

Can landscape metrics help determine the *Culicoides imicola* distribution in Italy?

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Abstract. *Culicoides imicola* is considered to be one of the main vectors of bluetongue disease in the Mediterranean Basin. However, local variations occur. For example, in Italy, *C. imicola* is a stable and abundant population in Sardinia and is widely distributed across the island, whilst in Tuscany on the Italian mainland, it ranges from low abundance in the west and coastal areas to absence in the eastern part of the region. Entomological surveillance data collected over 10 years were used to classify 52 sites as low to medium or high *C. imicola* abundance in Sardinia, and 59 sites as either positive or negative in Tuscany. The land cover was mapped from high-resolution remote sensing images using an object-based image analysis approach and a set of landscape metrics with 500 m buffers around each site. Multivariate analysis was used to test the statistical association of landscape metrics to *C. imicola* presence and abundance together with other eco-climatic and topographic variables. In Sardinia, 75% of the sites were correctly classified based on altitude alone and the inclusion of landscape-related variables did not improve the classification. In Tuscany, the mean annual temperature allowed classifying 70% of the positive/negative sites correctly. When landscape metrics was included in the multivariate model, an improvement up to 80% was obtained. The presence of riparian vegetation and water was found to be positively correlated with *C. imicola* presence, whilst forest (including the edge between the forest and cultivated areas) was found to be negatively related to the presence of *C. imicola*.

Keywords: landscape metrics, remote sensing, *Culicoides imicola*, bluetongue, Italy.

Introduction

Over the past 10 years, bluetongue (BT) virus has spread throughout southern and northern Europe and several species of midges of the genus *Culicoides* are considered to be responsible. Understanding environmental conditions that are favourable for the presence of the vector is an important step in the definition of areas at risk of BT spread and persistence (Mellor and Leake, 2000). In the past decade, most research aimed at identifying those factors has focused on environmental correlates of *C. imicola* and on the presence and degree of abundance of the *Obsoletus* complex, in areas where these species are considered to be the principal vectors of the BT virus. Many studies in the

Mediterranean Basin have previously used statistical modelling to predict the presence and abundance of BT vectors using variables gathered at the ground level (Wittmann et al., 2001; Calistri et al., 2003; Conte et al., 2003) or remotely sensed measurements. Among the factors that helped predict the BT vectors there were several low to medium resolution remotely sensed variables, such as land surface temperature (LST), the normalized difference vegetation index (NDVI) and the middle infra-red reflectance (MIR) (Baylis et al., 2001; Tatem et al., 2003; Purse et al., 2004; Calvete et al., 2008; Acevedo et al., 2010).

A large proportion of the variability in vector abundance has been correctly predicted by previous models but certain degrees of variability remained unexplained. There were also a number of areas where models based on eco-climatic variables failed to predict the species distribution accurately. Pili et al. (2006), for example, found several discrepancies when they compared the predicted distribution and abundance of *C. imicola* and other vectors obtained by such models with field data from southern Sardinia,

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Italy. Temperature and humidity are certainly important factors in determining the presence of the vector on a broad scale, but they are not necessarily sufficient to provide accurate predictive maps on a finer geographic scale. The spatial range of *Culicoides* caught in light traps is relatively small (Rigot and Gilbert, 2012) and many important processes operating on this scale cannot be predicted by eco-climatic variables measured by remote sensing at low to medium resolution (0.25-1 km).

Remotely sensed data providing high spatial resolution (pixel sizes ranging from 0.6 m to 30 m) facilitates the description of the environment and identify landscape characteristics that may be associated with high populations of vectors, and hence influence disease spatial dynamics. This type of analysis deals with the mosaic structure of the landscape (Forman and Gordon, 1986; Kitron, 1998) and focuses on the effect of dynamics of spatial heterogeneity on biotic processes (Turner, 2005). The parameters considered to describe the environment can be summarised as follows:

- (i) patch (an area that differs in some way from the surrounding landscape);
- (ii) composition (the amount of area covered and the number of different patches);
- (iii) configuration (the shape of the patch and the spatial arrangement of the elements);
- (iv) connectivity (the continuity of an habitat spanning the landscape); and
- (v) fragmentation (the break-up of the landscape into patches or spots).

The use of landscape composition and configuration metrics to study disease dynamics is relatively recent (Ostfeld et al., 2005) and offers prospects to improve our understanding of several eco-epidemiological processes (Li and Wu, 2004). Landscape analysis has been applied to investigate the relationship between the environment and vectors for different diseases (Estrada-Peña, 2002, 2009; Linard et al., 2007; Ducheyne et al., 2009; Estrada-Peña et al., 2010). In the particular case of BT, very few studies have focussed on the effect of landscape factors. Guis et al. (2007) studied the statistical association between landscape metrics and BT-free and BT-infected farms in southern Corsica, with three sizes of buffer around the farms (500 m, 1 km and 2 km radius). The results revealed an association between BT-infection status and some landscape metrics, more specifically those corresponding to woodlands and open prairies. In another study conducted by Durand et al. (2010), edge densities between pastures and forests, and between forests and arable lands, were identified as risk factors

for seropositivity to BT in cattle in France. Boyer et al. (2010) used climate and environmental data to estimate the risk of testing positive for antibodies to the BT virus and epizootic haemorrhagic disease viruses in cattle in Illinois and western Indiana in the United States of America over three seasons (from 2000 to 2002). They found that the risks of BT virus and epizootic haemorrhagic disease viruses seropositivity were positively associated with temperature during every year of the study, and that seropositivity was positively associated with forest patchiness in two of those years. No significant association was found between BT virus seropositivity and forest patchiness. Purse et al. (2012) tested different models to investigate the influence of landscape, host and remotely sensed climate factors on species and groups of *Culicoides* in Scotland. The best models were those based on climatic variables, followed by models developed using landscape predictors (proportions of land cover).

The objective of this study was to determine whether landscape metrics could help explaining the variability in *C. imicola* distribution. We aimed to investigate if landscape metrics, together with other eco-climatic variables, could be significant descriptors for the explanation and prediction of the *C. imicola* distribution in Sardinia and Tuscany. These two Italian regions show different patterns of abundance and distribution of this vector: Sardinia is considered "endemic" for *C. imicola*, with a probability of 80% of detecting the species when a single insect collection is performed (Goffredo et al., 2003, 2004; Conte et al., 2009). In Tuscany, in contrast, the highest abundance of *C. imicola* is observed along the western coast with decreasing abundance in the East.

Materials and methods

Study regions and entomological data

An extensive dataset on *C. imicola* in Sardinia and Tuscany, covering 10 years of entomological surveillance activity (2000-2009), was analysed for presence/absence and abundance of *C. imicola*. The *Culicoides* collections were performed according to standardised surveillance procedures (Goffredo and Meiswinkel, 2004) in geo-referenced farms. To avoid potential, false-negative results, only sites with at least three collections made between May and November (the most favourable period for *C. imicola*), covered by satellite imagery, were selected for the analyses. The most abundant collection across the survey period in each site was considered to be representative for abundance.

In Sardinia, 7,217 catches were used to classify 52 sites of *C. imicola* abundance as low to medium (<1,000 specimens) or high ($\geq 1,000$ specimens). In Tuscany, where *C. imicola* was less abundant, 5,967 catches were used to classify 59 sites for *C. imicola* presence or absence.

A circular buffer of 500 m radius was created around the selected farms and the values of all predictors were referred to this geographical unit. The 500 m radius buffer was chosen as being representative of the activity range of *C. imicola*. In the literature, the flight range of *C. imicola* is considered to vary from a few hundred metres to a few kilometres, but its active dispersal depends on the availability of blood meals and breeding sites. Usually, *C. imicola* is strictly linked to livestock with breeding sites at the farm level (Braverman et al., 1974; Meiswinkel et al., 2004).

Satellite imagery and land-cover classification

With sampling sites distributed throughout Sardinia and Tuscany and considering that we initially aimed to classify the study area at the regional level, high-resolution images were preferred to very-high resolution images as being more appropriate and less costly to cover. A set of SPOT-5 (<https://directory.eoportal.org/web/eoportal/satellite-missions/s/spot-5>) XS images acquired during the spring was used. These images have a spatial resolution of 10 m in the very near infrared bands and 20 m in the short wave infrared (SWIR) band. They were obtained with the "Optimising Access to Spot Infrastructure for Science" (OASIS) programme (http://www.eoportal.org/directory/info_OASISOptimisingAccessToSpotInfrastructureForScience.html) financed by the European Commission. A set of images from the ASTER sensor (<http://www.satimagingcorp.com/satellite-sensors/aster.html>), also acquired in the spring season and covering as much as possible of the same areas, was used as a complement to enable greater discrimination of the different vegetation types. ASTER has 14 spectral bands but a slightly coarser spatial resolution than SPOT-5, i.e. 15 m in the very near infrared (VNIR) bands, 30 m in SWIR bands and 90 m in the thermal infrared (TIR) bands. The SPOT-5 and ASTER images were processed using atmospheric correction 2 (ATCOR2) (<http://www.geosystems.pl/upload/zalaczniki/ATCOR%20Product%20Description.pdf>), orthorectified, mosaicked and clipped to their matching extent. Furthermore, a MODIS 250 m, 16-day, NDVI product time series (MOD13Q1, version 5, NDVI) was used to help discriminate classes with a

specific vegetation dynamics pattern. These data are distributed by the Land Processes Distributed Active Archive Center (LP DAAC), located at the United States Geological Survey (USGS), Earth Resources Observation and Science (EROS) Center (Warehouse Inventory Search Tool). A 10-class legend (Table 1) was designed, taking into account the resolution of the imagery, observations made during field visits and overall compliance with existing classification schemes, such as the CORINE land cover project (EEA Technical Report, 2007).

A semi-automatic classification method using data from the three different sensors was designed and applied. Object-based image analysis was selected due to the high heterogeneity of the vegetation classes. The delimitation of the image objects was performed using the multi-resolution segmentation algorithm embedded in the eCognition® software. Further details on the methods are provided in Vanhuysse et al. (2010). Moreover, a visual interpretation of the landscape around the selected sampling sites was then made to reduce the classification errors. This interpretation was based on the set of SPOT-5 XS images and supported by knowledge of the field and data collected during a 3-week field survey.

Landscape metrics

The 10 classes initially defined were subsequently grouped according to *C. imicola* habitat suitability into five main classes, as follows:

- (i) urban areas (UA);
- (ii) vegetated areas (VA);
- (iii) cultivated areas (CA);
- (iv) coniferous forested areas (FA); and
- (v) riparian vegetation-water body areas (RWA) (Table 1).

Two main landscape metrics were included in the analysis, namely:

- (i) the surface of each land cover class in the 500 m radius buffer around farms, i.e. UA; VA; CA; FA; and RWA.
- (ii) the length of the edge between the cultivated land and each other class in the buffer, i.e. cultivated/vegetated areas (EDCV); cultivated/coniferous areas (EDCF); cultivated/riparian-water areas (EDCRW); and cultivated/urban areas (EDCU).

CA were chosen as a reference class in edge metrics for two main reasons: (i) it is where animals breed; and (ii) it was the predominant class in the buffers in the two regions (it covers 71.3% of buffers area in Sardinia and 70.2% in Tuscany).

Table 1. Land-cover classes derived from high resolution images classification and class group definition used in the analysis.

Class name	Description	Class group
Artificial surfaces and open spaces with little or no vegetation	Urban fabric, industrial and commercial units, quarries, beaches, bare rock, scattered high-altitude vegetation	Urban areas (UA)
Arable land and pastures	Cultivated areas and pastures (used for grazing or harvesting); includes bare fields	Cultivated areas (CA)
Natural grasslands	Natural low-productivity grasslands	Vegetated areas (VA)
Irrigated permanent crops and complex cultivation patterns	Vineyards, other permanent crops and juxtaposition of small parcels of diverse annual crops, pasture and/or permanent crops that are permanently or periodically irrigated	Cultivated areas (CA)
Non-irrigated permanent crops and complex cultivation patterns	Olive groves, other permanent crops and juxtaposition of small parcels of diverse annual crops, pastures and/or permanent crops that are not irrigated	Cultivated areas (CA)
High maquis and broad-leaf forests	Dense vegetation association composed mainly of sclerophyllous vegetation and/or broad-leaf trees	Vegetation areas (VA)
Low maquis and garrigue	Bushy sclerophyllous vegetation association, discontinuous sclerophyllous vegetation associations	Vegetation areas (VA)
Coniferous forests	Vegetation formation composed principally of trees, where coniferous species predominate	Coniferous forested areas (FA)
Riparian vegetation	Hydrophilic plant associations at the land/stream interface	Riparian and water areas (RWA)
Water bodies	Inland and marine waters	Riparian and water areas (RWA)

Environmental and climatic variables

The NASA MODIS Land Surface Temperature/Emissivity (LST), 8-day Global 1 km resolution for nine complete years (2001-2009) and the NASA MODIS EVI, 16-day L3 Global 250 m resolution (Warehouse Inventory Search Tool) were used to extract the predictor variables. Both datasets were subject to temporal Fourier analysis to estimate the mean value, the annual, bi-annual amplitudes and phases of the time series (Scharlemann et al., 2008).

The mean and standard deviation of elevation and slope were also derived from the Digital Elevation Model 20 m spatial resolution of Italian territory. The mean values of LST and EVI amplitudes and phases, together with the statistics for elevation and slope, were calculated in the 500 m radius buffer around each site and included in the analysis.

Statistical analyses

Univariate and multivariate analyses were used to test if landscape metrics and the other eco-climatic and topographic variables were statistically related to *C. imicola* abundance in Sardinia or its presence/absence in Tuscany. The first step was to

identify the model that best explained the relationship between *C. imicola* abundance and environmental-climatic variables. Secondly, the addition of landscape metrics was tested to assess how they could complement other variables to improve the model.

Considering the high correlation among predictors, univariate, discriminant analysis was used as a first step. Principal component analysis (PCA) was used to identify redundancies between landscape metrics. Finally, discriminant analysis was used to identify the set of landscape metrics together with the best environmental variables, associated with the low-medium and high *C. imicola* populations in Sardinia and its presence or absence in Tuscany. SPSS Statistics 17.0 software was used for the statistical analysis.

Results

Land-cover classification

All classes presented in Table 1 were discriminated by object-based image analysis (OBIA). An accuracy assessment using 290 validation points showed a *k*-index of agreement (KIA) of 82%. The best classification results were obtained for “water bodies”, “artificial surfaces and open spaces with little or no vege-

tation”, “coniferous forest” and “riparian vegetation”. The main misclassifications occurred between the “high maquis and broad-leaved forest” and the “low maquis and garrigue”. The OBIA results were not only assessed through quantitative indicators, they were also superimposed on true-colour composites of very-high resolution images from Google Earth™ and visually checked, particularly in the buffers around the entomological sites.

Considering that misclassifications could have a negative impact on subsequent analyses, a visual interpretation of the SPOT-5 images from the buffers was performed to improve the results. It was supported by

knowledge of the field and data collected during a 3-week field survey. The difference between the OBIA classification and the improved classification, together with examples of the final results, are given in Figs. 1 and 2.

Relationship between variables/metrics and entomological data

In Sardinia, 24 sites of low to medium abundance (<1,000 specimens) and 28 as areas of high abundance (≥1,000 specimens) were identified (Fig. 3). The best discriminant model based on environmental variables

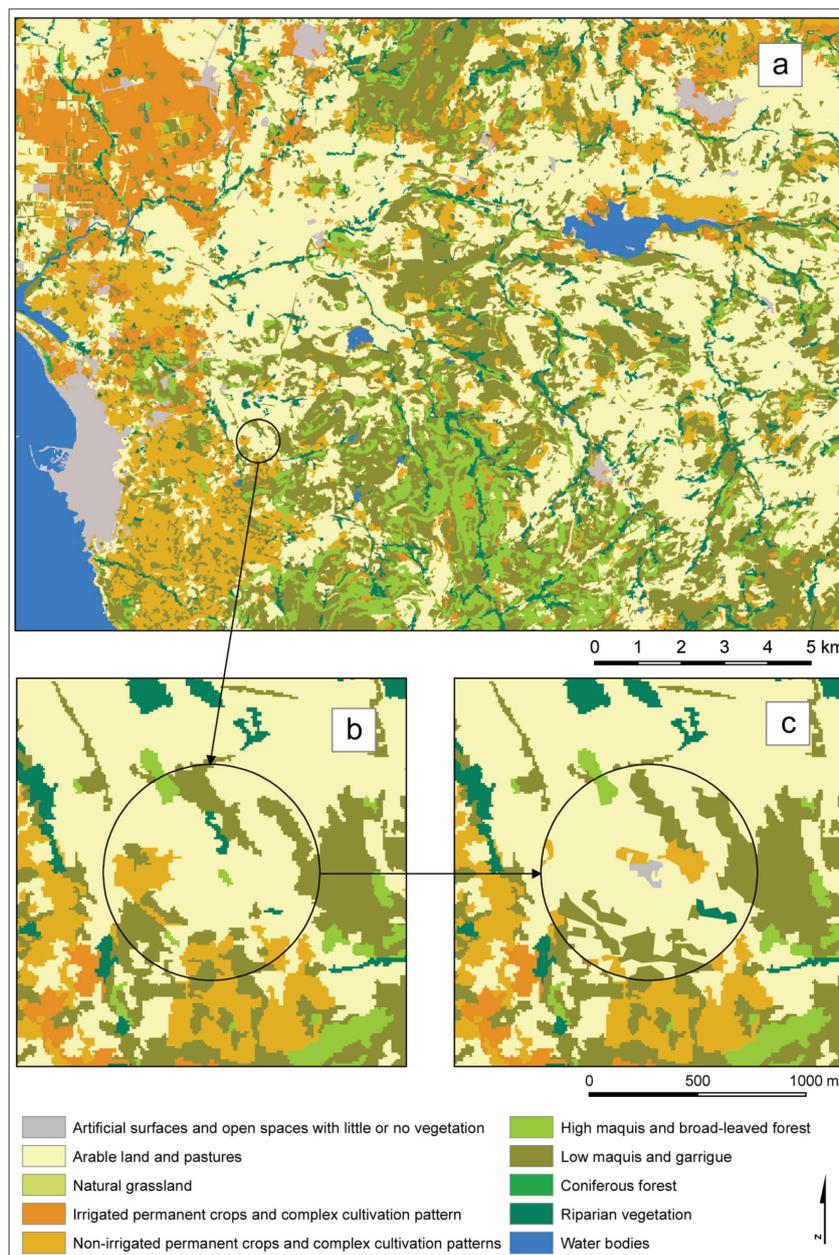


Fig. 1. Example of land-use classification resulting from object-based image analysis (a and b) and further improvements (c).

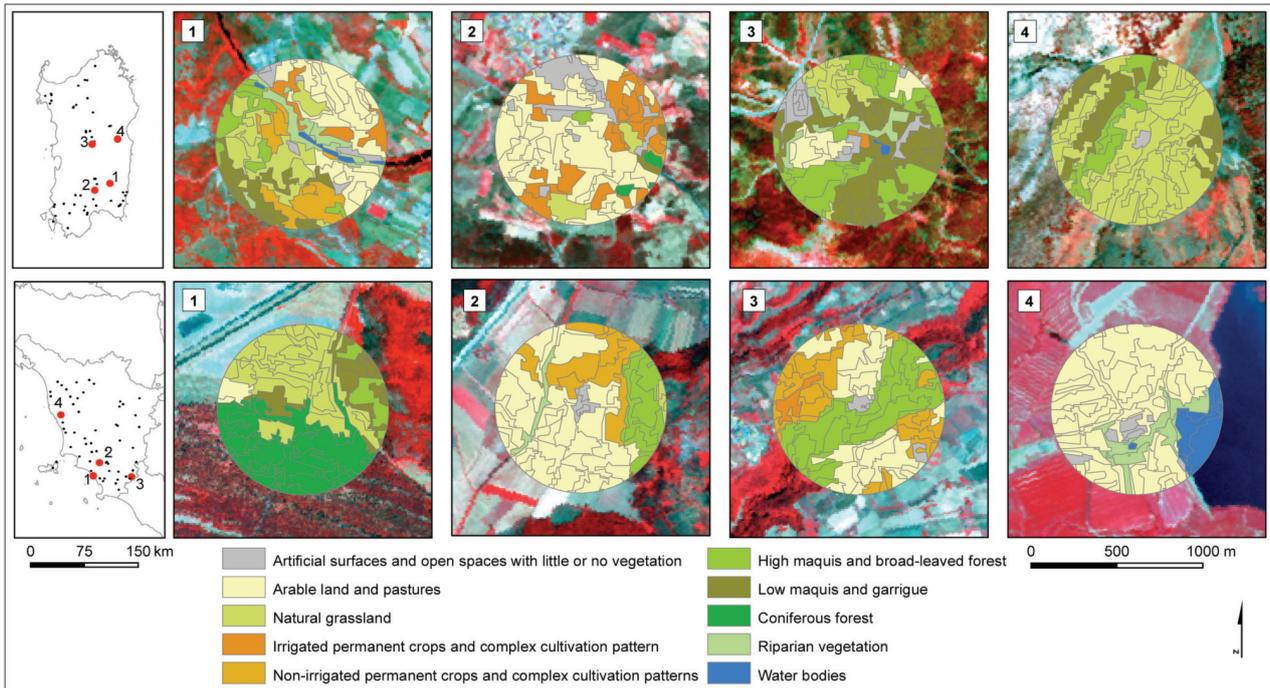


Fig. 2. Examples of entomological sites in Sardinia and Tuscany and 500 m-radius buffers land-use classification.

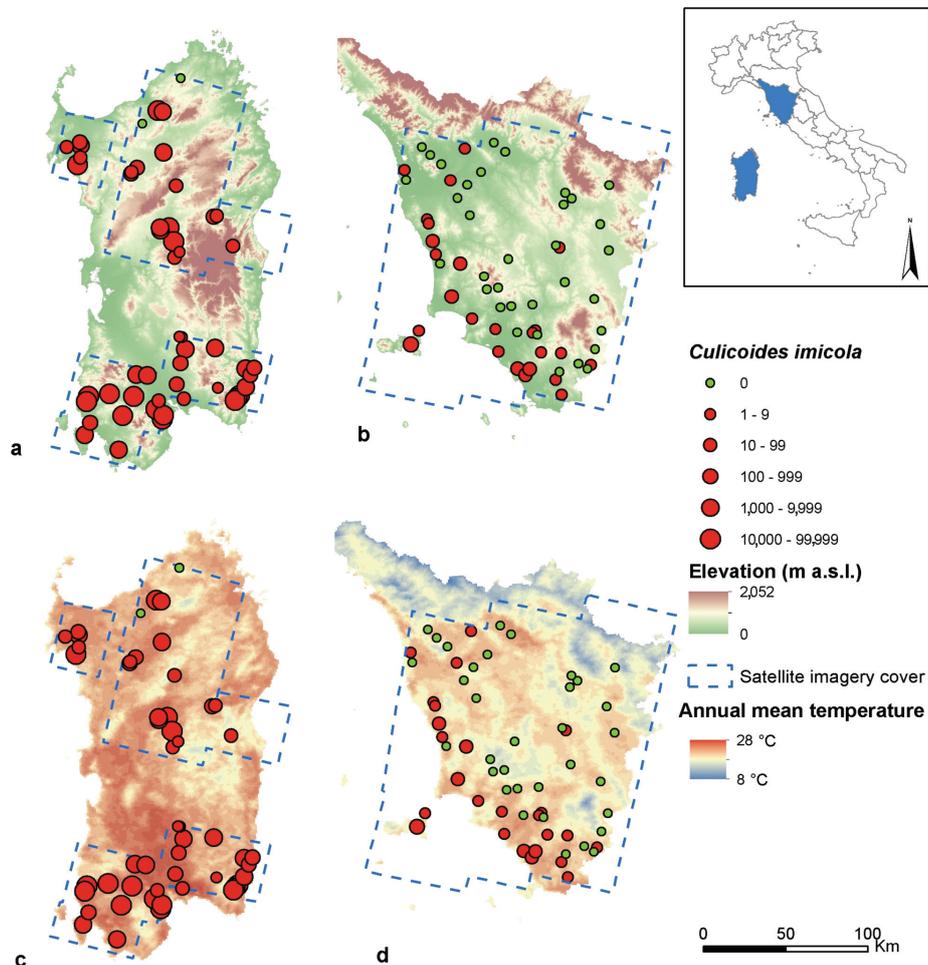


Fig. 3. Trap sites in the area covered by satellite imagery and *C. imicola* abundance in Sardinia (left) and Tuscany (right).

Table 2. Contingency table for the classification of *C. imicola* abundance in Sardinia using elevation as predictor.

		Predicted		Total
		Low/medium	High	
Observed	Low/medium	13	11	24
	High	2	26	28
Total		15	37	52

included elevation above the mean sea level (MSL) as the only predictor, which enabled the correct classification of 75% of the sites (Table 2). The factor analysis of the landscape metrics identified four factors (Table 3), but when these factors were included with elevation in a multivariate discriminant analysis, a decline of 69.2% in the overall correct classification was noted (six sites observed as highly abundant for *C. imicola* were classified as having low to medium abundance, while 10 observed low to medium sites were reported as highly abundant).

In Tuscany, 59 sites (34 negative and 25 positive) were used in the analysis (Fig. 3). The maximum number of *C. imicola* specimens found in a night collection totalled 271. The environmental variables model that best discriminated between the two groups was the one with “mean annual temperature” as the predictor, correctly classifying 69.5% of the sites (Table 4a). Factor analysis on the landscape metrics identified three factors (Table 5) and when included with the “mean annual temperature” in a multivariate discriminant analysis, the proportion of correct classification increased to 79.7% (Table 4b). The standardised coefficient of the discriminant function and the structure matrix gave the highest discriminating power to the “annual mean temperature” (0.780), followed by “riparian vegetation and water bodies factor” (0.436)

Table 4. Contingency table for *C. imicola* classification in the region of Tuscany using annual mean temperature only (a) and annual mean temperature plus landscape factors as predictors (b).

		Predicted		Total
		Absence	Presence	
Observed	Absence	23 a	11 a	34
		27 b	7 b	
	Presence	7 a	18 a	25
		5 b	20 b	
Total		30 a	29 a	59
		32 b	27 b	

Table 3. Landscape factors in the region of Sardinia identified by factor analysis - varimax rotated matrix.

	Factors			
	1	2	3	4
CA	-0.968	0.006	-0.158	-0.048
VA	0.952	-0.042	-0.096	-0.141
EDCV	0.566	-0.167	-0.271	-0.012
EDCF	-0.077	0.994	-0.011	0.017
FA	-0.089	0.992	0.019	-0.003
EDCU	-0.254	0.035	0.902	-0.104
UA	0.094	-0.034	0.953	0.006
EDCRW	-0.192	0.019	-0.108	0.883
RWA	0.098	-0.005	0.020	0.920

and “coniferous forest” (-0.237). In particular, the “riparian vegetation and water bodies” factor contributed positively, indicating the preference of *C. imicola* for this type of landscape; the presence of forest is a factor negatively associated with the presence of *C. imicola*.

Discussion

Our results highlight that the inclusion of landscape metrics can only marginally help in explaining and predicting the *C. imicola* distribution in Italy. Part of the variability both in Sardinia and Tuscany remains unexplained. Our findings should be seen in light of the general distribution of the species and habitat preferences.

C. imicola is widely distributed across the world, ranging from South Africa to the Mediterranean and from the Iberian Peninsula to the Middle and Far East, including southern People’s Republic of China, Lao PDR and Vietnam (Meiswinkel et al., 2004). Within this range, however, the distribution is not

Table 5. Landscape factors in the region of Tuscany identified by factor analysis - varimax rotated matrix.

	Factors		
	1	2	3
EDCU	0.905	-0.107	0.183
UA	0.690	-0.176	0.428
VA	-0.797	-0.431	0.254
CA	0.728	0.321	-0.384
EDCRW	0.029	0.902	-0.118
RWA	-0.008	0.905	-0.073
EDCV	-0.483	-0.554	-0.103
EDCF	0.120	-0.012	0.822
FA	-0.077	-0.082	0.863

continuous but appears in patches with areas of low abundance (Venter and Meiswinkel, 1994) or even with zones completely free of *C. imicola*. These zones are probably due to local presence of sandy textures with fast drainage leading to poor nutrient status of the soil (Meiswinkel, 1997; Conte et al., 2007b). The immature stages of *C. imicola* require a semi-moist soil enriched with organic matter but, as the pupae are not able to float on the water surface, such areas cannot be waterlogged. These conditions restrict the species to flat and slow-draining regions with soils of the clay type. Furthermore, *C. imicola* breeds in areas where sunny surfaces prevail together with low vegetation; they avoid areas covered by forest and thus differ in this aspect from other European vector species, for example those belonging to the *Obsoletus* complex (Meiswinkel et al., 2004; Conte et al., 2007a).

In Italy, in very similar areas with comparable and favourable conditions in terms of temperature and other eco-climatic factors, the abundance of *C. imicola* ranges from very high to almost absence (e.g. in the eastern and western coasts of the region of Calabria, where the soil type is the discriminating factor as previously discussed by Calistri et al. (2003) and Conte et al. (2007b)). However, the regions of Sardinia and Tuscany present a very different pattern in terms of distribution and abundance of this vector. In Sardinia the maximum number of *C. imicola* ever

recorded in 10 years of entomological surveillance was as high as 64,145, while it reached only 302 in Tuscany. The difference is also observed in terms of spatial distribution of positive catches: it is evenly spread in Sardinia but, in Tuscany, concentrated along the coast. These differences in abundance and distribution between the two regions appear not to be explained by topography (Figs. 3a and 3b), but rather by the climatic conditions (Figs. 3c and 3d). As shown in Fig. 4, and comparing Figs. 3c and 3d, the differences between the annual mean temperature in Sardinia and Tuscany seem relevant. On average, there is a 4 °C difference over the year, which peaks at approximately 7 °C in early summer. However, temperature alone can not explain all of the difference. For example, a longitudinal population model accounting for temperature and trained with data from Sardinia failed to accurately predict *C. imicola* population dynamics when applied to Tuscany (Rigot et al., 2012). Furthermore, in addition to these eco-climatic differences, the two regions present very different animal populations in terms of species raised and the size of flocks and herds. Sardinian farms are more livestock-oriented than those in Tuscany; specifically they are more in the ovine sector. In 2005, it was estimated that approximately 3.9 million sheep and goats were raised in Sardinia in comparison to only 0.6 million in Tuscany (ISTAT, 2005). At the farm level, this converts to an average of 240 sheep



Fig. 4. Annual mean temperature in the regions of Sardinia and Tuscany and differences (in °C) throughout the year.

per farm in Sardinia compared to 120 in Tuscany, whilst the number of bovines per farm (about 38 head) is comparable in the two regions. Consequently, more hosts, and more hosts per farm, might provide a further explanation for the difference in *C. imicola* populations between Sardinia and Tuscany.

The association between eco-climatic variable and *C. imicola* distribution, both in Sardinia and Tuscany, can mainly be explained by a single predictor (elevation in Sardinia and the mean annual temperature in Tuscany) that was able to correctly classify 75.0% and the 69.5% of the sites in the two regions, respectively. The inclusion of landscape factors in the model, quantified through area and edge metrics, did not lead to any improvement in classification in Sardinia, where *C. imicola* is widespread and abundant. This might be due to the fact that *C. imicola* is “endemic” (meaning that the vector has already found a suitable habitat) and the relative abundance might rather depend on factors other than landscape predictors.

In Tuscany, where the comparison was done between positive and negative sites only, the discriminant analysis with the inclusion of landscape variables (area and edge metrics) identified riparian vegetation, stream water and coniferous woodland as significant factors, improving the classification of sites by up to 80%. Riparian vegetation develops alongside rivers and small streams where the muddy banks, in the drier and sunnier sections, can potentially provide suitable breeding habitats for *C. imicola*. It is worth noting that polygons representing such factors, characterised by thin and long patches, were identified by the fine-scale classification used in this study, but not detectable using different sources (e.g. Corine land cover data, level III). We also found factors related to coniferous woodland (area and edge between forests and cultivated areas) to be negatively related to the presence of *C. imicola*. These factors can be linked to the breeding preference of *C. imicola* in sunny areas with low vegetation. However, it can also be a confounding factor correlated to elevated, steeply sloped and colder places that are all less suitable for this species. We only tested area and edge metrics with the hypothesis that they directly influence the vector populations. Many other landscape metrics could have been estimated and tested, e.g. the connectivity or fragmentation of the landscape. However, given the multiplicity of indices used in landscape ecology and their frequent multi-collinearity, there is now a strong consensus that landscape metrics should be used with parsimony. In addition, testing many variables in a multivari-

ate framework would dramatically increase the risk of identifying statistically significant, but spurious associations. As we had no hypothesis on the potential effect of the other metrics on *C. imicola* populations, those other factors were not tested.

In both the regions, about 20% of variability remained unexplained, even with the inclusion of the landscape metrics. The distribution of local and small patches of soil matching the ecological requirements of breeding sites (humidity, organic matter content, texture and pH) may be factors that explain this variability in trap catches. These small patches can potentially be found within land of natural or semi-natural vegetation. However, they are believed to be relatively frequent on farms; firstly because the water spill around livestock drinking points, or small leakages in irrigation systems often create local patches of semi-moist soil in an otherwise extremely dry environment (Braverman et al., 1974; Meiswinkel et al., 2004; Pili et al., 2006) or, secondly, the amount of organic matter that can be found on the ground on farms can be very significant. Moreover, the concentration of vertebrate hosts provides blood meals for the insect population and then influences the proliferation of insects. Man-made influence, host animal population and soil texture might therefore play an important role in the explanation of *C. imicola* distribution, but they should be quantified using alternative approaches.

In contrast to our results, other studies have found landscape factors to be associated with BT vectors or BT outbreaks (Durand et al., 2010; Purse et al., 2012). However, both these studies were performed in different eco-climatic conditions and in regions where BT virus transmission involves different vector species with different habitat preferences. In a Mediterranean context, the only study that examined the influence of landscape factors on BT virus was conducted by Guis et al. (2007). However, this study analysed the statistical relationship between landscape indices and BT outbreaks, rather than vector abundance or vector presence. Furthermore, the landscape factors identified in the study were difficult to interpret in biological terms and tended to vary with the buffer size radius considered (500 m, 1 km and 2 km). Consequently, the results we present here cannot be directly compared to previously published studies in a useful way. Indeed, the scale of our classification and its level of accuracy verified and validated site by site are unprecedented in similar studies; yet the effect of some landscape variables was found to be limited, suggesting that, landscape-related variables might have a minor impact on *C. imicola* populations.

Conclusions

A large part of the variability in *C. imicola* abundance in Sardinia, or in its presence/absence in Tuscany, can be predicted by temperature or elevation alone. The limited influence of landscape variables on *C. imicola* populations may be related to specific situations. In Sardinia, the accuracy provided by landscape-related variables cannot help in discriminating between various levels of abundance, while, in Tuscany, landscape metrics associated with natural breeding sites in the areas surrounding the farm provided a 10% improvement of the discriminant model in the classification of positive and negative sites. The marginal improvement obtained with the inclusion of landscape metrics as predictors of *C. imicola* distribution do not therefore justify, at least in some parts of Italy, reliance on detailed land-use classification. More attention should be given to *ad hoc* site information that might influence the biology of the vector.

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