Modelling the distribution of outbreaks and *Culicoides* vectors in Sicily: towards predictive risk maps for Italy

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Summary

Vector (911 light-trap catches from 269 sites) and serological surveillance data were obtained during recent bluetongue (BT) outbreaks in Sicily. The distributions of *Culicoides* vectors are compared with that of bluetongue virus (BTV) to determine the relative role of different vectors in BTV transmission in Sicily. The 'best' climatic predictors of distribution for each vector species were selected from 40 remotely-sensed variables and altitude at a 1 km spatial resolution using discriminant analysis. These models were used to predict species presence in unsampled pixels across Italy. Although *Culicoides imicola*, the main European vector, was found in only 12% of sites, there was close correspondence between its spatial distribution and that of the 2000 and 2001 outbreaks. All three candidate vectors *C. pulicaris*, *C. newsteadi* and *C. obsoletus* group were widespread across 2002 outbreak sites but *C. newsteadi* was significantly less prevalent in outbreak versus non-outbreak sites in Messina and BTV has been isolated from wild-caught adults of both *C. pulicaris* and *C. obsoletus* in Italy. The yearly distribution and intensity of outbreaks is attributable to the distribution and abundance of the vectors operating in each year. Outbreaks were few and coastal in 2000 and 2001 due to the low abundance and prevalence of the vector, *C. imicola*. They were numerous and widespread in 2002, following hand-over of the virus to more prevalent and abundant novel vector species, *C. pulicaris* and *C. obsoletus*.

Climatic determinants of distribution were species-specific, with those of *C. obsoletus* group and *C. newsteadi* predicted by temperature variables, and those of *C. pulicaris* and *C. imicola* determined mainly by normalised difference vegetation index (NDVI), a variable correlated with soil moisture, vegetation biomass and productivity. The predicted continuous presence of *C. pulicaris* along the Appenine mountains, from north to south Italy, suggests BTV transmission may be possible in a large proportion of this region and that seasonal transhumance between *C. imicola*-free areas should not generally be considered safe. Future distribution models for *C. imicola* in Sicily should include non-climatic environmental variables that may influence breeding site suitability such as soil type.

Keywords

Bluetongue – Climate – *Culicoides imicola* – *Culicoides obsoletus* – *Culicoides pulicaris* – Discriminant analysis – Distribution – Risk map – Sicily – Vector.

Introduction

Since 1998, an unprecedented epidemic of bluetongue (BT) has affected many countries in the Mediterranean Basin (east and west) including regions where the main vector, *Culicoides imicola*, is absent (6). This indicates that novel vector species are involved in BT virus (BTV) transmission, and recently, BTV has been isolated from wild-caught adults of *C. pulicaris* in Sicily (15) and from those of the *C. obsoletus* group in other regions of Italy (28).

Although present in all nine BT-affected regions of Italy (11), *C. imicola* is abundant only in Sardinia and

eastern Calabria (12, 19). In Sicily, one of the first regions of Italy to be affected by BT (13 October 2000), *C. imicola* is particularly scarce. Given these factors and the isolations described above, Sicily is particularly suitable for the investigation of the relative role of different *Culicoides* vector species. In collaboration with the National Reference Centre for Exotic Diseases (CESME: *Centro Studi Malattie Esotiche*) in Teramo, a vector and serological surveillance programme across the entire landmass of Sicily was established in 2000. These data are used here to quantitatively compare vector distributions with that of the virus to investigate the relative role of these vectors in BTV transmission in Sicily.

In addition, it is essential to model the relationship between environmental factors and the distribution of different vectors (3, 4, 5) so that risk-maps can be formulated for unsampled regions on the basis of their environment. Although all *Culicoides* species share the same basic habitat requirements, i.e. presence of host for blood-meals and breeding sites for egg and larval development (22), they differ in their life-history characteristics, and in turn, the extent to which their distribution and abundance is affected by environmental factors. The distributions of different vectors in Sicily are modelled in relation to satellite-derived climate variables and the climatic determinants of distribution are compared between vectors. These relationships are extrapolated to predict the distribution of these vectors across Italy.

Materials and methods

From 2000 to 2002, a total of 911 light-trap collections were made using Onderstepoort-type blacklight traps in 269 trap-sites across Sicily between May and December (1 to 88 catches per site). Individuals of *C. imicola* were counted and the presence of *C. obsoletus* group (hereafter referred to as *C. obsoletus*) and *C. pulicaris*, *C. newsteadi* and *C. circumscriptus* species scored in each sample. The analyses of distribution are restricted to the summer-autumn period since, in Europe, this period corresponds with outbreaks of *Culicoides*-borne disease and peak *Culicoides* abundance (25). Maximum catches in this period are also consistently related to the annual abundance of *Culicoides* across sites in Morocco (2). For *C. imicola*, the analysis was further restricted to 767 catches from 248 sites sampled between July and December since this species reaches peak numbers later than the other species.

Serological and sentinel surveillance, i.e. sampling of unvaccinated sentinel bovines, began across all Sicilian provinces in November 2000 and is on-going

(14). The geographical co-ordinates of trap sites and outbreaks were determined using a Garmin GPS 12 receiver (those of 16 outbreaks were obtained from websites (1, 23). Altitudes were derived from the 1 km × 1 km spatial resolution global topography (GTOPO30) digital elevation model (17).

Four variables of environmental significance were considered in the development of climate models of species distribution, namely: normalised difference vegetation index (NDVI), middle infra-red reflectance (MIR), land surface temperature (LST) and air temperature (TAIR). NDVI specifically measures chlorophyll abundance and light absorption, but is correlated with soil moisture, rainfall and vegetation biomass, coverage and productivity (13). MIR is correlated with the water content, surface temperature and structure of vegetation canopies (9). LST is a general index of the apparent environmental surface temperature (whether soil or vegetation) and TAIR is an estimate of the air temperature a few metres above the land surface (18).

The method by which these four variables are obtained from 1 km ×1 km spatial resolution Pathfinder AVHRR imagery (17) and fourier-processed to summarise their seasonal variations (producing 40 remotely sensed variables and altitude in all) are given elsewhere (26, 29). The discriminant analysis procedure used to select (by forward step-wise selection) the 10 environmental variables that best divide trap sites into presence-absence classes for each species, and then to predict species presence in unsampled pixels is described by Rogers (27). Four methods of internal validation were used for assessment (see 'Results').

Results and discussion

Relationship between the distribution of *C. imicola* and the distribution of outbreaks

Between 2000 and 2002, 74 outbreaks of bluetongue were reported in Sicily (Fig. 1). In the first two years, there were few outbreaks (11 in 2000, 6 in 2001) and these were restricted to coastal regions (<9 km from the sea). In 2000, they occurred in the provinces closest to Sardinia. In 2001, outbreaks began in Messina and Siracusa, over 175 km from the 2000 outbreak sites. This suggests there was a second incursion of virus in 2001 rather than overwintering of the virus in the 2000 outbreak sites by continual host-to-midge cycling. In 2002, outbreaks were much more numerous and widespread, occurring in five provinces and further inland (>40 km from the sea). They commenced in sites near (<12 km) the outbreak sites of the previous year, suggesting that

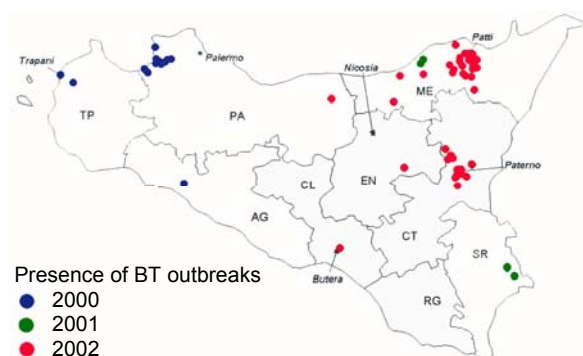the virus probably overwintered between 2001 and 2002.

Figure 1
Distribution of bluetongue outbreaks each year in Sicily
Key to provinces: TP = Trapani; PA = Palermo;
ME = Messina; EN = Enna, CT = Catania;
AG = Agrigento; CL = Catanisetta; RG = Ragusa;
SR = Siracusa

Culicoides imicola occurred in only 12% of trap sites (mostly in coastal areas <30 km from the sea) and generally in low abundances (Fig. 2, Table I). However, it was the only vector found in all 2000 and 2001 outbreak sites and was particularly abundant in Palermo and Siracusa. In stepwise logistic regression models comparing sites in which seroconversion to BTV occurred in 2000 to those in which seroconversion did not occur, the presence of *C. imicola* was the only species presence variable added to the model (model χ^2=10.6, p=0.001, 1 d.f.). Thus, *C. imicola* was probably the vector responsible for most BTV transmission in the first two years of the outbreak in Sicily. However, it was trapped at only a few 2002 outbreak sites in Paterno, Catania. Thus, in 2002, other vectors must have been responsible for BTV transmission.

Table I
Maximum catches and prevalence of different vector species across different subsets of trap sites in Sicily, 2002

| Species | Prevalence No. (%) of sites | | |
	All sites (sampled May to December)	2002 outbreak sites n =42	2002 Messina outbreak sites n =18
C. imicola	33 (12)	8 (19)	0 (0)
C. obsoletus	161 (60)	24 (57)	13 (72)
C. pulicaris	103 (38)	22 (52)	14 (78)
C. newsteadi	176 (65)	25 (60)	13 (72)
C. circumscriptus	138 (51)	13 (31)	7 (39)

Maximum catch
of *C. imicola*
● 500-2 000
● 101-500
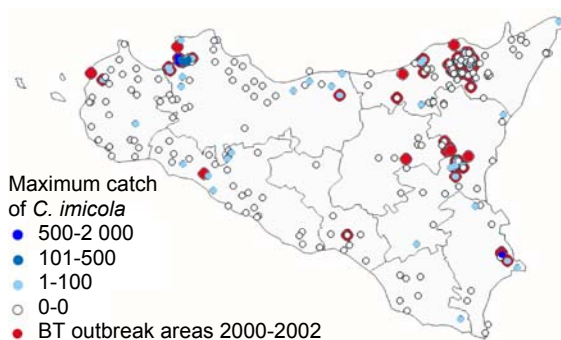● 1-100
○ 0-0
● BT outbreak areas 2000-2002

Figure 2
Distribution of *Culicoides imicola* across sites in Sicily from catches made between June and December, from 2000 to 2002

Relationship between the distribution of potential novel vector groups and the distribution of outbreaks

Culicoides pulicaris, *C. obsoletus* and *C. newsteadi* were widespread across trap sites in Sicily including inland and high altitudes sites (Fig. 3, Table I) and were all present in over 50% of the 2002 outbreak sites. However, the four vector species were rarely present alone in a trap site, complicating the detection of statistical relationship between the distribution of one particular candidate vector and that of outbreaks. For example, *C. obsoletus* was the sole vector species in only 6% of sites positive for this species whilst it was found with two or more other potential vectors in 41% of such sites. Thus, no species presence variables were added to a stepwise logistic regression model comparing 2002 outbreak and non-outbreak sites. *Culicoides newsteadi*, being less prevalent in outbreak versus non-outbreak sites in Messina (χ^2 = 4.8, 1 d.f., p =0.028), was the least likely candidate vector species.

Relationship between vector distribution of potential novel vector groups and environmental variables

The climatic determinants of distribution differ between *Culicoides* species in Sicily, as indicated by the differing rank and order of climatic variables added to the presence-absence models (Table II). The proportion of correct predictions from the presence-absence models were high (range: 60% to 96% across species) and kappa values of around 0.6 indicated substantial agreement between observed and predicted species presence (Table III).
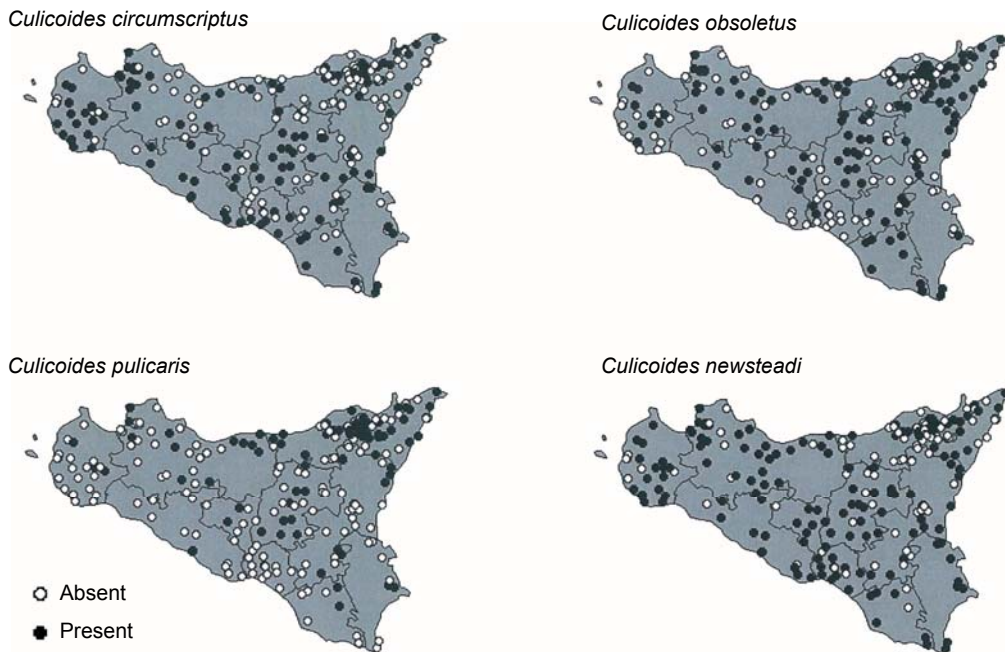
Figure 3
Distribution of *Culicoides circumscriptus*, *C. obsoletus*, *C. pulicaris* and *C. newsteadi* across sites in Sicily
from catches made between May and December, from 2000 to 2002

Table II
The first four of ten variables, ranked in order of importance, which best allocated the Sicilian trap sites to the
observed *Culicoides* species presence-absence classes

Rank	*C. obsoletus*	K	*C. newsteadi*	K	*C. pulicaris*	K	*C. imicola*	K
1	LST mean	0.24	MIR mean	0.26	NDVI mean	0.42	LST tri-ann. phase	0.04
2	LST variance	0.32	LST ann. amp.	0.30	NDVI tri-ann. amp.	0.47	MIR tri-ann. phase	0.26
3	MIR bi-ann. amp.	0.38	TAIR min.	0.37	NDVI variance	0.50	NDVI ann. phase	0.45
4	TAIR min.	0.41	LST min.	0.39	LST variance	0.48	NDVI mean	0.49

K	kappa value for the model at the step when the variable was added	LST	land surface temperature
NDVI	normalised difference vegetation index	MIR	middle infrared reflectance
TAIR	air temperature x m above ground	DEM	altitude derived from digital elevation model
amp.	amplitude	ann.	annual
bi-ann.	bi-annual	tri-ann.	tri-annual

Table III
Kappa coefficients, sensitivity, specificity and consumer accuracy for each species presence-absence model

Validation method	*C. obsoletus*	*C. newsteadi*	*C. pulicaris*	*C. imicola*
Sensitivity[a]	76.3	77.7	78.4	91.7
Specificity[b]	78.7	72.0	79.5	81.1
Consumer accuracy[c]	84.1	84.0	70.2	62.3
Kappa-coefficient	0.54	0.55	0.62	0.68

a) percentage of positive observations predicted correctly, equivalent to the producer's accuracy

b) percentage of negative observations predicted correctly

c) percentage of predictions observed to be correct

Kappa coefficient (estimate of the agreement between two variables, accounting for the degree of overlap expected by chance)
Key to levels of agreement indicated by kappa values from Landis and Koch (20):
K= 0.0-0.20 – slight; K = 0.21-0.40 – fair; K = 0.41-0.60 – moderate; K = 0.61-0.80 – substantial; K = 0.81-1.00 – near perfect

The distributions of *C. obsoletus* and *C. newsteadi* were primarily related to remotely-sensed temperature variables in Sicily since mostly LST or RAIR variables were added to their distribution models (and made up 5 and 7 of 10 variables added respectively). For *C. obsoletus*, both median LST and minimum TAIR was slightly higher in absence than presence sites (LST medians: 43.1°C versus 42.9°C, $W = 20121$, $p = 0.01$; TAIR medians: 16.0°C versus 14.3°C; $W = 19744$, $p = 0.001$) whilst the variance in LST was slightly lower (medians 9.2°C versus 9.6°C; $W = 13097$, $p = 0.01$). This preference for warmer, less variable temperature regimes may reflect the fact that *C. obsoletus* is a northern Palaearctic species (i.e. primarily found in Europe, North Asia and North Africa) on the southern margin of its range in Sicily. It is probably adapted to cold, requiring relatively low temperatures for optimal development and survival.

For *C. newsteadi*, the mean MIR (medians 42.7 versus 43.4, $W = 9957$, $p<0.001$), the minimum TAIR sites (medians 13.3°C versus 15.5°C; $W = 9911.0$, $p<0.001$) and the minimum LST (medians 40.3 versus 40.7, $W = 10105$, $p<0.001$) were all higher in presence versus absence sites. This association with high values of MIR and minimum temperatures may reflect high thermal requirements for development in this southern Palaearctic species.

NDVI variables were the most important determinants of the distribution of *C. pulicaris* (six NDVI variables were added to the model). The mean NDVI was much higher (medians 0.34 versus 0.43; $W = 18\,241$, $p<0.001$) and the variance in NDVI was lower (medians 0.009 versus 0.006, $W = 11374$, $p<0.001$) in presence versus absence sites. This indicates that *C. pulicaris* prefers microclimates with high, stable levels of moisture for optimal survival and development. This is consistent with its breeding site requirements which are listed as wet soil and bogs (8).

Although an LST variable was the most important predictor of *C. imicola* distribution, eight other variables added to the model were NDVI or MIR variables, suggesting again that moisture levels as well as temperature are important for survival of this species. The close positive relationship between NDVI and *C. imicola* distribution is consistent with previous models (3, 5, 7, 29) and arises because microclimate conditions that favour vegetation growth (indicated by high NDVI) also permit breeding of *C. imicola*. This species breeds in water-saturated soils that are high in organic matter (10).

Predicted distributions of *C. imicola* and potential novel vectors

The large differences in the predicted ranges of the different vectors (Fig. 4) have important implications for disease surveillance and control. *C. pulicaris* was

a) *Culicoides pulicaris*
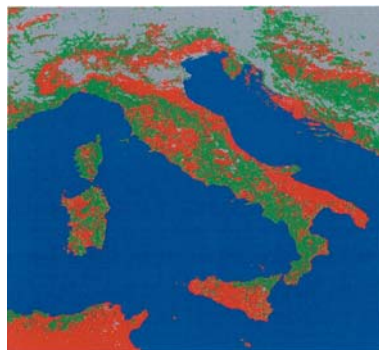
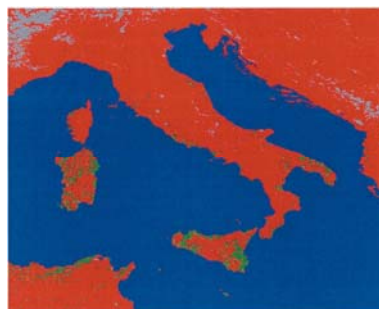b) *Culicoides obsoletus*

c) *Culicoides imicola*

Figure 4
Predicted distribution of *Culicoides* species in Sicily and Italy
For a and b: from a model derived from the observed presence/absence data for each species at 268 sites in Sicily, sampled between May and October (2000-2002)
For c: from a model derived from the observed presence/absence data for *C. imicola* at 142 sites in Sicily, sampled between July and October (2000-2002)
Key: Green = model prediction of species presence
Red = model prediction of species absence
Grey = no prediction since Mahalanobis distance between a pixel and its assigned probability or class was two or more times greater than the maximum distance observed between any one of the sites in Sicily and the classes to which they belonged

predicted to be present almost continuously across the Appenine mountains, from north to south Italy (see Fig. 5 for locations of mountains and provinces in Italy).

Conte *et al.* (16) asserted that the identification of mountainous *C. imicola*-free areas in central Italy could facilitate safer transhumance. Livestock are moved annually at the beginning of summer, from lowland winter pastures to highland areas until late autumn. However, such movement of infected livestock between coastal areas (areas of BTV transmission by *C. imicola*) to mountainous areas at the time of peak *C. pulicaris* abundance could provide a mechanism for hand-over of BTV between the main and the novel vector (21). BTV could then spread along the Appenines via passive wind dispersal of midges and cycle through *C. pulicaris* and host populations. *Culicoides obsoletus* is predicted to be widespread across Italy, including areas where *C. pulicaris* is predicted to be absent or rare, i.e. along the Adriatic coast and in Tuscany. If this species is also widely implicated in BTV transmission in Italy and our model predictions of its range are accurate, this further increases the area of Italy at risk of BTV.

When compared to the distribution observed by Conte *et al.* (16), our model correctly predicts the presence of *C. imicola* in coastal regions of Lazio and Tuscany and along the Ionian cost (Calabria, Basilicata and Puglia). However in Sardinia, the predicted distribution is much patchier than that observed. Whilst this could be due to the disparity in resolution between the two maps (1 km × 1 km pixels versus 10 km × 10 km pixels) (16), our predicted distribution of *C. imicola* is generally much less extensive than those derived from previous satellite-driven models (7, 29). *Culicoides imicola* occurs in a few sites in Sicily and so only a narrow portion of the potential niche of this species (in terms of environmental conditions) was represented in the model training set and, in turn, only a portion of its potential distribution could then be predicted. Models where the training set included a large numbers of sites from a core area of the species distribution perform better when extrapolated to unsampled areas (4).

For Sicily, predictions both from satellite-driven climate models and models based on interpolated weather station data predict *C. imicola* to be much more widely distributed across Sicily than it is observed to be. Thus it is more likely that, in Sicily, the relative absence of *C. imicola* is caused not by climatic factors but by other environmental factors that may influence its breeding sites. For example, a

Figure 5
Map of Italy showing provinces and mountains (closed triangles)

negative relationship was found between *C. imicola* abundance and soil sandiness in South Africa (5). Similarly, Calistri *et al.* (12) suggest that the porous, freely draining volcanic soils with poor moisture content that predominate in Sicily are unsuitable as *C. imicola* breeding sites.

Conclusions and future directions

During the 2000 and 2001 outbreaks, BTV appeared to be transmitted primarily by *C. imicola* and, in 2002 outbreaks, primarily by *C. pulicaris* with a contribution by *C. obsoletus*. The operation of different vectors, with different distributions and abundances, produced pronounced differences in the patterns of outbreaks between years.

The climatic determinants of distribution are species-specific in Sicily and these determinants seem to correlate to some extent with breeding site requirements and species ranges. Thus, predictive risk maps for BT derived entirely from distribution data for the main European vector, *C. imicola*, will omit extensive regions at risk of transmission via novel vector species.

A fuller examination of the relative role of different vectors and their climatic requirements requires models of species abundance rather than presence based on data collected regularly throughout outbreak periods. In the future, the distribution and abundance of these species will be re-modelled using vector surveillance data from a current European Union project collected according to a standardised protocol across a large area of the distribution of all species. Future spatial models of *C. imicola* distribution in Sicily should include additional environmental variables such as soil type.

Acknowledgements

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