ORIGINAL PAPER

Genetic characterization, distribution and prevalence of avian pox and avian malaria in the Berthelot's pipit (*Anthus berthelotii*) in Macaronesia

**Juan Carlos Illera · Brent C. Emerson ·
David S. Richardson**

Abstract Exotic pathogens have been implicated in the decline and extinction of various native-island-bird species. Despite the fact that there is increasing concern about the introduction of diseases in island ecosystems, little is known about parasites in the islands of Macaronesia. We focus on Berthelot's pipit (*Anthus berthelotii*), an endemic and widespread Macaronesian bird species, using a combination of field studies and molecular techniques to determine: (1) the range and prevalence of avian pox and malaria in Berthelot's pipits throughout the species' distribution, (2) the genetic characterization of both parasites in order to ascertain the level of host specificity. We sampled 447 pipits across the 12 islands inhabited by this species. Overall, 8% of all individuals showed evidence of pox lesions and 16% were infected with avian malaria, respectively. We observed marked differences in the prevalence of parasites among islands both within and between archipelagos. Avian pox prevalence varied between 0–54% within and between archipelagos and avian malaria prevalence varied between 0–64% within and between archipelagos. The diversity of pathogens detected was low: only two genetic lineages of avian malaria and one lineage of avian pox were found to infect the pipit throughout its range. Interestingly, both avian malaria parasites found were *Plasmodium* spp. that had not been previously reported in the Macaronesian avifauna (but that had been observed in the lesser kestrel *Falco naumannii*), while the avian pox was a host specific lineage that had previously been reported on two of the Canary Islands.

J. C. Illera (✉)
Island Ecology and Evolution Research Group, IPNA, CSIC,
C/ Astrofísico Francisco Sánchez, 3,
E-38206 La Laguna, Tenerife, Canary Islands, Spain
e-mail: jcillera@ipna.csic.es

B. C. Emerson · D. S. Richardson
Centre for Ecology, Evolution and Conservation,
School of Biological Sciences, University of East Anglia,
Norwich NR4 7TJ, UK

Introduction

Although identifying unambiguous causal relationships between the introduction of pathogens and extinction has proved elusive, pathogens have been implicated in the extinction of various island bird species, (Wikelski et al. 2004; Smith et al. 2006). Avian pox and avian malaria are two pathogens that have been identified as major problems for the conservation of endemic bird species inhabiting islands (Warner 1968; van Riper et al. 1986, 2002; Kleindorfer and Dudaniec 2006). Both pox and malaria have been implicated in the decline and extinction of a number of endemic bird species (e.g., van Riper et al. 1986, 2002; Vargas 1987), and the impact of their introduction on the avifauna of archipelagos, such as the Galápagos and Hawaii, has been disastrous (Warner 1968; van Riper et al. 1986, 2002). However, the negative effects of infection on a range of parameters such as antipredator defenses (Laiolo et al. 2004, 2007), male pairing success (Kleindorfer and Dudaniec 2006), average productivity (Carrete et al. 2008) and individual survival (Møller and Nielsen 2007) has only recently been investigated.

Understanding the identity, prevalence and host range of parasites is essential to understand the limits of their transmission, host specificity and virulence, but also to provide insights into the impact of invasive diseases on native species through population monitoring. In addition, this information will help asses risk management for such

activities as the transfer of host species to new habitats and islands.

The Macaronesian region (i.e., Azores, Madeira, Selvagens, Canaries and Cape Verde) has been the subject of considerable ornithological research (e.g. Hazevoet 1995; Martín and Lorenzo 2001; Oliveira and Menezes 2004; Lorenzo 2007). However, while the presence of pathogens has been documented in the avifauna, this work is very limited and/or anecdotal. Little work has been done on the genetic identity, prevalence, geographic distribution or impact of pathogens. For example, avian pox has only been reported in three wild species across three islands in the Canaries (Medina et al. 2004; Smits et al. 2005) while avian malaria (*Haemoproteus columbae*) has only ever been reported in common pigeons (*Columba livia*) living in the capital of Tenerife (Foronda et al. 2004). Given the diversity and prevalence of pathogens across bird populations around the world (Waldenström et al. 2002; Wikelski et al. 2004; Beadell et al. 2004, 2006; Pérez-Tris et al. 2007; Thomas et al. 2007), there is little doubt that similar pathogens will be prevalent throughout the Macaronesian islands. Gaining detailed information on the identity, distribution and impact of these pathogens across these isolated archipelagos is of scientific interest as it will allow us to investigate processes such as within-host parasite speciation and host resistance evolution across multiple populations of the same species (Bonneaud et al. 2006; Pérez-Tris et al. 2007; Loiseau et al. 2008). Gaining such information should also be a conservation priority due to the high number of endemic bird species that inhabit these islands (Stattersfield et al. 1998).

Berthelot's pipit (*Anthus berthelotii*) is an endemic passerine inhabiting the Canary, Selvagen and Madeiran islands, where it is considered one of the most common bird species inhabiting Macaronesia (Oliveira and Menezes 2004; Illera 2007). The presence and prevalence of avian pox has recently been documented in the pipit on two of the Canary Islands (Smits et al. 2005), but there is no information regarding the rest of its range. No studies have been undertaken on avian malaria in this species. The aim of the present study is to: (1) determine the prevalence of avian pox and malaria in Berthelot's pipit throughout the species' distribution (2) use molecular techniques to identify and characterize the strain/species of these pathogens infecting the pipit and, (3) to ascertain the level of host specificity of both diseases infecting this bird.

Materials and methods

Berthelot's pipit is a small sedentary passerine (≈16 g weight) that inhabits open and semi-arid habitats on all the main islands and islets (18 in total) of the Canary Islands,

Madeira and Selvagens archipelagos (Fig. 1, Snow and Perrins 1998). It is thought to have initially colonized the Atlantic archipelagos during the late Pliocene (≈2.5 million years, Voelker 1999) but appears to have only recently dispersed (from south to north) across the Macaronesian islands (Illera et al. 2007). The islands of these archipelagos, which are volcanic and have never been connected with the mainland, vary widely in altitude. At 163 m above sea level, Selvagen Grande is the lowest lying while Tenerife, reaching a peak of 3,718 m, is the highest. Berthelot's pipit is especially abundant on the coastal habitats of the main islands, but it can be also found in alpine habitats at altitudes of up to ≈3,700 m above sea level (Illera 2007).

Pipits were trapped on all the main islands across their range (12 islands, ≥24 individuals per island). Birds were captured using clap nets and ringed with a numbered aluminum ring from the relevant Spanish or Portuguese Environmental Ministries. Juveniles and adults were differentiated on the basis of feather molt pattern (Cramp 1988). Birds were meticulously examined for evidence of pox lesions, which primary consisted of swollen growths on the legs, feet or face (Smits et al. 2005). When found, a small portion of one of the larger lesions was excised using a sterile scalpel and placed into 800 μl of 100% ethanol in a screw-cap microfuge tube that was subsequently stored at room temperature. A small (approx 25 μl) blood sample was also collected by venipuncture from each bird and likewise preserved in 800 μl of 100% ethanol.
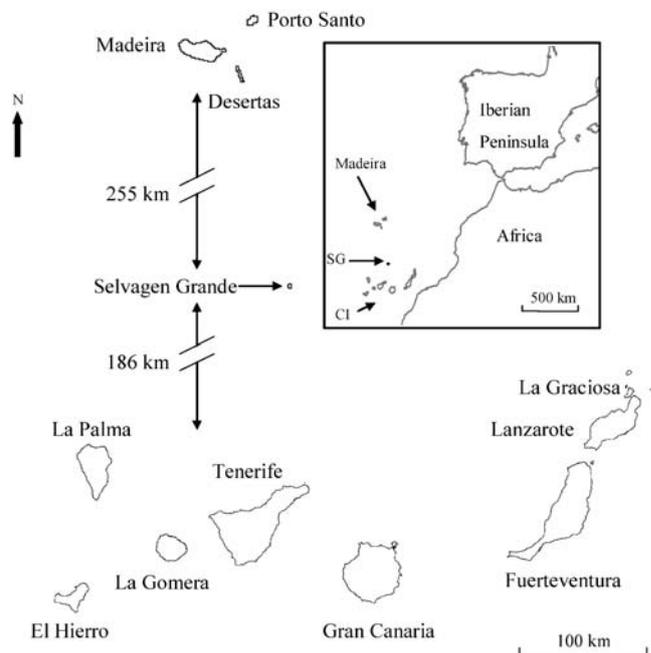

Fig. 1 Geographical location of the Macaronesian islands studied. *SG* Selvagen Grande, *CI* Canary Islands

DNA extraction was undertaken using the salt method (Sunnucks and Hales 1996; Aljanabi and Martínez 1997). Individuals were sexed using the molecular method described in Griffiths et al. (1998). To detect avian pox infections we used the P1 and P2 primers described in Lee and Lee (1997), which encompass a region within the fowl avian pox 4b core protein gene in order to amplify a fragment of 498 base pairs (bp). The polymerase chain reactions (PCR) were set up in 10 μl total volume including, 5 μl of 2× ReddyMix PCR Master Mix (Abgene), 0.5 μl (10 mM) of each primer, 1.0 μl MgCl$_2$ (25 mM) and 1.5 μl of DNA (25 ng/μl), and performed on a Tetrad 2 thermocycler using the following conditions: initial denaturation at 94°C for 5 min followed by 37 cycles of denaturation at 94°C for 30 s, with an annealing temperature of 60°C for 30 s, and extension at 72°C for 1 min and a final extension at 72°C for 10 min. Sequencing reactions were performed using the Perkin Elmer BigDye terminator reaction mix in a volume of 10 μl using 1 μl of PCR product and the primer P1 with the following conditions: initial denaturation at 94°C for 2 min followed by 25 cycles of denaturation at 94°C for 30 s, an annealing temperature of 50°C for 30 s, and extension at 60°C for 2 min, followed by a final extension at 60°C for 1 min.

To detect malaria infections, we used a nested PCR that amplifies a 422 (bp) fragment of the cytochrome *b* gene (Hellgren et al. 2004; Waldenström et al. 2004). Reactions of the initial PCR were set up in 10 μl total volume including 5 μl of 2x ReddyMix PCR Master Mix (Abgene), 0.4 μl (10 mM) of primers HaemNFI and HaemNR3 (Hellgren et al. 2004), 0.4 μl MgCl$_2$ (25 mM) and 0.8 μl of DNA (25 ng/μl). Reactions were performed on a Tetrad 2 thermocycler following conditions suggested by Waldenström et al. (2004). The final PCR was set up in 25 μl total volumes including 12.5 μl of 2× ReddyMix PCR Master Mix (Abgene), 0.5 μl (10 mM) of primers HAEMNF and HAEMNR2 (Waldenström et al. 2004), 1.5 μl MgCl$_2$ (25 mM) and 1.0 μl of amplicon from the initial PCR (25 ng/μl). Reaction conditions for the final PCR were as described in Waldenström et al. (2004). Sequencing reactions were performed using the Perkin Elmer BigDye terminator reaction mix in a volume of 10 μl using 1 μl of PCR product and the primer HAEMNF with the same conditions as used for the sequencing of the avian pox.

Sequences were edited and aligned by eye against homologous sequences from other species/strains of avian malaria or avian pox published in the National Centre for Biotechnology Information (NCBI) gene bank database (Table 1), plus one extra pox sequence obtained from a Canary blue tit (authors unpublished data), using BIOEDIT (version 7.01, Hall 1999), except the Molluscipoxvirus sequence which was aligned using Clustal W program (Chenna et al. 2003). Neighbor-joining trees were con-

structed with MEGA (version 4, Tamura et al. 2007) using the Kimura 2 parameter model to ascertain phylogenetic relationships between the strains of pathogens found in the pipits and the other known avian malaria and avian pox strains (Table 1). The avian pox and avian malaria trees were rooted with sequences from one Molluscipoxvirus species (*Molluscum contagiosum virus*, genbank accession number U60315) and one *Haemoproteus* species (*H. sylvae*, AY099040), respectively. Node support was assessed with 10,000 bootstrap replicates.

Statistical procedures

In order to test whether sample size could be biased by sex or age, two-way ANOVAs were performed to compare avian pox and malaria prevalence in relation to sex and age between islands. Arcsine square root transformed prevalence data was the dependent variable and sex and age were the fixed factors. Islands without pathogens were excluded from the analyses. Statistical analyses were performed using SPSS 14.01.

Results

Geographical distribution of diseases

Of the 447 pipits sampled across the 12 islands, 33 (8%) showed evidence of pox lesions while avian malaria (*Plasmodium* sp.) was detected in 68 individuals (16%). The prevalence of both diseases varied greatly across the Macaronesian islands. We did not detect any malaria or pox case on three islands: Selvagen Grande, Desertas, and Madeira Island (Fig. 2). Overall, the distribution of avian
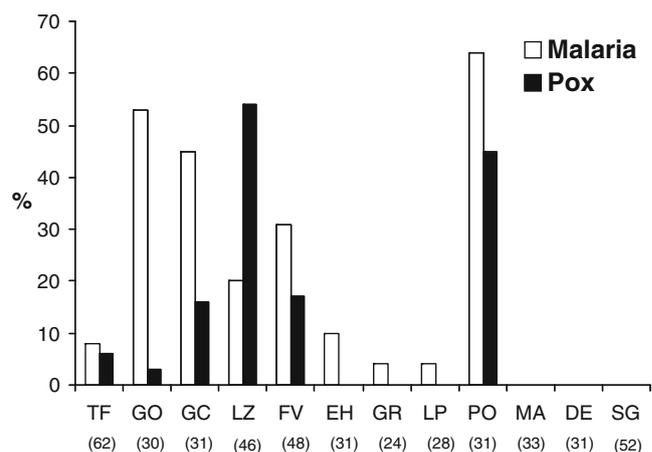
Fig. 2 Prevalence (%) of individuals infected with avian pox and malaria per island. *TF* Tenerife, *GO* Gomera, *GC* Gran Canaria, *LZ* Lanzarote, *FV* Fuerteventura, *EH* El Hierro, *GR* La Graciosa, *LP* La Palma, *PO* Porto Santo, *MA* Madeira, *DE* Desertas, *SG* Selvagen Grande. Sample sizes are in brackets

pox was restricted being found on only five islands within the Canaries and one island (Porto Santo) within the Madeiran archipelago (see Fig. 2). In contrast, avian malaria was recorded within all the Canary Island populations and on one island of the Madeiran archipelago (Porto Santo). Considering only those islands that contained pox or malaria, the prevalence within infected populations was 18% and 28% for avian pox and malaria, respectively. Pipits inhabiting Porto Santo had the highest prevalence values of avian malaria and the second highest of avian pox. However, we did not find a clear geographical pattern of pathogen prevalence across Macaronesia in relation to the proximity to other islands. For example, Madeira, Desertas, and Selvagens, the three islands closest to Porto Santo were free of both pathogens, despite the fact that Porto Santo had the highest overall levels of infection. Nevertheless, within the Canaries, avian pox prevalence level showed a clear gradient east to west from the highest to the lowest values, but no such pattern was found for avian malaria.

There was no relationship between avian pox or malaria infection and either sex or with age across Macaronesia. For avian pox, two-way ANOVA, Sex, $F_{1,21}=0.00$, $P=0.98$; Age, $F_{1,21}=0.29$, $P=0.59$. For avian malaria, two-way ANOVA, Sex, $F_{1,33}=0.37$, $P=0.54$; Age, $F_{1,33}=1.32$, $P=0.26$.

Genetic characterization

Sequences have been deposit in the National Centre for Biotechnology Information (NCBI) gene bank database under the accession numbers EU883532–EU883533 for avian pox and EU883534–EU883535 for malaria.

Avian pox

We were able to extract and sequence avian pox DNA from lesions taken from seven individuals (six from Porto Santo and one from Lanzarote). All seven sequences were identical. In a neighbor-joining phylogenetic analysis, the Berthelot's pipit's avian pox sequence (named as V5PO) was placed (with moderate bootstrap support, 78%) within a clade of pox lineages that mainly parasitize passerines (Fig. 3, node A). Measured across 428 bp of aligned sequence, the genetic similarity between the Berthelot's pipit pox and the canarypox virus was 92.5%, with pigeonpox, it was 84.6%, and with fowlpox, it was 79.7%.

Malaria

Overall, 28 positive samples (between two and eight samples per infected island) were sequenced. Two strains of avian malaria (*Plasmodium* sp.; named as TF413 and PAL282) were detected in the Berthelot's pipit. The two

strains differ from each other at three base pairs (0.7% of the length). We did not find any individual infected with more than one strain. TF413 ($n=26$) was most common and was distributed throughout all islands except La Palma, where the only infected individual found contained the strain PAL282. This PAL282 strain was also found in one individual from El Hierro Island but nowhere else.
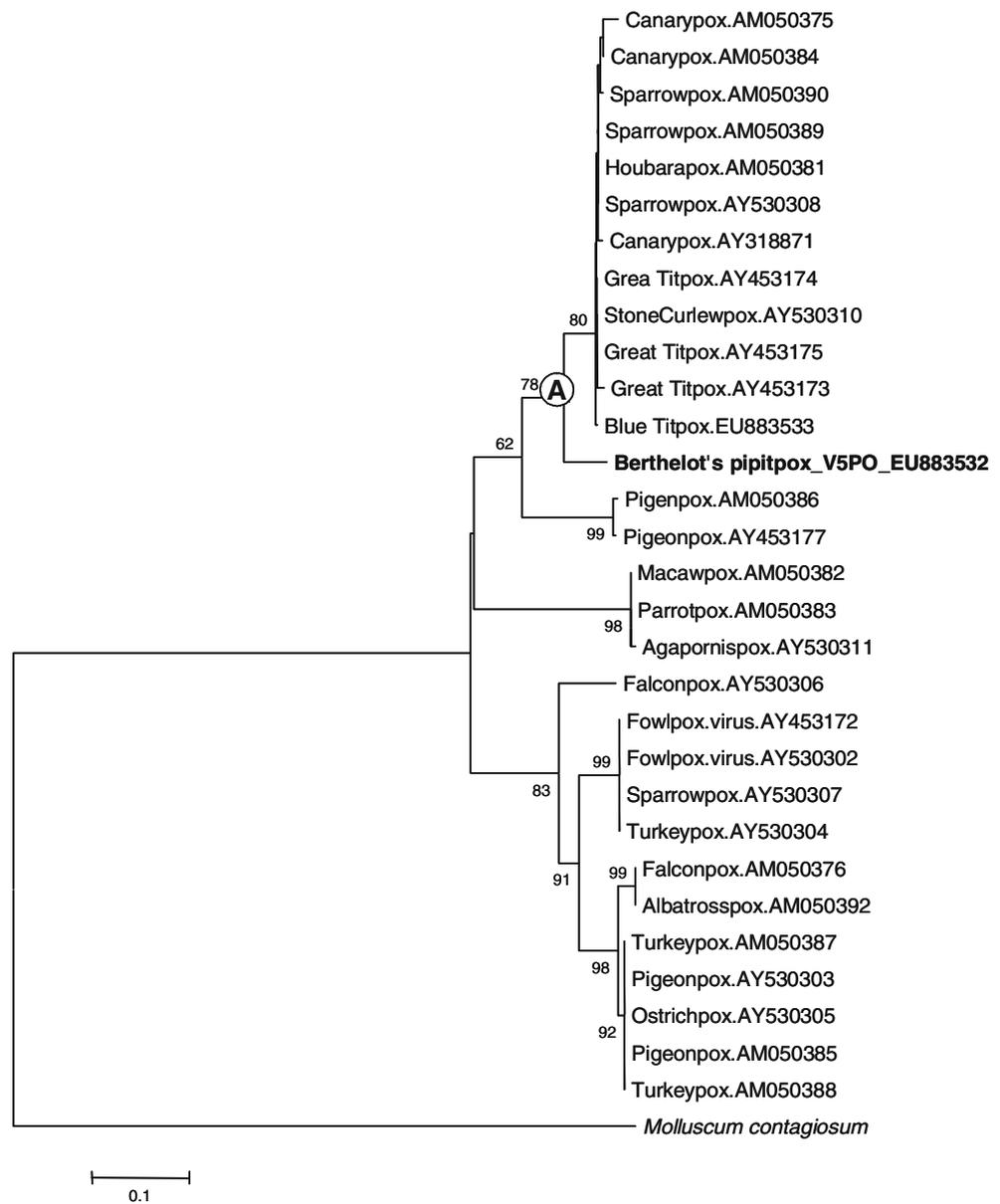
In a neighbor-joining analysis, the two pipit malaria strains were grouped together with high bootstrap support (96%) with two strains (LK5 and LK6) recently isolated from the Lesser Kestrel, (*Falco naumanni*) a small raptor breeding throughout Eurasia and wintering in sub-Saharan Africa (Fig. 4, node A; Ortego et al. 2007). These two pipit and two kestrel strains plus *P. rouxi* and two other unnamed *Plasmodium* species form a monophyletic group with high (92%) bootstrap support (Fig. 4, node B). The 411 base pairs that could be aligned between the kestrel/pipit strains all matched exactly: LK5/PAL282 and LK6/TF413, strongly suggesting that these paired strains are the same.

Discussion

We found a low overall prevalence of avian pox and avian malaria (8% and 16%, respectively) in populations of Berthelot's pipit across Macaronesia compared to other species (Beadell et al. 2004; Atkinson et al. 2005; Fallon et al. 2005; Pérez-Tris et al. 2007). However, there was considerable variation in prevalence between archipelagos and between islands within archipelagos (0–54%; 0–64%), for pox and malaria, respectively. The Canary Islands had the highest overall level of infections—all islands were infected with malaria and 5/9 of them by avian pox. Only one island of the Madeiran archipelago (Porto Santo) contained pipits infected by both malaria and pox, while the Selvagen Grande population was free of both these pathogens (Fig. 2).

The reason(s) behind the variation in pathogen prevalence among populations is difficult to ascertain based on the information currently available. It is plausible that the potential for transmission may differ between islands (van Riper et al. 1986, 2002; Freed et al. 2005). The main vector known to transmit both diseases in Macaronesia is the arthropod *Culex pipiens* (introduced by man in at an unknown date), which is distributed throughout all the islands included in this study except Desertas and Selvagens (Capela 1982; Báez and García 2004). This result could explain the absence of both parasites in these islands but not in Madeira. Unfortunately, information available about the relative abundance of *C. pipiens* among islands or its distribution and abundance between habitats within islands is scarce. Work is needed to determine if and how the abundance of vector populations differs between population

Fig. 3 Neighbor-joining (NJ) tree (428 bp of 4b core protein gene) for avian pox sequences constructed using Kimura two-parameter distances. *Numbers* indicate NJ bootstrap support (>60%). The placement of the sequence from Berthelot's pipit is highlighted in bold. *Letters* correspond to nodes discussed in the text

Canarypox.AM050375
Canarypox.AM050384
Sparrowpox.AM050390
Sparrowpox.AM050389
Houbarapox.AM050381
Sparrowpox.AY530308
Canarypox.AY318871
Grea Titpox.AY453174
StoneCurlewpox.AY530310
Great Titpox.AY453175
Great Titpox.AY453173
Blue Titpox.EU883533
80
78 (A)
Berthelot's pipitpox_V5PO_EU883532
62
Pigenpox.AM050386
Pigeonpox.AY453177
99
Macawpox.AM050382
Parrotpox.AM050383
Agapornispox.AY530311
98
Falconpox.AY530306
Fowlpox.virus.AY453172
Fowlpox.virus.AY530302
Sparrowpox.AY530307
Turkeypox.AY530304
99
Falconpox.AM050376
Albatrosspox.AM050392
99
Turkeypox.AM050387
Pigeonpox.AY530303
Ostrichpox.AY530305
98
Pigeonpox.AM050385
Turkeypox.AM050388
92
91
83
Molluscum contagiosum

0.1

(and habitats) and how this relates to infection rates. However, various factors, including levels of rainfall and temperature, have been associated with differences in mosquito abundance and, consequently, the prevalence of avian pox and avian malaria in other studies (Freed et al. 2005; van Riper et al. 2002; Vander Werf et al. 2006). There are very significant differences in rainfall (but not in temperature) between the Macaronesian islands (e.g., García et al. 2001). For instance, precipitation in the Canary Islands increases from east to west. La Palma and El Hierro islands are the wettest (>700 mm/annum) while Fuerteventura and Lanzarote are the most arid (<200 mm/per annum, García et al. 2001). Such patterns of rainfall could, potentially, explain the variation in pathogen prevalence observed, however this does not appear to be the case. Indeed, the pattern of avian

pox prevalence was the opposite of what would be expected based on rainfall, while malaria prevalence was irregular across this gradient—of the three wettest most westerly islands, one (La Gomera) had the highest prevalence of malaria while the other two other (La Palma and El Hierro) had the lowest prevalence (Fig. 2).

In contrast to results from other studies on wild birds (Buenestado et al. 2004; Atkinson et al. 2005), our investigation found that the likelihood of being infected with avian pox or malaria did not differ in relation to either sex or age. Specifically, our results contrast with those of Smits et al. (2005), whose findings indicate that in Fuerteventura and Lanzarote adult Berthelot's pipits had a higher prevalence of avian pox than juveniles. However, the study of Smits et al. (2005) was focused on only two

Fig. 4 Neighbor-joining (NJ) tree (387 bp mtDNA) for avian malaria sequences constructed using Kimura two-parameter distances. *Numbers* indicate NJ bootstrap support (>60%). The placement of sequences from Berthelot's pipit are highlighted in *bold*. *Letters* correspond to nodes discussed in the text
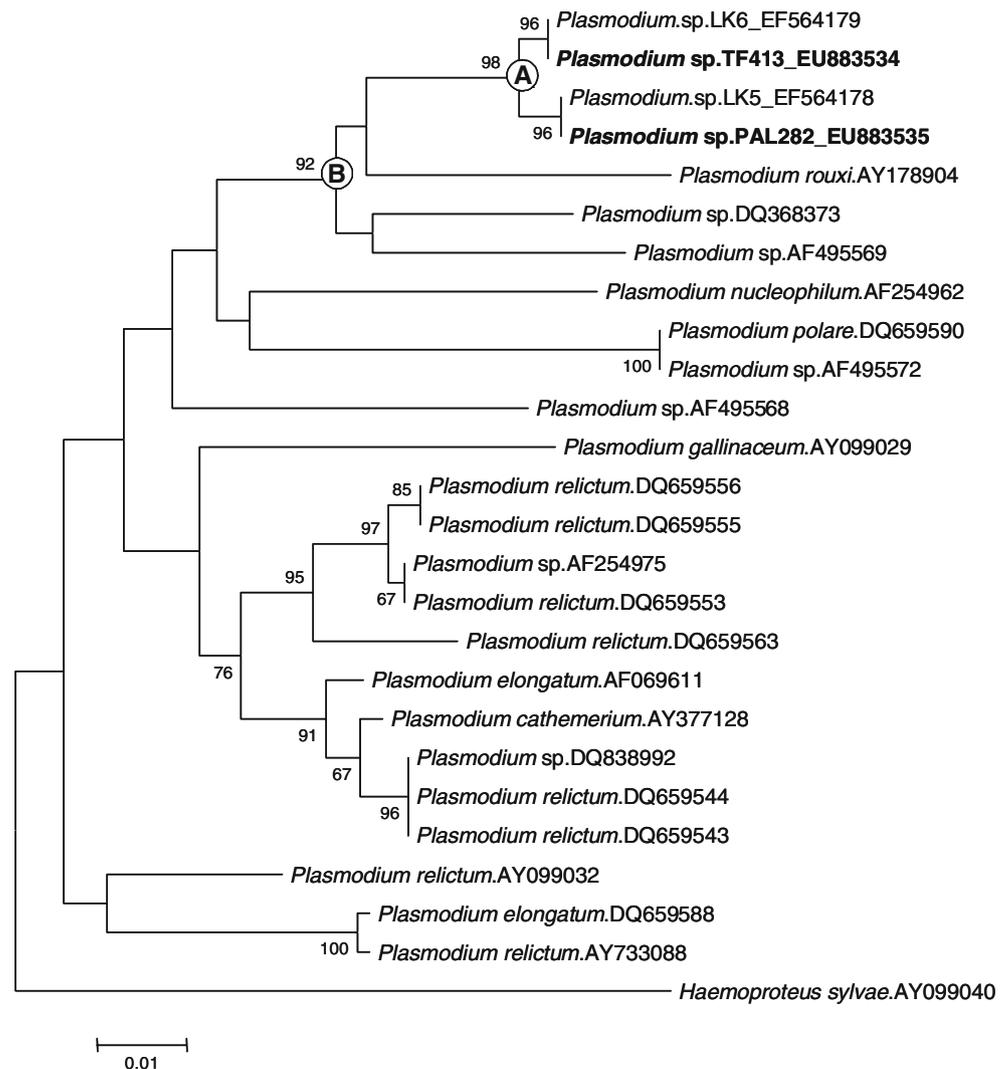
islands with a limited sample size ($n=139$). We screened more than three times as many individuals ($n=447$) throughout all islands of the three archipelagos (≥24 birds per island) and so, are confident that our results are not an artifact of the sampling method used (Jovani and Tella 2006). Nevertheless, we cannot exclude the possibility that other parameters such as the timing or number of individuals caught per season and differing meteorological conditions between sampled years could be the causative agents of the differences detected.

Genetic characterization

We found only two strains of avian malaria infecting the pipits across all the Macaronesian islands (one of these was restricted to the Canaries) and one strain of avian pox. These are low levels of lineage diversity compared to other bird species parasitized (Fallon et al. 2005; Beadell et al. 2006; Jarmin et al. 2006; Pérez-Tris et al. 2007). Unfortunately, there is no information on the distribution or

prevalence of avian malaria and limited information on pox lineages for other Macaronesian bird species, which prevents us from constructing any overall hypotheses for the low diversity in the Berthelot's pipit.

The avian pox lineage found in this study is, as far as we know, exclusive to the Berthelot's pipit (Fig. 3). It is different from the pox lineage found in one individual of the Canary blue tit (Fig. 3) and also from that identified in the short-toed lark (*Calandrella rufescens*), another endemic passerine inhabiting the same semi-arid habitats in the central and eastern islands of the Canary Islands (Smits et al. 2005). One puzzling result was that we found a different level of similarity between the pipit avian pox and strains of canarypox and fowlpox virus compared to that found by Smits et al. (2005) who also identified a pipit pox virus. Unfortunately, it was not possible to compare the sequence they found with the one we found, as theirs has not been made publicly available. However, the most likely explanation is that the previous study mistakenly transposed the values reported for the Berthelot's pipit and

short-toed lark as the values match up exactly when switched around. Although our results contrast with the absence of host specificity in *Avipoxvirus* strains found in the Galápagos Islands (Thiel et al. 2005), sequencing of more species parasitized across the Macaronesian Islands will provide more conclusive information about the full extent of host avian pox specificity within the region.

The two lineages of *Plasmodium* sp. found in Berthelot's pipit (Fig. 4) have also been identified in lesser kestrels (*Falco naumannii*, Ortego et al. 2007). Recent molecular studies have demonstrated that avian blood parasites of the genera *Haemoproteus* and *Plasmodium* show a lower degree of host specific than was previously thought (Bench et al. 2000; Waldenström et al. 2002; Szymanski and Lovette 2005), although it is lower in *Plasmodium* than in *Haemoproteus* parasites (Beadell et al. 2004; Fallon et al. 2005). Our results are consistent with these findings in that the *Plasmodium* parasites we detected do not appear to be constrained by the phylogenetic relationships of their hosts. As the lesser kestrel does not breed on any of the Macaronesian islands, but is a trans-Saharan migrant that only occasionally arrives as a vagrant (Martín and Lorenzo 2001); the possibility of cross-species transmission is limited. However, the fact those identical lineages can be found in both species means that either there is transmission between these two hosts or that this is a relatively common strain of malaria that has just yet to be detected in other species that interact with both the kestrel and the pipit.

The low diversity of the avian malaria parasite lineages found in the Berthelot's pipit is striking. It contrasts, for example, with six avian malaria lineages (*n*=586 individuals screened) found in the lesser kestrel and 26 (*n*=415) observed in the blackcap *Sylvia atricapilla* (Ortego et al. 2007; Pérez-Tris et al. 2007). The Canary Islands are close to African mainland sources of host and parasite populations and receive a lot of migrants such as the blackcap every year that could elevate parasite richness. Also striking is the restricted distribution of avian malarial parasite diversity within Berthelot's pipit. The only population hosting the two avian malaria strains was that of El Hierro, the more distant of the Macaronesian Islands from Africa. Future screening of more native bird species inhabiting Macaronesia will help our understanding of the full breadth of parasite diversity within the region, and factors influencing the taxonomic, geographic, and ecological structuring of this diversity.

Conclusion

Our results show that both avian pox and malarial infection (*Plasmodium* sp.) occurs throughout the pipit's range. This is the first time that avian malarial infection has been documented within a bird species endemic to Macaronesia. The prevalence of avian pox and malaria was generally low across pipit populations, but levels varied widely, with more than 50% of individuals infected within some populations. The high density of Berthelot's pipit in Macaronesia (Illera 2007) means that it is unlikely these pathogens threaten the local extinction of island pipit populations, at least, in the short term. However, we cannot exclude the possibility that those islands not infected may be more susceptible to these parasites. Our conclusion is that avian pox is endemic within the Berthelot's pipit but malaria is not host specific. Although they may impact on individual health and fitness, they apparently do not limit the population.

Appendix

Table 1 Parasite sequences used in this study

Parasite	Host	Abbreviation	GeneBank
Canarypox	*Serinus canaria*		AM050375
Canarypox	*Serinus canaria*		AM050384
Sparrowpox	*Passer domesticus*		AM050390
Sparrowpox	*Passer domesticus*		AM050389
Houbarapox	*Chlamydotis undulata*		AM050381
Sparrowpox	*Passer domesticus*		AY530308
Canarypox	*Serinus canaria*		AY318871
Stone curlewpox virus	*Burhinus oedicnemus*		AY530310
Great Titpox virus	*Parus major*		AY453174
Great Titpox virus	*Parus major*		AY453175
Great Titpox virus	*Parus major*		AY453173
Blue titpox virus	*Parus teneriffae*		EU883533
Berthelot's pipitpox virus	*Anthus berthelotii*	V5PO	EU883532
Pigeonpox	*Columba livia*		AM050386
Pigeonpox	*Columba livia*		AY453177
Macawpox	*Ara spp.*		AM050382
Parrotpox	*Amazona spp.*		AM050383
Agapornispox	*Agapornis spp.*		AY530311

Table 1 (continued)

Parasite	Host	Abbreviation	GeneBank
Falconpox	*Falco spp.*		AY530306
Fowlpox virus	*Gallus gallus*		AY530302
Turkeypox	*Meleagris gallopavo*		AY530304
Fowlpox virus	*Gallus gallus*		AY453172
Sparrowpox	*Passer domesticus*		AY530307
Falconpox	*Falco spp.*		AM050376
Albatrosspox	*Diomedea melanophis*		AM050392
Turkeypox	*Meleagris gallopavo*		AM050387
Pigeonpox	*Columba livia*		AY530303
Ostrichpox	*Struthio camelus*		AY530305
Pigeonpox	*Columba livia*		AM050385
Turkeypox	*Meleagris gallopavo*		AM050388
Molluscum contagiosum virus	*Homo sapiens*		U60315
Plasmodium sp.	*Falco naumanni*	LK6	EF564179
Plasmodium sp.	*Anthus berthelotii*	TF413	EU883534
Plasmodium sp.	*Falco naumanni*	LK5	EF564178
Plasmodium sp.	*Anthus berthelotii*	PAL282	EU883535
Plasmodium rouxi	*Unknown*		AY178904
Plasmodium sp.	*Anthus trivialis*		DQ368373
Plasmodium sp.	*Cercotrichas galactotes*		AF495569
Plasmodium nucleophilum	*Acrocephalus arundinaceus*		AF254962
Plasmodium polare	*Parus major*		DQ659590
Plasmodium sp.	*Acrocephalus schoenobaenus*		AF495572
Plasmodium sp.	*Cercotrichas galactotes*		AF495568
Plasmodium gallinaceum	*Gallus gallus*		AY099029
Plasmodium relictum	*Sula capensis*		DQ659556
Plasmodium relictum	*Ploceus velatus*		DQ659555
Plasmodium sp.	*Acrocephalus arundinaceus*		AF254975
Plasmodium relictum	*Hemignathus virens*		DQ659553
Plasmodium relictum	*Carduelis chloris*		DQ659563
Plasmodium elongatum	*Passer domesticus*		AF069611
Plasmodium cathemerium	*Serinus canaria*		AY377128
Plasmodium sp.	*Anthus hodgsoni*		DQ838992
Plasmodium relictum	*Corvus corone*		DQ659544
Plasmodium relictum	*Luscinia svecica*		DQ659543
Plasmodium relictum	*Zeneida macroura*		AY099032
Plasmodium elongatum	*Ardea herodias*		DQ659588
Plasmodium relictum	*Spheniscus demersus*		AY733088
Haemoproteus sylvae	*Acrocephalus arundinaceus*		AY099040

Avian pox correspond to a 498-bp fragment of 4b core protein gene. Avian malaria sequences correspond to a 422-bp fragment of the cytochrome *b* gene

References

Aljanabi SM, Martinez I (1997) Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Res 25:4692–4693

Atkinson CT, Lease JK, Dusek RJ, Samuel MD (2005) Prevalence of pox-like lesions and malaria in forest bird communities on leeward Mauna Loa Volcano, Hawaii. Condor 107:537–546

Báez M, García A (2004) Diptera. In: Izquierdo I, Martín JL, Zurita N, Arechavaleta M (eds) Lista de especies silvestres de Canarias (hongos, plantas y animales terrestres) 2004. Consejería de Medio Ambiente y Ordenación Territorial, Gobierno de Canarias, pp 149–154

Beadell JS, Gering E, Austin J et al (2004) Prevalence and differential host-specificity of two avian blood parasite genera in the Australo-Papuan region. Mol Ecol 13:3829–3844

Beadell JS, Ishtiaq F, Covas R et al (2006) Global phylogeography limits of Hawaii's avian malaria. Proc R Soc B 273:2935–2944

Bench S, Stjernman M, Hasselquist D et al (2000) Host specificity in avian blood parasites: a study of *Plasmodium* and *Haemoproteus* mitochondrial DNA amplified from birds. Proc R Soc B 267:1583–1589

Bonneaud C, Pérez-Tris J, Federico P, Chastel O, Sorci G (2006) Major histocompatibility alleles associated with local resistance to malaria in a passerine. Evolution 60:383–389

Buenestado F, Gortázar C, Millán J, Höfle U, Villafuerte R (2004) Descriptive study of an avian pox outbreak in wild red-legged partridges (*Alectoris rufa*) in Spain. Epidemiol Infect 132:369–374

Capela RA (1982) Contribuição para o conhecimento dos mosquitos (Diptera, Culicidae) dos arquipélagos da Madeira e das Selvagens. Bol Museu Mun Funchal 34:105–123

Carrete M, Serrano D, Illera JC, López G, Vögeli M, Delgado A, Tella JL (2008) Goats, birds and emergent diseases: apparent and hidden effects of an exotic species in an island environment. Ecol Appl 00:000-000, in press

Chenna R, Hideaki S, Koike T, Rodrigo L, Gibson TJ, Higgins DG, Thompson JD (2003) Multiple sequence alignment with the Clustal series of programs. Nucleic Acids Res 31:3497–3500

Cramp S (1988) The birds of the Western Peleartic, vol 5. Oxford University Press, London

Fallon SM, Birmingham E, Ricklefs RE (2005) Host specialization and geographic localization of avian malaria parasites: a regional analysis in the Lesser Antilles. Am Nat 165:466–480

Foronda P, Valladares B, Rivera-Medina JA, Figueruelo E, Abreu N, Casanova JC (2004) Parasites of *Columba livia* (Aves: Columbiformes) in Tenerife (Canary Islands) and their role in the conservation biology of the Laurel pigeons. Parasite 11: 311–316

Freed LA, Cann RL, Goff ML, Kuntz WA, Bodner GR (2005) Increase in avian malaria at upper elevation in Hawai'i. Condor 107:753–764

García R, Gallego D, Hernández E (2001) Influence of the north Atlantic oscillation on the Canary Islands precipitation. J Climate 14:3889–3903

Griffiths R, Double MC, Orr K, Dawson RJG (1998) A DNA test to sex most birds. Mol Ecol 7:1071–1075

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acids Symp Ser 41:95–98

Hazevoet CJ (1995) The birds of the Cape Verde Islands. B.O.U. Check-list No. 13. British Ornithologist's Union, UK

Hellgren O, Waldenström J, Bench S (2004) A new PCR assay for simultaneous studies of Leucocytozoon, *Plasmodium*, and *Haemoproteus* from avian blood. J. Parasitol 90:797–802

Illera JC (2007) Bisbita Caminero *Anthus berthelotii*. In: Lorenzo JA (ed) Atlas de las aves nidificantes en el archipiélago Canario (1997–2003). Dirección General de Conservación de la Naturaleza-Sociedad Española de Ornitología, Madrid, pp 344–347

Illera JC, Emerson BC, Richardson DS (2007) Population history of Berthelot's pipit: colonisation, gene flow and morphological divergence in Macaronesia. Mol Ecol 16:4599–4612

Jarmin S, Manvell R, Gough RE, Laidlaw SM, Skinner MA (2006) *Avipoxvirus* phylogenetics: identification of a PCR length polymorphism that discriminates between the two major clades. J General Virol 87:2191–2201

Jovani R, Tella JL (2006) Parasite prevalence and sample size: misconceptions and solutions. TRENDS Parasitol 22:214–218

Kleindorfer S, Dudaniec RY (2006) Increasing prevalence of avian poxvirus in Darwin's finches and its effect on male pairing success. J Avian Biol 37:69–76

Laiolo P, Tella JL, Carrete M, Serrano D, López G (2004) Distress calls may honestly signal bird quality to predators. Proc R Soc B 271:S513–S515

Laiolo P, Serrano D, Tella JL, Carrete M, López G, Navarro C (2007) Distress calls reflect poxvirus infection in lesser short-toed lark *Calandrella rufescens*. Behav Ecol 18:507–512

Lee LH, Lee KH (1997) Application of the polymerase chain reaction for the diagnosis of fowl poxvirus infection. J Virol Methods 63:113–119

Loiseau C, Zoorob R, Garnier S, Birard J, Federici P, Julliard R, Sorci G (2008) Antagonistic effects of a Mhc class I allele on malaria-infected house sparrows. Ecol Letters 11:258–265

Lorenzo JA (Ed) (2007) Atlas de las aves nidificantes en el archipiélago canario (1997–2003). Dirección General de Conservación de la Naturaleza-Sociedad Española de Ornitología. Madrid

Martín A, Lorenzo JA (2001) Aves del Archipiélago Canario. Lemus, La Laguna

Medina FM, Ramírez GA, Hernández A (2004) Avian pox in white-tailed laurel-pigeons from the Canary Islands. J Wildlife Diseases 40:351–355

Møller AP, Nielsen JT (2007) Malaria and risk of predation: a comparative study of birds. Ecology 88:871–881

Oliveira P, Menezes D (2004) Birds of the archipelago of Madeira. Serviço do Parque Natural da Madeira/Arquipélago Verde produtos promocionais, Lda, Funchal

Ortego J, Calabuig G, Cordero PJ, Aparicio JM (2007) Genetic characterization of avian malaria (Protozoa) in the endangered lesser kestrel, *Falco naumanni*. Parasitol Res 101:1153–1156

Pérez-Tris J, Hellgren O, Krizanauskiene A, Waldenström J, Secondi J, Bonneaud C, Fjeldsa J, Hasselquist D, Bench S (2007) Within-host speciation of malaria parasites. PLoSONE 2:e235

Smith KF, Sax DF, Lafferty KD (2006) Evidence for the role of infectious disease in species extinction and endangerment. Cons Biol 20:1349–1357

Smits J, Tella JL, Carrete M, Serrano D, López G (2005) An epizootic of avian pox in endemic short-toed larks (*Calandrella rufescens*) and Berthelot's pipits (*Anthus berthelotti*) in the Canary Islands, Spain. Vet Path 42:59–65

Snow DW, Perrins CM (1998) The birds of the western palearctic. Concise Edition. Vol. 2. Passerines. Oxford University Press, Oxford

Stattersfield AJ, Crosby MJ, Long AJ, Wege DC (1998) Endemic bird areas of the world. BirdLife conservation, Series 7. BirdLife, Cambridge

Sunnucks P, Hales DF (1996) Numerous transposed sequences of mitochondrial cytochrome oxidase I–II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). Mol Biol Evol 13:51–524

Szymanski MM, Lovette IJ (2005) High lineage diversity and host sharing of malarial parasites in a local avian assemblage. J Parasitol 91:768–774

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599

Thiel T, Whiteman NK, Tirapé A, Baquero MI, Cedeño V, Walsh T, Uzcátegui GJ, Parker PG (2005) Characterization of canarypox-like viruses infecting endemic birds in the Galápagos islands. J Wild Diseases 41:342–353

Thomas NJ, Hunter DB, Atkinson CT (2007) Infectious diseases of wild birds. Blackwell, Oxford

VanderWerf EA, Buró MD, Rohrer JL, Mosher SM (2006) Distribution and prevalence of mosquito-borne diseases in O' Ahu 'Elepaio. Condor 108:770–777

van Riper C III, van Riper SG, Goff ML, Laird M (1986) The epizootiology and ecological significance of malaria in Hawaiian land birds. Ecol Monographs 56:327–344

van Riper C III, van Riper SG, Hansen WR (2002) Epizootiology and effect of avian pox on Hawaiian forest birds. Auk 119:929–942

Vargas H (1987) Frequency and effect of pox-like lesions in Galápagos mockingbirds. J Field Ornithol 58:101–102

Voelker G (1999) Dispersal, vicariance, and clocks: historical biogeography and speciation in a cosmopolitan passerine genus (*Anthus*: Motacillidae). Evolution 53:1536–1552

Waldenström J, Bench S, Kiboi S, Hasselquist D, Ottosson U (2002) Cross-species infection of blood parasites between resident and migratory songbirds in Africa. Mol Ecol 11:1545–1554

Waldenström J, Bench S, Hasselquist D, Östman Ö (2004) A new nested polymerase chain reaction method very efficient in detecting *Plasmodium* and *Haemoproteus* infections from avian blood. J Parasitol 90:191–194

Warner RE (1968) The role of introduced diseases in the extinction of the endemic Hawaiian avifauna. Condor 70:101–120

Wikelski M, Foufopoulos J, Vargas H, Snell H (2004) Galápagos birds and diseases: invasive pathogens as threats for island species. Ecol Society 9 5. [online] URL: http://www.ecologyandsociety.org/vol9/iss1/art5