



Urbanization correlates with the prevalence and richness of blood parasites in Eurasian Blackbirds (*Turdus merula*)

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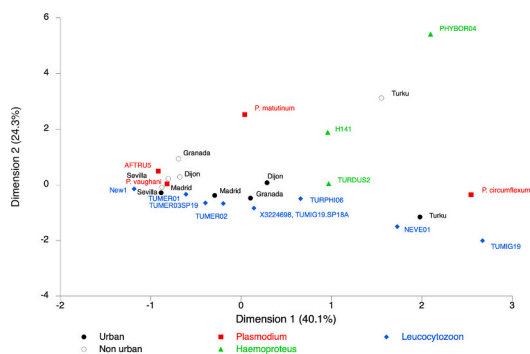
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GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Rafael Mateo

Keywords:

Avian malaria
Haematozoa prevalence
Host-pathogen interactions
Parasite diversity
Parasite richness
Vector-borne pathogens

ABSTRACT

Urbanization is increasing worldwide, producing severe environmental impacts. Biodiversity is affected by the expansion of cities, with many species being unable to cope with the different human-induced stressors present in these landscapes. However, this knowledge is mainly based on research from taxa such as plants or vertebrates, while other organisms like protozoa have been less studied in this context. The impact of urbanization on the transmission of vector-borne pathogens in wildlife is still unclear despite its relevance for animal and human health. Here, we investigated whether cities are associated with changes in the prevalence and richness of lineages of three vector-borne protozoans (*Plasmodium*, *Haemoproteus* and *Leucocytozoon*) in Eurasian blackbirds (*Turdus merula*) from multiple urban and forest areas in Europe. Our results show important species-specific differences between these two habitat types. We found a significant lower prevalence of *Leucocytozoon* in urban birds compared to forest birds, but no differences for *Plasmodium* and *Haemoproteus*. Furthermore, the richness of parasite lineages in European cities was higher for *Plasmodium* but lower for *Leucocytozoon* than in

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<https://doi.org/10.1016/j.scitotenv.2024.171303>

Received 6 October 2023; Received in revised form 13 February 2024; Accepted 25 February 2024

Available online 27 February 2024

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forests. We also found one *Plasmodium* lineage exclusively from cities while another of *Leucocytozoon* was only found in forests suggesting a certain level of habitat specialization for these protozoan vectors. Overall, our findings show that cities provide contrasting opportunities for the transmission of different vector-borne pathogens and generate new scenarios for the interactions between hosts, vectors and parasites.

1. Introduction

Urbanization, the expansion of urban areas, has rapidly increased in recent decades (McIntyre, 2021; United Nations, 2019). This landscape change is tightly linked with the sociological change by which humans leave rural areas to live in towns and cities. Only 10 % of the world population was living in cities in 1900, and by the start of the 21st century the proportion had increased to 50 % (Grimm et al., 2008). These changes continue nowadays and different projections indicate that urban areas will continue their expansion in the near future (Angel et al., 2011; Seto et al., 2012; United Nations, 2019).

Urbanization strongly affects the wildlife communities living in these human-altered areas (Aronson et al., 2014; Evans, 2010; Ibáñez-Alamo et al., 2017; McKinney, 2006). Only a few animal species are able to survive in urban habitats and consequently urban communities are often characterized by the presence of a reduced number of species (Chace and Walsh, 2006; McKinney, 2006). However, some species, typically known as 'urban exploiter/adaptor species' (Blair, 1996), thrive in the urban areas and present important populations in cities (Isaksson, 2018). Urban ecology studies have focussed on the impact of urbanization on the diversity of different clades including plants, birds or mammals (e.g. Chace and Walsh, 2006; Ibáñez-Alamo et al., 2017; Kondratyeva et al., 2020; McKinney, 2008), but much less attention has been paid to its impact on interactions between species and, especially, host parasite interactions (Awoyemi and Ibáñez-Alamo, 2023; Delgado-V and French, 2012).

Understanding the ecology of wildlife pathogens in urban areas is increasingly important for managing disease risk to wildlife and humans (Bradley and Altizer, 2006). A recent meta-analysis concluded that some types of health risks, such as exposure to toxicants, were more important for wildlife living in urban areas (Murray et al., 2019). Their conclusions were less clear for exposure to parasites, and an effect of urbanization was only detectable on the prevalence of contact-transmitted pathogens. The transmission of vector-borne diseases to wildlife may be reduced in urban areas as these areas have less suitable breeding sites for hematophagous insects (Bradley and Altizer, 2006; Delgado-V and French, 2012), although the effect of urbanization on parasite dynamics may also depend on parasite ecology and environmental conditions (e.g. interannual variation) (Jiménez-Peñuela et al., 2021; Murray et al., 2019). For instance, studies in Europe suggested that the abundance and species richness of mosquitoes is lower in urban areas (Ferraguti et al., 2016) and the prevalence of vector-borne pathogens is positively related to vector density (Ferraguti et al., 2018; Martínez-de la Puente et al., 2013; Martínez-de la Puente et al., 2018). However, some species of mosquitoes are able to breed in the small water containers that are present in urban areas and consequently, may also present important populations in cities (e.g., *Culex pipiens*, an important vector of avian *Plasmodium*; Cornet et al., 2013; Gutiérrez-López et al., 2020; Pigeault et al., 2015). Moreover, invasive species of mosquitoes in Europe and North America such as *Aedes albopictus* also reach high populations in urban areas (Ferraguti et al., 2023; Kache et al., 2020), although this species may be less important for avian *Plasmodium* transmission likely due to its preference to feed on mammals (Martínez-de la Puente et al., 2020).

Here, we analyse how urbanization affects the prevalence and richness of blood parasites transmitted by different groups of haematophagous insects in Europe. Avian malaria parasites (genus *Plasmodium*) and

the related haemosporidians of the genera *Haemoproteus* and *Leucocytozoon* are common model systems in studies of host-parasite ecology and evolution (Rivero and Gandon, 2018). Mosquitoes are the vectors of *Plasmodium* parasites, while *Haemoproteus* parasites are transmitted by *Culicoides* and hippoboscids, and parasites of the genus *Leucocytozoon* are mainly transmitted by blackflies (Valkiunas, 2005). We hypothesize that haemoparasite prevalence will be lower in urban areas, due to the expected lower abundance of vectors (Bradley and Altizer, 2006; Ferraguti et al., 2016). In addition, given that we expect that vertebrate and vector communities in urban areas will be more reduced in size and complexity (Delgado-V and French, 2012; Ferraguti et al., 2016), we expect the richness of haemoparasite lineages to be lower in cities than in forests. Contrasting patterns could be expected between parasite genera based on the differential environmental requirements of the vectors of these parasites.

We compared the prevalence, richness and community composition of the three genera of vector-borne haemoparasites in five urban and five forest populations of Eurasian blackbirds (*Turdus merula*, blackbird hereafter) across Europe. Blackbirds are well adapted to live in cities and are the second most common urban bird in Europe (Morelli et al., 2021), but are also commonly found in forests, their original habitat (Luniak et al., 1990). Blackbirds are an excellent model system for the study of urbanization effects. Blackbirds living in cities appear to experience senescence earlier in life, as measured by their red blood cell telomere length (Ibáñez-Alamo et al., 2018), but show decreased physiological stress compared to their forest conspecifics (Ibáñez-Alamo et al., 2020). Regarding haemoparasites, Evans et al. (2009) investigated avian malaria prevalence (combining *Plasmodium* and *Haemoproteus* data) in multiple blackbird populations finding higher malaria prevalence in non-urban than urban habitats. Their study included both natural (i.e., forests) and different types of agricultural areas as non-urban habitats making it very difficult to identify avian malaria prevalence differences between their natural, original habitat and true urban habitat. Furthermore, their study did not check for other vector-borne parasites (e.g., *Leucocytozoon*), did not explore for urban-associated variation in parasitic richness, and did not investigate the community composition of parasitic lineages in urban and non-urban habitats. Therefore, we still lack crucial information from this model species to correctly understand the effect of urbanization on their vector-borne diseases.

2. Materials and methods

2.1. Study design and sampling

Blackbirds were captured at 10 different localities across Europe, which included paired urban and non-urban sites in Finland, France and Spain (Fig. 1). Therefore, five localities were located in urban parks and gardens inside cities (Turku, Dijon, Madrid, Granada and Seville), and the other five in nearby protected forested areas outside each city, with minimum human activity and at a considerable distance to secure the independence of each blackbird population (mean distance \pm s.e.: 29,8 \pm 3,8 km; Paradis et al., 1998). To further characterize differences in the level of anthropization between these two habitat types, an urbanization score was obtained for each locality following a widely used methodology (Liker et al., 2008; Seress et al., 2014). In each pair of urban/forest locations the number of cells with high vegetation density was higher in the forest locations (range: 91–100, mean: 97,8; urban, range: 7–47,

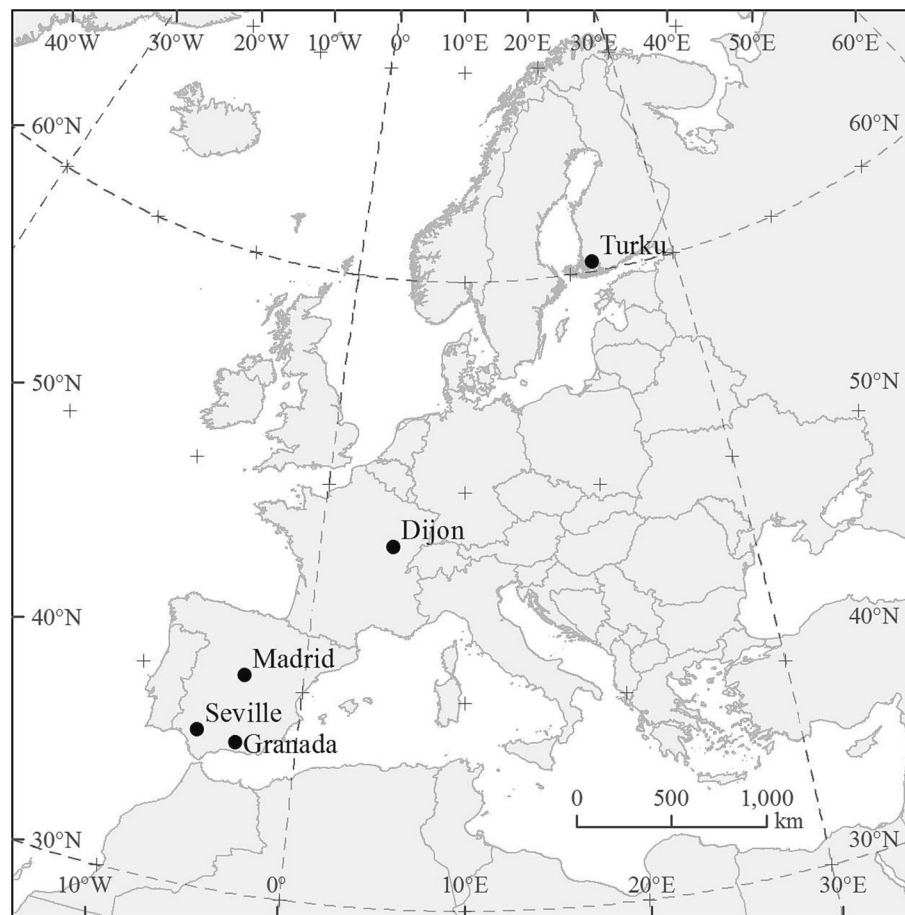


Fig. 1. Geographical distribution of the sampling localities. Each dot represents a paired urban and forest sampling sites (dyad).

mean: 22,2) while the number of cells with high building density was higher in the urban ones (range: 11–50, mean: 23,8; forest, range: 0–5, mean: 1). The urbanization score further confirmed the visual scoring of urban and forest areas. At each locality, blackbirds were captured using mist nets in March–July 2015, sexed and classified as either born in the previous season or older birds (1-year-old vs. older individuals) based on their plumage (Jenni and Winkler, 1994). So, yearlings, individuals born in the current season were excluded from this study because were captured in low numbers in many of the localities. We capture blackbirds during the breeding period of each locality according to local researchers and previous information from the sampling area. All captures were done in actively breeding territories as shown by singing males (100 %) and breeding females (i.e. 95 % showed brood patch). Captures spanned between 30 March and 12 June in Sevilla (Spain), 12 March and 24 April in Granada (Spain), 27 April and 17 May in Madrid (Spain), 24 May and 4 June in Dijon (France) and 7 June and 1 July in Turku (Finland). Immediately after capture, blood samples (350–450 μ l) were taken from the brachial vein, kept at 4 °C for up to 5 h until centrifugation (15 min at 5000g), after which the red blood cells were frozen at -80 °C. Genomic DNA was isolated from the cellular fraction of the blood sample using the Maxwell® 16 LEV system Research (Promega, Madison, WI) (Gutiérrez-López et al., 2015). Parasites of the genus *Plasmodium*, *Haemoproteus* and *Leucocytozoon* were identified according to Hellgren et al. (2004) based on the amplification of a 478 bp fragment of the mitochondrial cytochrome *b* (cyt *b*) gene. Analyses of negative samples were repeated to avoid false negatives (McClintock et al., 2010). Negative controls for PCR reactions (at least one per plate) and DNA extraction (one per 15 samples) were included in the analyses. PCR amplifications were resolved in 1.2 % agarose gels and positive samples

were sequenced uni-directionally from the 5' end using the MacroGen Sequencing Service (MacroGen Inc., The Netherlands).

Blood samples from 268 individuals were analysed, infections were reported in 242 individuals and multiple infections were observed at 107 samples. 96 of these double infections could be resolved because these corresponded to coinfections by different genus or were combinations of lineages already detected in other individuals, but the lineages present in 11 coinfections and 7 single infections remained unidentified. Sequences were edited using Sequencher™ v.4.9 (Gene Codes Corp, Ann Arbor, MI, USA) and lineages were identified by comparison with DNA sequences in Genbank and MalAvi databases (Bensch et al., 2009).

2.2. Statistical analyses

Infections by each parasite genus (prevalence) were analysed with separate Generalized Linear models with binomial error distribution and logit link function. Sampling location nested within dyad (each pair of sites) was included as a random factor. In that way we controlled for large scale geographic factors that may differ between localities in different geographic areas. Age (1 year vs ≥ 2 years old individuals), sex (male vs female) and habitat (urban vs forest) were included as fixed factors. We controlled for age because this may affect exposure to vectors and hematozoan infection prevalence (e.g., Ferraguti et al., 2021; Jiménez-Peñuela et al., 2019) and sex because blood parasite prevalence is related to sexual dimorphic traits (Hamilton and Zuk, 1982). Number of genera infecting each individual was analysed with a similar model but with Poisson distributed errors and log link function. Statistical analyses were done in SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

Estimation of lineage richness is problematic because estimates are strongly biased by sample size. Consequently, we used rarefaction analyses to compare the richness of lineages in urban and forest areas while controlling for sample size. Rarefaction analyses use lineage accumulation curves to estimate richness independent of sample size. Rarefaction curves were fitted using the function *rarefy* in the R package ‘vegan’ (Oksanen et al., 2008), and lineage richness estimates were compared between urban and forest populations using paired *t*-test. Finally, to compare the composition of blood parasite communities across habitats and sites, we analysed lineage frequency at each locality using correspondence analyses with the R package ‘CA’ (Nenadic and Greenacre, 2007). This correspondence analysis is a multivariate analysis that summarises community composition data allowing for the simultaneous representation of both habitats and lineages in a two dimensions plane (Legendre and Legendre, 2012). Therefore, sites with similar parasite communities will be closer between them and to the main lineages that characterize these communities.

3. Results

Of the 268 individuals analysed, 126 were captured in forests and 142 in cities. The numbers analysed per locality ranged between 14 and 34, being higher than 21 in nine of the ten localities studied.

3.1. Parasite prevalence

All the individuals captured at the forests were infected by at least one parasite genus while in the urban areas 116 individuals (82 %) were infected. The effect of age on pathogen prevalence was not homogeneous across haematozoa genera (Table 1). While older blackbirds had higher *Plasmodium* prevalence (79 % vs 44 %; Fig. 2A), the opposite occurred for *Haemoproteus* (14 % vs 30 %; Fig. 2B). In contrast, we found no age differences for *Leucocytozoon*. No differences in prevalence by sex were found for any of the genera studied (Table 1). Finally, neither *Plasmodium* nor *Haemoproteus* prevalence differed between urban and forest habitats (Table 1), but *Leucocytozoon* prevalence was significantly lower in urban (9 %) than in forest areas (69 %; Fig. 2C). We also found that blackbirds living in urban areas were infected by a lower number of haematozoa genera (0.93 ± 0.28 ; Fig. 2D) than those inhabiting European forests (1.65 ± 0.44). This variable was not influenced by age or sex (Table 1).

3.2. Parasite diversity

Differences in parasite lineage identity (Table 2) were found in birds from urban and forest areas (Fig. 3). The first axis of the correspondence analysis explained 40.8 % of variance and separated the localities according to the geographical distribution with higher scores for localities at higher latitudes. However, all the urban localities tended to cluster together with the only exception of the more geographically distant locality in Finland. The second axis of the correspondence analysis explained 24.7 % of variance and clearly separated urban (with higher

scores) from forest areas. All the *Leucocytozoon* lineages appeared closer to the forest localities, while most *Plasmodium* lineages appeared closer to the urban localities (Fig. 3). Two lineages were clearly associated with habitat characteristics (Table 3). Lineage pLINN1, that belongs to the species *Plasmodium matutinum*, was detected in the five urban areas but in none of the forest areas. The *Leucocytozoon* lineage NEVE01 was detected in all five forest areas but never in urban individuals.

Urban populations of blackbirds hosted a higher number of *Plasmodium* lineages (observed: $t_4 = 6$, $p = 0.004$; estimated: $t_4 = 4.42$, $p = 0.01$, Table 3) but a lower number of *Leucocytozoon* lineages (observed: $t_4 = -2.59$, $p = 0.06$; estimated: $t_4 = -3.74$, $p = 0.02$). No differences in the number of *Haemoproteus* lineages were found between urban and forest populations (observed: $t_4 = 0.78$, $p = 0.48$; estimated: $t_4 = -0.10$, $p = 0.93$).

4. Discussion

We found important effects of urbanization on the prevalence and diversity of parasite infection patterns of wild birds from 10 populations distributed south to north across Europe. Our results show that the impact of urbanization is not the same for all parasites, which could be due to the differential environmental requirements of their vectors. This supports previous work showing variable impacts of urbanization on avian parasites (e.g. Evans et al., 2009; Ferraguti et al., 2021; Fokidis et al., 2008; Murray et al., 2019; Tinajero et al., 2019).

We found significantly lower prevalence and species richness in urban areas for *Leucocytozoon*, a genus of parasites transmitted by blackflies, an insect that breeds preferentially in running water (Currie and Adler, 2008). However, no differences in pathogen prevalence between urban and natural areas were found for the mosquito-borne *Plasmodium* and *Culicoides*-borne *Haemoproteus*. These results are in line with those found for *Plasmodium* prevalence in French house sparrows (*Passer domesticus*; Bichet et al., 2014) but contrast with the higher prevalence of *Plasmodium*-*Haemoproteus* in a non-urban area in Mexico city (Santiago-Alarcon et al., 2020). Evans et al. (2009) also reported a higher malaria prevalence in non-urban than urban blackbirds. These contrasting findings could be explained because of the different habitat comparisons between the different studies. For example, in the case of blackbirds, our study compared urban vs well-preserved forested areas while Evans et al.’s study compared urban vs a mix of non-urban habitats including forests and different types of agricultural areas. Avian malaria can be differentially influenced by natural or agricultural areas (Ferraguti et al., 2018), potentially explaining these between-study variations. Our prevalence results do not comply with our initial prediction that blood parasites can explain the significantly shorter telomeres in urban blackbirds (Ibáñez-Alamo et al., 2018) and indicate that other mechanistic reasons should explain urban vs forest changes in this important health biomarker.

Vector abundance is an important predictor of the prevalence of vector borne pathogens (Ferraguti et al., 2021; Martínez-de la Puente et al., 2018), and we only found differences between cities and forests in the prevalence of infection for *Leucocytozoon*, but not for *Plasmodium*

Table 1

Results of Generalised Linear models testing the effects of age, sex and urbanization on the infection rates by *Plasmodium*, *Haemoproteus*, *Leucocytozoon* or by any of the three genera of haematozoa analysed in Eurasian blackbirds ($n = 268$). For each model the parameter estimates and standard error, the F values and associated *p*-values are given. Statistically significant factors are marked in bold.

| | <i>Plasmodium</i> | | | <i>Haemoproteus</i> | | | <i>Leucocytozoon</i> | | | N genera | | |
|------------------|-----------------------|------------------|-------------|----------------------|------------------|-------------|----------------------|------------------|-------------|----------------------|------------------|--------------|
| | | F _{1,4} | p | | F _{1,4} | p | | F _{1,4} | p | | F _{1,4} | p |
| Age (1 year) | -1.574 ± 0.354 | 19.79 | 0.01 | 1.001 ± 0.359 | 7.77 | 0.05 | -0.550 ± 0.363 | 2.30 | 0.20 | -0.162 ± 0.116 | 1.95 | 0.24 |
| Sex (Female) | 0.238 ± 0.316 | 0.57 | 0.49 | -0.078 ± 0.337 | 0.05 | 0.83 | -0.224 ± 0.333 | 0.45 | 0.54 | -0.032 ± 0.109 | 0.09 | 0.78 |
| Urbanization (F) | 0.025 ± 1.025 | 0.00 | 0.98 | 1.431 ± 1.018 | 1.98 | 0.23 | 3.054 ± 0.914 | 11.17 | 0.03 | 0.571 ± 0.111 | 26.47 | 0.007 |
| Overdispersion | 0.87 | | | 0.89 | | | 0.85 | | | 0.24 | | |

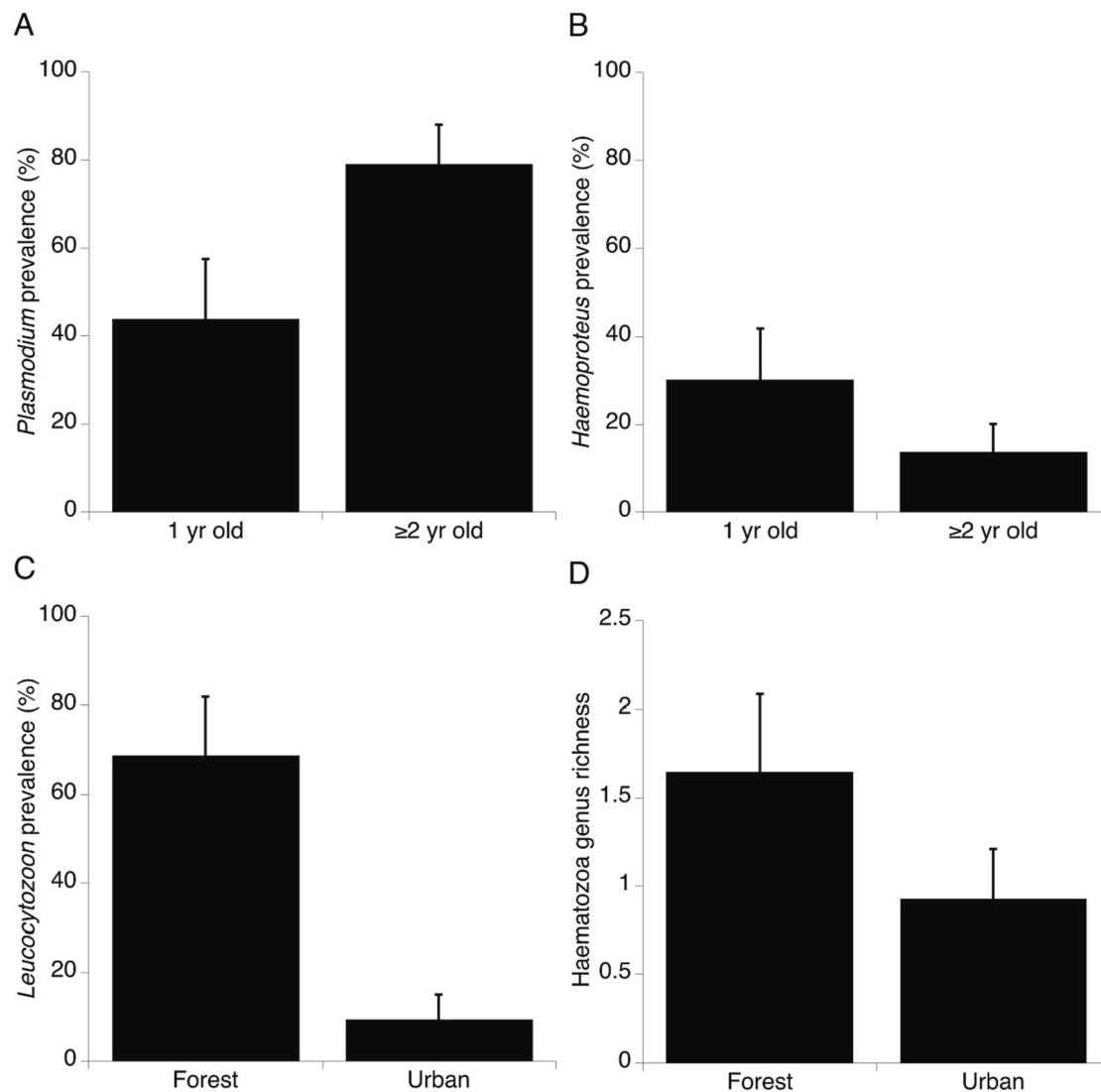


Fig. 2. Mean prevalence of *Plasmodium* (A), and *Haemoprotheus* (B) in 1 year old and older Eurasian blackbirds, mean prevalence of *Leucocytozoon* in forest and urban localities (C) and mean number of Haematozoa genera infecting each individual in forest and urban localities (D). Bars represent S.E.

and *Haemoprotheus*. A recent meta-analysis showed that Diptera diversity but not their abundance is reduced in urban areas (Fenoglio et al., 2020). All vectors for these blood parasites are Diptera and, while mosquitoes are generally less abundant in urban areas than in natural areas, some mosquito species are well adapted to urban areas and can maintain important populations (e.g. Ferraguti et al., 2016). This is the case of *Cx. pipiens*, a well-known vector of avian malaria worldwide (Ferraguti et al., 2016; Gutiérrez-López et al., 2020). Less information is available on how urbanization affects the specific abundance of *Culicoides*, the main vectors of *Haemoprotheus* infecting European blackbirds. But available information suggests little differences between urban and non-urban habitats. For example, Möhlmann et al. (2018) reported that in peri-urban areas *Culicoides* diversity was higher or similar to that found in wetlands and farms, although their abundance was lower. According to our results *Haemoprotheus* prevalence and lineage richness was similar in the cities and the forests. So, it is possible that urban wooded areas may represent suitable habitats for the maintenance of ornithophilic *Culicoides* species allowing the transmission of *Haemoprotheus* parasites in these human-altered areas (Santiago-Alarcon et al., 2013). Unfortunately, to the best of our knowledge the abundance and diversity of

Culicoides in urban and forest areas has not been compared yet. No information is also available for the case of blackflies, but these insects require running freshwater areas that are not very common in the studied urban areas so it will not be surprising that they are less abundant in these cities potentially explaining the lower prevalence of *Leucocytozoon* in urban blackbirds. Furthermore, the lower prevalence is also associated with a reduced richness of lineages in the city, and one of the lineages (NEVE01) was not found in any urban sites. The most plausible reason for this finding is the absence or the very low density of competent vectors for this lineage in the studied cities. However, we cannot exclude at this moment alternative explanations such as differences in the immune system and the genetic resistance of pathogens because the process of urbanization has been associated to homogeneous adaptive processes in this and other urban-dweller species (Mueller et al., 2013; Salmon et al., 2021).

Interestingly, the richness of *Plasmodium* lineages in urban areas was larger than in natural areas. In particular, the *Plasmodium matutinum* lineage LINN1 was only detected in urban blackbirds. This lineage has been associated to important exo-erythrocytic infections potentially causing the death of many blackbirds in central Europe (Himmel et al.,

Table 2
 Number of Eurasian blackbirds infected by the different lineages of *Plasmodium*, *Haemoproteus* and *Leucocytozoon*. For each pathogen the number of individuals infected in each locality presented as number in forest/number in urban is given, together with the overall number of tested individuals. The last column indicates the number of individuals not infected by any of the parasites analysed. The infections in 18 individuals could not be identified to lineage level and for this reason the number of Total infected by *Plasmodium*, *Haemoproteus* or *Leucocytozoon* may be higher than the sum of those infected by each lineage. Note that a same individual may be also infected by more than one genera or lineages of parasites.

| Locality | <i>Plasmodium</i> | | <i>Haemoproteus</i> | | <i>Leucocytozoon</i> | | Total inf. | Number Not sampled | Total inf. Leuco. | | | | | | | |
|-----------------|--|--|--|---|---|-----------------|------------|--------------------|-------------------|------------|-------------|--------------|-------------|-----------|------------|-------------|
| | <i>P. circumflexum</i> (Rinshi-2)BT7 SYAT05) | <i>P. vaughani</i> AFTRU5P. matutinum (pLINN1) | Total <i>H. minutus</i> (TUCHR01) inf. | Total <i>H. minutus</i> (TURDUS2) (PHYBOR04) inf. | Total 3,224,698 TUPHI06 NEVE01 TUMER03SP19 TUMIG19. SP18A | TUMER02 TUMIG19 | | | | | | | | | | |
| Turku (Finland) | 7/2 | 0/5 | 8/7 | 0/1 | 17/10 | 0/5 | 19/17 | 1/0 | 16/0 | 1/0 | 1/0 | 20/0 | 27/34 | 0/10 | | |
| Dijon (France) | | 7/14 | 0/1 | 8/16 | 2/0 | 3/3 | 6/3 | 1/0 | 2/0 | 0/1 | 0/1 | 3/1 | 14/22 | 0/3 | | |
| Madrid (Spain) | | 18/20 | 0/1 | 21/27 | 8/1 | 8/1 | 8/1 | 1/0 | 3/0 | 11/8 | 2/3 | 17/12 | 30/29 | 0/1 | | |
| Granada (Spain) | | 9/14 | 0/6 | 10/20 | 19/1 | 19/1 | 19/1 | 2/0 | 2/0 | 14/5 | 10/0 | 29/5 | 30/29 | 0/8 | | |
| Sevilla (Spain) | | 25/19 | 0/2 | 25/21 | 0/1 | 0/1 | 0/2 | 0/1 | 1/0 | 14/3 | 2/1 | 18/5 | 25/28 | 0/4 | | |
| Total | 7/2 | 0/15 | 91 | 2/1 | 47/16 | 0/5 | 24 | 2/0 | 24/0 | 1/0 | 15/4 | 40/17 | 15/4 | 23 | 142 | 0/26 |

2020). Screening for *Plasmodium* in mosquitoes detected this lineage in a wide range of mosquitoes, including *Cx. pipiens* (Ferraguti et al., 2013; Martínez-de la Puente et al., 2015), the most common mosquito present in urban areas in Europe (Rizzoli et al., 2015). Consequently, despite the lower abundance and diversity of birds and mosquitoes in cities, the presence of *Cx. pipiens* may provide an adequate environment for the successful transmission of avian *Plasmodium* in European urban areas, especially considering the relatively low specificity found between avian *Plasmodium* and mosquito species (Kimura et al., 2010). Additionally, we cannot exclude that the relative higher densities of blackbirds in cities compared with forests (Schnack, 1991) may also facilitate the maintenance of *Plasmodium* lineages in urban blackbird populations, as it has been reported in more dense great tit *Parus major* populations (Isaksson et al., 2013). This lineage has been found in previous studies infecting juvenile blackbirds and mosquitoes in natural areas in southern Spain, note that names pSPHJ and LINN1 correspond to the same genetic lineages (Diez-Fernández et al., 2021; Ferraguti et al., 2013).

In addition to the urban variation, we found important age differences regarding *Plasmodium* and *Haemoproteus* prevalence. As life-time infections, the prevalence of these two protozoans is expected to increase with age, because the exposure to potentially infected vectors will increase with lifespan (Wood et al., 2007). While this could be the reason explaining the positive association between age and *Plasmodium* prevalence, other reasons should be behind the opposite pattern found for *Haemoproteus* infections. These discrepancies could be due to different factors including differences in the virulence between these two parasite genera with a higher mortality rate in *Haemoproteus* infected individuals compared to those infected by *Plasmodium*. For instance, field experimental evidence supports that *Haemoproteus* infection significantly reduce host survival (Martínez-de la Puente et al., 2010), while observational data indicate that chronic *Plasmodium* infection also reduce survival (Lachish et al., 2011) and that dual infections by *Haemoproteus* and *Plasmodium* increase host mortality (Marzal et al., 2008). In particular, the *Haemoproteus minutus* lineage hTURDUS2, which is widespread in European blackbirds (i.e. the most prevalent in our studied individuals), have been identified as very pathogenic for exotic species producing high mortalities in other bird groups such as parrots (Olias et al., 2011). If this is the case for blackbirds too, then the lower prevalence of *Haemoproteus* in adults may reflect such differences. Thus, we could speculate that the lower prevalence of *Haemoproteus* in older blackbirds may also reflect a higher immune capacity to fight *Haemoproteus* proliferation. In contrast, this explanation is not valid regarding the higher prevalence of *Plasmodium* in older birds could be that they have experienced higher mosquito abundance in previous years when younger adults would have not even been born yet. Annual variation in vector abundance can be large under certain circumstances (Roiz et al., 2014). Finally, the lack of sexual differences in haematzoa prevalence is not surprising given that a recent meta-analysis did not find any sex bias in the prevalence of *Plasmodium*, *Haemoproteus* nor *Leucocytozoon* (Valdebenito et al., 2020).

To sum up, urbanization is associated with important changes in the prevalence and diversity of pathogens that are not homogenous and vary depending on the parasite genera. While urban birds had a reduced infection prevalence by *Leucocytozoon*, no differences were found for *Plasmodium* and *Haemoproteus*. A higher number of *Plasmodium* lineages were present in blackbirds living in European cities compared with nearby natural forests, but the opposite pattern was found for *Leucocytozoon*. Among other factors, the capacity of the vectors to sustain significant populations in cities may explain such taxon-dependent results, highlighting the need to consider the different ecologies of these three genera in future studies (Jiménez-Peñuela et al., 2021; Pérez-Rodríguez et al., 2013). Interestingly, some particular parasitic lineages were exclusively detected in birds from one of the two habitat types suggesting that urbanization can play an important role modifying the interactions between birds, vectors and parasites.

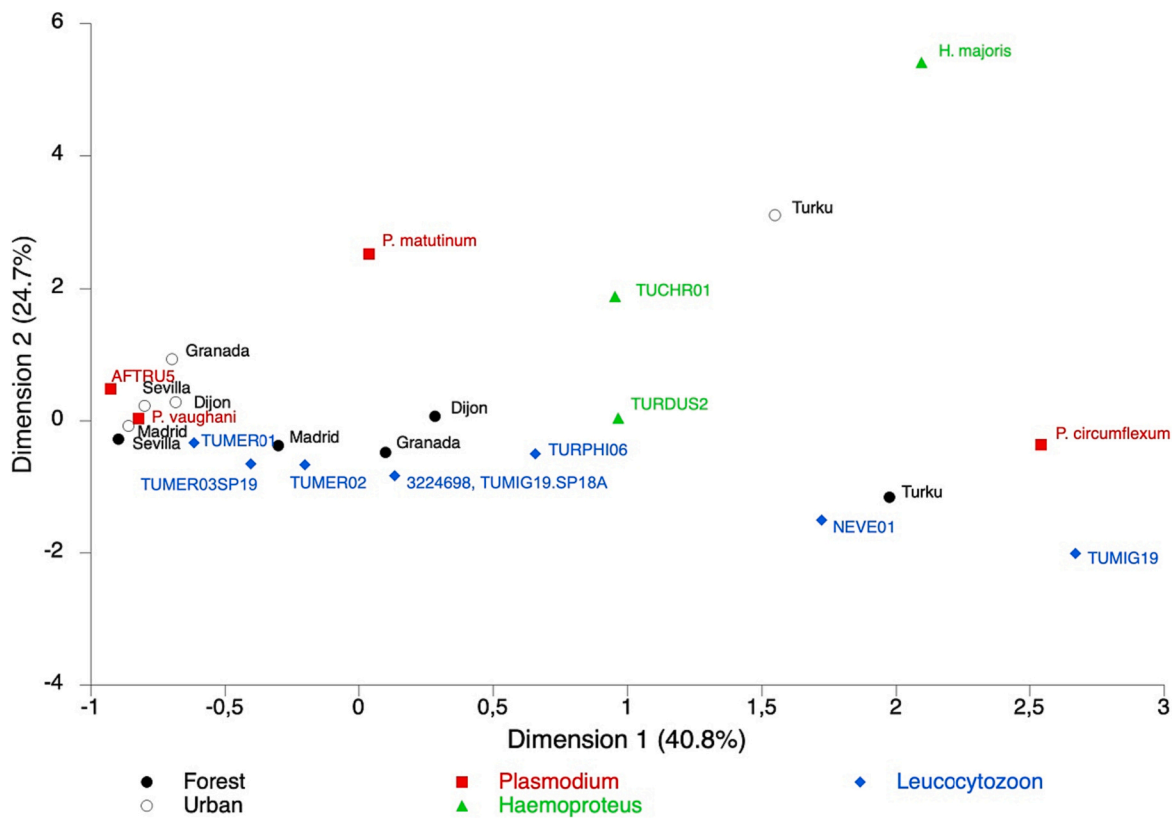


Fig. 3. Plot of the first two axis of a correspondence analysis of the *Plasmodium*, *Haemoproteus* and *Leucocytozoon* lineages found in the five paired urban and forest localities in Europe. Each locality symbol is plot near the more frequently detected lineages. The first dimension of the correspondence analyses was related to the geographical distribution of the localities, while the second dimension was lower for the forest than for the urban localities.

Table 3

Number of lineages of *Plasmodium*, *Haemoproteus* and *Leucocytozoon* found at each forest/urban locality and lineage richness estimated through rarefaction curves.

| Locality | <i>Plasmodium</i> | | <i>Haemoproteus</i> | | <i>Leucocytozoon</i> | |
|-----------------|-------------------|-----------|---------------------|-----------|----------------------|-----------|
| | Obs. | Estimated | Obs. | Estimated | Obs. | Estimated |
| Turku (Finland) | 1/2 | 1/1.61 | 1/3 | 1/2.35 | 5/0 | 3.07/0 |
| Dijon (France) | 1/3 | 1/2.27 | 2/1 | 2/0.96 | 2/1 | 2/0.64 |
| Madrid (Spain) | 1/2 | 1/1.48 | 1/1 | 1/0.48 | 4/2 | 3.05/1.89 |
| Granada (Spain) | 1/2 | 1/1.99 | 1/1 | 1/0.48 | 5/1 | 3.38/0.97 |
| Sevilla (Spain) | 1/2 | 0/1.76 | 0/1 | 0/0.52 | 3/3 | 2.42/1.89 |

Ethic statement

Bird trapping was carried out with all the necessary permits from regional and national authorities. Our procedures were approved by the Ethical Committee of the Consejo Superior de Investigaciones Científicas (CSIC) with the reference number 258/2015.

CRediT authorship contribution statement

Jordi Figuerola: Conceptualization, Formal analysis, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Josué Martínez-de la Puente:** Conceptualization, Investigation, Supervision, Validation, Writing – review & editing. **Alazne Díez-Fernández:** Investigation, Validation, Writing – review & editing. **Robert L. Thomson:** Investigation, Resources, Writing – review & editing. **José I. Aguirre:** Investigation, Resources, Writing – review & editing. **Bruno Faivre:** Investigation, Resources, Writing – review & editing. **Juan Diego Ibañez-Alamo:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We thank F. Espinosa, I. Hernandez, A. Pastoriza, and M. Vázquez for their help capturing blackbirds. J.D.I. was funded by a postdoctoral contract (TAHUB-104) from the program ‘Andalucía Talent Hub’ (Marie Skłodowska Curie actions - COFUND). A.D.F. was funded by the ‘Severo Ochoa’ grant (SVP-2014-068571) from MICINN (Spain). J.A. was funded by a grant from the Universidad Complutense de Madrid (CT45/15-CT46/15). During the writing of this manuscript, J.F. was supported by the MICINN project PID2021-123761OB-I00 supported by FEDER funds, J.M.P. by the MCIN/AEI/10.13039/501100011033 [grant number

PID2020-118205GB-I00] and by the Junta de Andalucía, Consejería de Universidad, Investigación e Innovación (Proyecto: P21_00049), while J.D.I. was supported by the Spanish Ministry of Science and Innovation (PID2019-107423GA-I00 / SRA (State Research Agency / 10.13039/501100011033).

Funding

J.D.I. was funded by a postdoctoral contract (TAHUB-104) from the program 'Andalucía Talent Hub' (Marie Skłodowska Curie actions - COFUND). A.D.F. was funded by the 'Severo Ochoa' grant (SVP-2014-068571) from MICINN (Spain). J.A. was funded by a grant from the Universidad Complutense de Madrid (CT45/15-CT46/15). During the writing of this manuscript, J.F. was supported by the MICINN project PID2021-123761OB-I00 supported by FEDER funds, J.M.P. by the MCIN/AEI/10.13039/501100011033 [grant number PID2020-118205GB-I00] and by the Junta de Andalucía, Consejería de Universidad, Investigación e Innovación (Proyecto: P21_00049), while J.D.I. was supported by the Spanish Ministry of Science and Innovation (PID2019-107423GA-I00 / SRA (State Research Agency / 10.13039/501100011033).

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