

ORIGINAL PAPER

PREDICTORS OF RED FOX (*VULPES VULPES*) HELMINTH PARASITE DIVERSITY IN THE PROVINCES OF SPAIN

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Keywords

Diversity indices, geographical trends, human influence, variation partitioning.

Abstract

We analysed the viscera of 321 red foxes collected over the last 30 years in 34 of the 47 provinces of peninsular Spain, and identified their helminth parasites. We measured parasite diversity in each sampled province using four diversity indices: Species richness, Margalef's species richness index, Shannon's species diversity index, and inverse Simpson's index. In order to find geographical, environmental, and/or human-related predictors of fox parasite diversity, we recorded 45 variables related to topography, climate, lithology, habitat heterogeneity, land use, spatial situation, human activity, sampling effort, and fox presence probability (obtained after environmental modelling of fox distribution). We then performed a stepwise linear regression of each diversity index on these variables, to find a minimal subset of statistically significant variables that account for the variation in each diversity index. We found that most parasite diversity indices increase with the mean distance to urban centres, or in other words, foxes in more rural provinces have a more diverse helminth fauna. Sampling effort and fox presence probability (probably related to fox density) also appeared as conditioning variables for some indices, as well as soil permeability (related with water availability). We then extrapolated the models to predict these fox parasite diversity indices in non-sampled provinces and have a view of their geographical trends.

Introduction

Understanding the factors that determine the diversity of parasite communities is an important problem in ecology, as parasites have a significant effect on the structure and stability of natural communities [1-3]. Hence, identifying the factors that govern parasite occurrence is critical for managing wild populations and mitigating risks to human and domestic animal health [4].

The red fox (*Vulpes vulpes* L., 1758) is the most widespread and abundant wild carnivore inhabiting Spain, as it occurs practically all over its continental territory. This canid is also broadly spread in the rest of the European continent, although the form that inhabits the Iberian Peninsula is considered an endemic subspecies, *V. v. silacea* Miller, 1907, whose northern distribution limit is the Pyrenean mountain range [5,6]. The red fox is an opportunistic carnivore with a broad food spectrum that includes small and medium-sized mammals and birds, invertebrates, fruits, carrion, and rubbish [6,7]. It is often associated with human population nuclei, as it finds abundant and easily available food supplies in rubbish dumps and livestock

carrion. It is also a game species, the main vector of rabies in Europe [8], and a reservoir host to numerous helminth species that are parasites of the domestic dog, the wolf and, in some cases, humans [9].

The endoparasitic helminthfauna of *V. vulpes* is reasonably well characterized throughout Europe [10-15]. Several studies have also addressed the helminthfauna of *V. vulpes* in several areas of the Iberian Peninsula [9,16-19]. More recently, an ecological analysis of the helminth fauna of the red fox throughout most of Spain, Andorra, and the Malcata Natural Park (Portugal) was published [20].

Our aims were to measure the diversity of red fox helminth parasites in a number of sampled provinces of Spain, using different diversity indices; to find geographical, environmental, and/or human-related predictors of this diversity; and to predict parasite diversity in all of the Spanish provinces and have an overview of its geographical trends.

Methods

The study area, peninsular Spain, covers approximately 493,000 km², nearly 85% of the Iberian Peninsula, in the southwestern end of Europe. It has heterogeneous climate and orography, and a patent peninsular character, as the isthmus that connects it with the rest of the European continent is relatively narrow (about two-fifths of its northern border) and is crossed by the Pyrenees, which complicate biotic and abiotic exchanges with the adjacent territories.

Peninsular Spain is divided into 47 administrative provinces with surface areas ranging from 2,000 to 22,000 km² approximately (mean area \approx 10,500 km²). The use of political territory divisions as a basis for biogeographical studies is sometimes criticized, but these provinces have been considered the best lattice for obtaining biotic regionalisations for ferns in Spain when compared to river basins, natural regions, physiographic and geological regions, and mountains and plains [21]. Besides, human activity patterns, whose influence on fox parasite diversity we also intended to analyze, often are shaped by political limits, and most of the variables that reflect them are only available on a political-unit basis. Political territorial units have been used successfully in a number of other biogeographical studies [22-24].

Official institutions provided the viscera of 321 red foxes collected over the last 30 years in 34 of the 47 provinces of peninsular Spain. We systematically checked all viscera of each individual for helminth parasites using standard helminthological methods. All helminths found were removed and processed according to the species, and identified on the basis of previous descriptions. All aspects concerning the methodology employed have been previously described in detail [20].

We calculated four indices of parasite infection diversity for each sampled province. Indices were calculated as described in the literature [25] using the number of infections (one infection = presence of one parasite species in one individual of red fox) instead of the number of individuals of each parasite species, in order to avoid over-representation of species with greater parasite intensity in the diversity indices.

The indices used were:

a) Species richness:

S = number of species;

b) Margalef's species richness index:

$$R = \frac{S - 1}{\ln(n)},$$

where S is the number of parasite species and n is the total number of infections;

c) Shannon's species diversity index:

$$H' = -\sum p_i \cdot \ln(p_i),$$

where p_i is the proportion of infections by species i in the total of infections;

d) Inverse Simpson's index:

$$\frac{1}{D} = \frac{1}{\sum(p_i)^2},$$

where p_i is the same as in Shannon's index.

In order to find out the factors that best predict the diversity of red fox helminth parasites in the Spanish provinces, we recorded a series of variables related with topography, climate, lithology, habitat heterogeneity, land use, spatial situation, and human activity. We also included sampling effort (in the form of number of foxes analysed) and fox presence probability (related with fox abundance and density; see Discussion for details), to find out if these factors produced an effect on the diversity of parasites observed. The 45 variables used and their sources are listed in Table 1.

Table 1. Variables used to model the diversity of red fox (*Vulpes vulpes*) helminth parasites in the administrative provinces of peninsular Spain.

Code	Variable
<i>Alti</i> *	Mean altitude (m) [26]
<i>AMax</i>	Maximum altitude (m)
<i>AMin</i>	Minimum altitude (m)
<i>AltR</i> *	Altitude range (m) (= <i>AMax-AMin</i>)
<i>Slop</i> *	Slope (degrees) (calculated from <i>Alti</i>)
<i>HJan</i> *	Mean relative air humidity in January at 07:00 hours (%) [27,28]
<i>HJul</i> *	Mean relative air humidity in July at 07:00 hours (%) [27,28]
<i>HRan</i> *	Annual relative air humidity range (%) (= <i>HJan-HJul</i>)
<i>PET</i> *	Mean annual potential evapotranspiration (mm) [27,28]
<i>AET</i> *	Mean annual actual evapotranspiration (mm) (= min (<i>PET</i> ; <i>Prec</i>))
<i>Inso</i> *	Mean annual insolation (hours/year) [27,28]
<i>SRad</i> *	Mean annual solar radiation (kwh/m ² /day) [27,28]
<i>TJan</i> *	Mean temperature in January (°C) [27,28]
<i>TJul</i> *	Mean temperature in July (°C) [27,28]
<i>Temp</i> *	Mean annual temperature (°C) [27,28]
<i>TRan</i> *	Annual temperature range (°C) (= <i>TJul-TJan</i>)
<i>DFro</i> *	Mean annual number of frost days (minimum temperature ≤ 0°C) [27,28]
<i>DPre</i> *	Mean annual number of days with precipitation ≥ 0,1 mm [27,28]
<i>Prec</i> *	Mean annual precipitation (mm) [27,28]
<i>MP24</i> *	Maximum precipitation in 24 hours (mm) [27,28]
<i>RMP</i> *	Relative maximum precipitation (= <i>MP24/Prec</i>)

Table 1 (Cont.)

Code	Variable
<i>Cont</i>	Continental index [28]
<i>Humi</i>	Humidity index [28]
<i>PIrr*</i>	Pluviometric irregularity [29]
<i>ROff*</i>	Mean annual run-off (mm) [30]
<i>Lati*</i>	Latitude (degrees N) [31]
<i>Long*</i>	Longitude (degrees E) [31]
<i>Perm*</i>	Soil permeability [30]
<i>DHi*</i>	Mean distance to the nearest highway (km) [31]
<i>U100*</i>	Mean distance to the nearest town with more than 100,000 inhabitants (km) [31,32]
<i>U500*</i>	Mean distance to the nearest town with more than 500,000 inhabitants (km) [31,32]
<i>DPyr</i>	Mean distance to the Pyrenees (km)
<i>NFlo</i>	Number of bioclimatic floors [33]
<i>NReg</i>	Number of fitogeographic regions [33]
<i>HPD</i>	Human population density (inhabitants/km ²) [34]
<i>Indu</i>	Industry density (factories/km ²) [35]
<i>HDen</i>	Highway density (m/km ²) [36]
<i>RDen</i>	Regular road density (m/km ²) [36]
<i>HRD</i>	Highway+road density (m/km ²) [36]
<i>VDen</i>	Vehicle density (vehicles/km ²) [37]
<i>Wood</i>	Woodland area (%) [36]
<i>Crop</i>	Cropland area (%) [38]
<i>Past</i>	Pasture area (%) [38]
<i>SEff</i>	Sampling effort (number of infected foxes analysed)
<i>FPP</i>	Fox presence probability

Isoline variables (*HJan* through *Long*) were digitized using CartaLinx 1.2, and isoline vertices were interpolated in Idrisi32 from a triangulated irregular network performing parabolic bridge and tunnel edge removal. Secondary variables, defined in Table 1 by an algebraic operation in parentheses, were calculated from primary variables using the Idrisi Image Calculator. *Perm* was obtained from a categorical map of synthesis of ground-water aquifers with three different degrees of soil permeability. We determined *Perm* by calculating the mean of the permeability values assigned to all pixels within each province. Distance variables (*DHi* through *DPyr*) were calculated using the Idrisi DISTANCE module. Density and percentage data (*HPD* through *Past*) were calculated dividing the absolute values obtained from the literature by the area of each province. Fox presence probability was calculated by logistic regression of presence/absence data (taken from [39]) on a set of environmental, spatial, and human-related variables, using UTM 10x10-km squares as operational geographic units. We extrapolated the logistic regression model to 1x1 km squares, and then calculated mean fox presence probability for each province. The methodology used to obtain and extrapolate fox presence probability has been described for the European otter [40].

We then performed a stepwise linear regression of each diversity index on the 45 variables, to find a minimal subset of statistically significant variables that account for the variation in fox parasite diversity. Interactions between factors often result in an overlaid effect in space, so the sum of the amounts of variation explained by each

variable included in a model is usually different from the total amount explained by the whole model [41,42]. Consequently, in the cases where more than one variable entered the regression equation, we performed a Partial Regression Analysis [42], a variance partitioning procedure designed to specify how much of the variation in diversity explained by each of the variables corresponds to its pure effect, and which proportions are attributable to interactions between variables.

Results

We found 34 helminth parasite species in the foxes analysed (Table 2). The diversity indices obtained for the 34 sampled provinces are represented in Figure 1.

Table 2: Helminth parasite species found in the red foxes analysed, and the number of provinces (N) in which each of them was recorded (total number of analysed provinces = 34).

Group and species	N
TREMATODA	
<i>Alaria alata</i>	4
<i>Brachylaima</i> sp.	3
<i>Metorchis bilis</i>	2
<i>Opistorchis felineus</i>	1
CESTODA	
<i>Taenia crassiceps</i>	6
<i>T. hydatigena</i>	3
<i>T. polyacantha</i>	12
<i>T. taeniaeformis</i>	1
<i>T. pisiformis</i>	8
<i>Joyeuxiella pasqualei</i>	6
<i>J. echinorhynchoides</i>	9
<i>Diplopylidium noelleri</i>	1
<i>Mesocestoides</i> sp.	24
NEMATODA	
<i>Aonchotheca putorii</i>	1
<i>Eucoleus aerophilus</i>	22
<i>Pearsonema plica</i>	14
<i>Trichuris vulpis</i>	7
<i>Dirofilaria immitis</i>	1
<i>Toxocara canis</i>	19
<i>T. cati</i>	1
<i>Toxascaris leonina</i>	21
<i>Strongyloides</i> sp.	1
<i>Uncinaria stenocephala</i>	23
<i>Vigisospirura potekhiniae</i>	1
<i>Angiostrongylus vasorum</i>	13
<i>Crenosoma vulpis</i>	16
<i>Pterygodermatites affinis</i>	23
<i>Molineus patens</i>	3
<i>M. legerae</i>	3
<i>Physaloptera sibirica</i>	8
<i>Cyathospirura</i> sp.	1
<i>Spirocerca lupi</i>	7
<i>Mastophorus muris</i>	2
ACANTHOCEPHALA	
<i>Macracanthorhynchus catulinus</i>	2

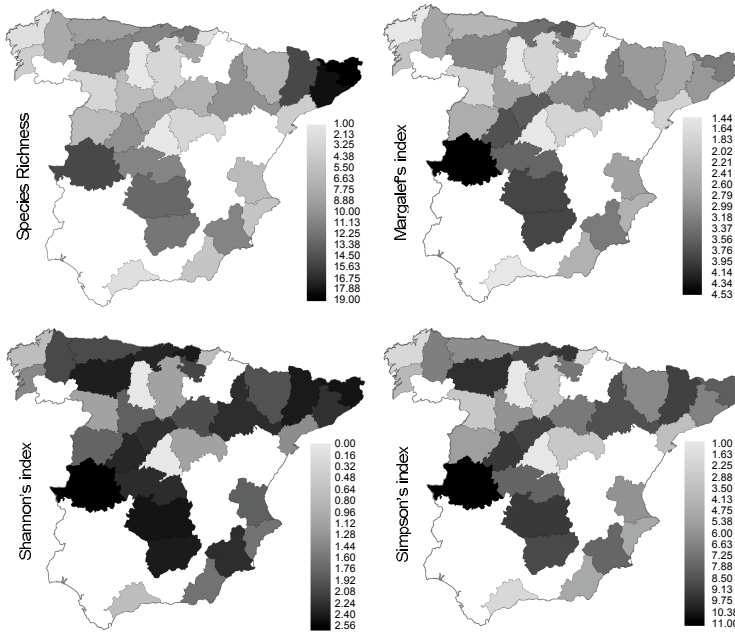


Fig. 1. Diversity indices obtained for the helminth parasites of red fox (*Vulpes vulpes*) in the administrative provinces of peninsular Spain. Formulas are shown in the text. Only the sampled provinces are displayed.

The stepwise logistic regression of fox presence/absence data [39] on the variables marked in Table 1 yielded the following model:

$$FPP = 1 - (1 / (1 + \text{EXP} (2.29 - 0.00060 \text{ Alti} + 0.010 \text{ Area} + 0.0012 \text{ AltR} + 0.0057 \text{ DHi} + 0.24 \text{ Long} + 0.41 \text{ Perm} - 0.12 \text{ TJul} - 0.010 \text{ U100} + 0.0019 \text{ U500} + 0.035 \text{ HRan})))$$

Fox presence probability on 10x10-km squares is shown in Figure 2.

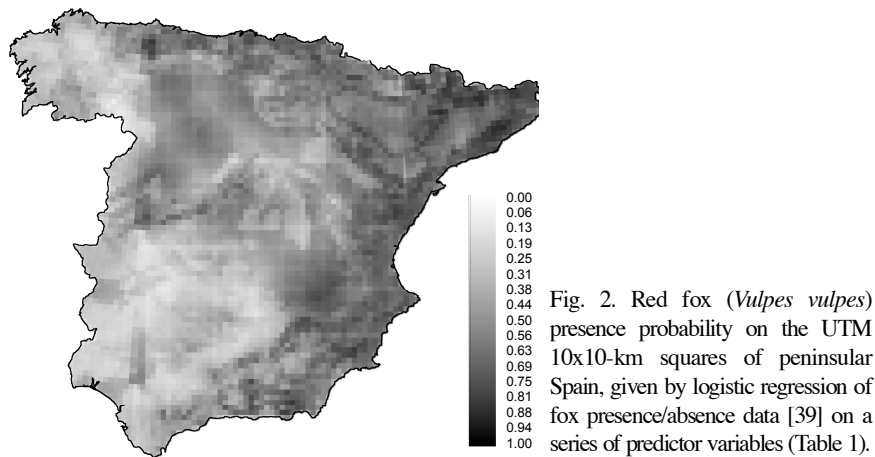


Fig. 2. Red fox (*Vulpes vulpes*) presence probability on the UTM 10x10-km squares of peninsular Spain, given by logistic regression of fox presence/absence data [39] on a series of predictor variables (Table 1).

The stepwise linear regressions of the parasite diversity indices on the variables listed in Table 1 yielded the following equations:

$$\text{Species richness} = 2.43 + 0.14 SEff + 0.078 U100$$

$$R^2 = 0.548; p < 0,001$$

$$\text{Margalef's index} = 1.91 + 0.017 U100$$

$$R^2 = 0.234; p = 0.005$$

$$\text{Shannon's index} = -1.001 + 4.33 FPP - 0.56 Perm$$

$$R^2 = 0.281; p = 0.006$$

$$\text{Simpson's index} = 3.46 + 0.049 U100$$

$$R^2 = 0.170; p = 0.016$$

The predicted values for each diversity index, according to the regression models obtained, for all peninsular Spanish provinces are shown in Figure 3.

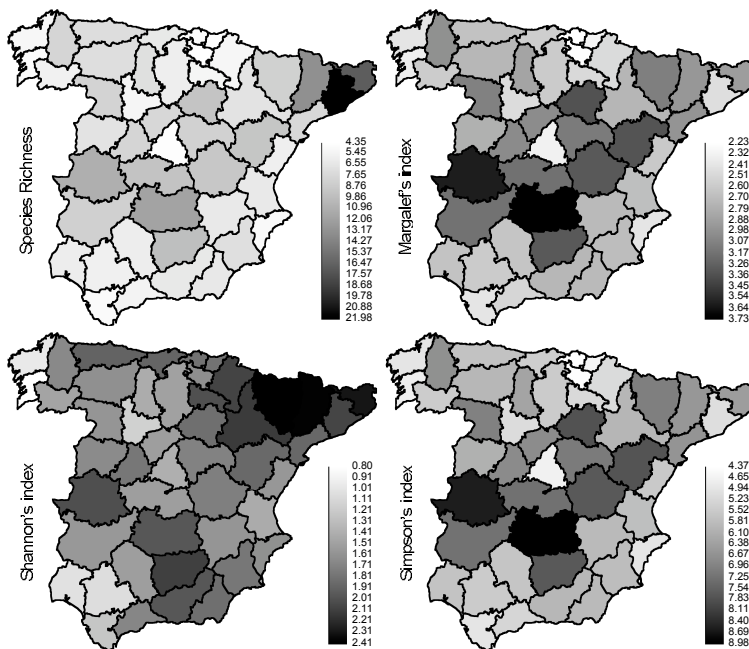


Fig. 3. Predicted values for each fox parasite diversity index in all administrative provinces of peninsular Spain.

The results of the variance partitioning for species richness and for Shannon's diversity index are summarized in Figure 4. Over 40% of the variation in species richness (S) was explained solely by sampling effort, whereas ca. 14% was attributed to the distance to urban centres. The total amount of variation explained by the model (R^2), $\approx 55\%$, is lower than the sum of the amounts of variation

explained exclusively by each of the two variables and, consequently, the shared effect of both variables on species richness is negative. For Shannon's diversity index (H'), 20% of the variation is due exclusively to fox presence probability, and 13% to soil permeability, with a negative shared effect.

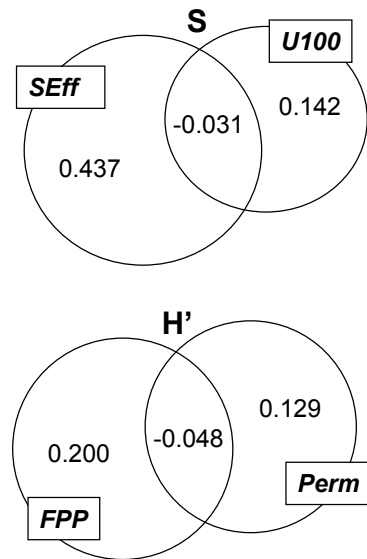


Fig. 4. Results of the variance partitioning procedure for species richness (S) and for Shannon's diversity index (H') of fox helminth parasites. Values in the diagrams are the proportions of the variation attributable to the pure effect of each of the variables included in the stepwise regression model, and to the interaction between their effects. Variable codes as in Table 1.

Discussion

The predicted geographical variations in Margalef's and Simpson's indices are remarkably similar, as the distance to urban centres was the only variable selected for both models. Species richness is predicted by the same variable but also by sampling effort, so there are common traits between the geographical trends observed, but a clear effect of the differences in sampling effort is noticed. In contrast, Shannon's index is predicted by two other variables and thus exhibits different geographical trends.

Parasite species richness has been previously related with factors such as host density, host diet, and habitat diversity, among others (e.g. [4,43,44]). It is also strongly conditioned by sampling effort [45-47]. In a study of parasite diversity of Iberian rodents, species richness was found to correlate only with host sample size [48]. In our study, no diversity measure was sufficiently explained by sampling effort. Although sampling effort does significantly affect the total number of parasite species found, the mean distance to urban centres was also selected as a significant variable. This is particularly important, since sampling effort is negatively correlated with the distance to urban centres (Pearson's correlation, $r=-0.07$), which is why the variation in S explained simultaneously by *SEff* and *U100* is negative (see Fig. 4).

From a mathematical point of view, when some of the relations among multiple variables are negative, the magnitude of co-variation may be limited by the positive semi-definite (PSD) criterion, which is a property of all correlation matrices that constrains all *eigenvalues* and the determinant of each principal minor in a correlation matrix to values greater than or equal to zero [49]. In a system with three variables where two of the correlations are positive and one is negative, as it is the case here, an implication of the PSD criterion is that the shared effect of two of the variables on the other one is negative (e.g. [24,50]). In other words, sampling effort and distance to urban centres cause fox parasite diversity to vary in divergent directions. The pure effect of *U100* on *S* (Fig. 4), which can be obtained by using the residuals of the regression of parasite species richness on the number of foxes analysed as the target variable [41,42,51], is then higher than the effect attributable to *U100* regardless of the sampling effort. The importance of the distance to urban centres is also highlighted by the fact that it is the only variable which significantly explains both Margalef's and Simpson's indices. A higher mean distance to urban centres characterizes predominantly rural provinces. The fact that fox parasite diversity is higher in rural environments can be partially attributed to a possible higher diversity of intermediate and paratenic host species that can transmit more parasite species to foxes [52]. On the other hand, in the vicinity of urban centres, foxes feed largely on rubbish and less on potential intermediate or paratenic hosts [53], thus decreasing transmission of parasite species by prey.

Shannon's index, however, is best predicted by fox presence probability, which can be roughly related to fox abundance or density [54], and by soil permeability. Host density is often considered a key factor affecting parasite species richness [3,4,43], due to its positive effect on the transmission rates of monoxenous parasites. However, its effect can be masked by the existence of heteroxenous species among the parasite community under study, since their diversity is affected not only by the density of the final host but also by those of the intermediate ones [3]. Soil permeability has a negative effect on Shannon's diversity index ($r=-0.27$), even when it correlates positively with fox presence probability ($r=0.179$), which in turn affects Shannon's diversity index positively. Soil permeability is negatively related with superficial freshwater availability (more permeable substrates retain less water), which may be an important conditioning factor for many of these parasites or their intermediate hosts, thus conditioning parasite diversity as well. Significant differences have been detected among the fox parasite communities of two different types of habitat within the Ebro valley (E Spain), as some helminth species seemed to prefer irrigated lands, while others selected semiarid ones [6].

A further development of this work will be to search for differences in the predictors and geographical trends of the diversity of parasites with different types of life cycle, separating monoxenous species from those that use different groups of intermediate hosts (e.g. vertebrates vs. invertebrates).

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