b), but all parasites followed linear trends for richness and prevalence when minor changes were assumed (B2b scenario; Table 3, Figure 16). When the relationships between current and future expectations of high parasite impact were linear, the slope of



**Figure 15.** Variation between present and future conditions in the probability that haplotype richness (a) or prevalence (b) of each parasite genus score values above the median of present-day values, as obtained from 1000 randomly selected points sampled on the Iberian Peninsula (dotted lines: *Haemoproteus*; solid lines: *Plasmodium*; dashed lines: *Leucocytozoon*).

the regression line was always significantly lower than one (Table 3), although slopes were close to one for *Plasmodium* and lowest for *Haemoproteus* (Figure 16).

## Discussion

Our results show that MaxEnt models based on climate and topography can make statistically reliable predictions on the current distribution of areas of high impact of *Plasmodium*, *Haemoproteus* and *Leucocytozoon*. These results reinforce the usefulness of SDM to make predictions on the future spread and potential impact on host

populations of this group of vector-borne parasites. Regardless of the scenario of global warming considered, our models agree in foreseeing a situation in which both *Haemoproteus* and *Leucocytozoon* will experience a progressive loss of areas of great prevalence and richness among Iberian blackcap populations. Conversely, *Plasmodium* is expected to be favoured by climate warming, gaining areas of high impact in the future. Parasite impact is thus expected to vary in different ways for the three parasite genera investigated, so that in the future the expected situation entails a generalized reorganization of local

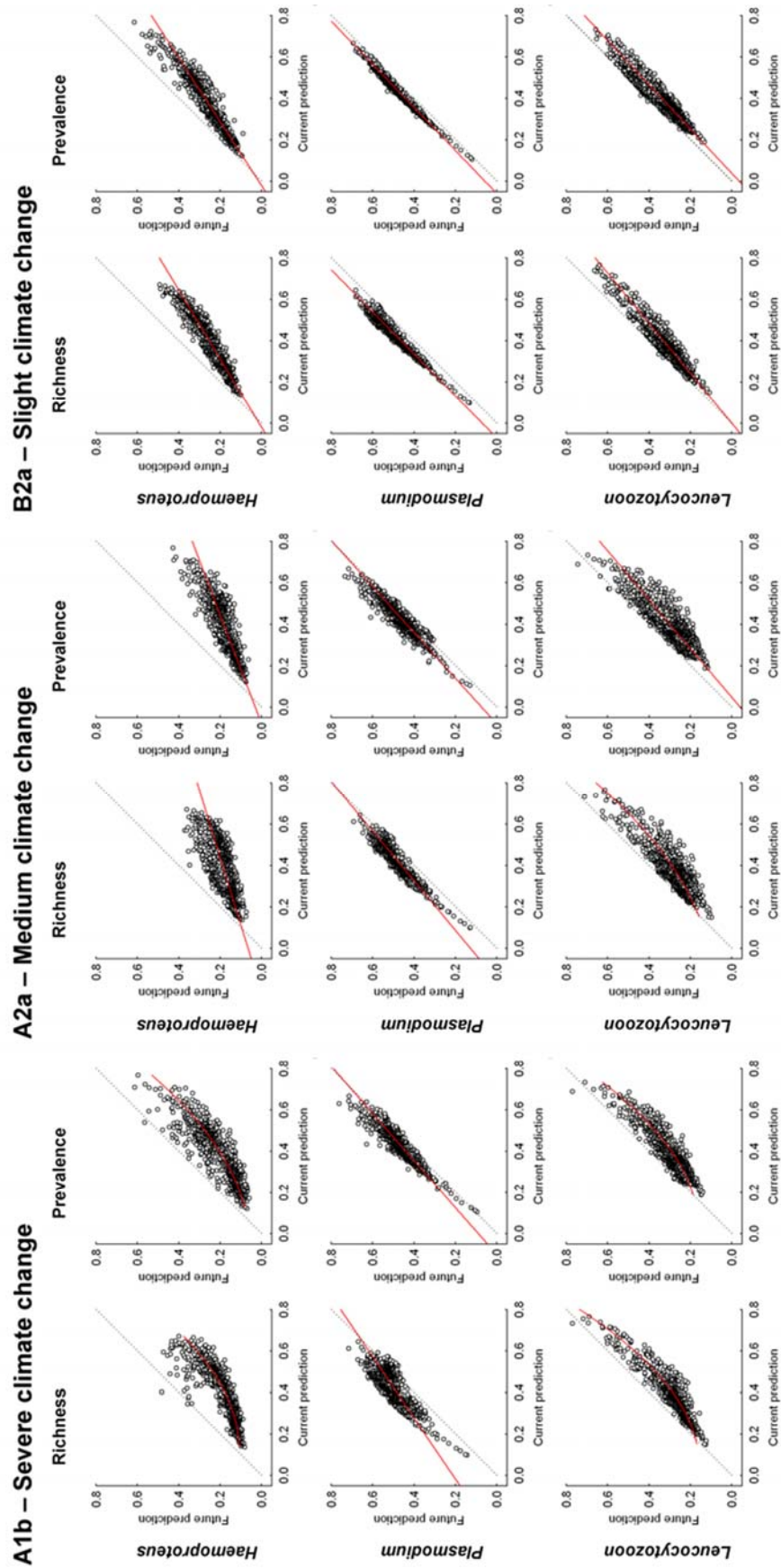
**Table 3.** Relationships between current and future predictions of high parasite impact (as measured by richness and prevalence above current average values) for three scenarios of climate change. Variance explained by linear (lin  $R^2$ ) and non-linear effects (nlin  $R^2$ ) were compared using a repeated measures ANOVA of absolute residual values. The magnitude (expressed as a percentage residual difference, RD) and the statistical significance of these differences is indicated. When the linear effect showed an equal or better fit than the quadratic effect, the slope of the relationship (b) and its standard error are indicated (all differed significantly from  $b = 1$ ).

|                      |            | lin $R^2$ | nlin $R^2$ | RD (%) | P       | b    | SE    |
|----------------------|------------|-----------|------------|--------|---------|------|-------|
| <b>A1b scenario</b>  |            |           |            |        |         |      |       |
| <i>Haemoproteus</i>  | Richness   | 0.711     | 0.740      | 11.3   | < 0.001 |      |       |
|                      | Prevalence | 0.767     | 0.782      | 7.2    | < 0.001 |      |       |
| <i>Plasmodium</i>    | Richness   | 0.668     | 0.608      | 5.1    | < 0.001 | 0.68 | 0.015 |
|                      | Prevalence | 0.857     | 0.824      | 6.0    | < 0.001 | 0.88 | 0.011 |
| <i>Leucocytozoon</i> | Richness   | 0.842     | 0.878      | 11.5   | < 0.001 |      |       |
|                      | Prevalence | 0.801     | 0.819      | 2.8    | 0.010   |      |       |
| <b>A2a scenario</b>  |            |           |            |        |         |      |       |
| <i>Haemoproteus</i>  | Richness   | 0.529     | 0.526      | 0.3    | 0.573   | 0.31 | 0.009 |
|                      | Prevalence | 0.752     | 0.757      | 0.4    | 0.701   | 0.38 | 0.007 |
| <i>Plasmodium</i>    | Richness   | 0.890     | 0.841      | 12.8   | < 0.001 | 0.83 | 0.009 |
|                      | Prevalence | 0.870     | 0.857      | 3.1    | 0.033   | 0.90 | 0.011 |
| <i>Leucocytozoon</i> | Richness   | 0.735     | 0.745      | 2.8    | 0.007   |      |       |
|                      | Prevalence | 0.738     | 0.740      | 0.4    | 0.586   | 0.85 | 0.016 |
| <b>B2a scenario</b>  |            |           |            |        |         |      |       |
| <i>Haemoproteus</i>  | Richness   | 0.904     | 0.906      | 0.1    | 0.961   | 0.60 | 0.006 |
|                      | Prevalence | 0.911     | 0.909      | 3.8    | 0.049   | 0.65 | 0.007 |
| <i>Plasmodium</i>    | Richness   | 0.952     | 0.917      | 19.3   | < 0.001 | 0.98 | 0.007 |
|                      | Prevalence | 0.980     | 0.944      | 31.2   | < 0.001 | 0.96 | 0.004 |
| <i>Leucocytozoon</i> | Richness   | 0.894     | 0.879      | 6.0    | < 0.001 | 0.86 | 0.009 |
|                      | Prevalence | 0.889     | 0.871      | 4.0    | 0.003   | 0.94 | 0.010 |

parasite component communities, what may have important consequences for host populations.

The different relationships between present and future predictions of local high parasite impact reveal further elements of change of parasite communities, both among parasite genera and climate change scenarios. In our particular case, fittings falling down the slope = 1 (a common feature of the predictions for *Haemoproteus* across the three considered scenarios) suggest that current differences among localities in the prospects of high parasite impact

will collapse in the future for this parasite genus. Under a scenario of intense climate change, only the areas which currently hold the greater probabilities of sustaining high levels of parasite impact, as a result of a particularly suited combination of environmental features, are expected to remain facing a stronger impact of *Haemoproteus*, which nevertheless is predicted to be lower than the one observed today. Remarkably, *Haemoproteus* parasites make the bulk of the haemosporidian diversity found in blackcaps; therefore, the drop of prevalence and the loss of lineage



**Figure 16.** Relationship between current and future predictions of parasite impact for the three global warming scenarios.



richness of *Haemoproteus* will likely change the future arena of host-parasite interactions for the blackcap, which may be invaded by opportunistic parasites that may be currently outcompeted by a large diversity of *Haemoproteus*. In fact, blackcaps that lost most *Haemoproteus* parasites after colonization of Macaronesian islands have acquired parasites that were unknown for continental populations (Chapter V). Therefore, the future decline in parasite richness and prevalence associated to the environmental constraints affecting the distribution of *Haemoproteus* parasites, which is expected to be exacerbated by less pronounced but similar trends followed by *Leucocytozoon*, is likely to affect not only the regime of parasite-mediated selection operating on Iberian blackcap populations, but also the actors involved in the geographic mosaic of blackcap-parasite interactions.

Our interpretations of the meaning of the shape of relationships between current and future expectations of high parasite impact are further supported by the different trends observed under different climate change scenarios. Thus, as the projected effects of climate change become more intense (A1b scenario), uncertainty of future predictions of high parasite impact rise up, the relationship between current and future predictions becomes less linear, and slopes tend to decrease. Altogether, these results suggest a stronger effect of local factors under more intense climate change. At any rate, *Plasmodium* is expected to experience strong and broadly homogeneous rises in its predictions of future high impact, whereas both *Haemoproteus* and *Leucocytozoon* might be more dependent on “parasite refuges” to sustain in the future areas of high impact, which in addition are prone to be few and patchily distributed.

Notably, all relationships between current and future expectations of high parasite impact had a slope below one, meaning that the difference in the expectation of high parasite impact between blackcap populations will decrease in the future. However, whereas this change will likely decrease the chances that populations that are currently most affected by *Haemoproteus* and *Leucocytozoon* will remain highly impacted by these parasites in the future, for *Plasmodium* the change will rather represent an increase of expected impact in populations that are currently less affected. In any case, our results predict a homogenisation of expected impacts among populations for all three parasite genera, which is likely to have evolutionary consequences if host populations that currently occupy areas where parasite impact is low eventually lose this advantage. A future scenario of more balanced parasite impacts may affect the evolution of blackcap population attributes that are candidate to be affected by parasites, such as migratory behaviour, life expectancy, or breeding success (Arriero & Møller, 2008; Møller & Szep, 2011; Garamszegi & Møller, 2012; Westerdahl *et al.*, 2012). How these changes may in turn affect the population dynamics of the species remains an important open question, which may be extrapolated to the many bird species that are affected by blood parasites in the Mediterranean region.

The predicted changes in the areas of high impact of haemosporidian parasites can be interpreted as a likely consequence of the different ecological requirements of these parasites and their vectors (Santiago-Alarcón *et al.*, 2012). *Culicoides* midges (Diptera, Ceratopogonidae), which are the vectors of passerine *Haemoproteus*, are generally considered to be among the

organisms that will be favoured by climate change (Wilson & Mellor, 2008), but more specific studies show that not all *Culicoides* species thrive in the same environmental conditions, and therefore the idea that all species may expand their geographic range with climate change is unrealistic (Calvete *et al.*, 2008). According to our predictions, one such exceptions may be the *Culicoides impunctatus* species group, which is the vector of *Haemoproteus parabelopolskyi* (Valkiūnas & Iezhova, 2004), the morphospecies that putatively makes the bulk of *Haemoproteus* haplotype diversity in blackcaps (Pérez-Tris *et al.* 2007; Valkiūnas *et al.*, 2007; Križanauskienė *et al.*, 2010). Likewise, previous studies indicate that blackflies (Diptera, Simuliidae; the vectors of *Leucocytozoon*) are likely to benefit from the expected environmental shifting in southern Europe towards more seasonal, torrential streams with warmer waters (Bonada *et al.*, 2007; Feio *et al.*, 2010). But again our predictions for the end of the century suggest that the projected situation could be above the suitability threshold for those vectors in many areas of the Iberian Peninsula. *Plasmodium* is in turn the only one of the three parasite genera that is expected to increase its range of high impact in the Iberian Peninsula. This situation is even more probable given that all *Plasmodium* haplotypes detected in blackcaps in our survey are both host and vector generalists (many species of culicine mosquitoes; Diptera, Culicidae), so that they could easily skip local extinctions of a given vector or blackcap population and keep expanding their ranges (Woolhouse *et al.*, 2001).

According to our results, and independently of the particular predictions linked to specific scenarios of climate change, the general trend for

the end of the century is one of simplified parasite component communities among Iberian blackcap populations, driven by reduced prevalence and richness of *Haemoproteus* and *Leucocytozoon*. On the other hand, our results show that the areas where *Plasmodium* parasites reach high prevalence and richness have the potential to expand in the future. The spread of this particularly virulent parasite genus, together with the expected general loss of other parasites, anticipates a changing role of parasites on host population regulation (Dobson *et al.*, 2008). For instance, given that parasite diversity may drive the evolution of resistance mechanisms (Westerdahl *et al.*, 2005; Bonneaud *et al.*, 2006; Loiseau *et al.*, 2011b), a simplification of parasite communities may induce the evolution of simplified host resistance mechanisms (Goüy de Bellocq *et al.*, 2008). This could in turn hamper the ability of host populations to deal with novel, emerging pathogens over evolutionary time. At the same time, diversity loss among parasite component communities may simplify local competitive interactions among parasites, which may also end up modifying parasite impacts. For example, in-host competition among different parasite strains promotes virulence in malaria parasites (De Roode *et al.*, 2003; Råberg *et al.*, 2006; Wargo *et al.*, 2007), but the question remains as to what extent the expected change in structure of parasite component communities is likely to affect within-host parasite assemblages. The retreat of *Haemoproteus* (the most frequently found involved in co-infections in blackcaps) should ease competition among parasites, a circumstance which somewhat predicts lower virulence in the future (De Roode *et al.*, 2003). However, the expansion of *Plasmodium* rather foretells an increase in virulence of future parasite

component communities, an effect that may be exacerbated by in-host competition among more virulent parasites associated with the increase of *Plasmodium* haplotype richness and prevalence. As the hosts themselves are expected to be subjected to putative stressing effects of climate change (Doswald *et al.*, 2009), an enhanced *Plasmodium* activity could entail even worse parasite impacts on wild populations.

We are aware that our predictions are to be taken with caution, as our models by no means explain all the variance in the variables used to describe parasite distribution. Given the preeminent role that vectors play on determining parasite geographic ranges, ideally parasite modelling should take into account how the abundance and diversity of vectors are expected to change in the future. For example, the incidence of human malaria is known to be dependent on vector diversity and activity (Gil *et al.*, 2003; Fouque *et al.*, 2010), but similar knowledge is nearly absent for avian malaria vectors (Kim & Tsuda, 2010; Santiago-Alarcón *et al.*, 2012). In fact, competent vectors and their current distributions are virtually unknown for most haemosporidian parasites of birds, as the studies on the subject are still scarce (Imura *et al.*, 2010; Njabo *et al.*, 2010; Martínez-de la Puente *et al.*, 2011; Santiago-Alarcón *et al.*, 2012), which makes modelling vector distributions unaffordable for the time being. Besides, because parasites are also intimately linked to their hosts, it is highly desirable to count on models of host distribution to predict parasite range shifts. Unfortunately, available future distribution models for European blackcaps do not have enough resolution to be used to inform our models of parasite distribution at the Iberian scale (Huntley *et al.*, 2007; Doswald *et al.*, 2009).

The broad picture shown by our study highlights the power of the available tools to make predictions about future parasite distributions. Forecasting parasite abundance on the contrary has revealed itself to be critically dependent of large databases, which usually rely on requirements of resources and time well beyond the reach of most wildlife monitoring programs (Fuller *et al.*, 2012). Nevertheless, we have shown here that quantitative data can be adjusted to be used on a presence/absence frame in a reliable way. Most studies about emerging diseases and the interactions between diseases and climate change focus on potential consequences to humans and livestock (Gage *et al.*, 2008; Tabachnick, 2010; Rohr *et al.*, 2011), but parasites have and will continue to have a major impact on wildlife as well (Daszak *et al.*, 2000; Thompson *et al.*, 2010). By studying a diverse group of parasites infecting a convenient host species, we foresee geographic shifts of areas of high parasite impact in the future, highlighting how such changes are likely to vary among parasite types, which implications such differences may have on the impact of parasitism on future host populations, and how future parasite pressures can noticeably vary according to the environmental concerns involved in the different future scenarios of climate change. But even if our models of parasite distribution may be insightful, they still suffer from the lack of relevant information about hosts and vectors, which could contribute to produce even better predictive models. We make thus a plea for basic research on the distribution of wildlife pathogens and disease vectors, as these are key components to make meaningful predictions about what the combination of disease risk and global change has in store for the natural world in the years to come.



Huge numbers of blackcaps coming from all Europe congregate during winter in open Mediterranean shrubland areas dominated by mastics (in the picture) and wild olive trees, whose fruits ripen during that season. These shrubby patches are occupied mostly by subordinate birds; whereas the adjacent woodland areas, which offer both more cover against predators and a richer variety of berry-bearing plants (including ivy, myrtle or sarsaparille among others), are favoured by dominant migrant birds, as well as by the sedentary birds that endure on the woodlands year round.

This chapter is based upon the manuscript: **Pérez-Rodríguez, A.**, De la Hera, I., Bensch, S., Pérez-Tris, J. Evolution of patterns of seasonal transmission in avian blood parasites. *In preparation*

## CHAPTER IV: SEASONAL TRANSMISSION IN AVIAN BLOOD PARASITES

*Abstract:* In temperate regions, many vector-borne parasites maximize their transmission prospects by adjusting reproduction to seasonal cycles of host susceptibility and vector availability. Nevertheless, in regions where environmental conditions are favourable during the whole year, parasites could benefit from a year round transmission strategy. We analysed how different transmission strategies (strict summer transmission, extended summer transmission including spring and fall, and year round transmission) have evolved throughout the phylogeny of a diverse clade of avian blood parasites shared by three sister species of passerine hosts. Our results indicate that the ancestral state of this clade of parasites is summer transmission with the blackcap (*Sylvia atricapilla*) as the host, and that year round transmission and switches to the other host species (*S. abyssinica* and *S. borin*) evolved recently several independent times. However, parasites that developed longer periods of transmission did not seem to diversify as much as summer transmitted parasites, suggesting that, although non-seasonal transmission may be ecologically successful at present-time, seasonal transmission may be more stable over evolutionary time. Switches from seasonal to partial or to full year round transmission strategies could have ecological consequences if they promote the spread of parasites among distantly located regions, assisted by migrating birds. Therefore, a deep knowledge of transmission strategies of parasites affecting birds in temperate areas is essential for understanding disease emergence risks.

*Keywords:* *Haemoproteus parabelopolskyi*, Host switching, Parasite transmission, Seasonality, *Sylvia atricapilla*.

### Introduction

Parasites depend on efficient transmission mechanisms to thrive in their host populations, and consequently natural selection should favour parasite strategies that maximize transmission opportunities (Combes, 2005). In those situations when a greater number of parasite offspring increases the chances of infecting new hosts, parasite transmission prospects increase with increasing parasite reproductive investment. But this comes at the cost of an increase in host exploitation, which

in turn may negatively affect parasite fitness by causing early host death (Antia *et al.*, 1994). This is why host exploitation strategies of parasites may have been shaped by a trade-off between current and future parasite fitness, so that optimal strategies should maximize parasite lifetime transmission success (Antia *et al.*, 1994; Day, 2002). From this point of view, parasites submitted to seasonal changes in transmission success should as well adapt their life cycles in order to harm their hosts as little as possible during the periods of no transmission, so as to

maximize the chances of the hosts to survive until the next transmission season (Altizer *et al.*, 2006).

In seasonal environments, a way of maximizing parasite exploitation strategies could be to adjust parasite reproduction to the cycles of host susceptibility and/or vector availability (Altizer *et al.*, 2006). Many parasites spend part of the year as dormant forms and then reappear when transmission conditions are favourable. Typically, the transmission window peaks during or shortly after host reproduction, as it coincides with periods when susceptible hosts are abundant (immunologically naïve juveniles as well as reproductive adults in which immunity is constrained by reproductive physiological investments; Dowell, 2001; Altizer *et al.*, 2006). For vector borne parasites the transmission window is moreover ultimately set by the presence of competent vectors that typically show peak abundances during humid and warm seasons. Parasites follow thus seasonal cycles of covert and overt presence, which can be interpreted as the consequence of an optimal fitting of parasite reproduction to seasonal constraints (Sorell *et al.*, 2009). Nevertheless, the processes leading to the evolution of diverse annual patterns of transmission among closely-related parasites, and the evolutionary significance of such strategies in terms of promoting parasite diversification or switches to new host species, remain to be clarified.

Avian haemosporidians, a group of vector-borne blood parasites including avian-infecting *Plasmodium spp.* -the causal agent of avian malaria- and related organisms, have become subject of intense research in recent times (Bensch *et al.*, 2009). These parasites offer an excellent opportunity to test how different transmission types

have changed during parasite evolutionary history. Although some of the parasites of this group, such as several *Plasmodium relictum* strains, show a cosmopolitan distribution (Bensch *et al.*, 2009), most of them are geographically restricted (Hellgren *et al.*, 2007). For parasites infecting birds in temperate regions, winter represents a season during which transmission is highly compromised: insect vectors are usually absent and, due to the shortage of resources, the potential damage that parasites may cause could result in host death (Wobeser, 2008). Probably because of this reason, haemosporidians from temperate regions show two major patterns of seasonal transmission. One strategy is typical of parasites that infect resident and some migrant species; these parasites often overcome the winter by retreating from circulating blood to the internal organs, where they survive in a latent stage until spring. Then parasites relapse into the bloodstream in a process mediated by day-length increase (Valkiūnas *et al.*, 2004). Another strategy, typical of parasites infecting long distance migrant species, is that of being transmitted only in the tropical wintering grounds, where the limiting factors operating on the temperate breeding regions are no longer present (Hasselquist *et al.*, 2007). Despite the billions of birds that commute every year between breeding and wintering grounds, parasite exchange among biogeographical areas seems to be a rare evolutionary event, suggesting that both types of seasonal transmission are stable over evolutionary time (Hellgren *et al.*, 2007).

The life cycles of parasites in temperate regions have been shaped by processes operating at broad scale (seasonality), and natural selection may favour traits that promote parasite dispersal among host populations

(Pérez-Tris & Bensch, 2005b). Within this framework, a third strategy of parasite seasonal transmission has been described, which involves the continuous presence of the parasite in the bloodstream throughout the year, thus enabling parasite transmission at any time. This strategy has evolved in parasites of short-distance migrant birds that spend winter in warm areas of the temperate region (such as the Mediterranean region in the Palaearctic), where birds from different geographic origins share non-breeding habitat in an environment whose mild climatic conditions allow the activity of insect vectors during winter. Parasites transmitted only during the breeding season could find themselves limited to a number of more or less isolated host populations if transmission is restricted within the range of vector movements, but if a seasonally transmitted parasite manages to switch to a non-seasonal transmission strategy, it may get access to hosts from different locations that coexist during the wintering season, as stated. This process could result in an advantageous increase in the dispersal capacity of the parasite through the host species' range (Pérez-Tris & Bensch, 2005b).

*Haemoproteus parabelopolskyi* (Valkiūnas *et al.*, 2007) is a perfect model on which to examine the evolution of different seasonal parasite transmission strategies. Under the umbrella of this morphospecies, there are several closely related parasite lineages identified by distinct cytochrome *b* DNA haplotypes. These parasite lineages behave as biological species (as shown by matching phylogenies of mtDNA and nDNA markers; Bensch *et al.*, 2004; Pérez-Tris *et al.*, 2007) and display diverse transmission strategies (Pérez-Tris & Bensch, 2005b). *H. parabelopolskyi* infects just three bird species which

constitute a monophyletic group (Bensch *et al.*, 2009): the African hill babbler *Sylvia abyssinica* (an Afrotropical resident species), the garden warbler *S. borin* (a Palaearctic long-distance migrant which spends winter in the Afrotropics) and the blackcap *S. atricapilla* (Pérez-Tris *et al.*, 2007; Križanauskienė *et al.*, 2010). The blackcap, the host species in which *H. parabelopolskyi* has experienced its greatest diversification (Pérez-Tris *et al.*, 2007), is the Palaearctic bird with the widest display of migratory behaviours known for a single species, ranging from long-distance migration to sedentarism (Berthold, 2001). Each host species harbours its own lineages of *H. parabelopolskyi* (the only exception being H-SYAT01, which is shared by *S. abyssinica* and *S. atricapilla*; Pérez-Tris *et al.*, 2007), so that their different distribution ranges face parasites with a diverse array of seasonal environments. This allows for studying the evolutionary transitions between strategies of seasonal transmission during the evolutionary history of the group. Besides, *Haemoproteus spp.* parasites present the advantage that only gametocytes (the sexual parasite stage) are present in peripheral blood (Valkiūnas, 2005), which means that any period of parasite presence in blood may be unambiguously viewed as a period of parasite reproductive investment (Valkiūnas, 2005).

The main purpose of this study is to investigate how the temporal distribution of parasite reproductive investment in temperate regions has evolved. We aim to test two specific ideas. Firstly, we seek to find out how transmission strategies may have arisen among different parasite lineages, determining the amount of phylogenetic signal of each transmission class, how recently they have appeared, and how persistent they are during parasite



evolutionary history. Secondly, and as an obligate step towards fulfilling our first goal, we want to know how variation in seasonal transmission is expressed by parasites in temperate areas. There are two main possible scenarios: either (1) parasites that are able to be transmitted outside optimal periods (generally speaking the breeding season) have just skipped the mechanisms regulating refrain and relapse, so that their prevalence is expected not to change year round; or (2) parasites have evolved extended periods of presence in blood, remaining accessible to vectors during autumn beyond the post-breeding period and/or relapsing in spring well before the breeding season (in both cases covering the wintering period, during which birds coming from different origins could putatively be found together), but still keeping seasonal life cycles by retreating from blood during the harshest periods of the winter. This would combine the best of both strategies, thereby promoting parasite survival: parasites remain in blood during periods of coexistence of different host populations (which increases dispersal prospects) but still host exploitation is kept low (decreasing during the most restrictive periods for vectors, when transmission is likely reduced and host physiology may be more compromised). In a changing world where emergent infectious diseases have revealed themselves as major threats to biodiversity and human health (Daszak *et al.* 2000; Jones *et al.*, 2008; Thompson *et al.*, 2010), tracing the evolutionary history of changes in parasite transmission strategies becomes a crucial tool to understand, foresee and prevent current and future disease risks (Morgan *et al.*, 2012).

## Material and methods

### *Phylogenetic analyses*

To determine which *Haemoproteus spp.* lineages belong to the *H. parabelopolskyi* clade, a Maximum Likelihood tree with 10,000 bootstrap replicates including all *Haemoproteus spp.* sequences registered in the MalAvi database (Bensch *et al.*, 2009; last accession February 2013) was created using MEGA 5.1 (Tamura *et al.*, 2011) using the HKY85+G+I model of molecular evolution, as selected with jModelTest 0.1.1 (Posada, 2008). From that tree all lineages belonging to a well defined clade including all lineages previously matched with the *H. parabelopolskyi* morphospecies (Valkiūnas *et al.*, 2007) were considered as the target parasites (26 lineages in total; Table 4).

The precise phylogeny of the *H. parabelopolskyi* clade was estimated by Bayesian inference using BayesPhylogenies1.1 (Pagel & Meade, 2004). A run of four Markov chains (one cold and three heated) was performed for 10 million generations and sampled every 1000 generations for a total of 10,000 trees. After discarding the first 20% generations as the burn-in period, the remaining trees were used to calculate both the posterior probability distribution of the 50% majority rule consensus tree reflecting the phylogeny of the *H. parabelopolskyi* clade, and the Bayesian estimation of both the ancestral host and ancestral state of parasite transmission seasonality using BayesTraits 2.0 (Pagel *et al.*, 2004). Parasite host and current states of parasite transmission were obtained from the literature (Table 4) or derived from our own data (see below). Two different analyses were performed: a conservative one considering only those current states that are known with confidence, and another one assigning the most reasonable putative current state to all the parasites in the clade,

**Table 4.** Parasite lineages belonging to the *Haemoproteus parabelopolskyi* clade. Parasite host species and current transmission states were retrieved from the literature (1: Pérez-Tris & Bensch, 2005a. 2: Križanauskienė *et al.*, 2006. 3: Hellgren *et al.*, 2007. 4: Pérez-Tris *et al.*, 2007. 5: Santiago-Alarcón *et al.*, 2011. 6: Hellgren *et al.*, 2013. 7: Chapter II. 8: this chapter). Transmission states marked with an asterisk (\*) were considered unknown in the conservative ancestral state reconstruction analysis.

| Parasite lineage | Genbank<br>Acc. number | Transmission state   | Host species                                  | Source  |
|------------------|------------------------|----------------------|---|---------|
| H-PABY04         | DQ368355               | Year round (Africa)* | <i>S. abyssinica</i>                          | 3       |
| H-PABY06         | DQ368356               | Year round (Africa)* | <i>S. abyssinica</i>                          | 3       |
| H-SYAT01         | AY831750               | Year round           | <i>S. atricapilla</i><br><i>S. abyssinica</i> | 1, 3, 8 |
| H-SYAT02         | AY831751               | Extended             | <i>S. atricapilla</i>                         | 1, 8    |
| H-SYAT04         | AY831753               | Summer               | <i>S. atricapilla</i>                         | 1       |
| H-SYAT07         | AY831754               | Year round           | <i>S. atricapilla</i>                         | 1, 8    |
| H-SYAT09         | AY831756               | Summer               | <i>S. atricapilla</i>                         | 1       |
| H-SYAT10         | AY831757               | Year round           | <i>S. atricapilla</i>                         | 1, 8    |
| H-SYAT11         | AY831758               | Summer               | <i>S. atricapilla</i>                         | 1       |
| H-SYAT12         | AY831759               | Summer               | <i>S. atricapilla</i>                         | 1       |
| H-SYAT13         | AY831760               | Extended             | <i>S. atricapilla</i>                         | 1, 8    |
| H-SYAT14         | AY831761               | Summer               | <i>S. atricapilla</i>                         | 1, 8    |
| H-SYAT16         | AY831762               | Summer               | <i>S. atricapilla</i>                         | 1, 8    |
| H-SYAT17         | AY831763               | Summer               | <i>S. atricapilla</i>                         | 1       |
| H-SYAT19         | AY831765               | Summer               | <i>S. atricapilla</i>                         | 1       |
| H-SYAT21         | AY831766               | Summer               | <i>S. atricapilla</i>                         | 1       |
| H-SYAT26         | AY831767               | Year round*          | <i>S. atricapilla</i>                         | 1       |
| H-SYAT28         | AY831768               | Summer               | <i>S. atricapilla</i>                         | 1       |
| H-SYAT29         | AY831769               | Summer               | <i>S. atricapilla</i>                         | 1       |
| H-SYAT30         | GU784849               | Summer*              | <i>S. atricapilla</i>                         | 5       |
| H-SYAT36         | JN164702               | Year round*          | <i>S. atricapilla</i>                         | 8       |
| H-SYAT37         | JN164703               | Year round*          | <i>S. atricapilla</i>                         | 8       |
| H-SYAT41         | JQ670873               | Summer*              | <i>S. atricapilla</i>                         | 7       |
| H-SYBOR1         | AF495575               | Year round           | <i>S. borin</i>                               | 2, 3, 6 |
| H-SYBOR3         | DQ368365               | Summer               | <i>S. borin</i>                               | 2, 6    |
| H-SYBOR4         | DQ368366               | Summer               | <i>S. borin</i>                               | 4, 6    |

according to available knowledge of their occurrence among hosts, geographic regions and times of year. Parasites categorized as “unknown” in the first analysis were both African lineages restricted to *S. abyssinica* and those which were detected just once according to the available references. We assumed the African lineages to be year round transmitted because of the

putative lack of climatic limitations in the tropics. The poorly sampled lineages were classified as summer transmitted or year round transmitted based on the season during which they were detected (Table 4). To calculate ancestral states of the Most Recent Common Ancestor (MRCA) at each phylogeny node, a Markov chain was allowed to run for 11 million generations, and rate

coefficients and ancestral states were sampled every 1000 generations after discarding the first 1 million generations as the burn-in period. Transition rate parameters were adjusted using a gamma-distributed hyperprior. The intervals of the mean and the variance of the prior distribution altogether with the deviance rate were adjusted to get an acceptance rate between 20 and 40% (Pagel *et al.*, 2004).

We used the BiSSE method (Maddison *et al.*, 2007) to test whether the rates of lineage diversification within the *H. parabelopolskyi* clade depended on the transmission strategy or the host species of parasites. The procedure estimates the probability that the clade would have evolved with the observed character states at branch tips assuming two different evolution models. One model is constrained to assume equal rates of speciation associated to each character state, and the other one allows for speciation rates freely differing with respect to the state of the corresponding character. For each model, estimates of speciation rates are computed for phylogenetic lineages that have alternative character states, and the likelihood of the model is computed. Thus, a significant difference in likelihood between constrained and unconstrained models indicates that speciation rates are associated with character states. We also estimated if there was a significant association between host shifts and changes in transmission strategy, for which we used Pagel's character correlation analysis (Pagel, 1994). Again, this method tests for significant differences in likelihood between two models: one which considers that the two characters evolve independently, and another one which considers them to show correlated changes along the phylogeny. Because these two analyses only allow

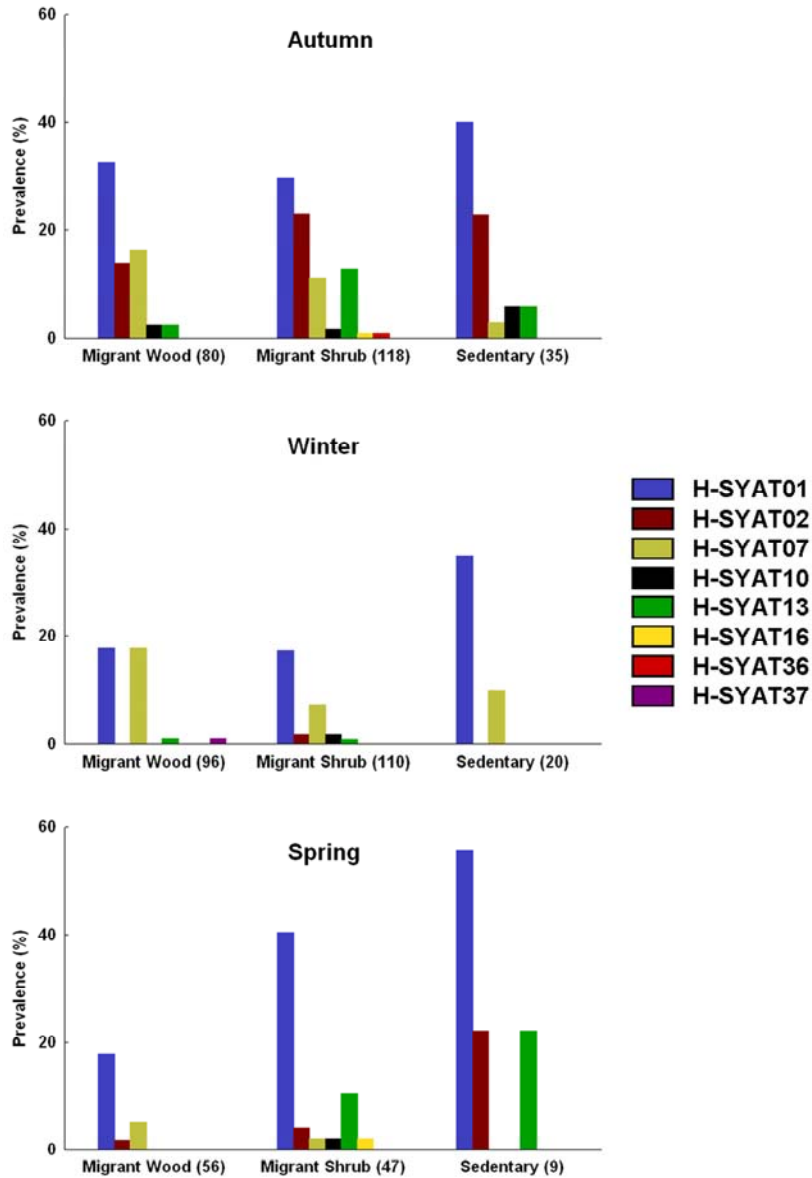
for testing binary characters (Pagel, 1994; Maddison *et al.*, 2007), we considered two alternative character states for type of transmission (in summer only or other) and two for host species (blackcap only or other). Calculations were done with the *Correl* and *Diverse* packages implemented in Mesquite 2.75 (Maddison & Maddison, 2011).

#### *Field sampling and Statistical analyses*

To complete our knowledge of the different seasonal transmission strategies of *H. parabelopolskyi*, and to search for evidence of extended transmission periods of seasonally transmitted parasites, we performed an extensive parasite screening of blackcaps captured during the whole wintering season. The variation in prevalence of the most frequently found *H. parabelopolskyi* lineages according to season (autumn, winter or spring) and their assignment to any of the three possible groups of birds (migrants in shrubland, migrants in woodland or sedentary birds in woodland, as the latter never occurred in shrubland), was assessed by log-linear analyses. The log-linear models were obtained proceeding hierarchically by fitting all interactions of order N to the corresponding null hypotheses that all of them are simultaneously zero. As soon as the reduction in N caused a lack of fit, the terms of that order or lower which significantly contributed to explain the distribution of frequencies were selected, thus generating the final model that best fitted the data (StatSoft Inc., 2013). All statistical analyses were performed with STATISTICA 7.0 (StatSoft Inc., 2004).

### **Results**

#### *Parasite screening and seasonal distribution*



**Figure 17.** Seasonal variation in prevalence of *Haemoproteus parabelopolskyi* lineages. Variation in prevalence of the eight most frequently found *Haemoproteus parabelopolskyi* lineages among the three studied seasons (autumn, winter or spring) and the three blackcap groups (migratory or sedentary blackcaps in woodland, and migratory blackcaps in shrubland). Number of sampled birds per group is stated between parentheses.

During the sampling period we captured a total of 571 blackcaps: 64 sedentary birds and 507 migratory ones (275 in woodland and 232 in shrubland). We were able to identify a total of 16 different parasite lineages, with an overall prevalence of 49% for *Haemoproteus spp.* (principally *H. parabelopolskyi*) and 6.1% for *Plasmodium spp.* (Table S6). We found no multiple infections comprising more

than two parasite lineages, and in all cases we could assess the identity of the parasites involved in them.

The prevalence of the different year round transmitted parasites varied across the study period, and this seasonal variation was the strongest effect detected in the log-linear analyses (Figure 17, Table S7). The prevalence of H-SYAT07 remained quite stable,

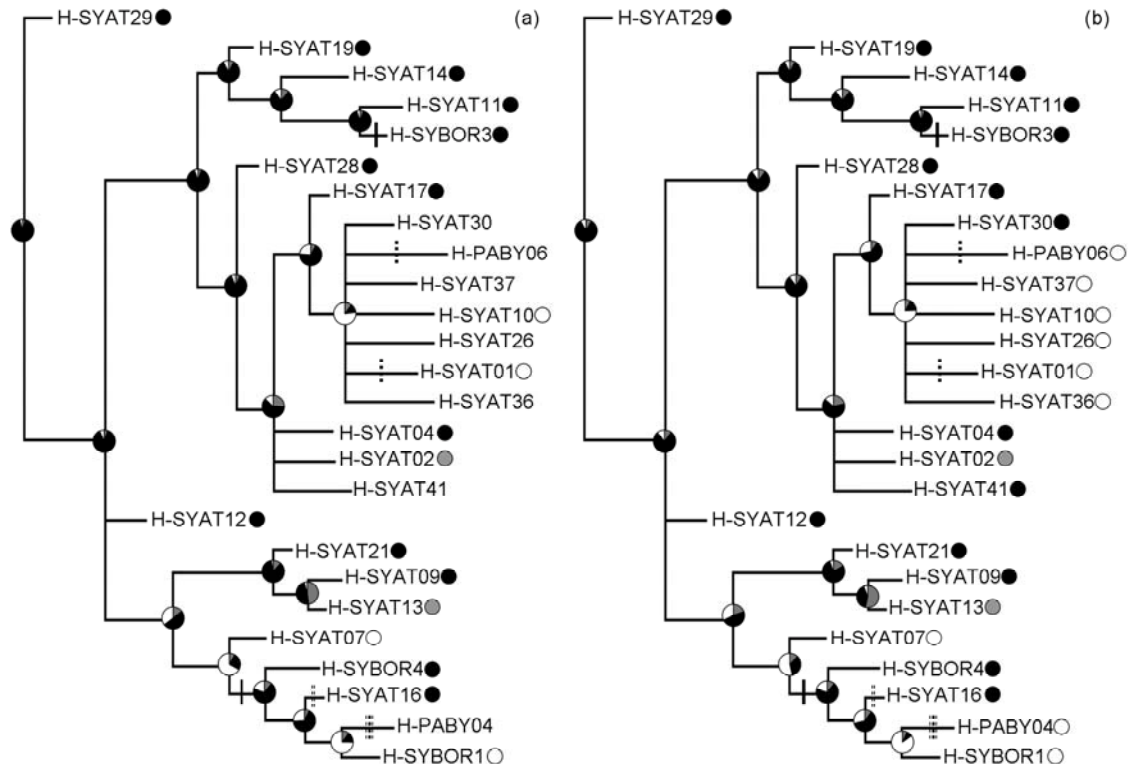
whereas H-SYAT02 and H-SYAT13 showed high prevalence in autumn, then they were almost absent during winter, and eventually they reappeared in spring. Meanwhile, the prevalence of H-SYAT01, by far the most frequent parasite in the sample, decreased during winter, but still remained comparatively high. Regarding the three different categories of wintering blackcaps, the prevalence of H-SYAT01 was higher among sedentary birds than in the migratory ones, regardless of the habitat they occupied. H-SYAT02 and H-SYAT13 showed instead significant variation between habitat types in prevalence, which in both cases was higher in woodlands than in shrublands.

#### *Phylogenetic analyses*

Both reconstructions of the ancestral state of host species and parasite strategies of transmission season (including all lineages or only those with unambiguous character states) yielded highly congruent results (Figure 18): the two reconstructions considered with a high posterior probability that the *H. parabelopolskyi* clade originated in blackcaps (posterior probability of 0.93) from a summer transmitted common ancestor (posterior probabilities of 0.92 for the conservative analysis and of 0.84 for the less conservative one). According to this general picture, the strategy of an extended transmission period would have recently arisen in two terminal, unrelated nodes of the *H. parabelopolskyi* phylogeny. Year round transmission would have arisen several times in the parasite phylogeny as well, but whereas the unresolved clade of seven parasite lineages including H-SYAT01 seems to originate in a recent year round transmitted MRCA, uncertainty related to the lack of knowledge of the current states complicates the interpretation of the acquisition of year round transmission

at other point of the phylogeny. Both reconstructions suggest the existence of a year round transmitted MRCA for the clade of five parasite lineages including H-SYAT07, although this is strongly dependent of this late parasite being of unseasonal transmission. Two possible scenarios arise in this clade thus: either from a year round transmitted parasite followed a reversion to summer transmission and back to year round transmission later in the evolution of the clade, coincident with several host shifts (assuming a different scenario instead in which H-PABY04 would be seasonally transmitted, then the switch to year round transmission would have happened just at two tips within the clade); or the whole clade would be instead of summer transmission, and year round transmission would have appeared just at the tips, which seems to be the most parsimonious explanation. With the sole exception of an early switch from blackcaps to garden warblers in this clade, host changes in turn were always of recent origin in the evolutionary history of the group.

Speciation rates were estimated to be 2.3 times higher for parasite lineages that were transmitted in summer than for parasites with other types of transmission ( $\lambda$  for summer transmission = 107.3; for other transmission types = 47.4), although these differences were not statistically significant ( $\chi^2_{(1)} = 1.05$   $p = 0.31$ ). We did not find the same difference in speciation rates between parasite lineages infecting blackcaps ( $\lambda = 89.4$ ) or other host species ( $\lambda = 76.8$ ;  $\chi^2_{(1)} = 0.08$   $p = 0.77$ ). We also did not find a significant association between host switches and switches of transmission strategy (Pagel's character correlation analysis:  $p = 0.21$ ).



**Figure 18.** Reconstruction of ancestral states. Reconstruction of ancestral states of seasonal transmission strategy and host species of parasites belonging to the *Haemoproteus parabelopolskyi* clade, either considering only those character states known with confidence (a), or assigning a current character state to all the parasite lineages (b). Current transmission states and estimated posterior probabilities for the transmission state of the Most Recent Common Ancestor are identified by colours (Black: summer transmission. Grey: extended seasonal transmission. White: year round transmission. The shaded percentage of each circle indicates the probability of the MRCA showing that trait). Host shifts are distinguished by vertical bars (Solid line: from *Sylvia atricapilla* to *S. borin*. Dotted line: from *S. atricapilla* to *S. abyssinica*. Double dotted line: from *S. borin* to *S. atricapilla*. Triple dotted line: from *S. borin* to *S. abyssinica*).

## Discussion

Our results strongly support the existence of the different seasonal transmission strategies described by Pérez-Tris & Bensch (2005b), as each parasite lineage detected in our study scored the same transmission dynamics as previously observed with an independent dataset (Pérez-Tris & Bensch, 2005b). Parasites such as H-SYAT14 or H-SYAT16, which are quite common in blackcaps during summer (Pérez-Tris & Bensch, 2005b; Pérez-Rodríguez *et al.*, 2013), were

absent or almost absent among the birds sampled during the non-breeding season, regardless of the fact that the climatic conditions of early autumn or spring would remain highly favourable for parasite transmission. This points towards a suppression of gametocyte production of these parasites probably mediated by day length (as the spring relapse is; Valkiūnas *et al.*, 2004), independent thus of weather changes or host physiological adjustments related to migration activity, given that the disappearance of these parasites takes place in migratory and sedentary birds

altogether. Our study allowed us to detect the existence of variation in the transmission strategy by parasites that are present in blood during blackcap non-breeding periods, notably the distinction between strict year round transmission (lack of noticeable variation in prevalence throughout the year, as for H-SYAT07 and to a lesser extent H-SYAT01) and extended seasonal transmission (high prevalence in autumn that drops in winter and quickly recovers in early spring, thereby allowing transmission during periods of coexistence of host populations with different geographical origins in sympatric wintering grounds, as for H-SYAT02 and H-SYAT13).

The lack of statistical support to the existence of different speciation rates between transmission strategies was somehow to be expected given the small size of our phylogeny. In fact, the BiSSE method requires much larger phylogenies, of the order of hundreds of extant species, to achieve sufficient statistical power (Maddison *et al.*, 2007). Therefore, our estimates of higher speciation rates in parasites with summer transmission deserve comment, especially because extended or year round transmission of these parasites has been shown to promote range expansion and local infection success (Pérez-Tris & Bensch, 2005b). Thus, the question stands out as why all the events of switching from summer transmission to other strategies seems to appear as recent phenomena in the evolutionary history of these parasites. Changes in transmission strategy are not rare events in the phylogeny of *H. parabelopolskyi*, so that an intrinsic limitation (a very tight genetic control of gametocyte seasonal production, for instance) does not appear to be the most likely explanation for the observed pattern. Natural selection acting on the long term against enlarged transmission

periods may be a more probable cause. In principle, the longer a parasite is affecting its host outside the breeding season, the more chances it has of being targeted by the host immune system, which may be particularly efficient against parasites that have a longer history of interaction with the host species (Altizer *et al.*, 2003; Woodworth *et al.*, 2005). In a context of competition with highly diverse and closely related parasite lineages (such as in *H. parabelopolskyi* parasites of blackcaps), any selective disadvantage for parasites that are highly conspicuous to host immunity may promote extinction or reversion to a summer transmission strategy (as it could have happened in the H-SYAT07 clade). The lineage-rich clade including H-SYAT01 could be considered to be a successful episode in which a change in strategy of seasonal transmission promotes parasite diversification as well, but all the parasites included in it (with the sole exception of H-SYAT01) are actually very scarce (see references in Table 4), perhaps as a consequence of an ongoing process of counter selection and/or the competitive advantage of other, seasonal parasites. Notwithstanding the depicted advantages of year round transmission, the fact is that in temperate areas most haemosporidians seem to retain seasonal transmission (Hellgren *et al.*, 2007). In fact, true year round transmission could not be as beneficial as previously thought, given that parasite lineages which follow the extended summer transmission strategy have greater ranges and higher prevalence than those with year round transmission *sensu stricto*, such as H-SYAT07 (Pérez-Tris & Bensch, 2005b; Pérez-Rodríguez *et al.*, 2013). Host switches appear also as recent events scattered throughout the phylogeny of *H. parabelopolskyi*, indicating that once a host shift has taken place, parasite diversification seems to have stopped;

but again we lack enough statistical power to unambiguously support this hypothesis. Interestingly, diversification increased again in one case (among the descendants of the common ancestor of SYAT07 and the clade of four lineages including H-SYBOR4) in which parasites recurrently switched among garden warblers, blackcaps and African hill babblers. Diversification throughout recurrent host switching is a process common to many parasites (Schluter, 2000; de Vienne, 2012) which has been important in the evolutionary history of avian haemosporidians (Ricklefs & Fallon, 2002, Ricklefs *et al.*, 2004; Jenkins *et al.*, 2012) as it seems to be the case here as well.

Our results support the idea that bird origin rather than habitat type is the primary factor shaping the distribution of parasites between blackcap wintering habitats. Thus, relative differences in prevalence of each parasite among host types (migratory or sedentary) and habitat types (woodlands and shrublands) remained unchanged during the study period for the eight parasites that were detected in the area. This observation could suggest that parasite transmission may occur at similar rates in all habitats, which is however unlikely because forests and shrublands differ in most attributes affecting the distribution of vectors. Or it may indicate instead that parasite transmission during the non-breeding season is a rare occurrence after all, despite parasite gametocytes being circulating in birds' blood through the year. Which of the above alternatives are true remains to be investigated. Nevertheless, a few events of transmission of parasites during periods of coexistence of migratory and sedentary blackcaps may be enough for their consequences to be detected over evolutionary time, as only a few immigrants per generation may allow

parasites reaching the level of gene flow necessary to obtain fitness benefits associated with increased local infectivity (Pérez-Tris & Bensch, 2005b).

These results widen our knowledge about the processes regulating parasite transmission throughout the year, and open new questions for future research. It would be interesting to know the precise mechanisms regulating parasite retreat from the peripheral blood for the distinct transmission strategies uncovered in this study. Future research considering more study years could for instance look for relationships between specific environmental conditions and variation in prevalence of the different parasite lineages across the wintering season, which could be behind the scarcity of parasites with an extended summer transmission in mid-winter. If temperature happens to have a detectable effect on the average winter prevalence of a given parasite, then a process of global warming could modify parasite abundances by affecting parasite transmission on the wintering grounds and not only on the breeding grounds, where studies addressing this issue are usually focussed (Møller, 2010; Garamszegi, 2011; Loiseau *et al.*, 2013; Pérez-Rodríguez *et al.*, 2013). Other temperate host species should be studied as well, to know if this set of different transmission strategies is exclusive of blackcap haemosporidians, or if on the contrary it is widespread across parasites infecting other birds. The reconstruction of the ancestral transmission seasonality stages indicates that, although the changes between transmission strategies do not show much stability over evolutionary time, they can arise frequently and temporarily achieve great ecological success (Pérez-Tris & Bensch, 2005b). The unpredictability of these switches



claims in favour of parasite monitoring surveys addressed to analyse several host species across both seasons and years (Fuller *et al.*, 2012), in order to determine how widespread different transmission strategies are and to detect any possible hazard that, in a context of fast and unpredictable global change, might threaten previously safe host species.



The moister areas of the Macaronesian archipelagos still maintain remnants of the laurel forest that once covered most of the Mediterranean region, before the Quaternary Glaciations. These forests, which abound in tree species with fleshy fruits, were colonised by migrating blackcaps after the Last Glacial Maximum. Blackcaps became sedentary on the Macaronesia; although their wing morphology is still more similar to that of migratory blackcaps than to that of the sedentary ones of the SW of the Iberian Peninsula. A number of Macaronesic blackcaps, as the one depicted, are melanistic; a trait particularly frequent on Madeiran birds in comparison with mainland blackcaps.

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## CHAPTER V: BLACKCAP HAEMOSPORIDIANS IN THE MACARONESIA

*Abstract:* The study of parasite biogeography on islands is important for our understanding of both the processes involved in the evolution of parasite assemblages worldwide, and the ecology and conservation of insular communities. By studying the haemosporidian blood parasites of a bird that has recently colonised a number of oceanic islands, we were able to test hypotheses relating to the processes involved in parasite colonization and community assembly prior to the permanent isolation of host species on islands. We used cytochrome *b* DNA sequences to determine the prevalence and richness of parasites of the genera *Haemoproteus*, *Plasmodium* and *Leucocytozoon* in blackcaps, *Sylvia atricapilla*, a widespread passerine which colonized these Atlantic archipelagos during the Last Glacial Maximum. We compared insular blackcap parasite assemblages with those observed in 37 blackcap populations sampled on mainland Europe. The insular parasite assemblage was impoverished, containing ca.10% of the parasites found on the continent. None of the parasites observed on the islands were blackcap specific. Some of the observed parasites appear to have switched from blackcaps to other Macaronesian host species, while others were of Afrotropical origin and were acquired after blackcaps colonised the islands. The prevalence of parasites in the island populations of blackcaps was lower than in mainland blackcap populations and parasite richness decreased with increasing island distance to the continent. Macaronesian blackcaps do not face the strong parasite load encountered by their mainland counterparts despite the fact that blackcap migration from the continent may directly transport mainland blackcap parasites to the islands. These results support the idea that normal mainland host-parasite associations are compromised on islands, and that parasite island syndromes (low richness, frequent host-switching, and reduced specialization) evolve even before insular host populations become completely isolated from their mainland counterparts.

*Keywords:* Avian haemosporidians, Blackcap *Sylvia atricapilla*, Canary Islands, Host specificity, Island colonization, Island syndromes, Madeira, Parasite biogeography

### Introduction

Oceanic islands, those which have never been linked to a continent, have been the focus of biogeographical research because of the opportunities they offer to understand the processes involved in

species' range expansion and the evolutionary consequences of population isolation (Whittaker & Fernández-Palacios, 2007). Although parasite biogeography is still an emerging field, a number of studies have analysed the patterns of island

colonization (Ishtiaq *et al.*, 2010; Cornuault *et al.*, 2012), host-parasite coevolution across different islands (Fallon *et al.*, 2003), or the development of island syndromes by parasites (changes in life-history, behaviour, or physiology after island colonization; Nieberding *et al.*, 2006). The island biogeography of parasites is also important for understanding the risk and patterns of disease introduction into new areas. Various studies have addressed what enables a parasite to be a successful invader (Ewen *et al.* 2012), or instead why some are lost during host range expansion (MacLeod *et al.*, 2010). The implications of this research are many; for instance, the introduction of exotic parasites offers dramatic examples of parasite-driven extinction in insular faunas (Wikelski *et al.*, 2004; Atkinson & LaPointe, 2009).

So far, the study of parasites on islands has mainly focused either on isolated host species, usually insular endemics (Fallon *et al.*, 2005; Illera *et al.*, 2008; Valkiūnas *et al.*, 2010; Howe *et al.*, 2012), or on the arrival of novel parasites to naïve insular faunas because of anthropogenic effects (Atkinson & LaPointe, 2009; Ewen *et al.*, 2012). Although undeniably relevant to our understanding of the evolution of parasitism on islands, these studies involve either too long, or too short, time-scales to capture the natural processes involved in the assembly of insular parasite communities. Insular endemics are no longer affected by parasites arriving with conspecific hosts from the continent, consequently the host-parasite interactions on such islands are already independent from the interactions maintained before island colonization. Meanwhile, anthropogenic parasite introductions may provide valuable information on the earliest stages of parasite colonization, but are too recent to allow

an assessment of the chances of a parasite's long-term establishment on the insular fauna. An important, yet infrequently used comparison in studies of insular parasites is between mainland and island parasite assemblages of host species that have recently (evolutionary speaking) colonized oceanic islands, and still remain linked to the original continental populations by migration. Such comparisons may allow us to test whether the evolution of the typical characteristics of insular parasite assemblages (i.e. parasite island syndromes which typically involve low richness, frequent host-switching, and reduced host specialization) is only possible after long periods of isolation on islands, or if they can evolve in the face of sustained contact between insular and mainland host populations.

Avian haemosporidians (order Haemosporida, phylum Apicomplexa) are blood parasites transmitted by the bite of several families of blood-sucking dipteran vectors. They are a diverse group of parasites that infect most bird species (Valkiūnas, 2005). The development of PCR-based detection methods has greatly facilitated the analysis of haemosporidians in ecological and evolutionary studies (reviewed in LaPointe *et al.*, 2012), and consequently they have become a preferred model in island parasite biogeography (Fallon *et al.*, 2003; Beadell *et al.*, 2007; Ishtiaq *et al.*, 2010; Ricklefs *et al.*, 2011; Cornuault *et al.*, 2012).

We studied the haemosporidian parasites of blackcaps (*Sylvia atricapilla*) on the Macaronesian archipelagos of Madeira and the Canary Islands. Macaronesia is the collective name of four Atlantic archipelagos (Azores, Madeira, Canary Islands and Cape Verde) of independent volcanic origin but shared biotic features.

Whether geographically isolated (Azores), or near the Saharan African coast, their biotas show clear links with the Mediterranean region (Whittaker & Fernández-Palacios, 2007; Fernández-Palacios *et al.*, 2011; Illera *et al.* 2012). Until recently these islands had been largely ignored by evolutionary biologists in comparison to the attention given to other archipelagos such as Hawaii or Galapagos (Fernández-Palacios *et al.*, 2011; Illera *et al.* 2012). Consequently, their avian haemosporidian communities still remain relatively poorly studied (Foronda *et al.*, 2004; Hille *et al.*, 2007; Illera *et al.*, 2008; Alcaide *et al.*, 2010; Hellgren *et al.*, 2011; Spurgin *et al.*, 2012). This paucity of research does not reflect the great interest shown to the Macaronesian avifauna which includes both locally evolved ancient endemics and recent colonizer species with broader geographical ranges outside the islands (Illera *et al.*, 2012), and provides an excellent range of different host models for the analysis of parasitism on oceanic islands.

Blackcaps are recent colonizers of the Macaronesian islands. Their arrival on these islands has been broadly dated between 4000 - 40 000 years BP (Pérez-Tris *et al.* 2004; Dietzen *et al.*, 2008), and most likely took place during the last 13,000 years through several independent colonization events by migrants from continental Europe (Pérez-Tris *et al.* 2004; Dietzen *et al.*, 2008). Blackcap populations are resident in Madeira and all of the Canary Islands except the two easternmost ones, but co-exist with a regular, though poorly studied, annual influx of migrating and overwintering European blackcaps, which is greater the closer the island is to the continent (Shirihai *et al.*, 2001). Although blackcaps harbour an exceptionally diverse community of avian

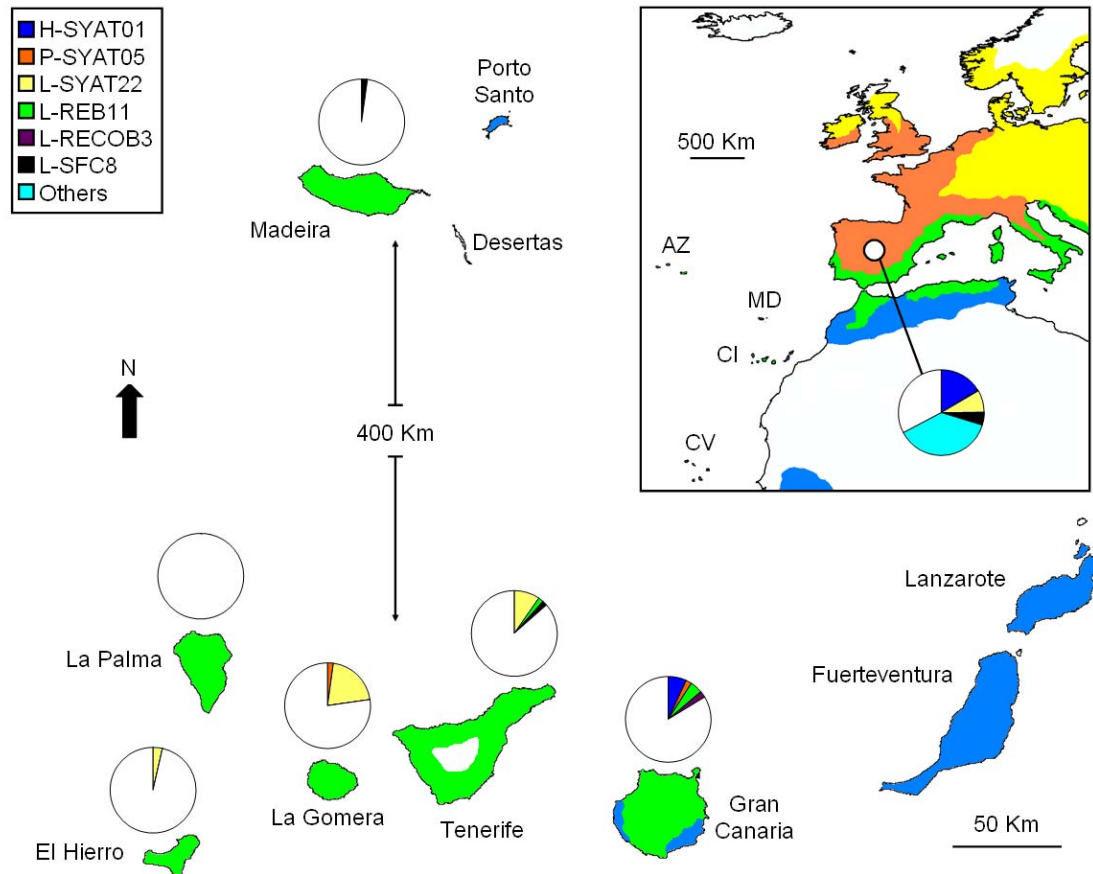
haemosporidians (Pérez-Tris *et al.*, 2007; Križanauskienė *et al.*, 2010), the only study that has so far analysed blackcap parasite diversity within Macaronesia (on São Miguel Island, Azores) found no infected birds among the 74 examined (Hellgren *et al.*, 2011).

We studied haemosporidian infections of blackcaps on the Canary Islands, Madeira and the Iberian Peninsula to test four predictions derived from current hypotheses on the factors promoting parasite arrival, establishment and loss associated to island colonization:

1. *Parasites that are common on the mainland are more likely to occur on islands.* Because the number of individuals involved in a colonization event is usually small (Whittaker & Fernández-Palacios, 2007), we predict that those parasites that are common in mainland blackcaps are most likely to be transported to islands with colonizing hosts (Ewen *et al.* 2012).

2. *Generalist parasites will be more likely to occur on islands than specialists.* A scarcity of preferred hosts, particularly at the beginning of the colonization process, should be a handicap for specialist parasites that cannot infect alternative host species (MacLeod *et al.*, 2010; Ewen *et al.* 2012). Therefore, we expect parasites that can infect both blackcaps and other host species to be more frequent on the islands than blackcap-specific parasites.

3. *The parasite diversity of a specific host should be positively correlated with that hosts' population size on the islands.* If any blackcap-specific parasites made it to the islands they would depend on the availability of blackcaps to thrive. Therefore, we expect the diversity of such parasites to be highest on the islands most populated



**Figure 19.** Blackcap distribution on the Macaronesian archipelagos (main map) and across the species' Western Palaearctic range (inset map; modified from Shirihai *et al.*, 2001). Population status is shown in different colours (yellow: summer visitor; orange: present year-round, breeding populations migratory; green: present year-round, breeding populations sedentary; blue: winter visitor). The geographic location of the four Macaronesian archipelagos is also shown on the inset map (AZ: Azores Islands, MD: Madeira Islands, CI: Canary Islands, CV: Cape Verde Islands). The pie charts represent the prevalence of the six insular parasite haplotypes (represented by different colours; white areas represent the percentage of uninfected birds) in each of the six sampled islands (data of 2007) and in the Iberian Populations ('others' are those not found on islands –Chapter II-, which have been grouped). Parasite genera are distinguished by the initial of haplotype names (H: *Haemoproteus*, P: *Plasmodium*, L: *Leucocytozoon*).

with blackcaps and lowest on those that are least populated (Altizer *et al.*, 2007).

4. *The parasite diversity on islands will be positively correlated with their proximity to the continent.* The number of migratory continental blackcaps that arrive on an island increases with island proximity to the African coast, thus increasing the chances of parasites being locally

established, either because of repeated historical colonization events from the continent, or because of contemporary cross infection from visiting continental hosts. Consequently, parasite assemblages should be structured according to the expectations of island biogeography theory (MacArthur & Wilson, 1967; Fallon *et al.* 2005).

**Table 5** Sample size per year and parasite screening results. Genbank accession numbers of parasite haplotypes are given between parentheses.

| Island       | Year | <i>n</i> | Parasite haplotypes    |                        |                       |                        |                      |                        |
|--------------|------|----------|------------------------|------------------------|-----------------------|------------------------|----------------------|------------------------|
|              |      |          | H-SYAT01<br>(AY831750) | L-SYAT22<br>(DQ847236) | L-REB11<br>(DQ847223) | L-RECOB3<br>(DQ847221) | L-SFC8<br>(DQ847234) | P-SYAT05<br>(DQ847271) |
| Madeira      | 2007 | 43       | 0                      | 0                      | 0                     | 0                      | 1                    | 0                      |
| Gran Canaria | 2003 | 21       | 0                      | 0                      | 0                     | 0                      | 0                    | 0                      |
|              | 2007 | 43       | 3                      | 0                      | 2                     | 1                      | 0                    | 1                      |
| Tenerife     | 2003 | 18       | 0                      | 0                      | 0                     | 0                      | 0                    | 0                      |
|              | 2007 | 51       | 0                      | 5                      | 1                     | 0                      | 1                    | 0                      |
| La Gomera    | 2004 | 16       | 0                      | 0                      | 1                     | 0                      | 0                    | 0                      |
|              | 2007 | 44       | 0                      | 9                      | 0                     | 0                      | 0                    | 1                      |
| La Palma     | 2004 | 23       | 0                      | 0                      | 0                     | 0                      | 0                    | 0                      |
|              | 2007 | 43       | 0                      | 0                      | 0                     | 0                      | 0                    | 0                      |
| El Hierro    | 2003 | 50       | 0                      | 0                      | 0                     | 0                      | 0                    | 0                      |
|              | 2007 | 28       | 0                      | 1                      | 0                     | 0                      | 0                    | 0                      |

## Material and Methods

### *Statistical analyses*

We used simple regression to test for correlations between the number of parasite haplotypes per island and (1) island distance to mainland (as a correlate of colonization impediment) or (2) size of the blackcap's range on each island (as a surrogate of host population size). We conducted these analyses using only the Canary Islands, excluding Madeira as belonging to a different archipelago, which was independently colonised by blackcaps (Pérez-Tris *et al.* 2004) and thus disconnected from any stepping-stone pattern of colonisation of the Canary Islands. We deliberately avoided simultaneously testing for distance and range size effects because of small sample size ( $n = 5$  islands). We used the number of UTM squares ( $10 \text{ Km}^2$ ) with confirmed breeding blackcaps on each island (Carbonell, 2003; Equipa Atlas, 2008) as a measure of the blackcap's range on each island. All variables were log-transformed to increase normality of residuals. Because of the small sample size, we also performed Spearman rank

correlations to substantiate our conclusions using two different approaches.

We compared parasite diversity found in the island blackcaps with mainland blackcaps using a sample of 882 birds from 37 breeding populations scattered throughout the Iberian Peninsula (Chapter II). Iberian blackcap populations accurately represent the parasite assemblages typical for the species in continental Europe (Pérez-Tris & Bensch, 2005b; Križanauskienė *et al.*, 2010). To control for different sampling effort between island and mainland, we constructed curves of cumulative parasite richness using the software EstimateS 7.5.1 (Colwell, 1999). From these curves we obtained the average number of parasites found after scoring twenty five infections ( $R_{25}$ ), which we used as a comparable estimate of parasite richness between islands and mainland. We derived average curves and  $R_{25}$  values from 10 000 richness curves constructed by randomly selecting the order in which individual infections were scored.



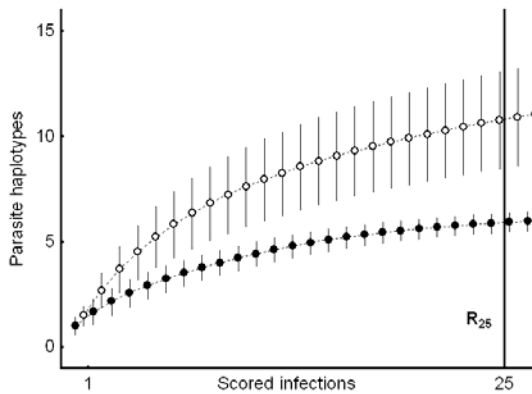
**Table 6.** Prevalence of the identified insular parasites in island and mainland blackcap populations. For each parasite haplotype, prevalence (mean  $\pm$  SD) has been estimated from 1000 bootstrap replicates of blackcaps sampled in populations where the corresponding parasite occurred (the number of re-sampled individuals in each case is shown in brackets). Estimates for L-SFC8 changed slightly depending on whether Madeira was included (all islands) or excluded (Canary Islands).

| Parasite haplotype | All islands            | Canary Islands         | Iberian Peninsula      |
|--------------------|------------------------|------------------------|------------------------|
| H-SYAT01           | 6.58 $\pm$ 0.23 (43)   | 6.58 $\pm$ 0.23 (43)   | 17.42 $\pm$ 0.08 (755) |
| L-SYAT22           | 12.06 $\pm$ 0.18 (123) | 12.06 $\pm$ 0.18 (123) | 16.97 $\pm$ 0.12 (397) |
| L-REB11            | 3.08 $\pm$ 0.11 (94)   | 3.08 $\pm$ 0.11 (94)   | 0                      |
| L-RECOB3           | 2.25 $\pm$ 0.14 (43)   | 2.25 $\pm$ 0.14 (43)   | 0                      |
| L-SFC8             | 2.09 $\pm$ 0.09 (94)   | 1.95 $\pm$ 0.12 (51)   | 9.36 $\pm$ 0.09 (431)  |
| P-SYAT05           | 2.30 $\pm$ 0.10 (87)   | 2.30 $\pm$ 0.10 (87)   | 4.07 $\pm$ 0.13 (96)   |

We tested whether island and mainland blackcap's parasite communities (as opposed to prevalence) differed significantly. To this end, we compared the parasite observed community on islands with the community expected, under the assumption that the occurrence of a given parasite in a sample of insular blackcaps equals its probability of occurrence in the continent regardless of variation in overall parasite prevalence. To generate a null distribution we simulated a population of 100 000 infected blackcaps, in which each parasite haplotype occurred with the same frequency as observed across all Iberian blackcap populations. This is a conservative approach, because it includes a number of locations where any given parasite was absent, a circumstance which lowers expected probabilities of parasite occurrence. From the simulated population, we randomly selected 10 000 groups of twenty seven parasite infections (equalling the number of infections retrieved from insular blackcaps; see Results), and measured the prevalence of each parasite in each random trial. We used these simulations to estimate the probability of finding particular parasite haplotypes on islands by

random sampling of the diversity of mainland blackcap parasites.

We also tested whether parasite haplotypes shared between island and mainland blackcap populations had similar prevalence in both geographical scenarios. We first estimated prevalence of each parasite haplotype on islands and Iberian localities where the parasite was found. (i.e., where we were certain the parasite thrives). Given that the prevalence of specific parasites varies among blackcap populations, we computed bootstrap estimates of prevalence for each parasite by resampling all screened blackcaps from the populations in which that specific parasite had been observed. From each sample, we obtained 1000 random samples of the same size (with repetition), which were used to compute the prevalence of each parasite. These estimates were then used to test whether the prevalence of the parasites that were found in insular and Iberian blackcaps was positively correlated between islands and mainland. We also assessed whether the prevalence of these parasites differed overall between islands and mainland, using repeated-measures ANOVA with parasite haplotype as a within-subject effect.



**Figure 20.** Cumulative parasite haplotype richness in insular (black dots) and mainland (open circles) blackcap populations. The curves represent mean richness ( $\pm$  SD) with increasing number of infections scored. The vertical line represents the sampling effort at which parasite richness was compared between islands and mainland.

## Results

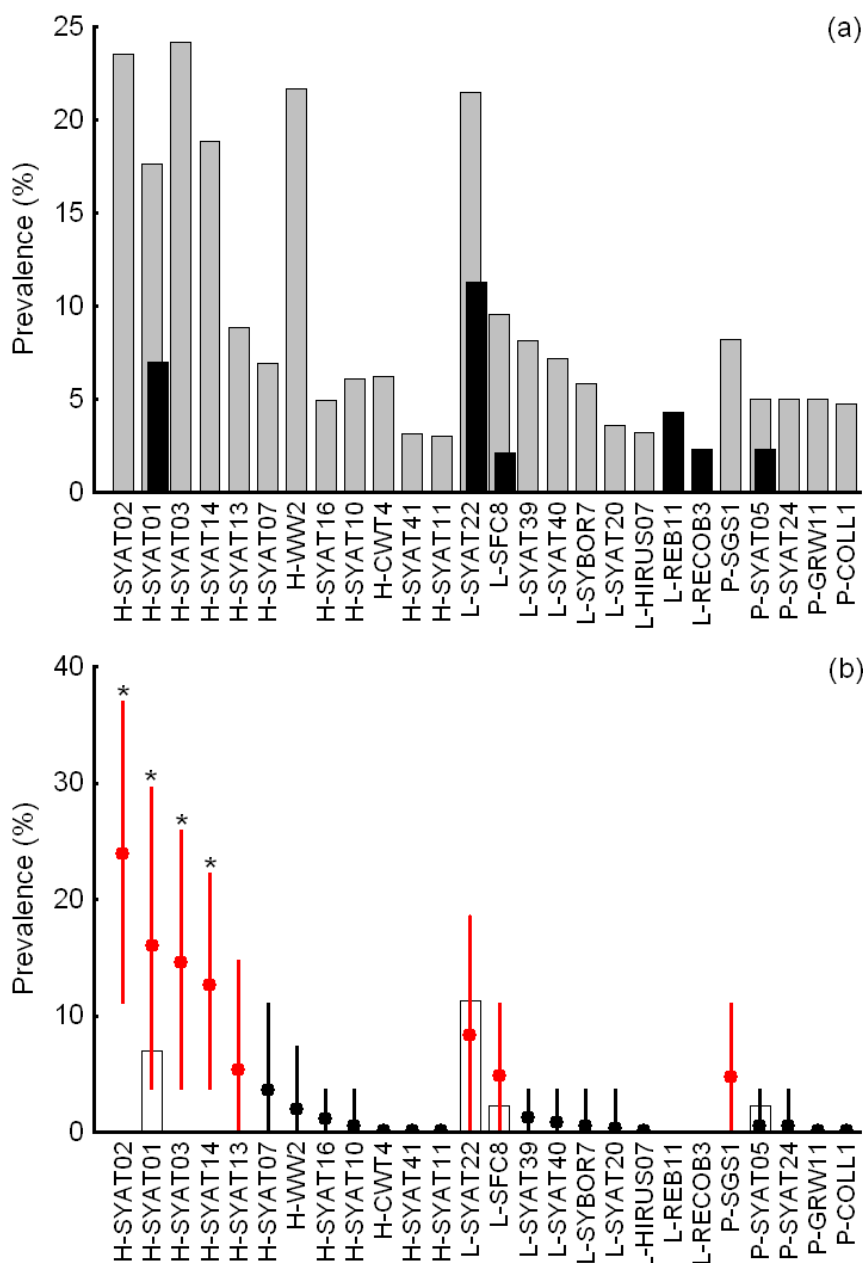
We found six different parasite haplotypes in blackcaps from Madeira and the Canary islands (Figure 19, Table 5). The insular parasite assemblage was poor compared with that found in Iberian blackcap populations (Chapter 2). Insular parasites comprised four haplotypes commonly observed among mainland blackcaps, as well as two haplotypes not previously found infecting blackcaps. Although 54.2% of the parasites found in the Iberian Peninsula were blackcap-specific (Chapter II), none of these host specialists were present among the six insular parasites. The known host and geographic ranges of these parasites, according to the MalAvi database (Bensch et al. 2009), are provided in Chapter II.

Twenty seven out of 380 blackcaps were found to be infected on the islands (Figure 19, Table 5). The observed average parasite prevalence (7.1% overall, 10.3% using only the 2007 data) was very low compared to

that observed in mainland populations (mean  $\pm$  SE =  $65.3 \pm 4.61\%$ ,  $n = 37$  Iberian localities). Parasite prevalence varied greatly among years, with no infections found in 2003, one observed in 2004, and 26 in 2007 (Table 5). Overall parasite prevalence also varied significantly among islands (chi-square test with data from 2007:  $\chi^2_{(5)} = 18.91$ ,  $P = 0.002$ ; the results of this test remained significant using birds from all years or excluding Madeira), ranging from 0% in La Palma to 22.7% in La Gomera in 2007.

All parasites shared between insular and continental blackcaps had lower prevalence in the island populations (Table 6), with differences for a given parasite between 29 - 79% less than the prevalence of that same parasite observed on the Iberian Peninsula (repeated-measures ANOVA:  $F_{1,3} = 11.16$ ,  $P = 0.044$ ; excluding Madeira:  $F_{1,3} = 10.37$ ,  $P = 0.049$ ). Nevertheless, the prevalence estimates of parasites that were found on island blackcaps were still positively correlated with those in the Iberian populations (Pearson's  $r = 0.80$ ; excluding Madeira:  $r = 0.79$ ; Table 6). Although these correlations were not significant due to small sample sizes ( $P = 0.19$  and  $0.21$  respectively,  $n = 4$  haplotypes; Spearman rank correlations, both  $r = 0.60$ ,  $P = 0.40$ ), they support our hypothesis that relative differences in prevalence among blackcap parasites on islands were similar to those observed on the continent.

The impoverishment of the insular parasite assemblage compared to that on mainland Europe was further supported by the difference observed in haplotype richness when controlling for sampling effort (mean  $\pm$  SD: islands:  $R_{25} = 5.93 \pm 0.48$ ; mainland:  $R_{25} = 10.95 \pm 2.33$ ;  $t = 284.26$ ,  $n = 10\ 000$  curves,  $P < 0.001$ ; Fig. 20). The random



**Figure 21.** (a) Prevalence of haemosporidian parasites infecting blackcaps in the mainland populations (grey bars) and on islands (black bars), computed using populations/islands where each parasite occurred. (b) Expected island parasite prevalence of each mainland parasite (dots represent means, with 95% CI) computed from 10 000 Monte Carlo simulations. Based on individual probabilities of occurrence ( $P_0$ ) obtained from these simulations, three parasites were predicted to occur on islands (marked with stars), and eight showed  $P_0 > 0.69$  (in red), which is the average probability of occurrence computed for mainland parasites that were found on islands (their prevalence is shown with open bars). Parasite genera are distinguished by the initial of haplotype names (H: *Haemoproteus*, P: *Plasmodium*, L: *Leucocytozoon*).

loss of rare parasites alone did not fully explain the impoverished island parasite assemblages, as shown by the fact that

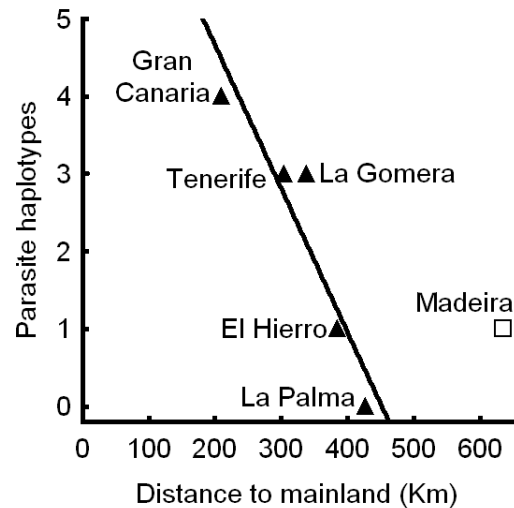
three *Haemoproteus* haplotypes (H-SYAT02, H-SYAT03 and H-SYAT14) that were extremely likely to be present

on the islands (given that they were very common on the mainland) were not found (Fig. 21). Many other haplotypes reached high prevalence in the Iberian Peninsula but were not found on islands, although the possibility that these would have been detected with a larger sample size cannot be excluded. Nevertheless, two of these parasites scored probabilities of occurrence above 0.69, which is the average estimated for the four mainland blackcap parasites that were found on the islands (Fig. 21).

Parasite richness was not correlated with the size of the blackcap's range on each island (Pearson's  $r = 0.31$ ,  $P = 0.60$ ; Spearman rank  $r = 0.41$ ,  $P = 0.49$ ;  $n = 5$ ). However, we found a negative correlation between parasite richness and island distance to the continent (Fig. 22). When all data were considered, this effect was significant only when using non-parametric statistics (Pearson's  $r = -0.83$ ,  $P = 0.084$ ; Spearman rank  $r = -0.97$ ,  $P = 0.005$ ;  $n = 5$ ). This correlation was clearer when tested considering only the more extensive data from 2007 (Pearson's  $r = -0.83$ ,  $r = -0.88$ ,  $P = 0.047$ ; Spearman rank  $r = -1$ ,  $P < 0.001$ ;  $n = 5$ ).

## Discussion

The assemblage of haemosporidian parasites found in Macaronesian blackcaps was very different from the one observed in mainland populations of the same species. The insular parasite assemblage was clearly impoverished, barely exceeding 10% of the parasite diversity known for mainland blackcaps. In addition, the structuring of the insular parasite assemblage was unusual, with *Leucocytozoon* dominating in terms of prevalence and



**Figure 22.** Correlation between parasite haplotype richness and island distance to the continent. The regression line was obtained excluding Madeira (open square), which is shown in the graph for comparative purposes only. Statistical analyses were performed with log-transformed values.

haplotype richness, whereas in mainland Europe *Haemoproteus* is the

predominant haemosporidian genus in blackcaps (Križanauskienė *et al.*, 2010; Santiago-Alarcón *et al.*, 2011; this study). As predicted, the island parasite assemblage was composed of parasites which were not specific to blackcaps, despite the fact that most parasites infecting continental blackcaps are host specific. Moreover, several of the parasites we found in insular blackcaps have already been shown to infect other species on the Atlantic Islands, despite the paucity of research on Macaronesian haemosporidians.

Our observation, that parasite richness was uncorrelated with the size of blackcaps' range on each island, was to be expected given that the parasites identified were not blackcap specific, and such generalist parasites can rely on other host species to thrive. However, we found a negative correlation between distance from the continent and

parasite richness. This correlation may result from various, not mutually exclusive processes: (1) sequential founder population bottlenecks in the parasites associated with the blackcaps (or other European host species) having first colonized the easternmost islands then spread westwards across islands (or with multiple colonization events affecting the easternmost islands more frequently; Dietzen *et al.*, 2008), (2) recurrent parasite colonization favoured by seasonal migration of blackcaps from Europe (which concentrate on the easternmost islands; Shirihai *et al.*, 2001), or (3) generalist parasites being transported from Africa and spreading westwards across the archipelago according to a stepping-stone pattern of island colonization. Whatever the mechanisms, our observation supports the basic tenet of island biogeography that the balance between colonization and extinction favours greater richness on islands that are located near the putative source of colonizers (MacArthur & Wilson, 1967), a process which could also act indirectly on parasite diversity through its effects on hosts or vectors.

Together with the observed loss of parasite richness, overall parasite prevalence also drops from 65% on the mainland to around 10% on those islands that had parasites. This difference cannot be explained solely by the absence on islands of the many blackcap parasites that raise overall prevalence on the mainland, because all parasites that were shared between island and mainland blackcaps showed lower prevalence on islands. Apart from being less prevalent, parasites demonstrated apparent temporal instability in blackcap island populations, an observation which is at odds with previous studies showing temporal consistency in the structure of haemosporidian assemblages (Pérez-

Tris & Bensch, 2005b; Durrant *et al.*, 2008; Spurgin *et al.*, 2012; but see Bensch & Åkesson, 2003). Low and unstable parasite prevalence might indicate that parasites on the blackcaps on the islands are prone to extinction. This idea is supported by the composition of the parasite assemblage (comprising non-specific parasites capable of exploiting multiple hosts), which may benefit from the exploitation of alternative host species in years when particular hosts (blackcaps in our case) may for some reason be less available. Decreasing richness of parasites with increasing island distance to the continent (a putatively uninterrupted source of parasite colonisation) may be evidence of frequent parasite extinction on islands, with parasite communities being re-seeded from the continent. Why parasite prevalence changes so much between years on islands, and whether parasites persist in other host species during periods of low prevalence in blackcaps, remain questions for future research.

An obvious candidate to explain the low richness and altered prevalence patterns observed on islands could be a reduced availability of appropriate vectors. This may be particularly relevant to the many blackcap-specific *Haemoproteus* haplotypes known from continental populations. *Haemoproteus* parasites are often vector-specific (Martínez-de la Puente *et al.*, 2011), so the apparent inability of blackcap-specific parasites to thrive on islands could be associated with their specific vectors (*Culicoides* biting midges, Diptera: Ceratopogonidae) having failed to colonize Macaronesian archipelagos. If the midge species that have colonised the islands do not target blackcaps, or if they are not competent to transmit blackcap-specific *Haemoproteus* parasites, this would explain the absence of these parasites from the

islands. Nevertheless, until the attributes of Macaronesian vector-bird-parasite interactions are thoroughly studied, we can only speculate on the possible role of vectors in driving the distribution of haemosporidians in insular blackcaps.

The only *Haemoproteus* parasite we found on insular blackcaps, *H. parabelopolskyi* H-SYAT01, is very common in mainland populations (Pérez-Tris & Bensch, 2005b; Santiago-Alarcón *et al.*, 2011; this study), which could have favoured its transport to the islands. However, other parasites lineages with higher prevalence than H-SYAT01 in mainland blackcaps were not found on islands (most notably H-SYAT02; Pérez-Tris *et al.*, 2007). H-SYAT01 is known to also infect a related species, the African hill-babbler *Sylvia abyssinica*, an Afrotropical resident (Pérez-Tris *et al.*, 2007). Therefore, it is possible that H-SYAT01 might have benefited from its ability to thrive in hosts other than the blackcap, or from being transmitted by vectors of African origin. It is also worth noting that H-SYAT01 was only found in Gran Canaria, the insular blackcap population closest to the continent. This provides additional support for the idea that migrating blackcaps may permanently enhance parasite diversity on easternmost islands.

We found low diversity and prevalence of *Plasmodium* in the insular blackcap populations, with just two infections of *P. vaughani* P-SYAT05 on two different islands. This result was unexpected because, probably as a consequence of broad host and vector range, *Plasmodium* is the most abundant haemosporidian parasite genus on oceanic islands worldwide (Wikelski *et al.*, 2004; Beadell *et al.*, 2006; Howe *et al.*, 2012; Spurgin *et al.*, 2012). However, mainland blackcap populations do not show great

prevalence or diversity of *Plasmodium* parasites. Therefore, given that parasite colonization success depends on the relative parasite frequency on the mainland (Fig. 21) the limited occurrence of this genus on the islands was to be expected. On the other hand, among *Plasmodium* haplotypes previously known for blackcaps P-SYAT05 was perhaps likely to be found on Macaronesia, because it is a host generalist that usually scores high prevalence in island avifaunas (Hellgren *et al.* 2011; Ewen *et al.*, 2012).

The parasites of the genus *Leucocytozoon* found in insular blackcap populations offer interesting examples of parasite biogeography. Based on the abundant studies of European haemosporidians conducted so far (Bensch *et al.*, 2009), we can safely assume that L-SYAT22 behaves as a blackcap-specialist in Europe. However, this is not the case in Macaronesia, where it also infects Berthelot's pipits (Spurgin *et al.*, 2012). The observation of L-SYAT22 in Berthelot's pipits may be evidence of ecological fitting (Agosta & Klemens, 2008). In this particular case, blackcaps could be acting as 'parasite reservoirs' from which L-SYAT22 spills over into other host species (a similar example is provided by blackbirds *Turdus merula* and their parasites on São Miguel Island, Azores; Hellgren *et al.*, 2011). Conversely, both L-REB11 and L-RECOB3 are *Leucocytozoon* haplotypes of putative tropical African adscription, which have been detected in several sedentary species in Nigeria. Outside mainland Africa, the haplotype L-REB11 has been found in Berthelot's pipits, while L-RECOB3 has been found in continental Europe (Ukraine) infecting garden warblers *Sylvia borin*, although only in adult individuals which had already visited tropical Africa (Hellgren *et al.* 2007a). After colonizing

Macaronesia, these parasites had the opportunity to access host species of Palearctic origin, e.g. blackcaps, as evidenced on our present study. Based on the previous records of these two parasites, their ability to infect a greater range of hosts may be constrained by strict environmental requirements or vectors that may not occur outside a narrow geographic range. Parasites like these are potential emergent disease threats if constraints on their range expansion are removed, for instance by vector species introductions or global warming (Patz *et al.*, 2000). In addition, in our study these parasites represent clear cases of local parasite acquisition by a host species that lost other parasites after range expansion (Marzal *et al.*, 2011).

The broad picture unveiled in our study is that Macaronesian blackcaps have escaped the heavy parasite burden faced by their mainland counterparts. This may be partially interpreted as the likely outcome of ‘missing the boat’ (if parasites fail to arrive with its host to the new area) or ‘drowning on arrival’ (if they do arrive but fail to become established) processes (MacLeod *et al.*, 2010) during island colonization. However, blackcaps have not only lost their usual continental parasites after island

colonization, they have also gained new parasites from those that were present in insular populations of other species. We are aware that these patterns cannot be completely understood without extensive knowledge of community-level bird-parasite interactions on the Macaronesian islands. However, our study adds to recent literature showing the impoverishment of insular parasite assemblages compared to mainland populations of the same host species (Hellgren *et al.*, 2011). In the blackcap this pattern occurs despite the potential influx of diverse parasites through seasonal host migration. Therefore, our results support the idea that the typical characteristics of insular parasite assemblages (low lineage richness, frequent host-switching, or reduced specialization of host-parasite relationships; Fallon *et al.*, 2005) may evolve before insular host populations become completely isolated from their mainland counterparts. Regardless of its causes, reduced parasite burden in insular populations of blackcaps and other species could have had evolutionary consequences (such as changes in bird immune resistance; Beadell *et al.*, 2007), which raise a cautionary word on the possible hazardous effects that the introduction of novel pathogens might have on yet another unique island bird community.



The blackcap is a widely used model organism on the study of migration; and as shown in this thesis, it is also particularly suited to the study of host-parasite relationships. For the time being, blackcaps are frequent and familiar birds throughout their wide distribution range... but we cannot take for granted, involved as we are in a process of global change, which even the most common species will remain so in the future without long term policies taking into account not only economical profit, but also environment preservation.





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## SUPPORTING INFORMATION

### Contents of this section (in order of appearance in the main text)

#### CHAPTER II

- **Table S1.** Parasite screening results per sampling locality.
- **Table S2.** Prevalence and geographic range per parasite genus.
- **Table S3.**  $R^2$  values of PLS models with and without non-climatic variables.
- **Table S4.** PLS regression predictor weights (complete table).

#### CHAPTER III

- **Appendix S1.** Parasite screening results per sampling locality.
- **Table S5.** Repeated measures ANOVA for the effects shown in Figure 15.
- **Figures S1 & S2.** Future changes on parasite richness and prevalence under scenarios A1b and B2a.

#### CHAPTER IV

- **Table S6.** Wintering season parasite screening results.
- **Table S7.** Log-linear analyses of parasite seasonal distribution in blackcap wintering grounds.



SUPPORTING INFORMATION

**Table S1.** Parasite screening results per sampling locality. For each locality, the table shows sampling year, number of blackcaps screened for parasites (*n*), and assigned host behaviour (migratory or sedentary). Richness represents the number of cytochrome *b* haplotypes of each parasite genus found in each locality. Prevalence is given as percentage of individuals infected by parasites of each genus in each locality. Locality identity numbers (ID) are the same as in Figure 3.

| ID | Year | <i>n</i> | Behaviour | <i>Haemoproteus</i> |            | <i>Plasmodium</i> |            | <i>Leucocytozoon</i> |            |
|----|------|----------|-----------|---------------------|------------|-------------------|------------|----------------------|------------|
|    |      |          |           | Richness            | Prevalence | Richness          | Prevalence | Richness             | Prevalence |
| 1  | 2009 | 22       | Migratory | 5                   | 72.7       | 1                 | 9.1        | 2                    | 13.6       |
| 2  | 2008 | 25       | Migratory | 4                   | 60         | 1                 | 32         | 4                    | 64         |
| 3  | 2009 | 24       | Migratory | 2                   | 12.5       | 1                 | 12.5       | 0                    | 0          |
| 4  | 2009 | 20       | Migratory | 3                   | 15         | 3                 | 35         | 0                    | 0          |
| 5  | 2011 | 19       | Migratory | 5                   | 100        | 0                 | 0          | 1                    | 5.3        |
| 6  | 2008 | 33       | Migratory | 7                   | 78.8       | 1                 | 12.1       | 2                    | 12.1       |
| 7  | 2009 | 21       | Migratory | 4                   | 61.9       | 1                 | 9.5        | 2                    | 9.5        |
| 8  | 2011 | 38       | Sedentary | 0                   | 0          | 1                 | 23.7       | 0                    | 0          |
| 9  | 2010 | 19       | Migratory | 2                   | 15.8       | 2                 | 10.5       | 0                    | 0          |
| 10 | 2009 | 22       | Migratory | 2                   | 13.6       | 1                 | 9.1        | 0                    | 0          |
| 11 | 2008 | 31       | Sedentary | 4                   | 29         | 2                 | 38.7       | 4                    | 16.1       |
| 12 | 2011 | 32       | Migratory | 6                   | 46.9       | 1                 | 3.1        | 2                    | 15.6       |
| 13 | 2009 | 22       | Migratory | 2                   | 36.4       | 1                 | 4.5        | 0                    | 0          |
| 14 | 2008 | 16       | Sedentary | 7                   | 93.8       | 1                 | 12.5       | 2                    | 68.8       |
| 15 | 2011 | 29       | Migratory | 6                   | 82.8       | 0                 | 0          | 2                    | 13.8       |
| 16 | 2009 | 22       | Migratory | 7                   | 90.9       | 1                 | 22.7       | 4                    | 27.3       |
| 17 | 2011 | 26       | Migratory | 6                   | 80.8       | 0                 | 0          | 4                    | 42.3       |
| 18 | 2011 | 21       | Migratory | 2                   | 52.4       | 0                 | 0          | 0                    | 0          |
| 19 | 2008 | 13       | Sedentary | 6                   | 100        | 1                 | 23.1       | 2                    | 61.5       |
| 20 | 2008 | 38       | Migratory | 6                   | 86.8       | 1                 | 21.1       | 0                    | 0          |
| 21 | 2008 | 13       | Sedentary | 6                   | 92.3       | 0                 | 0          | 2                    | 100        |
| 22 | 2011 | 25       | Migratory | 7                   | 80         | 0                 | 0          | 5                    | 52         |
| 23 | 2010 | 15       | Migratory | 6                   | 73.3       | 0                 | 0          | 1                    | 13.3       |
| 24 | 2008 | 20       | Migratory | 6                   | 90         | 0                 | 0          | 2                    | 20         |
| 25 | 2011 | 25       | Migratory | 3                   | 28         | 2                 | 28         | 0                    | 0          |
| 26 | 2011 | 32       | Migratory | 6                   | 65.6       | 1                 | 6.3        | 0                    | 0          |
| 27 | 2009 | 23       | Migratory | 3                   | 30.4       | 0                 | 0          | 1                    | 8.7        |
| 28 | 2009 | 30       | Migratory | 5                   | 90         | 1                 | 23.3       | 0                    | 0          |
| 29 | 2011 | 24       | Sedentary | 4                   | 75         | 0                 | 0          | 5                    | 45.8       |
| 30 | 2009 | 19       | Migratory | 2                   | 15.8       | 1                 | 10.5       | 0                    | 0          |
| 31 | 2008 | 13       | Sedentary | 4                   | 61.5       | 2                 | 46.2       | 1                    | 15.4       |
| 32 | 2009 | 25       | Migratory | 2                   | 8          | 1                 | 12         | 0                    | 0          |
| 33 | 2009 | 20       | Migratory | 6                   | 45         | 1                 | 10         | 1                    | 5          |
| 34 | 2011 | 21       | Migratory | 5                   | 71.4       | 2                 | 9.5        | 0                    | 0          |
| 35 | 2009 | 43       | Migratory | 5                   | 39.5       | 2                 | 9.3        | 1                    | 2.3        |
| 36 | 2008 | 20       | Migratory | 6                   | 65         | 1                 | 20         | 1                    | 10         |
| 37 | 2009 | 21       | Migratory | 7                   | 90.5       | 1                 | 38.1       | 1                    | 4.8        |

**Table S2.** Prevalence and geographic range per parasite haplotype. For each parasite cytochrome *b* haplotype found in the study, the table shows sequence Genbank accession number, taxonomic identity (if known) and number of known host species (n = 1 implies that the parasite is blackcap-specific according to current knowledge) according to the MalAvi database (see Bensch *et al.*, 2009; cited in the paper), total prevalence across localities, and geographic range (number of populations where each parasite lineage occurred).

| Haplotype                         | Genbank accession number | Parasite morphospecies              | Number of hosts | Number of infections | Total prevalence (%) | Number of populations |
|-----------------------------------|--------------------------|-------------------------------------|-----------------|----------------------|----------------------|-----------------------|
| H-SYAT01                          | AY831750                 | <i>Haemoproteus parabelopolskyi</i> | 2               | 132                  | 15.0                 | 32                    |
| H-SYAT02                          | AY831751                 | <i>Haemoproteus parabelopolskyi</i> | 1               | 194                  | 22.0                 | 35                    |
| H-SYAT07                          | AY831754                 | <i>Haemoproteus parabelopolskyi</i> | 1               | 30                   | 3.4                  | 17                    |
| H-SYAT10                          | AY831757                 | <i>Haemoproteus parabelopolskyi</i> | 1               | 4                    | 0.5                  | 3                     |
| H-SYAT11                          | AY831758                 | <i>Haemoproteus parabelopolskyi</i> | 1               | 1                    | 0.1                  | 1                     |
| H-SYAT13                          | AY831760                 | <i>Haemoproteus parabelopolskyi</i> | 1               | 44                   | 5.0                  | 22                    |
| H-SYAT14                          | AY831761                 | <i>Haemoproteus parabelopolskyi</i> | 1               | 88                   | 10.0                 | 24                    |
| H-SYAT16                          | AY831762                 | <i>Haemoproteus parabelopolskyi</i> | 1               | 9                    | 1.0                  | 7                     |
| H-SYAT41                          | JQ670873                 | <i>Haemoproteus parabelopolskyi</i> | 1               | 1                    | 0.1                  | 1                     |
| H-SYAT03                          | AY831752                 | <i>Haemoproteus pallidulus</i>      | 1               | 120                  | 13.6                 | 22                    |
| H-WW2                             | AY831755                 | <i>Haemoproteus majoris</i>         | 17              | 16                   | 1.8                  | 4                     |
| H-CWT4                            | AY393805                 | <i>Haemoproteus majoris</i>         | 6               | 1                    | 0.1                  | 1                     |
| P-GRW11                           | AY831748                 | <i>Plasmodium relictum</i>          | 24              | 4                    | 0.5                  | 4                     |
| P-SGS1                            | AF495571                 | <i>Plasmodium relictum</i>          | 55              | 38                   | 4.3                  | 17                    |
| P-SYAT05                          | DQ847271                 | <i>Plasmodium vaughani</i>          | 11              | 4                    | 0.5                  | 4                     |
| P-SYAT24                          | AY831749                 | <i>Plasmodium sp.</i>               | 1               | 1                    | 0.1                  | 1                     |
| P-COLL1                           | AY831747                 | <i>Plasmodium sp.</i>               | 6               | 1                    | 0.1                  | 1                     |
| L-HIRUS07                         | JN164707                 | <i>Leucocytozoon sp.</i>            | 2               | 1                    | 0.1                  | 1                     |
| L-SYBOR7                          | DQ847238                 | <i>Leucocytozoon sp.</i>            | 2               | 3                    | 0.3                  | 2                     |
| L-SFC8                            | DQ847234                 | <i>Leucocytozoon sp.</i>            | 4               | 40                   | 4.5                  | 18                    |
| L-SYAT20                          | DQ847235                 | <i>Leucocytozoon sp.</i>            | 1               | 4                    | 0.5                  | 4                     |
| L-SYAT22                          | DQ847236                 | <i>Leucocytozoon sp.</i>            | 2               | 68                   | 7.7                  | 17                    |
| L-SYAT39                          | JN164705                 | <i>Leucocytozoon sp.</i>            | 1               | 10                   | 1.1                  | 6                     |
| L-SYAT40                          | JN164706                 | <i>Leucocytozoon sp.</i>            | 1               | 7                    | 0.8                  | 4                     |
| Unidentified <i>Haemoproteus</i>  |                          |                                     |                 | 57                   | 6.5                  | 15                    |
| Unidentified <i>Plasmodium</i>    |                          |                                     |                 | 71                   | 8.1                  | 22                    |
| Unidentified <i>Leucocytozoon</i> |                          |                                     |                 | 13                   | 1.5                  | 7                     |

**Table S3.**  $R^2$  values of PLS regression models with and without non-climatic variables. Different models were built with all 48 variables considered in the study (complete models), with all variables but the climatic ones (no-climate models), or with climatic variables alone (climate-only models). Some analyses retained no significant factor (NS). The statistical significance of the improvement of climate-only models (expressed as increment in  $R^2$  resulting from adding non-climatic variables) was computed by comparing both types of models in 100 databases obtained by bootstrap of the original data (with repetition).

|                                   | <i>Haemoproteus</i> |            | <i>Plasmodium</i> |            | <i>Leucocytozoon</i> |            |
|-----------------------------------|---------------------|------------|-------------------|------------|----------------------|------------|
|                                   | Richness            | Prevalence | Richness          | Prevalence | Richness             | Prevalence |
| <b>PLS <math>R^2</math></b>       |                     |            |                   |            |                      |            |
| Complete model                    | 0.391               | 0.387      | 0.341             | 0.379      | 0.456                | 0.581      |
| No-climate model                  | NS                  | NS         | NS                | NS         | NS                   | 0.455      |
| Climate-only model                | 0.308               | 0.264      | 0.229             | 0.264      | 0.253                | NS         |
| <b>Climatic model improvement</b> |                     |            |                   |            |                      |            |
| delta $R^2$                       | 0.081               | 0.119      | 0.122             | 0.112      | 0.167                | 0.232      |
| <i>p</i>                          | < 0.01              | < 0.01     | < 0.01            | < 0.01     | < 0.01               | < 0.01     |

**Table S4.** PLS regression predictor weights (complete table). Partial Least Squares regression weights of each predictor variable on the factors (columns) retained in the models obtained for each dependent variable (haplotype richness and prevalence of each parasite genus). In all cases, only one factor was retained in the final model. Independent variables have been grouped into different categories (whose joint contributions are shown in Figure 12, in Chapter II). The weights of variables that significantly contributed to each factor are highlighted in bold.  $R^2$  values of each model are indicated at the bottom of the table.

| Predictor variables<br>(grouped by type):       | <i>Haemoproteus</i> |               | <i>Plasmodium</i> |               | <i>Leucocytozoon</i> |               |
|---|---------------------|---------------|-------------------|---------------|----------------------|---------------|
|   | Richness            | Prevalence    | Richness          | Prevalence    | Richness             | Prevalence    |
| <b>Confounding variables</b>                    |                     |               |                   |               |                      |               |
| Sex ratio                                       | -0.036              | -0.109        | 0.140             | 0.078         | -0.005               | -0.028        |
| Proportion of adults                            | 0.079               | 0.038         | -0.081            | -0.084        | 0.108                | <b>0.151</b>  |
| Sampling year: 2008                             | <b>0.157</b>        | <b>0.184</b>  | <b>-0.172</b>     | <b>-0.197</b> | <b>-0.144</b>        | <b>0.254</b>  |
| Sampling year: 2009                             | -0.099              | <b>-0.170</b> | 0.135             | 0.139         | -0.069               | <b>-0.187</b> |
| Sampling year: 2010                             | -0.032              | -0.055        | 0.036             | 0.092         | 0.026                | -0.046        |
| Sampling year: 2011                             | -0.031              | 0.028         | -0.013            | -0.072        | <b>0.166</b>         | -0.027        |
| <b>Geographical effects</b>                     |                     |               |                   |               |                      |               |
| Longitude (X)                                   | -0.024              | -0.128        | 0.100             | -0.003        | -0.060               | -0.074        |
| Latitude (Y)                                    | 0.104               | 0.084         | -0.124            | <b>-0.160</b> | -0.065               | <b>-0.197</b> |
| X:Y   | -0.010              | -0.056        | 0.124             | <b>0.200</b>  | <b>0.160</b>         | 0.125         |
| X <sup>2</sup>                                  | -0.064              | -0.114        | 0.125             | 0.110         | -0.083               | -0.100        |
| Y <sup>2</sup>                                  | 0.009               | <b>0.148</b>  | 0.014             | 0.107         | <b>0.151</b>         | <b>0.237</b>  |
| X <sup>2</sup> :Y                               | 0.077               | 0.066         | -0.083            | -0.097        | -0.072               | -0.108        |
| X:Y <sup>2</sup>                                | 0.034               | -0.087        | -0.059            | <b>-0.147</b> | -0.030               | -0.056        |
| X <sup>3</sup>                                  | -0.097              | <b>-0.185</b> | 0.092             | 0.026         | -0.096               | -0.089        |
| Y <sup>3</sup>                                  | -0.007              | -0.038        | -0.129            | <b>-0.197</b> | -0.112               | <b>-0.233</b> |
| <b>Temperature</b>                              |                     |               |                   |               |                      |               |
| Annual mean temperature                         | <b>-0.275</b>       | <b>-0.221</b> | <b>0.232</b>      | <b>0.221</b>  | <b>-0.212</b>        | -0.137        |
| Maximum annual mean temperature                 | <b>-0.274</b>       | <b>-0.238</b> | <b>0.246</b>      | <b>0.233</b>  | <b>-0.211</b>        | -0.115        |
| Minimum annual mean temperature                 | <b>-0.253</b>       | <b>-0.188</b> | <b>0.207</b>      | <b>0.199</b>  | <b>-0.200</b>        | <b>-0.151</b> |
| Breeding mean temperature                       | <b>-0.265</b>       | <b>-0.226</b> | <b>0.247</b>      | <b>0.233</b>  | <b>-0.232</b>        | <b>-0.165</b> |
| Maximum breeding mean temperature               | <b>-0.262</b>       | <b>-0.247</b> | <b>0.266</b>      | <b>0.251</b>  | <b>-0.235</b>        | -0.150        |
| Minimum breeding mean temperature               | <b>-0.245</b>       | <b>-0.194</b> | <b>0.227</b>      | <b>0.217</b>  | <b>-0.207</b>        | <b>-0.165</b> |
| Maximum temperature of the warmest month        | -0.006              | -0.076        | 0.082             | 0.063         | -0.005               | 0.057         |
| Minimum temperature of the coldest month        | 0.069               | <b>0.169</b>  | 0.053             | 0.058         | 0.050                | 0.035         |
| Temperature annual range                        | -0.022              | <b>-0.147</b> | 0.052             | 0.023         | -0.030               | 0.018         |
| Temperature mean diurnal range                  | <b>-0.200</b>       | <b>-0.231</b> | <b>0.182</b>      | <b>0.169</b>  | <b>-0.145</b>        | -0.035        |
| Isothermality (mean diurnal range/annual range) | <b>-0.219</b>       | -0.136        | <b>0.161</b>      | <b>0.162</b>  | <b>-0.177</b>        | <b>-0.153</b> |
| Temperature seasonality (SD of isothermality)   | -0.020              | -0.120        | 0.063             | 0.042         | -0.015               | 0.048         |
| Mean temperature of the wettest quarter         | <b>-0.205</b>       | <b>-0.200</b> | <b>0.204</b>      | <b>0.144</b>  | <b>-0.208</b>        | <b>-0.211</b> |
| Mean temperature of the driest quarter          | -0.115              | -0.045        | -0.043            | 0.017         | -0.032               | 0.014         |
| Mean temperature of the warmest quarter         | <b>-0.271</b>       | <b>-0.271</b> | <b>0.230</b>      | <b>0.208</b>  | <b>-0.209</b>        | -0.106        |
| Mean temperature of the coldest quarter         | <b>-0.237</b>       | <b>-0.148</b> | <b>0.189</b>      | <b>0.184</b>  | <b>-0.174</b>        | -0.126        |
| <b>Rainfall</b>                                 |                     |               |                   |               |                      |               |
| Annual rainfall                                 | 0.056               | <b>0.143</b>  | -0.087            | -0.026        | <b>0.165</b>         | 0.109         |
| Breeding rainfall                               | 0.094               | <b>0.148</b>  | -0.122            | -0.080        | <b>0.199</b>         | 0.129         |
| Rainfall of the wettest month                   | -0.009              | 0.093         | -0.017            | 0.049         | 0.117                | 0.093         |
| Rainfall of the driest month                    | 0.123               | 0.093         | <b>-0.155</b>     | <b>-0.219</b> | -0.007               | -0.088        |
| Rainfall seasonality (coefficient of variation) | <b>-0.154</b>       | -0.088        | 0.119             | <b>0.174</b>  | -0.030               | 0.094         |
| Rainfall of the wettest quarter                 | 0.003               | 0.108         | -0.025            | 0.053         | <b>0.144</b>         | 0.121         |
| Rainfall of the driest quarter                  | 0.109               | 0.077         | -0.136            | <b>-0.197</b> | -0.004               | -0.108        |
| Rainfall of the warmest quarter                 | 0.099               | 0.073         | -0.115            | <b>-0.182</b> | -0.005               | -0.107        |
| Rainfall of the coldest quarter                 | 0.040               | <b>0.158</b>  | -0.079            | 0.028         | <b>0.187</b>         | <b>0.176</b>  |

Table S3 continues on the next page.

SUPPORTING INFORMATION

Table S3 continued from previous page.

| Predictor variables<br>(grouped by type):        | <i>Haemoproteus</i> |               | <i>Plasmodium</i> |               | <i>Leucocytozoon</i> |               |
|--|---------------------|---------------|-------------------|---------------|----------------------|---------------|
|  | Richness            | Prevalence    | Richness          | Prevalence    | Richness             | Prevalence    |
| <b>Landscape features</b>                        |                     |               |                   |               |                      |               |
| Altitude   | <b>0.158</b>        | 0.072         | -0.106            | -0.120        | <b>0.185</b>         | <b>0.169</b>  |
| Slope  | 0.042               | 0.096         | <b>-0.228</b>     | <b>-0.175</b> | <b>0.272</b>         | <b>0.309</b>  |
| Mean annual NDVI                                 | -0.091              | 0.013         | -0.113            | -0.048        | 0.034                | -0.019        |
| Mean breeding NDVI                               | -0.068              | 0.038         | -0.084            | -0.004        | 0.062                | -0.005        |
| Percentage of coniferous forest                  | -0.013              | -0.045        | -0.069            | -0.118        | 0.096                | 0.090         |
| Percentage of broadleaf forest                   | -0.012              | 0.079         | <b>-0.185</b>     | -0.094        | <b>0.181</b>         | 0.103         |
| Percentage of shrubland                          | <b>-0.269</b>       | <b>-0.264</b> | 0.102             | 0.080         | -0.019               | -0.040        |
| Percentage of open spaces                        | 0.126               | 0.075         | <b>0.153</b>      | 0.126         | -0.076               | -0.073        |
| Percentage of wooded croplands                   | 0.037               | 0.079         | -0.138            | -0.110        | 0.015                | -0.017        |
| Percentage of arable land                        | 0.130               | 0.021         | -0.052            | -0.041        | 0.024                | -0.021        |
| Percentage of water bodies                       | -0.032              | -0.081        | 0.086             | 0.019         | -0.053               | 0.041         |
| Percentage of urban areas                        | -0.073              | -0.089        | 0.028             | 0.010         | <b>-0.202</b>        | <b>-0.171</b> |
| <b>Behaviour</b>                                 |                     |               |                   |               |                      |               |
| Migration: Migration                             | 0.052               | -0.049        | 0.013             | -0.090        | <b>-0.150</b>        | <b>-0.256</b> |
| Migration: Sedentarism                           | -0.052              | 0.049         | -0.013            | 0.090         | <b>0.150</b>         | <b>0.256</b>  |
| <b>R2 of the model</b><br>(% variance explained) | <b>39.5</b>         | <b>40.0</b>   | <b>36.1</b>       | <b>38.8</b>   | <b>46.5</b>          | <b>60.6</b>   |

**Appendix S1.** Random Forest analyses and results.

Random Forest (Breiman, 2001) is a machine learning technique which has revealed itself as a very convenient tool in ecological studies (Cutler *et al.*, 2007), including its use as a SDM technique (Sehgal *et al.*, 2010; Bisrat *et al.*, 2012; Tôrres *et al.*, 2012). Random Forest fits many regression trees to a data set, and then combines the predictions from all the trees. The algorithm selects a given number of bootstrap samples (which typically include 2/3 of the original observations) from the dataset, fitting a regression tree to each of them, but using at each node only a subset of the available variables as potential splitters. Every tree is fully grown and used to predict the 1/3 of observations (called “out-of-bag”) not used in its construction. Accuracies and error rates for each observation are computed using the out-of-bag predictions, and then averaged over all observations. Given that the out-of-bag observations were not used in the fitting of the trees, the out-of-bag estimates are essentially cross-validated accuracy estimates. The combined information of all the obtained regression trees allows calculating both the relative importance of each variable and their reliability as predictors (Cutler *et al.*, 2007).

Random Forest analyses were performed using the Random Forest R package (Liaw & Wiener, 2002) implemented in ArcGIS through the Marine Geospatial Ecology Arctoolbox (Roberts *et al.*, 2010). Using as inputs the information of several GIS layers and the georeferenced values of the response variable, it gives as outputs at the same time the results of the R analyses (including the proportion of explained variance of each model - pseudo-R<sup>2</sup>-) and the predicted values as a new GIS layer; elaborated either from the original GIS layers or from different ones (the ones of different climate change scenarios in our case).

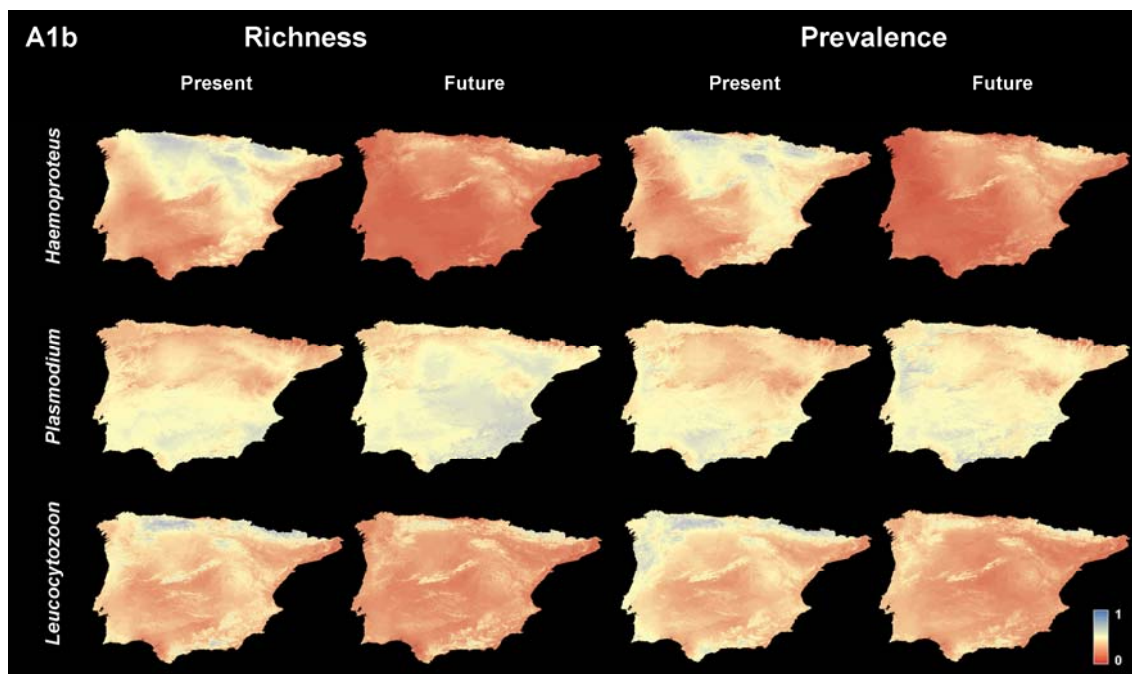
In spite of the great potential that Random Forest has as a SDM tool, the explanatory power of the models we obtained was very low (see table) and thus they were unsuitable to elaborate reliable predictions; arguably because of the difficulty to make accurate quantitative predictions of parasite prevalence or richness with a small number of sample points. Therefore we decided to use MaxEnt to make our predictions, given that it has been shown to perform remarkably well when only a small dataset is available (Bean *et al.*, 2012).

| <b>Response variable</b>        | <b>PseudoR<sup>2</sup></b> |
|---------------------------------|----------------------------|
| <i>Haemoproteus</i> richness    | 29.8                       |
| <i>Haemoproteus</i> prevalence  | 1.97                       |
| <i>Plasmodium</i> richness      | 4.38                       |
| <i>Plasmodium</i> prevalence    | 26                         |
| <i>Leucocytozoon</i> richness   | 20.4                       |
| <i>Leucocytozoon</i> prevalence | 38.2                       |

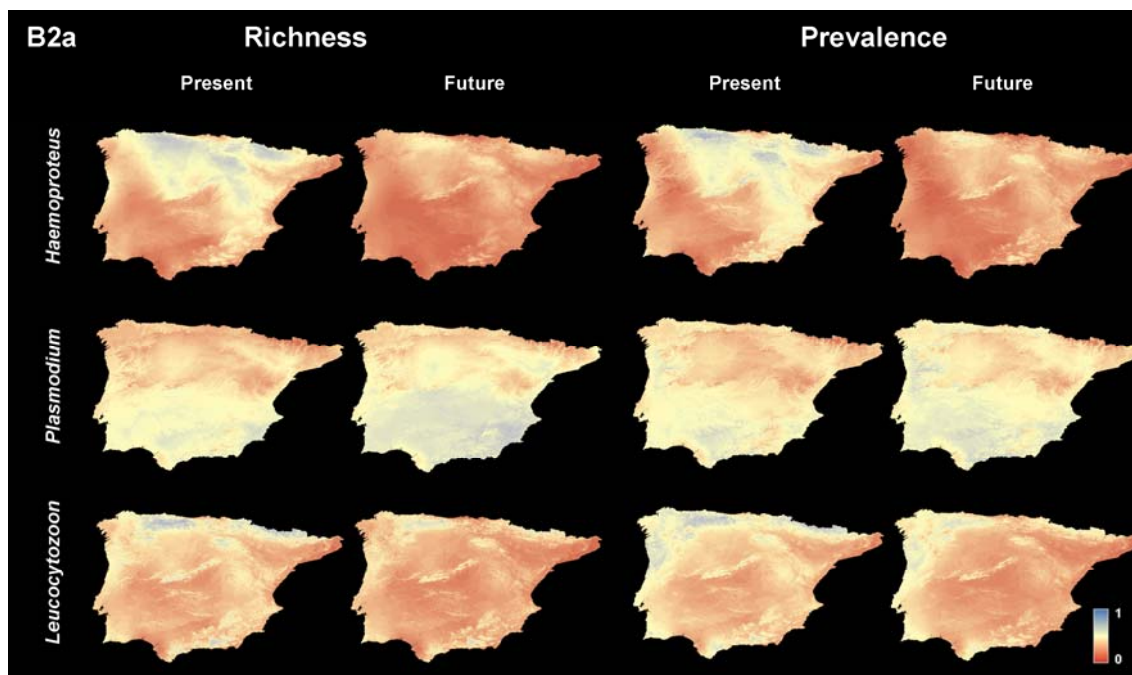
**Table S5.** Repeated measures ANOVA for variation between current and future conditions in the probability of high impact of each parasite (haplotype richness or prevalence above current average values), under the three climate change scenarios considered.

| Source of variation            | Parasite richness |          |        |         | Parasite prevalence |          |        |         |
|--------------------------------|-------------------|----------|--------|---------|---------------------|----------|--------|---------|
|                                | df effect         | df error | F      | P       | df effect           | df error | F      | P       |
| <b>Scenario</b>                | 2                 | 1984     | 747,6  | < 0.001 | 2                   | 1980     | 1504,4 | < 0.001 |
| <b>Genus</b>                   | 2                 | 1984     | 1002,5 | < 0.001 | 2                   | 1980     | 795,5  | < 0.001 |
| <b>Time</b>                    | 1                 | 992      | 4927,3 | < 0.001 | 1                   | 990      | 5937,5 | < 0.001 |
| <b>Scenario × Genus</b>        | 4                 | 3968     | 942,4  | < 0.001 | 4                   | 3960     | 911,9  | < 0.001 |
| <b>Scenario × Time</b>         | 2                 | 1984     | 747,6  | < 0.001 | 2                   | 1980     | 1504,4 | < 0.001 |
| <b>Genus × Time</b>            | 2                 | 1984     | 6294,1 | < 0.001 | 2                   | 1980     | 6480,4 | < 0.001 |
| <b>Scenario × Genus × Time</b> | 4                 | 3968     | 942,4  | < 0.001 | 4                   | 3960     | 911,9  | < 0.001 |

**Figure S1.** Present and future distribution of the probability of areas with high richness and prevalence of *Haemoproteus*, *Plasmodium* and *Leucocytozoon* in Iberian blackcaps. The colour range varies between red (0) and blue (1). Future distribution of probability is projected according to a scenario for future climate assuming severe changes (A1b emission scenario).



**Figure S2.** Present and future distribution of the probability of areas with high richness and prevalence of *Haemoproteus*, *Plasmodium* and *Leucocytozoon* in Iberian blackcaps. The colour range varies between red (0) and blue (1). Future distribution of probability is projected according to a scenario for future climate assuming light changes (B2a emission scenario).





SUPPORTING INFORMATION

**Table S6.** Wintering season parasite screening results. Parasite lineages detected and prevalences per month.

| Parasite morphospecies              | Lineage   | Genbank<br>Acces. Number | Number of<br>infections | Prevalences per month (in %; number of sampled birds is shown) |          |          |          |           |          |             |
|-------------------------------------|-----------|--------------------------|-------------------------|--|----------|----------|----------|-----------|----------|-------------|
|                                     |           |                          |                         | Sep (74)   | Oct (71) | Nov (88) | Dec (84) | Jan (142) | Feb (76) | Mar<br>(36) |
| <i>Haemoproteus parabelopolskyi</i> | H-SYAT01  | AY831750                 | 152                     | 31.1   | 39.4     | 27.3     | 19.0     | 19.0      | 22.4     | 47.2        |
| <i>Haemoproteus parabelopolskyi</i> | H-SYAT02  | AY831751                 | 53                      | 37.8   | 19.7     | 4.5      | 1.2      | 0.7       | 3.9      | 5.6         |
| <i>Haemoproteus parabelopolskyi</i> | H-SYAT07  | AY831754                 | 58                      | 5.4  | 18.3     | 11.4     | 15.5     | 9.9       | 3.9      | 2.8         |
| <i>Haemoproteus parabelopolskyi</i> | H-SYAT13  | AY831760                 | 28                      | 10.8   | 7.0      | 6.8      | 0.0      | 1.4       | 0.0      | 19.4        |
| <i>Haemoproteus parabelopolskyi</i> | H-SYAT10  | AY831757                 | 9                       | 4.1  | 0.0      | 3.4      | 1.2      | 0.7       | 0.0      | 2.8         |
| <i>Haemoproteus parabelopolskyi</i> | H-SYAT16  | AY831762                 | 2                       | 1.4  | 0.0      | 0.0      | 0.0      | 0.0       | 1.3      | 0.0         |
| <i>Haemoproteus parabelopolskyi</i> | H-SYAT36  | JN164702                 | 1                       | 0.0  | 1.4      | 0.0      | 0.0      | 0.0       | 0.0      | 0.0         |
| <i>Haemoproteus parabelopolskyi</i> | H-SYAT37  | JN164703                 | 1                       | 0.0  | 0.0      | 0.0      | 0.0      | 0.7       | 0.0      | 0.0         |
| <i>Haemoproteus pallidulus</i>      | H-SYAT03  | AY831752                 | 3                       | 1.4  | 0.0      | 0.0      | 0.0      | 0.0       | 0.0      | 5.6         |
| <i>Plasmodium relictum</i>          | P-SGS1    | AF495571                 | 29                      | 2.7  | 4.2      | 6.8      | 6.0      | 4.2       | 6.6      | 5.6         |
| <i>Plasmodium relictum</i>          | P-GRW11   | AY831748                 | 1                       | 0.0  | 0.0      | 0.0      | 0.0      | 0.7       | 0.0      | 0.0         |
| <i>Plasmodium relictum</i>          | P-COLL1   | AY831747                 | 1                       | 0.0  | 0.0      | 1.1      | 0.0      | 0.0       | 0.0      | 0.0         |
| <i>Plasmodium elongatum</i>         | P-GRW06   | DQ368381                 | 1                       | 0.0  | 0.0      | 0.0      | 0.0      | 0.0       | 0.0      | 2.8         |
| <i>Plasmodium sp.</i>               | P-SYAT24  | AY831749                 | 1                       | 1.4  | 0.0      | 0.0      | 0.0      | 0.0       | 0.0      | 0.0         |
| <i>Plasmodium sp.</i>               | P-SYAT38  | JN164704                 | 1                       | 0.0  | 0.0      | 0.0      | 1.2      | 0.0       | 0.0      | 0.0         |
| <i>Plasmodium circumflexum</i>      | P-TURDUS1 | AF495576                 | 1                       | 0.0  | 0.0      | 0.0      | 0.0      | 0.0       | 1.3      | 0.0         |

**Table S7.** Log-linear analyses of parasite seasonal distribution in blackcap wintering grounds. Relationships between the most frequently found *Haemoproteus parabelopolskyi* lineages and sampling season or host group (three different groups: sedentary or migratory blackcaps in woodlands and migratory blackcaps in shrublands). Each log-linear model was built from the three-way contingency table including infection status by the corresponding parasite, season and host group. Only significant two-way interactions involving infection status are reported for each model. All models had a reasonable fit to the data (maximum likelihood chi-square tests with  $P > 0.13$ ).

| Interactions in the model        | d.f. | Partial association |          | Marginal association |          |
|----------------------------------|------|---------------------|----------|----------------------|----------|
|                                  |      | $\chi^2$            | <i>P</i> | $\chi^2$             | <i>P</i> |
| Season × H-SYAT01                | 2    | 10.29               | 0.006    | 11.35                | 0.003    |
| Season × H-SYAT02                | 2    | 48.05               | < 0.001  | 51.69                | < 0.001  |
| Season × H-SYAT07                | 2    | 7.61                | 0.022    | 6.34                 | 0.042    |
| Season × H-SYAT13                | 2    | 13.67               | 0.001    | 14.26                | 0.001    |
| Host population group × H-SYAT01 | 2    | 6.88                | 0.032    | 7.95                 | 0.019    |
| Host population group × H-SYAT02 | 2    | 5.92                | 0.052    | 9.55                 | 0.008    |
| Host population group × H-SYAT07 | 2    | 7.64                | 0.022    | 6.37                 | 0.041    |
| Host population group × H-SYAT13 | 2    | 11.05               | 0.004    | 11.65                | 0.003    |



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