ORIGINAL ARTICLE

The role of ecology in the geographical separation of blood parasites infecting an insular bird

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ABSTRACT

Aim Niche modelling is increasingly used to predict species' geographical distributions or to infer the evolutionary or ecological processes that constrain them, but relatively few studies have examined the ecological processes governing the distributions of parasites. Among such processes, niche divergence is frequently invoked to explain species range variation. Here, we test whether the geographical distributions of two lineages of *Leucocytozoon* (Haemosporida) avian parasites are linked to climatic conditions and whether niche divergence can explain their geographical separation.

Location Réunion, Mascarene archipelago, south-western Indian Ocean.

Methods *Leucocytozoon* prevalence data were obtained by PCR screening of avian blood samples. Prevalence data and 20 environmental layers were used to build species distribution models (SDMs). SDMs were built by averaging the predictions of five different models: random forests (RF), generalized linear models (GLM), generalized additive models (GAM), multivariate adaptive regression splines (MARS) and support vector machines (SVM). Niche identity and background tests were used to test for a role of niche divergence in explaining parasite distributions.

Results The geographical ranges of the two lineages of *Leucocytozoon* under study showed little overlap. Species distribution modelling suggested that niche divergence may explain the spatial variation observed in *Leucocytozoon* distribution, implying that the geographical separation of parasites is linked to environmental conditions. The variables that best explained parasite distribution were all related to precipitation patterns.

Main conclusions As precipitation cannot directly affect endosymbiotic *Leucocytozoon* parasites, we suggest that the geographical separation of *Leucocytozoon* lineages is the result of an underlying geographical structure in the dipteran vectors that transmit these parasites. This illustrates the need to consider the ecology of vectors when predicting the distribution of vector-borne parasites. Our study also shows that different parasite lineages, contained within broadly defined parasitic taxa, may have very different ecologies, and that these differences should be taken into account when attempting to understand the ecological determinants of parasite distribution and disease emergence.

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INTRODUCTION

Closely related lineages are often found occupying geographically separated ranges whose boundaries are determined by environmental factors (e.g. Bridle *et al.*, 2001; Secondi *et al.*, 2006; Khimoun *et al.*, 2011). One process explaining geographical separation of closely related lineages is their adaptation to different climatic conditions (i.e. climatic niche

divergence), with each lineage having its own climatic niche envelope (Wiens & Graham, 2005). The opposite process of niche conservatism, i.e. the tendency of lineages to retain ancestral ecological requirements (Ricklefs & Latham, 1992; Wiens, 2004; Wiens & Graham, 2005), can also affect the geographical distribution of closely related lineages. In this case, all lineages require the same abiotic conditions and may have similar or broadly overlapping geographical distributions, or occupy geographically separated areas that share a similar climate (Wiens & Graham, 2005). Testing the contrasting hypotheses of niche conservatism or divergence thus provides insights into the evolutionary and ecological processes shaping species' distributions (Stephens & Wiens, 2009).

In recent years, new GIS tools and the availability of global climate data have triggered the development of ecological niche modelling, which aims to characterize the ecological niches of lineages and predict habitat suitability in unsampled areas (Guisan & Thuiller, 2005; Elith & Leathwick, 2009). Although such tools have been extensively used for investigating the potential distributions of a wide range of organisms (e.g. Elith & Leathwick, 2009; Cianfrani *et al.*, 2011), we still know relatively little about the processes constraining the distribution of parasites, although the distribution and abundance of many species may be primarily determined by host–parasite interactions (Ricklefs, 2011). To date, most studies that have modelled parasite niches have aimed to predict the risk of human (Martens *et al.*, 1995; Peterson *et al.*, 2004, 2005; Schur *et al.*, 2011) or wildlife (Smith *et al.*, 2002; Estrada-Peña, 2008; Haverkost *et al.*, 2010; Sehgal *et al.*, 2011; Martínez-Salazar *et al.*, 2012) diseases emerging in new areas. In contrast, we know of no studies that have used niche modelling to investigate how ecological and evolutionary processes may influence the relative geographical distributions of parasites.

The present study seeks to explain the geographical distribution at the regional scale of *Leucocytozoon* blood parasites (Leucocytozoidae), members of the order Haemosporida, a group that also includes parasites of the genera *Plasmodium* (malarial parasites) and *Haemoproteus* (Martinsen *et al.*, 2008). *Leucocytozoon* infect only avian hosts, in a life cycle that includes asexual reproduction in the host and sexual reproduction in the vector, mainly blackflies (Diptera: Simuliidae) (Valkiūnas, 2005). The distribution of hemosporidian parasites may be determined by the availability of resources (competent avian hosts and vectors) and/or by abiotic conditions. Several studies on human hemosporidians revealed that their distribution can be constrained by abiotic conditions, either directly (e.g. temperature influences the duration of parasite sporogony) or indirectly through modification of vector dynamics (Onori & Grab, 1980; Craig *et al.*, 1999).

Zosterops borbonicus (Boddaert, 1783) (Aves: Zosteropidae) is endemic to the Indian Ocean island of Réunion, where it occurs in all habitats (Gill, 1973), and harbours a *Leucocytozoon* community consisting almost exclusively of two distinct evolutionary lineages (Cornuault *et al.*, 2012). Here we characterize and compare the geographical distribution of the two *Leucocytozoon* lineages at the scale of the island. We then investigate the role of a combination of ecogeographical predictors in shaping the relative distributions of the two lineages. To this end, we determine whether the lineages may occupy different niches through the use of species distribution models (SDMs) and correlation analyses of parasite prevalence and environmental data. We also conduct a background test (Warren *et al.*, 2008) to assess the roles of niche conservatism and niche divergence in shaping parasite distribution.

MATERIALS AND METHODS

Geographical distribution of *Leucocytozoon* lineages

Prevalence data

Birds were mist-netted on Réunion between 2007 and 2010 and blood samples were collected by gently puncturing the sub-brachial vein. All manipulations were conducted under a ringing permit issued by the CRBPO Muséum d'Histoire Naturelle (Paris, France). A total of 496 individual birds from 33 localities distributed across all major habitat types were screened for *Leucocytozoon* infections by PCR amplification of a fragment of the parasite's cytochrome *b* (cyt *b*) gene (protocol in Hellgren *et al.*, 2004). Subsequent bidirectional sequencing was used to identify cyt *b* haplotypes (see Cornuault *et al.*, 2012, for details). From a total of 291 infected birds, we obtained *Leucocytozoon* sequences from 131 different individuals, which led to the identification of 17 haplotypes. Among these, seven and eight haplotypes were identified as belonging to Lineage A and Lineage B, respectively, following the classification published by Cornuault *et al.* (2012), wherein the two lineages were named Clade A′ and Clade B′. Details of the patterns of infection of a greater part of the Réunion avifauna by the two lineages, together with sequence accession numbers, can be found in Cornuault *et al.* (2012). These two lineages colonized Réunion independently and their presence on the island is ancient, with minimum colonization times of 0.66 Ma [95% confidence interval (CI): 0.31–1.07 Ma] for Lineage A, and 1.56 Ma (95% CI: 0.97–2.24 Ma) for Lineage B (Cornuault *et al.*, 2012). Thus, the colonization of Réunion by these *Leucocytozoon* lineages is likely to be a natural phenomenon, pre-dating human arrival (AD 1640; Cheke & Hume, 2008). They also appear to be specific to *Zosterops* on Réunion, as only one infection by Lineage B was recorded outside this reservoir (Cornuault *et al.*, 2012). The two Réunion *Zosterops* species (*Z. borbonicus* and *Z. olivaceus*) share the same parasite lineages (mainly lineages A and B) in the same proportions (Cornuault *et al.*, 2012) and their geographical distributions largely overlap (Gill, 1971). This biological system is therefore well suited to the investigation of intra-island biogeographical patterns of parasites, as the two lineages under study are known to have undergone separate evolutionary and colonization histories, despite their current presence in the same hosts on Réunion.

Only prevalence data in *Z. borbonicus* were used in this study, because our data for *Z. olivaceus* are limited. The prevalence of each lineage per locality was then calculated as the proportion of birds infected by each lineage. For localities in which we only obtained parasite sequences from a fraction of infected individuals, prevalence per lineage was estimated by multiplying the relative prevalence per lineage (the proportion of individuals for which we obtained sequences that are infected by each lineage) by the total prevalence (the proportion of birds infected by any *Leucocytozoon* lineage) observed at the locality.

Test for differences between geographical distributions of parasites

We used Syrjala's test (Syrjala, 1996) to determine whether the geographical distributions of the two lineages are significantly different. This test makes use of geolocated density measures (here prevalence) to test the null hypothesis that two populations have similar geographical distributions. Statistical significance is estimated through data permutation (Syrjala, 1996).

Species distribution models and differences in *Leucocytozoon* environmental niches

Eco-geographical predictors

We obtained 20 environmental data layers covering the whole island of Réunion (see Table 1 for a description of the variables and their spatial resolution). Seventeen of these layers, obtained from the French Meteorological Office (Météo-France, Toulouse), summarize climatic data reflecting different aspects of temperature and precipitation over the last 30 years. The three remaining data layers were elevation, and mean and within-year standard deviation of the normalized difference vegetation index (NDVI) for the year 2009. NDVI layers are based on Moderate Resolution Imaging Spectroradiometer (MODIS) data (Justice *et al.*, 1998). Mean NDVI is a useful proxy of standing biomass or vegetation cover (Myneni *et al.*, 1995). Mean NDVI was included because habitat openness has been shown to influence hemosporidian parasite prevalence, probably through effects on host or vector densities (Bonneaud *et al.*, 2009). Furthermore, it is known to influence the distribution of *Z. borbonicus* on Réunion (Gill, 1973).

SDM building and variable selection

Viney *et al.* (2009) and Grenouillet *et al.* (2011) showed that the predictive performance obtained by averaging the fitted values of several models is likely to outperform the predictive performance of a single model. We therefore used ensemble modelling (Viney *et al.*, 2009) to predict the prevalence of the two lineages at the scale of the island by averaging the predictions of five elementary models that can handle prevalence data (percentage): random forests (RF; Breiman, 2001),

Table 1 Description of environmental variables.

Variables*	Description	Resolution
BIO1	Annual mean temperature	132 m × 132 m
BIO4	Temperature seasonality (standard deviation × 100)	132 m × 132 m
BIO5	Maximum temperature of the warmest month	132 m × 132 m
BIO6	Minimum temperature of the coldest month	132 m × 132 m
BIO9	Mean temperature of the driest quarter	132 m × 132 m
BIO10	Mean temperature of the warmest quarter	132 m × 132 m
BIO11	Mean temperature of the coldest quarter	132 m × 132 m
Elevation	Elevation	132 m × 132 m
BIO3	Isothermality (BIO2/BIO7) (×100)	132 m × 132 m
BIO7	Temperature annual range (BIO5 − BIO6)	132 m × 132 m
BIO12	Annual precipitation	132 m × 132 m
BIO13	Precipitation of the wettest month	132 m × 132 m
BIO14	Precipitation of the driest month	132 m × 132 m
BIO15	Precipitation seasonality (coefficient of variation)	132 m × 132 m
BIO16	Precipitation of the wettest quarter	132 m × 132 m
BIO17	Precipitation of the driest quarter	132 m × 132 m
BIO18	Precipitation of the warmest quarter	132 m × 132 m
BIO19	Precipitation of the coldest quarter	132 m × 132 m
mean NDVI	Mean normalized difference vegetation index (NDVI) over the year 2009	1000 m × 1000 m
sd NDVI	Standard deviation of NDVI within the year 2009	1000 m × 1000 m

*Bioclimatic variables and elevation were obtained at high resolution from the French Meteorological Office (Météo-France, Toulouse). NDVI data were monitored by the MODIS device (NASA's Terra mission; http://e4eil01.cr.usgs.gov:22000/WebAccess/drill?attrib=esdt&esdt=MOD13A3.5&group=MOLT).

generalized linear models (GLM), generalized additive models (GAM), multivariate adaptive regression splines (MARS; Friedman, 1990) and support vector machines (SVM; Karatzoglou *et al.*, 2004). We carried out variable selection for GLM and GAM by selecting the best model under the Akaike information criterion (Akaike, 1974). One SDM was constructed for each lineage and then used for predicting prevalence over the whole island.

Assessment of SDM predictive performance

An important quality of an SDM is its predictive (or generalization) performance. Some models can fit some particular data sets well, but perform poorly when used on other data

(e.g. due to overfitting). To determine model performance and avoid overfitting, models are typically calibrated on a random partition of the initial data set (calibration set) and tested with the remaining data (test set). This is, however, inconvenient for small data sets, because the calibration data may be insufficient. A jackknife procedure can alternatively be used (Pearson *et al.*, 2007) – i.e. leaving a portion of the data out for testing and calibrating the model with the remaining data. If the data set consists of n localities, the procedure consists of an initial calibration of the model with $n − 1$ localities and further testing using the omitted datum, repeating the procedure until each data point has been omitted once. The testing procedure thus involves building n SDMs.

For a statistical assessment of whether an SDM performed better than the random expectation, we proceeded as follows. Let x_i be the observed predictive error of the i-th SDM, defined as $x_i = |o_i − p_i|$, with o_i and p_i the observed and predicted prevalence, respectively, for locality i (i.e. the locality that was omitted when training the i-th SDM during the jackknife procedure). Then, let P_i be the vector of predicted prevalence for the whole study area that was obtained with the i-th SDM. The length of P_i is the number of cells in the grid. The vector $X_i = |o_i − P_i|$ is then the vector of differences between the observed prevalence at locality i and random predicted prevalence. X_i represents random predictive errors. We then calculate the probability $\Pr_i(X_i < x_i)$ that a random predictive error is lower than the observed predictive error x_i. This probability is given by the proportion of X_i that satisfies $X_i < x_i$. It represents the probability of the i-th SDM having as low a predictive error as observed, purely by chance. Each of the n SDMs generated during the jackknife procedure thus received a value $\Pr_i(X_i < x_i)$ reflecting its predictive error, taking into account the distribution of P_i. To summarize this information across all localities, we used the following statistic (S):

$$S = \sum_{i=1}^{n} \Pr_i(X_i < x_i)$$

A null distribution of S was constructed by generating 1000 random vectors of n values of $\Pr_i(X_i < x_i)$. Each $\Pr_i(X_i < x_i)$ within such a vector was obtained by drawing within P_i one random value for p_i and proceeding to the calculation of S. A *P*-value was calculated as the proportion of the null distribution that was lower than the observed value of S. Our procedure for model evaluation is a continuous analogue of the procedures that Pearson *et al.* (2007) and Khimoun *et al.* (2013) developed for presence/absence data.

Variable importance

The use of ensemble modelling makes it more difficult to assess the importance of individual variables in predicting lineage prevalence, as each contributing model may differently rank the variables used. To overcome this issue, we used a jackknife procedure for assessing variable importance in our models. Each SDM was calibrated with only one variable and predictions were obtained for omitted localities (following the jackknife procedure described in the previous section). The importance of a given variable was then measured as Pearson's correlation coefficient between observed and predicted prevalence values.

Test of niche identity

This test allows a comparison of two SDMs, and estimates whether their predictions are significantly different. It was previously developed for presence-only data (Warren *et al.*, 2008) and consists of randomizing the identity of occurrence points (i.e. which lineage is found at the different sampled locations) and rebuilding new SDMs from these pseudodatasets. This procedure is repeated numerous times in order to generate a null hypothesis for two statistics measuring niche similarity: the I statistic (Warren *et al.*, 2008) and Schoener's D (Schoener, 1968). Here, this test was adapted to continuous data where each locality is associated with two measures of prevalence (one for each parasite lineage) instead of a binary variable (see Appendix S1 in Supporting Information). The null hypothesis consisted of 1000 replicates.

Test of niche divergence

It is important to note that observing two non-sympatric lineages in different niches does not necessarily mean support for a niche divergence hypothesis, because the apparent differences in the niche occupied by the lineages may be due merely to spatial autocorrelation (Luoto *et al.*, 2005). For example, historical and dispersal factors can influence lineage distributions if the two lineages do not occupy certain parts of the available geographical and niche spaces only because they have never reached them yet, not because they are unsuitable (Lobo *et al.*, 2010; Sillero, 2011). In this case, spatial autocorrelation of environmental conditions may result in the two lineages occupying different niches, even though environment plays no part in the geographical separation. The background test (the background is the area circumscribing all occurrences of a lineage) allows for the test of the hypotheses of niche divergence and conservatism despite these issues (Warren *et al.*, 2008). It determines whether one lineage occurs in a part of its background that is significantly similar (conservatism) or different (divergence) compared to the other lineage niche (Warren *et al.*, 2008). The procedure for this test was adapted to prevalence data and is detailed in Appendix S1.

All analyses were carried out in the R environment (R Development Core Team, 2011). Details of SDM construction and the computation of all tests are given in Appendix S1.

RESULTS

Geographical distributions

The geographical ranges of Lineage A and Lineage B are parapatric (i.e. geographically separated ranges abutting along common boundaries), with a narrow overlap zone
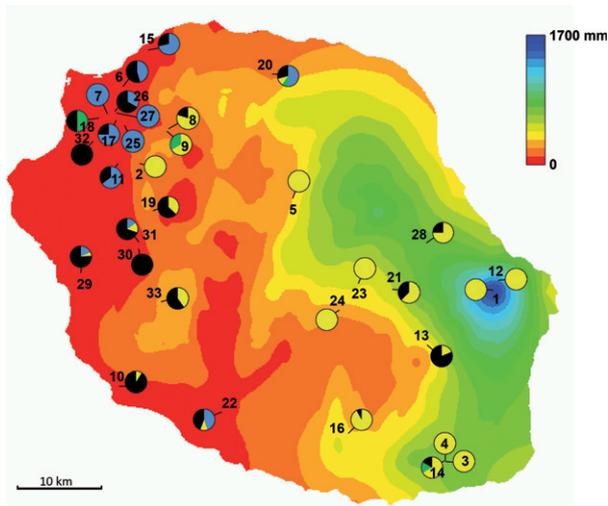
Figure 1 Geographical distribution of lineages A and B of *Leucocytozoon* infecting *Zosterops borbonicus* on the island of Réunion. Pie charts represent the relative proportions of birds infected by Lineage A (blue), Lineage B (yellow), both lineages A and B (green) or neither of these lineages (i.e. uninfected or infected by other lineages; black). The background colouring corresponds to the precipitation in the driest quarter (variable BIO17), one of the most important variables for the distribution of the lineages of this study.

(Fig. 1). Lineage A is mostly restricted to the north-western part of the island, whereas Lineage B is distributed across much of the remaining area. The two lineages coexist in only 7 out of 33 localities (Fig. 1, Appendix S2). The test for differences between the two ranges reveals a strong pattern of geographical separation between them ($\Psi = 2.34$; $P < 0.001$).

Predictive performance

For the two lineages, all elementary models predicted the observed prevalence better than by chance alone, with the exception of the GLM for Lineage A (Table 2). The average SDMs resulting from ensemble modelling also predicted better than chance and were used for all further analyses (Table 2).

Tests of niche identity and divergence

The predicted prevalence shows clear differences in niche characteristics and breadth for the two lineages (Fig. 2a,b). This analysis indicates that Lineage A should be restricted to small areas of low elevation (< 1000 m) in the north-west of the island, associated with a dry and warm climate. In contrast, Lineage B is expected to occur at high prevalence ($> 40\%$) on more than half the island, but is typically rare in Lineage A's range. Elevation does not appear to constrain the range of Lineage B, as it is predicted to occur from sea level to above 2000 m. The distinct geographical areas occupied by the two lineages are characterized by distinct environmental conditions, as modelling from environmental variables

Table 2 Evaluation of species distribution models (SDMs) for *Leucocytozoon* lineages infecting *Zosterops borbonicus* on Réunion.

	Predictive performance*	
Modelling technique	Lineage A ($n = 33$)	Lineage B ($n = 33$)
Average	0.72***	0.85***
Random forests (RF)	0.55**	0.83***
Generalized linear model (GLM)	0.32 n.s.	0.67**
Generalized additive model (GAM)	0.94***	0.94***
Multivariate adaptive regression splines (MARS)	0.35***	0.66***
Support vector machines (SVM)	0.72***	0.61***

*For each SDM (table cell), we report Pearson's correlation coefficient between observed prevalence and predictions obtained through the jackknife procedure. Asterisks indicate significance based on 1000 pseudoreplicates and show that virtually all SDMs predicted better than chance. n.s., $P > 0.05$; **$P < 0.01$; ***$P < 0.001$.

predicts parasite distributions consistent with observed prevalence (Fig. 1). The niche identity test is significant (Table 3), indicating that the difference between the environmental niches of the two lineages is greater than expected by chance and the background test is significant in favour of niche divergence. The background test is only significant when randomizing the occurrences of Lineage A, suggesting that Lineage A occurs in a part of its background where environmental conditions are particularly dissimilar to the environmental niche of Lineage B.

Variable importance

Precipitation variables rank first in the models for both lineages; the five most important variables for Lineage A and the eight most important variables for Lineage B all represent different aspects of precipitation (Fig. 3). In particular, variables BIO17 and BIO14 (precipitation of the driest quarter and month, respectively) rank among the top three variables for both lineages. There is a positive relationship between the prevalence of Lineage B and BIO17, indicating that the minimal amount of precipitation throughout year seems to be a limiting factor to the presence of Lineage B. In contrast, the inverse trend was observed for Lineage A (Fig. 3c).

DISCUSSION

A number of studies have investigated how environmental factors may affect the prevalence of malarial parasites and related Haemosporida. Most of them concern human parasites and aimed to predict the risk of the emergence of malaria in novel areas (Gemperli *et al.*, 2006; Guerra *et al.*, 2006; Patz & Olson, 2006; Kulkarni *et al.*, 2010; Lindsay *et al.*, 2010; Reid *et al.*, 2010; Riedel *et al.*, 2010). Other studies have provided evidence for the roles of elevation (Akhwale *et al.*, 2004; Atkinson *et al.*, 2005; Drakeley *et al.*,
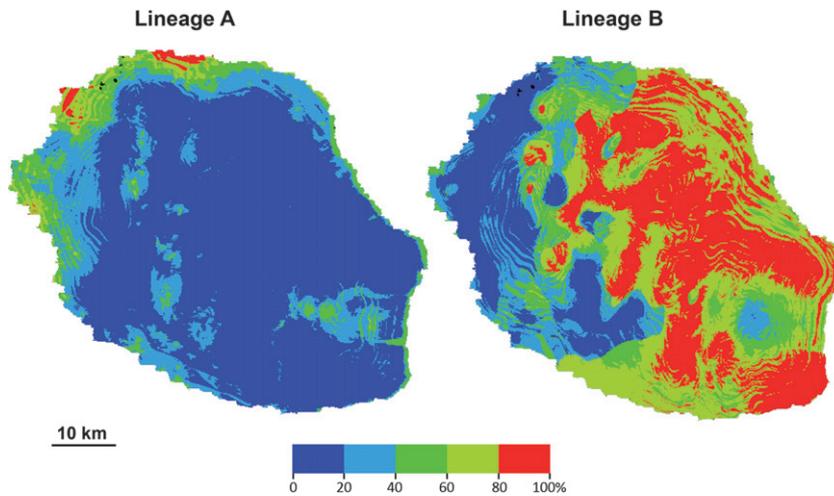
Figure 2 Predicted prevalence of *Leucocytozoon* Lineage A and Lineage B infecting *Zosterops borbonicus* on Réunion obtained from averaged species distribution models. Colouring indicates the predicted prevalence in the percentage of birds infected.

Table 3 Results of niche identity and background tests for *Leucocytozoon* lineages infecting *Zosterops borbonicus* on Réunion. These tests assess niche differences between *Leucocytozoon* lineages A and B. All tests were carried out with average models using 1000 pseudoreplicates.

	I	P	D	P
Niche identity test	0.65	< 0.001***	0.45	< 0.001***
Niche background test† with randomization of occurrences of Lineage A (*n* = 33)	0.65	< 0.001***‡	0.45	< 0.001***‡
Niche background test† with randomization of occurrences of Lineage B (*n* = 33)	0.65	0.07 n.s.‡	0.45	0.04 n.s.‡

n.s., $P > 0.05$; ***$P < 0.001$.

†This test is two-tailed. If the observed statistic is greater than (less than) the null distribution, there is evidence for niche conservatism (divergence). The *P*-values shown here represent the niche divergence significance; that is, they are the proportion of the null distribution which is less than the observed statistic.

‡The background of the lineage whose occurrence points were randomized was defined as the minimal convex polygons circumscribing all occurrence points. Two alternative methods for defining the background of Lineage A yielded similar results; details are given in Appendix S1.

2005; Bødker *et al.*, 2006; Shurulinkov & Chakarov, 2006; Savage *et al.*, 2009), precipitation and temperature (Møller, 2010; Garamszegi, 2011; Sehgal *et al.*, 2011) in predicting parasite prevalence. However, these studies examined specific parasite lineages or a whole hemosporidian genus and did not investigate the possible variation of ecological requirements between closely related, congeneric, lineages.

In our study, we found that a clear geographical separation of two lineages of *Leucocytozoon* occurs on Réunion, a small island of 2512 km² (Fig. 1). Through the use of ecological modelling, we showed that the environmental niches occupied by the two lineages are significantly different, with only a slight overlap (Table 3, Fig. 2). These results indicate that the two lineages occupy geographical areas with different environmental conditions. Such patterns of geographical and ecological segregation of the two lineages may be explained by different sets of processes.

Neutral processes such as dispersal limitation can prevent organisms from colonizing an area of suitable habitat in its entirety (Holt, 2003). Additionally, the history of colonization of Réunion by the two lineages may have shaped their distributions, with some parasites never reaching some parts of the island due to dispersal limitation (Lobo *et al.*, 2010). Under such a neutral process of dispersal limitation, the observed differences of environmental conditions between the two lineages' ranges would only be due to spatial autocorrelation. Although dispersal limitation seems unlikely on a small island such as Réunion, we carried out a niche background test, which allowed us to control for spatial autocorrelation and estimate whether differences in the niches occupied by the two parasite lineages are the product of (1) different environmental requirements (niche divergence; Graham *et al.*, 2004), or (2) other constraints, while environmental requirements are similar (niche conservatism; Pearman *et al.*, 2008). This test showed that the two lineages occur in parts of their backgrounds where environmental conditions are particularly different from the environmental niche of the other lineage, bringing evidence for niche divergence between the two lineages. Thus, dispersal limitation alone cannot account for the geographical and ecological separation of the two lineages.

The geographical separation of the two lineages could also be due to their infection of different avian hosts occurring in different areas (e.g. Bensch *et al.*, 2000; Ricklefs & Fallon, 2002; Fallon *et al.*, 2005; Ricklefs *et al.*, 2005; Križanauskienė *et al.*, 2006; Sehgal *et al.*, 2006; Hellgren *et al.*, 2007). The influence of non-*Zosterops* hosts is unlikely, because the two lineages appear to infect only *Zosterops* (Cornuault *et al.*, 2012). As for the *Zosterops* reservoir, *Z. olivaceus* primarily occurs in indigenous forests, and *Z. borbonicus* prefers disturbed and edge habitats (Gill, 1971). The difference between these two types of habitat would be best described by habitat
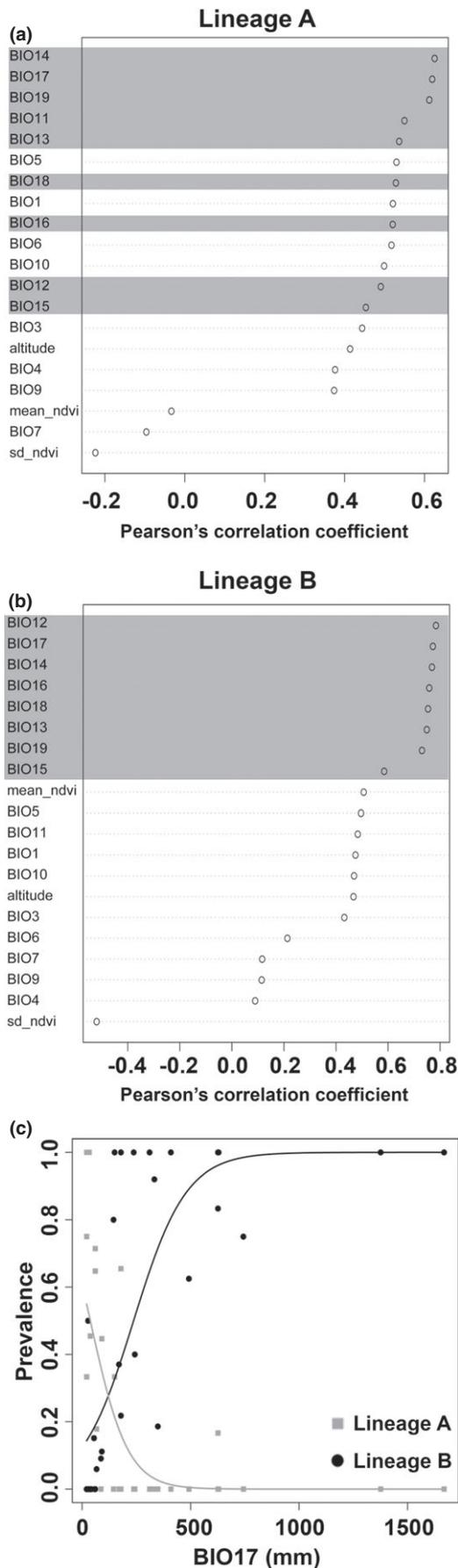
Figure 3 Estimation of variable importance in the species distribution models for *Leucocytozoon* lineages infecting *Zosterops borbonicus* on Réunion. (a) Lineage A, (b) Lineage B: Pearson's correlation coefficient between observed prevalence and prevalence predicted for omitted data for models with single variables. Precipitation variables (BIO12–BIO19) are shaded grey. (c) The relationship between the prevalence of the two lineages and BIO17 (precipitation of the driest quarter). Fitted curves were obtained with logistic regression.

fragmentation, although it should also be captured by the NDVI, which correlates with vegetation greenness. In any case, *Z. olivaceus* is seldom as abundant as the ubiquitous *Z. borbonicus* (Gill, 1971), and the latter species is likely to be of primary importance for the transmission of both parasite lineages. Moreover, the relative prevalence of the two lineages does not vary between their two *Zosterops* hosts (Cornuault *et al.*, 2012), and we consider it unlikely that *Zosterops* hosts have any effect on the geographical separation of parasites.

Alternatively, host sharing can induce the spatial and ecological segregation of parasites if competition for common resources leads to local competitive exclusion (Webb *et al.*, 2002). Under this scenario, however, we would not expect a correlation between parasite prevalence and environmental conditions, except if differential selection is at play and influences the outcome of competition. We found that among the environmental factors considered, precipitation is the best predictor of the spatial separation of *Leucocytozoon* parasites on Réunion. As precipitation per se cannot bear any direct selective impact on *Leucocytozoon* (because they are endosymbiotic parasites), a hypothesis of differential selection is not supported.

In contrast to the avian hosts, little is currently known about which vectors transmit lineages A and B. Giudicelli (2008) described four different species of potential vectors (*Simulium* spp.) on Réunion which are, interestingly, geographically separated, with two species restricted to lower elevations and two restricted to higher elevations. The precise distribution of these potential vectors along with their competence for transmitting the different parasite lineages are currently unknown, but it is possible that the two lineages are transmitted by distinct vector species and that the geographical separation of the vectors directly affects the parasites. Moreover, blackflies are known to be sensitive to variation in precipitation (Cilek & Schaediger, 2004; Oluwole *et al.*, 2009). Notably, precipitation limitation in the year's driest quarter certainly determines the minimal level of water in the streams where blackfly larvae develop, which may in turn affect the sustainability of blackfly populations. Therefore, the importance of precipitation limitation in predicting parasite prevalence could in fact reflect the impact of environment on the relative distributions of vector species, if some vector species differ in their climatic niche envelopes.

This study provides evidence of a clear geographical separation of two lineages of *Leucocytozoon* parasites at a very

small spatial scale during the erythrocytic (vertebrate-borne) phase. Through the use of species distribution models, we found a clear correlation between levels of precipitation and the relative prevalence of these lineages. We suggest that such a spatial pattern may reflect processes acting during the sporogonic (vector-borne) phase, because precipitation cannot act directly on parasites with no free-living stage. If so, the distribution of parasite lineages could reflect the ecological niches and distributions of vectors more closely than those of the hosts. Future work should test the effect of the distribution range and ecology of vectors on the distribution of parasites, while taking into consideration the immense diversity that can be contained within broadly defined parasitic taxa, such as hemosporidian genera. This seems important for predicting the risk of emergence of vector-transmitted diseases in novel areas or in regions where environmental conditions are altered by climate change.

ACKNOWLEDGEMENTS

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Details of species distribution models and associated statistical tests.
Appendix S2 Sample sizes and lineage prevalence per locality.

BIOSKETCH

Josselin Cornuault studies the processes underlying organismal biogeography and evolution, with a focus on host–parasite interactions and insular communities.

Author contributions: J.C., A.K., C.T. and P.H. conceived the ideas; B.M., J.C., C.T., Y.X.C.B and P.H. collected the data; J.C., A.K. and R.J.H analysed the data; and J.C. led the writing with significant contribution from all authors.

Editor: Lisa Manne