Monitoring of saproxylic beetles and other insects protected in the European Union

Edited by Alessandro Campanaro, Sönke Hardersen, Giuseppino Sabbatini Peverieri, Giuseppe Maria Carpaneto



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Introduction



Fourteen years have passed since the National Centre for Forest Biodiversity "Bosco Fontana" organized the International Symposium "Dead Wood: a Key to Biodiversity" in Mantua. In these years the National Centre has maintained its original identity, continuing its dedicated work on saproxylic invertebrates, convinced of their vital importance for forest ecosystems. This adventure began at the end of the 1980s, when Martin Speight, author of the famous "Report" of

the Council of Europe "Les invertébrés saproxyliques et leur protection", visited the reserve Bosco Fontana. This report was highly influential in informing the public and also forest managers on the importance of dead wood.

The recognition of the importance of dead wood has steadily increased; so much so that the European Union today considers this resource a "proxy" for the conservation of biodiversity in forests. What matters most is that finally the concept of conservation of the saproxylic fauna is also accepted by forestry sciences, traditionally a conservative discipline. In the last years new forest management techniques were developed, such as "islets of senescence" in France and Switzerland and "arteries for biodiversity" in Italy.

The European Workshop of the Life Project "Monitoring of Insects with public participation", aimed primarily at the development of standard monitoring techniques for five species of saproxylic beetles, is aiming to advance the recognition of the importance of dead wood with two publications: "Guidelines for the Monitoring of five species of saproxylic beetles "and the proceedings of the European workshop of the LIFE project.

The hope is that these two publications will help to consolidate the conservation of saproxylics in the daily practical work of forest managers, so that these concepts will not disappear in the "dusty drawer of good intentions".

> Franco Mason Project manager Life 11 NAT/IT/00252 "Monitoring Insects with public participation" Verona/Mantova, May 2017

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RESEARCH ARTICLE



Can we successfully monitor a population density decline of elusive invertebrates? A statistical power analysis on *Lucanus cervus*

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Abstract

Monitoring global biodiversity is essential for understanding and countering its current loss. However, monitoring of many species is hindered by their difficult detection due to crepuscular activity, hidden phases of the life cycle, short activity period and low population density. Few statistical power analyses of declining trends have been published for terrestrial invertebrates. Consequently, no knowledge exists of the success rate of monitoring elusive invertebrates. Here data from monitoring transects of the European stag beetle, *Lucanus cervus*, is used to investigate whether the population trend of this elusive species can be adequately monitored. Data from studies in UK, Switzerland and Germany were compiled to parameterize a simulation model explaining the stag beetle abundance as a function of temperature and seasonality. A Monte-Carlo simulation was used to evaluate the effort needed to detect a population abundance decline of 1%/year over a period of 12 years. To reveal such a decline, at least 240 1-hour transect walks on 40 to 100 transects need to be implemented in weekly intervals during warm evenings. It is concluded that monitoring of stag beetles is feasible and the effort is not greater than that which has been found for other invertebrates. Based on this example, it is assumed that many other elusive species with similar life history traits can be monitored with moderate efforts. As saproxylic invertebrates account for a large share of the forest biodiversity, although many are elusive, it is proposed that at least some flagship species are included in monitoring programmes.

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Keywords

Lucanus cervus, Natura 2000 monitoring, elusive saproxylic beetles, Monte-Carlo simulation, population decline

Introduction

Monitoring global biodiversity is essential for nature conservation in order to understand and counter its current loss due to anthropogenic disturbances (Jones et al. 2015; Lindenmayer and Likens 2010; Reynolds et al. 2011). However, it has often been argued that species selected for monitoring or conservation are biased towards more familiar species (Clark and May 2002; Franklin et al. 2011; Regan et al. 2008), while invertebrates are often under-represented (Cardoso et al. 2011; D'Amen et al. 2013; Leather 2013). Among other reasons, technical issues, i.e. the difficulty to monitor these species, have been argued. Bosso et al. (2013), for example, highlight the difficulty of monitoring the elusive Rosalia alpina due to difficult detection in forests and its short life span of adults while Roets et al. (2013) mention the nocturnal activity, hidden phases of the life cycle and short activity period for the Colophon stag beetle. A monitoring plan should be designed effectively and cost-efficiently (e.g. Lindenmayer and Likens 2010; Reynolds et al. 2011). Statistical power analysis is a widely acknowledged tool for that goal (Di Stefano 2003; La Morgia et al. 2015; Reynolds et al. 2011) in which a simulation is used to calculate the probability for correctly rejecting the null hypothesis (H0) when the alternative hypothesis (H1) is true with a given monitoring scenario. In other words, what is the chance of detecting a simulated decline? Monitoring of elusive species, in general, yields a low power (e.g. Jones et al. 2015; Steenweg et al. 2016; Williams and Thomas 2009). Unfortunately, only a few power analysis studies on the populations trends of terrestrial invertebrates have been published in the peer reviewed literature (Bried and Pellet 2012; Lang et al. 2016; Schmucki et al. 2016, all on butterflies). Consequently, the possibility of adequately monitoring the population trends of invertebrates in general and elusive invertebrates in particular has hardly been evaluated.

The European stag beetle, *Lucanus cervus* (further called the stag beetle), is a good model species to investigate whether the population trend of a strongly elusive terrestrial invertebrate can be adequately monitored. This saproxylic species is often considered as an umbrella species, representing the large saproxylic diversity inhabiting forests and half open landscapes (Luce 1996; Thomaes et al. 2008). The stag beetle is included in the second annex of the European Habitats Directive and consequently, species specific protection and monitoring is mandatory for every member state. As this species can only be observed during a very narrow time window (Campanaro et al. 2016; Harvey et al. 2011a), it can be argued that monitoring this species would yield insufficient data to evaluate its population trend. The stag beetle life cycle takes 3 to 5 years (Fremlin, Hendriks and Thomaes unpublished data, Rink and Sinsch 2008) which are spent mainly in underground dead wood. After eclosion in late summer, the adults overwinter in a quiescent stage and become active above ground for mating and dispersal next summer. After

emergence, males live for about 8 weeks while females can live up to 12 weeks (Harvey et al. 2011a). Even then, the species remains mostly hidden underground, being active during a short period around sunset (Campanaro et al. 2016; Rink and Sinsch 2007). Furthermore, the activity of adults strongly depends on temperature (Harvey et al. 2011a; Rink and Sinsch 2011; Sprecher-Uebersax 2001; Thomaes et al. 2008). Finally, relative humidity, rain, wind speed and other weather variables have been found to influence the stag beetles' activity (e.g. Fremlin and Fremlin 2010; Rink and Sinsch 2007). However, as these variables are likely to be related to temperature and none of them has been tested in combination with temperature, conclusions should be interpreted carefully. Finally, the stag beetles' activity is suggested to depend on the moon cycle (Mader 2009) but this has not been confirmed by Sprecher-Uebersax (2001) and Campanaro et al. (2016).

Different monitoring protocols have been evaluated for the stag beetle: acoustic larval detection (Harvey et al. 2011b), baited or unbaited traps (e.g. Chiari et al. 2014) and direct observations of living and/or dead beetles along transects (e.g. Campanaro et al. 2011; Campanaro et al. 2016; Fremlin and Fremlin 2010; Mader 2009; Sprecher-Uebersax 2001). Vrezec et al. (2012b) found detection efficiency of evening transects (>90%) to be higher than that of trunk surveys and pit fall traps in the ground or attached to a tree (about 30–50%). Other techniques of trapping or indirect monitoring have also yielded poor results (Chiari et al. 2014; Harvey et al. 2011b). Consequently, a walked transect in the evening currently seems to be the best available sampling technique. As it is a generally well known species, a citizen science approach with many simultaneous transects is a feasible monitoring strategy (www.stagbeetlemonitoring.org). Few transects have been followed up nearly daily. In most cases, a weekly follow up has been used with a fixed day (e.g. Campanaro et al. 2011) or with a variable day depending on the weather (Campanaro et al. 2016). Finally, it can be argued whether monitoring days should be concentrated around the short activity peak or over a longer period (Campanaro et al. 2016; Fremlin and Fremlin 2010; Vrezec et al. 2012a). Due to the short activity period within a day, only one evening transect can be walked per observer and, due to the short season, only a limited number of days per year are suitable for monitoring, especially under colder climatic conditions. Consequently, cost efficiency is low and the power of such monitoring can be questioned.

Here, data have been used from three transects in north-western Europe which have been monitored nearly daily for seven up to ten years to parameterize a simulation model that estimates the stag beetles' relative abundance. This model is then adjusted to include a population decline of 1%/year and used for a Monte-Carlo simulation. This decline was derived from European guidelines (European Topic Centre on Biological Diversity 2011) which state that a population decline of more than 1%/year within 12 years (short term) or 24 years (long term) should result in a negative report for this species. Finally, different monitoring scenarios are evaluated using the simulated data in order to determine the effort needed to successfully detect this population decline (power analysis). We hypothesize that despite a very narrow window of activity and a high variability in abundance, the stag beetle can still be successfully monitored with a moderate monitoring cost when the monitoring scenario is adapted to the phenology of this species.

Materials and methods

Abbreviations

- C-Season: Centred measurement for the day of the season which is equal in each year calculated as: (Julian date (1 to 365) 170)/30
- T-Season: Centred measurement for the day of the season but shifted based on the temperature of that specific year to accommodate a season that was triggered by a certain temperature calculated as: (Julian date (1 to 365) first day with 18°C or more + 1)/30
- MAB: Median absolute bias on the trend estimation calculated as the median value of the absolute difference between the trend introduced in the simulation and the trend estimated by the validation model.

Data

The data were compiled from three published studies on transects that have been monitored daily during the activity period of the stag beetle, i.e. mid-May till early July, for several years (Table 1, full data published as Thomaes et al. 2016). The first transect is located in Basel (Switzerland) and was monitored between 1991 and 2000. The transect was walked from 21:00h to 22:15h (see Sprecher-Uebersax 2001 for further details). The second transect lies in Colchester (UK) and was followed up from 2005 to 2011. Stag beetles were recorded along the transect from 21:00h till 22:00h (from 2008 onwards, the transect was shortened from 21:30h till 22:00h, see Fremlin and Fremlin 2010 for further details). The last transect lies in Tairnbach (Germany) and was followed up from 2008 to 2014. This transect was walked between 21:00h and 22:00h and stag beetles, amongst other insects, were recorded (see Mader 2009; Mader 2013 for further details). The three sites represent quite distant and extreme situations in north-western Europe: Colchester lies near the western distribution border of the species and comprises a very Atlantic climate; the site in Basel is situated at 262 m asl. and might represent a more mountainous population of this species while Tairnbach represents a more eastern situation for the populations in north-western Europe (although the species is found up to the Ural mountains). Furthermore, each site represents a different typical habitat for the species (Table 1). The methodology of slowly walking a short transect in about one hour is very similar for the three transects. However, these studies inevitably encompass small differences in monitoring protocol (e.g. length and duration of the transect walk and starting time) which were optimised to local conditions or needs. Nevertheless, it is believed that sampling methods were sufficiently consistent to provide reliable and comparable estimates of temporal variation in population abundance when the duration of the transect walk is used as offset. A similar method of combining monitoring data was used by Meyer et al. (2010). Weather data (air temperature, rain, wind, relative humidity and air pressure) during the transect walks were compiled from nearby weather stations (Basel: Lufthygieneamt

Location	Long (°E)	Lat (°N)	Alt (m)	start year	end year	Habitat	Duration of transect walk	Reference for protocol	#
Tairnbach (Germany)	8.75	49.25	191	2008	2014	Forest edge	1h	Mader (2009)	681
Basel (Switzerland)	7.58	47.57	262	1991	2000	Park	1.25h	Sprecher-Uebersax (2001)	510
Colchester (UK)	0.88	51.88	28	2005	2011	Urban	0.5–1h	Fremlin and Fremlin (2010)	459

Table 1. Metadata of the stag beetle transect walks including location, longitude, latitude, altitude, start, end year, habitat, duration and reference to the protocol and number of transect walks (#).

in Sprecher-Uebersax 2001, Colchester: http://www.tijou.co.uk in Fremlin and Fremlin 2010 & Tairnbach: http://www.worldweatheronline.com). Rain and air pressure data are not available for Basel. Moon cycle data were calculated as the visible part of the moon as a percentage (http://aa.usno.navy.mil/data/docs/MoonFraction.php). Data compilation resulted in 1610 transect walks.

Model selection

It is assumed that the number of individuals observed along the transects mirrors the local population density as there is no population density function available for this species. This situation is common for many monitored species (Reynolds et al. 2011). Two variants of a generalised additive model (GAM) with Poisson family and log link explaining the number of stag beetles observed during the 1610 transect walks were compared. The first part is identical for both model variants and includes an offset, the transect (Basel, Colchester, Tairnbach) and the year per transect interaction. The natural logarithm of the duration of the transect walk (in hours) was added as the offset term. In this way the models express the abundance per hour of monitoring rather than the observed abundance (Zuur et al. 2009). The interaction between year and transect will give the log-linear trend for each transect. The year was centred to the first year of the transect to enhance the numerical stability of the model.

As mentioned in the introduction, the stag beetle abundance shows a strong seasonal and temperature-dependent pattern. Therefore, the second part estimates the temperature effect using a spline smoother. This smoother was included to gain insight into the relationship between temperature and stag beetle abundance, being linear or multi-polynomial. The latter option is based on the observation that stag beetles' activity increases until they are fully active from 18°C onwards (Fremlin and Fremlin 2010; Harvey et al. 2011a; Hawes 2008; Rink and Sinsch 2007; Smit and Krekels 2008). To prevent that the smoother would fit every detailed random temperature effect, the maximum degrees of freedom was set to four. The third part of the model explains the seasonal effect using two different variants (C-Season or T-Season). C-Season represents a constant season over the different years. A centred measurement was used to enhance the numerical stability of the model calculated as the Julian date (1 to 365) minus 170 (as a mean value) and divided by 30 to get a result in approximated months. T-Season represents a shifted season based on the temperature of that specific year to accommodate a stag beetle season that is triggered by a certain temperature. Vrezec et al. (2012a) have argued that stag beetle emergence depends on the temperature resulting in such a shifting season. Therefore, the first day of T-Season was defined as the first transect day with 18°C or higher, resulting in a negative season before that day. Days of this shifted season were again divided by 30. C-Season or T-Season was included with a spline smoother (with a maximum of four degrees of freedom):

stag beetles ~ Intercept + offset (log(transect duration)) + transect + year:transect + s(temperature) + s(C-Season)

or

stag beetles ~ Intercept + offset (log(transect duration)) + transect + year:transect + s(temperature) + s(T-Season)

The two variants of the explanatory model were, in the first place, evaluated based on the analysis of the model residuals in relation to model variables. As both variants performed equally well, the best model was finally selected by the AIC criterion (see results). Model residuals were plotted against weather variables that were not used in the model (see section 2.1) and moon cycle data to detect any remaining variability and correlation coefficients were calculated to decide whether to include these variables in the explanatory model.

Monte-Carlo simulation of a 1% population decline

New data sets were created consisting of 10 up to 100 transects, with each transect simulated for 12 years (cfr. European Topic Centre on Biological Diversity 2011). Transects data was generated from 10 May till 4 July each year to accommodate an 8 weeks period that included the abundance peak of the stag beetle and that was mainly within the monitoring range of the original transects. The duration of the transect walk was set to 1 hour.

The observed temperature data was modelled with a generalised linear mixed model (GLMM) including season as a second degree polynomial as explanatory variable and transect and year as crossed random effects:

Temperature ~ Intercept-temperature + C-Season + C-Season² + (1|Transect) + (1|Year)

To generate simulated temperature data, the GLMM with the original coefficients was converted to a GLM by changing the random effects in normally distributed fixed effects with zero as mean and their sigma as variance. The auto-correlation was set to 0.7, based on visual and empirical interpretation (Suppl. material 1: Figure A.1). Fur-

ther, we added a normal distributed random part to the intercept with zero mean and a low standard deviation (σ =0.01) between simulations.

The number of stag beetles observed per transect walk was simulated based on the selected explanatory model from the model selection. To facilitate the simulation, this GAM model was simplified to a GLM simulation model including a second degree polynomial of temperature and third degree polynomial of C-Season, based on the degrees of freedom in the original model. The third degree of temperature was not used in the simulation model as its significance in the explanatory model depended on the year:transect interaction and is therefore not an overall population characteristic but a statistical compensation for this interaction. Moreover, the temperature effect of a second or third degree polynomial on the number of stag beetles observed remains very similar (Suppl. material 1: Figure A.2). Year and transect were included as normally distributed fixed effects with zero as mean and their variance based on the explanatory model. Each transect was given a fixed trend which encompasses a 1% population decline per year (cfr. European Topic Centre on Biological Diversity 2011). The model can be presented as:

stag beetles ~ rpois(Expected count /transect duration)

Log (Expected count /transect duration) = Intercept + transect_i + year_j + poly(temperature, 2) + poly(C-Season, 3) + $((1-0.12)^{1/12})$ * year

transect_i ~ Normal(0, sd_{transect}) year_i ~Normal(0, sd_{vear})

Statistical power analysis

Four monitoring scenarios (Weekly, Warmest of 7d, Peak temperature and Daily) were applied to the simulated data to comply with different monitoring protocols. In the Weekly scenario, each transect was monitored weekly during one up to eight weeks centred around the period with peak abundance. In the Warmest of 7d scenario, the transect was monitored on the warmest day of each week representing a monitoring protocol that depends on the weather forecast for the coming seven days. This is a simplification of the method proposed by Campanaro et al. (2016) where only the days from Monday till Thursday were used for monitoring. In the Peak temperature scenario, the monitoring started on the first day with a temperature of 18°C or higher and was continued for one up to eight consecutive days. These three scenarios (each including one up to eight days of monitoring per year and transect) were compared with a Daily scenario which includes daily monitoring during one up to eight weeks (7 to 56 days per year and transect).

On the subsets of data sampled with the different scenarios, a GLM validation model was fitted similar to the simulation model, but without year and transect effects

to improve the processing time (they could be left out as these were centred around zero). If the subset included data of less than four weeks, then C-Season was left out of the validation model as the period is too short to fit the season effect properly. When modelling data from the Peak temperature scenario, both temperature and C-Season were left out of the validation model for the same reason. From each validation model, the parameter estimate and p-value for year were extracted.

Simulations were run 1000 times for each of the different simulation options, i.e. 10 to 100 transects (sample size), one to eight days/weeks of monitoring per year and transect (frequency) and for each of the four scenarios. Power (1 - type II error) was calculated as the percentages of p<0.05 (type I error) with parameter estimate <1 (i.e. prediction of a declining trend) for each of the simulation options. Based on these results, the minimum effort (= frequency * sample size) needed to reach a power > 90% was assessed. A threshold of 90% has been repeatedly suggested for reliable trend detection (e.g. Meyer et al. 2010; Steidl et al. 1997) in order to balance type II and type I errors (Di Stefano 2003). The median absolute bias on the trend estimation (MAB) for each scenario was also calculated to evaluate the accuracy of the trend estimation. MAB is sometimes used as an alternative for the power to optimise the effort of monitoring (Jones et al. 2015; La Morgia et al. 2015).

Finally, the power of three existing monitoring protocols was calculated: two in Flanders and one in Slovenia. In Flanders (Northern Belgium) a monitoring protocol for this species was designed including 36 transects and eight weeks of monitoring during the presumed warmest day of the week (Thomaes 2014), further called the Flanders scenario. As a start-up, this protocol was downscaled to 15 transects with three to eight weeks of monitoring (scenario Warmest of 7d) and 30 other transects that would be monitored only once a year (Flanders start-up). This downscaling was due to the fact that few volunteers have experience with stag beetles. It was simulated as three transects with eight, seven and six weeks of monitoring, two transects with five, four and three and 30 transects with one yearly random monitoring in a three week period around the abundance peak. In Slovenia, the monitoring includes two transects that are walked yearly plus eight that are walked every two years with three assessments within a period of about five weeks (Al Vrezec, pers. comm.). To assess its power, this protocol was implemented as a five week period with monitoring in the first, third and fifth week with the scenario Warmest of 7d. Al statistics were performed in R 3.3.1 (R Core Team, 2015) with mgcv, lme4 and ggplot2 as libraries (Wood 2011; Bates et al. 2014; Wickham 2009).

Results

Model selection

The model with C-Season had a lower AIC (7971) than the model variant with T-season (AIC = 8743) meaning that the hypotheses presented in Vrezec et al. (2012a) explaining the emergence of stag beetles at a certain temperature threshold could not

Table 2. Coefficients of the explanatory GAM model variant with lowest AIC which explains the stag beetle abundance. The table includes coefficients and their significance, estimated degrees of freedom for the smoothers of C-season and temperature and percentage deviation explained by the model (%dev. expl.: % deviation explained).

		Coeffi		%dev. expl.				
Tr	ransect			Year x Trans	ect	s(Temp)	s(C-Season)	
Basel Colc	chester	Tairnbach	Basel	Colchester	Tairnbach			
-1.13*** 0.2	24***	-0.11	-0.12***	0.11***	0.26***	2.898***	2.991***	47.4

***: <0.001

be confirmed. Therefore, the model variant with C-Season (Table 2 and Suppl. material 1: Figure A.3) was used as a selected explanatory model. The smoother for temperature (Suppl. material 1: Figure A.2) confirmed the finding that stag beetles are fully active from about 18°C onwards (see earlier). Model residuals showed no relation with other weather variables or moon cycle data (Suppl. material 1: Figure A.4), so no updates were made to include these variables in the explanatory model. As all these weather data were correlated with temperature (Suppl. material 1: Figure A.5), it can be assumed that temperature is a robust variable of weather conditions in general.

Statistical power analysis

Only with three of the four scenarios, a power of 90% was achievable but the effort and number of transects needed differed (Figure 1). The lowest effort to reach this power corresponded to the Warmest of 7d scenario with 80 transects and 2 days per year and transect (resulting in 160 days/y) (Figure 2). With the same scenario, many other options were possible to yield a power of 90% with 30 up to 100 transects with respectively 8 and 2 days per year and transect resulting in an effort between 180 and 240 days/y. The Weekly scenario required a slightly higher effort, at least 240 days/y with a combination of 80 transects and 3 days per year and transect. Again, many other options were also possible and only needed a slightly higher effort. The Daily scenario had the highest effort needed to reach a power of 90%. Here, an effort of 420 days/y was needed with a combination of either 20, 30 or 60 transects and respectively 3, 2 or 1 week of monitoring per year and transect. However, it was the only scenario that allowed successful monitoring with 20 transects. With the Peak temperature scenario, it was not possible to reach a power of 90%; with 100 transects and 8 days per year and transect, a power of 88.5% was reached. Based on the fairly coincidental lines in Figure 2, it is clear that the scenario and effort are of main importance to optimise the power, while the individual combinations of number of transects and frequency are of lesser importance.

The MAB criteria provided very similar results compared to the power (Suppl. material 1: Figure A.6). All scenarios with a power above 90%, yielded a low MAB (i.e. 0.01 to 0.04 or 1.4–3.7% of the real trend) and vice versa (Suppl. material 1: Figure A.7).



Figure 1. Statistical power for different scenarios, number of transects and frequency as number of days (for Peak temperature, Weekly and Warmest of 7d) or weeks (for Daily) per year and transect for monitoring the stag beetle (*Lucanus cervus*).



Figure 2. Statistical power for different scenarios, efforts as number of days/year (limited to 1000) and number of transects for monitoring the stag beetle (*Lucanus cervus*).

Thus, the MAB criteria yielded little additional insight for selecting the optimal scenario and effort.

The original monitoring for Flanders, Flanders scenario, yielded a power of 95% within 12y. The Flanders start-up scenario still had a power of 79%. The scenario of Slovenia, with only ten transects, yielded a power of 23%. This was quite low but, for a period of 24y the power increased to 81%.

Discussion

With the statistical power analysis presented, it was shown that it is at least feasible to monitor population density changes of the stag beetle with an effort of 240 days/y. This effort can be applied successfully with different combinations of scenarios, number of transects (between 30 and 100) and frequency. Before concluding which monitoring strategy and effort is most advisable to employ, the impact and alternatives for the missing density function, the limitations of the data used and the consequences of methods used for the results of the power analysis will be discussed first.

One of the main methodological problems for population trend analysis is the use of a relative abundance measure (here number of stag beetles found along a transect) to estimate the absolute population size (e.g. Bried and Pellet 2012; LaCommare et al. 2012; Williams and Thomas 2009). In many cases, the relationship between them is unknown and consequently a linear relationship must be assumed (LaCommare et al. 2012). Although a linear relationship might often be reasonable, several exceptions have been mentioned including differential population declines across sexes or life-history stages (Shea et al. 2006; Reynolds et al. 2011). For example, it is known that stag beetle transect walks are biased towards observations of male adults (e.g. Vrezec et al. 2012b). Consequently, threats affecting males in a selective way might result in an overestimate of the real population decline. Solutions that have been suggested to cope with this are distance sampling, mark-recapture procedures and presence-absence methods. Distance sampling is not realistic to apply to flying insects and mark-recapture procedures have yielded little recaptures for stag beetles (e.g. Chiari et al. 2014). Joseph et al. (2006) determined that for low density and hard-to-detect species, presence-absence methods equated or outperformed abundance methods at tracking changes in population size. For the stag beetle, this does not seem to be the case as the effort suggested by Campanaro et al. (2016) to assess the presence is 3 transect walks per year and transect which is comparable to some of our conclusions. However, presence-absence methods in general need many more transects than abundance methods so the overall effort will be higher.

The between-site variation on the number of the stag beetles observed is difficult to assess as only three sites have been monitored. Bart et al. (2004) mention that the variability in habitat and environment between sites is important when balancing the number of sites and monitoring frequency per site. As our three sites represent quite distant locations and habitats in north-western Europe, the results can be interpreted as based on maximal between-site variation and, consequently, as estimates of the outer limits of effort needed for monitoring the species in north-western Europe or countries within this region. Outside this region, the species response to temperature and season might differ and possibly also the effort needed to monitor it. LaCommare et al. (2012), Pais et al. (2014) and Jones et al. (2015) also concluded that the optimal monitoring strategy might differ across locations. Consequently, care must be taken when applying the results in other parts of the range of the species. For example, it might be expected that the species is less temperature restricted in warmer climates and consequently lower effort is needed. Furthermore, if the monitoring covers a large area, more variability between sites is likely to be expected and therefore more transects should be selected (Meyer et al. 2010; Pollock et al. 2002).

The most efficient way of monitoring the stag beetle seems to involve a scenario with weekly transects walks during the warmest evening. The scenario with transect walks concentrated after a first evening with 18°C or higher seems to have missed the period with abundance peak resulting in a very low power. Possibly, the stag beetle emergence in this region is triggered by lower temperatures and this causes the mismatch. However, if this peak period can be predicted, then the power of such a monitoring scenario might be much higher. When T-season is used in the simulation model instead of C-season, the Peak temperature scenario has the lowest effort needed to reach a power of 90% (results not shown). This is due to the fact that the simulation model and data sampling are then ideally tuned as both are based on the same hypothesis i.e. the period with abundance peak starts on the first evening with a temperature of 18°C. In reality, the start of this peak might be more complex and therefore more difficult to predict. Especially in different regions, stag beetle emergence might be expected to respond differently and thus different monitoring protocols might be needed for each region if this were to be applied. Consequently, it might be difficult to organise a large network of transects and instruct volunteers if the monitoring differs at each transect depending on the local temperature or climate zone. In that case, it might be easier to have transects that need to be walked weekly on the warmest day or even on a fixed day.

The Warmest of 7d scenario is simulated with the simplifications of a perfect weather forecast (i.e. the warmest evening is known at the beginning of the week) and so, in reality, the power might decrease slightly due to an imperfect weather forecast. However, as the power of the Weekly scenario is quite similar, this effect is expected to be limited. For more southern locations, this effect might be even smaller as days with unsuitable weather become rare.

An advantage of the Warmest of 7d above the Peak temperature scenario is that the effect of season remains evaluated. By this, changes in seasonality can also be detected. For example, climate change is expected to negatively affect the activity period (Rink and Sinsch 2011) which might not be detected with a Peak temperature scenario. Furthermore, data sampled in other periods (due to different monitoring strategies) or additional transect walks can still be included in the analysis as season and temperature remain in the validation model. This is not possible for the Peak temperature scenario where season and temperature are left out of the validation model and consequently balanced data is needed, thus making this scenario less robust (cfr. Schmucki et al. 2016).

Daily sampling clearly results in oversampling of a site in terms of population trend detection and is therefore not advised when trying to optimise the monitoring effort. However, this sampling technique might be very useful when only a limited number of transects is available or to study other population parameters, e.g. gaining insight into the period with peak abundance.

When comparing different options with the same effort, it seems that, in the presented simulation, the number of transects and frequency has little additional impact on the power. Thus, different combinations can be used to bring this monitoring into practice. Due to some simplifications that were included in the simulation, e.g. constant seasonal effects at all locations and equal decline at all sites, it is not advisable to use the lowest sufficient effort calculated but rather select a more robust estimate of the effort needed. Therefore, it is concluded that any combination with the Warmest of 7d scenario and an effort of minimal 240 days per year and between 40 and 100 transects can be used to realise the monitoring of this species to detect the given trend. A higher number of transects only slightly improves the power (cfr. Meyer et al. 2010). However, it is also important to take into account the costs for selecting and installing additional transects and finding and training volunteers (cfr. Jones et al. 2015; Lang et al. 2016; Williams and Thomas 2009). Therefore, it might be more realistic to realise only 40 transects with 6 days of monitoring per year and transect than 80 transects with 3 days per year and transect.

When comparing our results with other studies, it is concluded that the effort needed to monitor this elusive stag beetle (240 surveys/y) is not higher than for other invertebrates. Bried and Pellet (2012) concluded that the minimum allowable effort for occupancy monitoring of the Karner blue butterfly was 360 (40 sites x 9 surveys) for the spring generation and 200 (20 sites x 10 surveys) for the summer generation. Keizer-Vlek et al. (2012) found that more than 1000 sites must be sampled to detect a 40% change in the frequency for monitoring rare river inhabiting macroinvertebrates (50 sites for common species). Lang et al. (2016) found the need for about 600 to 2200 transect and four survey events to detect a population decline of frequent and rare butterflies respectively. However, as the scales, methods and detection thresholds differ, a one to one comparison is not possible.

Based on the current study, it is assumed that many other elusive species with similar life history traits can likely be monitored with a similar magnitude of effort. Many other stag beetles species share the short activity period, crepuscular activity and temperature dependence (e.g. Roets et al. 2013) and thus it is likely that comparable efforts are needed to study them. Other saproxylic beetles, like *Rosalia alpina*, *Morimus asper* or *Cerambyx cerdo* (all European Habitats Directive species), also share these life history traits despite being mainly monitored by trapping (respectively Bosso et al. 2013; Buse et al. 2008; Vrezec et al. 2012b) and consequently might need efforts of comparable magnitude.

It is concluded that it is possible to monitor a rather small population density decline of 1% per year for the elusive stag beetle with a moderate monitoring cost of 240 transect walks per year. Based on this example, it is assumed that many other elusive species with similar life history traits can be monitored with moderate efforts. This finding is especially important as saproxylic insects represent a large share of the total forest biodiversity (e.g. Müller et al. 2008, Horak et al. 2012) although many are elusive. Based on the current finding, we propose that at least some flagship species of this group are included in species monitoring programmes as their monitoring seems feasible.

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Supplementary material I

Figures of statistical support

Authors: Arno Thomaes, Pieter Verschelde, Detlef Mader, Eva Sprecher-Uebersax, Maria Fremlin, Thierry Onkelinx, Marcos Méndez

Data type: statistical data

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RESEARCH ARTICLE



Patterns of distribution and landscape connectivity of the stag beetle in a human-dominated landscape

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Abstract

Urbanisation and the spread of agriculture have resulted in high levels of forest loss, habitat fragmentation and degradation in many regions of the world. In Italy, the Po Plain is the most human-dominated landscape of the country and, after decades of exploitation, old-growth forests have been reduced to small and isolated patches, often threatened by invasive tree species such as the black locust (*Robinia pseudoacacia*). In these habitats, the occurrence of many forest-dependent species is related to the quality and availability of suitable areas, as well as the connectivity between the remaining forested patches.

Thus, recently developed species distribution models have been applied, namely the Ensemble of Small Models (ESMs), to identify areas of occurrence for a rare and protected saproxylic beetle species, the stag beetle *Lucanus cervus* and the inverse of the resulting distribution maps as resistance maps have been used to estimate landscape connectivity for this species.

Response curves suggested that the probability of the stag beetle occurrence increased with habitat diversity, grassland coverage and native forests, especially oak and mixed forests. The other forest coverage, such as those with black locust, beech, chestnut and black cherry, showed a unimodal relationship peaking approximately at 70%, 8%, 55% and 13% respectively. The stag beetle occurrence was unimodal related to distance to watercourses and distance to human settlements and negatively related to shrub-lands, croplands, sparse and dense human settlements. Landscape connectivity showed similar patterns, except for oak forest coverage, which showed a negative relationship to landscape connectivity.

In conclusion, stag beetles can persist in a human dominated landscape only in the presence of forest patches, including those with black locust trees. It is also inferred that ESMs may be suitable for modelling rare species distributions and estimating landscape connectivity to promote species conservation.

Keywords

Circuitscape, invasive species, Lucanus cervus, Robinia pseudoacacia, Species Distribution Models

Introduction

Urbanisation and intensive agriculture have resulted in high levels of forest loss and natural landscape fragmentation in many areas of the world (Foley et al. 2005). In Italy, new human settlements (buildings, industrial and commercial areas, as well as infrastructure) take over 70 hectares of natural or agricultural land per day (Munafò and Tombolini 2014). The remaining natural areas are not only embedded in a highly disturbed matrix of human-transformed land cover, but often serve as a continuous source for non-native species and become population "sinks" that are unable to support self-sustaining populations of the native species (McKinney 2002).

In this context, many forest-dependent animals are at risk of extinction, especially those with limited dispersal abilities, such as many saproxylic insects. Usually, these species are highly specialised and are linked to specific forest resources such as the amount of dead wood and particular conditions of temperature, humidity and fungal associations (Ranius and Fahrig 2006, Stokland et al. 2014). Amongst forest species affected by habitat fragmentation, the stag beetle *Lucanus cervus* (Linnaeus, 1758) is one of the flagship species adopted for saproxylic insect guilds (Thomaes et al. 2008). Indeed, even though the stag beetle is able to disperse well, reaching about 700m with a single flight (Rink and Sinsch 2007), it is dependent on sites with high spatio-temporal continuity of habitat related to natural forests and it is therefore used as an indicator species for habitat continuity (Müller et al. 2015).

Thus, forest planning and management should explicitly include connectivity assessments, identifying the most suitable forest sites for the maintenance of habitat connectivity in human dominated landscapes (Pascual-Hortal and Saura 2007).

Species distribution models (SDMs) have helped conservation planning for threatened species by identifying sites in which environmental conditions are favourable, especially in those areas where the species is not present and where restoration programmes could therefore be focused (Guisan et al. 2013). However, their predictive accuracy decreases drastically due to model overfitting when species with limited occurrences (such as rare species) and multiple predictor variables are considered in the models (Lomba et al. 2010, Breiner et al. 2015). This problem has recently been overcome by a new set of models called "Ensemble of Small Models" (ESMs), which are able to limit model-overfitting for rare species and provide more accurate predictions compared to standard SDMs (Breiner et al. 2015).

Landscape connectivity is increasingly estimated through circuit theory-based methods (McRae et al. 2008) which conceptualise the landscape as a conductive surface within an electrical circuit. Species locations or favourable habitat patches often represent the nodes of the network and the connections between nodes are determined by underlying resistance surfaces, based on ecological assumptions about the move-

ments of the species within the landscape (Tournant et al. 2013). Due to limited information regarding factors affecting animal movement and dispersal, SDMs are increasingly and successfully used to develop resistance surfaces (Wang et al. 2008, Milanesi et al. 2016a, b) and attention recently shifted from ecological corridor identification amongst species locations to independent node-based models (Koen et al. 2014).

Although recent studies developed conservation frameworks using ESMs to identify high conservation priority areas (Breiner et al. 2015, D'Amen et al. 2015, Benito et al. 2016) and independent node-based models to estimate landscape connectivity (Koen et al. 2014, Pitman et al. 2016), to the authors' knowledge, a combination of ESMs and independent node-based models for threatened species conservation in human-dominated landscapes have never been carried out to date.

Thus, the aims of this paper were (i) to identify habitat requirements for the stag beetle in a human-dominated landscape by developing ESMs, (ii) to identify ecological corridors for this species using the resulting probability of occurrence map of ESMs as a resistance surface in independent node-based models and (iii) to verify whether the above mentioned invasive tree species are related to stag beetle occurrence and landscape connectivity.

The empirical data set consisted of the locations of the stag beetle from one of the most human-dominated landscapes in Europe, the Po Plain in Italy (Ingegnoli 2015). In fact, after decades of agriculture and industrial activity, the old-growth forests of the Po Plain have been reduced to small and isolated patches (Blondel and Aronson 1999) and the remaining forests are also greatly threatened by human-introduced invasive tree species such as the black locust (*Robinia pseudoacacia*) and the black cherry (*Prunus serotina*; Annighöferet al. 2015). Even if previous research has also shown saproxylic insect occurrence in habitats affected by the invasive black locust (Della Rocca et al. 2016), further tests on the effect of invasive species on native species occurrence and connectivity are needed.

Materials and methods

Study area

The study was conducted in an area of about 1,025 km² within the Varese Province in the Lombardy region of northern Italy. Elevation in the study area ranges from a few metres above sea level (a.s.l.) near the Ticino River to about 600 m a.s.l at the foothills of Campo dei Fiori, above the Varese lake. The climate is temperate sub-continental (Ferré et al. 2005), generally humid with a mean annual temperature of about 13°C and an annual precipitation of between 700mm and 1,200mm (Prigioni 1995). This area encompasses twelve Natura 2000 sites amongst which were included nine Sites of Community Importance (SCI) and three Special Protection Areas (SPA) (Fig. 1; Suppl. material 1: Table S.1). Due to its strategic geographical location, this area connects the most extensive biodiversity source of the Po Plain, represented by the Valle del Ticino, to the massive mountains



Figure 1. Study area and stag beetle sampling locations (black dots with white circles). Filled black dots indicate investigated sites where the species was not recorded. Black lines indicate the borders of the study area.

of Campo dei Fiori (Casale et al. 2015) and it is an important ecological corridor between the Alps and the Apennines (Casale and Brambilla 2008), the two major "reservoirs of biodiversity" present in the Italian peninsula and included amongst the worldwide Priority Ecoregions for Global Conservation (Olson and Dinerstein 1998). The role of the study area as an ecological corridor is confirmed by the fact that two very ecologically exigent forest species such as the pine marten (*Martes martes*) and the black woodpecker (*Dryocopus martius*), which only lived in mountainous and forest areas until a few years ago, recently began colonising lowland areas of the Po Valley (Casale et al. 2015).

Despite its important ecological role and the high biodiversity value of the Ticino Valley Regional Park, which has been acknowledged as the MAB Biosphere Reserve "Valle del Ticino" (UNESCO 2005), the study area crosses the most urbanised area of the country, including the Milano-Malpensa International Airport. The forests of

this area are very fragile, fragmented and mainly concentrated along the riverbanks. Wherever broad roads and clearings have been opened up, the dominant tree species, including the pedunculated oak (*Quercus robur*), black alder (*Alnus glutinosa*) and hornbeam (*Carpinus betulus*), have been rapidly replaced by a scrub of exotic species consisting of a dense undergrowth of black cherry (*Prunus serotina*), black locust (*Robinia pseudoacacia*), tree of heaven (*Ailanthus altissima*) and red oak (*Quercus rubra*) (Bruno et al. 2008).

Study species and data

The target species, the stag beetle, is considered as threatened in several countries within its geographical range (Harvey et al. 2011), listed in the IUCN Red List of Threatened Species as "near threatened" and in the EU Habitats Directive as a priority species of community interest (Appendix II) (Nieto and Alexander 2010). It is considered a focal species for the conservation of suitable habitats for saproxylic beetles (Thomaes et al. 2008) and as an umbrella species, as it represents a diverse and highly vulnerable fauna associated with a wide range of broad-leaved trees (Percy et al. 2000) and coarse woody material (Buse et al. 2008, Ducasse and Brustel 2008). Therefore, by directing management efforts toward the requirements of this species, the requirements of many cohabitant species that use the same habitat are addressed (Roberge and Angelstam 2004). The dataset consists of locations of stag beetles collected in the context of the TRANS INSUBRIA BIONET (TIB) LIFE project (LIFE10/NAT/IT/241) and the AMBROSIANO Regional project. Both of these projects aimed to identify, improve and protect ecological corridors for endangered species in highly fragmented and human-dominated areas. Species occurrences were investigated from 2012 to 2015 in 22 sites, from 2013 to 2015 in 6 sites and from 2014 to 2015 in 6 more sites for a total of 34 sites within the study area. Each site was surveyed every year, for two months (June and July), once a week. To minimise detection bias due to changes in stag beetle activity throughout the day, each site was surveyed in three different time periods: once a month at sunset and three times per month alternatively in the morning or in the afternoon. Each survey consisted of one transect, 500 m length and 10 m width, walking along roads or paths within wooded areas at a constant speed of 17m/min, for a total duration of 30 minutes in dry and low-windy days (Campanaro et al. 2016). The exact locations (X, Y coordinates) and the gender of all specimens observed flying or walking on the ground within transects were recorded.

Predictor variables

A set of 18 predictor variables were derived that were contiguously available for the entire study area (Table 1). Specifically, the percentage of oak forests, native broad-leaved forests, invasive broad-leaved forests, mixed forests, beech forests, chestnut woodlands,

Variable	VIF	Sampled sites	Presence sites		
Native broad-leaved forests (%)	2.854	26.35 (±38.21)	28.76 (±40.71)		
Mixed woods (broad-leaved and coniferous) (%)	2.507	19.06 (±38.25)	19.62 (±39.54)		
Oak forests (%)	1.081	9.87 (±23.74)	14.66 (±29.31)		
Beech woods (%)	1.001	6.34 (±26.93)	8.85 (±34.07)		
Chestnuts woods (%)	1.629	3.76 (±17.21)	6.11 (±21.75)		
Distance to watercourses (m)	1.416	804.24 (±576.81)	859.34 (±626.99)		
Shrub-lands (%)	1.121	7.85 (±6.11)	9.27 (±7.01)		
Grasslands (%)	1.843	14.12 (±22.02)	10.48 (±19.12)		
Croplands (%)	2.155	10.01 (±21.31)	10.67 (±23.13)		
Invasive broad-leaved forests (%)	1.002	3.14 (±2.25)	3.09 (±2.22)		
Black cherry woods (%)	1.006	3.12 (±2.41)	3.21 (±2.51)		
Black locust woods (%)	1.979	9.41 (±22.71)	8.95 (±23.32)		
Other woods (%)	1.011	4.56 (±12.36)	5.50 (±15.16)		
Shannon diversity index of habitats (unitless)	1.307	1.81 (±0.59)	1.71 (±0.56)		
Dense human settlements (%)	2.961	2.18 (±4.88)	1.91 (±6.15)		
Sparse human settlements (%)	2.989	4.59 (±13.47)	4.76 (±14.41)		
Distance to human settlements (m)	2.582	166.57 (±130.69)	180.35 (±148.06)		
Distance to roads (m)	1.701	221.89 (±257.03)	167.67 (±200.11)		

Table 1. Variables used in the development of stag beetle (*Lucanus cervus*) Ensemble of Small Models. Variables with Variance Inflation Factor (VIF) > 3 have to be removed due to multi-collinearity with other variables. Average values ± standard deviations at sampled and presence sites are also shown.

black cherry stands, black locust stands and other woods were derived from the map of forest types in Lombardy 2016 (vector data; http://www.geoportale.regione.lombardia.it/). The percentage of shrub-lands, croplands, grasslands and dense and sparse human settlements were derived from the map of destination of agricultural usage and forest soils of Lombardy 2012 (vector data; http://www.geoportale.regione.lombardia.it/). The Shannon diversity index of habitats was also calculated considering all predictors mentioned above. The distance to watercourses and to human settlements was calculated from the map of destination of agricultural usage and forest soils of Lombardy 2012, while the distance to roads was estimated from the Open Street Map (http://www.openstreetmap.org). All of these predictor variables were re-sampled at 100 × 100m scale resolution to match the average home-range size of the target species (Sprecher-Uebersax 2003; Thomaes et al. 2008).

To avoid multi-collinearity amongst predictors, the Variance Inflation Factor (VIF) was calculated. Following Zuur et al. (2010), predictor variables with VIF values > 3 (highly related to other predictors) should be removed.

Ensemble of small models

The ESMs approach is based on the development of all the possible bivariate models (only two predictors at a time out of a larger set of predictors), followed by their combination into an ensemble (Lomba et al. 2010, Breiner et al. 2015). ESMs were developed with seven widely used modelling techniques (see Appendix S.1). Similar to Breiner et al. (2015), ESMs were developed using locations where the species was recorded and 10,000 random points were generated to serve as pseudo-absence data (including sampled sites where the species was not recorded).

20-fold split sampling (90% training data and 10% test data) was used to evaluate the bivariate models and the resulting ESMs. Similarly to Breiner et al. (2015), all bivariate models with Somers' D (also known as Gini coefficient; i.e. $2 \times AUC-1$) values equal to or less than 0 (i.e. AUC \leq 0.5 and thus worse than a random model) were not included in the ESM. The area under the receiver operating characteristic curve (AUC) and the continuous Boyce index were used to evaluate model performance. ESM outputs in an ensemble prediction (EP) weighted by Somers' D values were averaged and thus the resulting map (ranging between 0 and 100) was used in the further analyses. Finally, considering the cells with values above the cut-off point identified by the EP, areas of potential presence for stag beetles were identified. The R package 'ECOSPAT' (Di Cola et al. 2017) in R version 3.2.4 (R Core Team 2013) was used to develop, evaluate and project ESMs.

Structural connectivity and ESMs

A resistance map was derived as the inverse (1 – probability of occurrence) of the resulting map from ESMs and thus it was combined with circuit theory to explore landscape connectivity (McRae et al. 2008). Circuit theory was chosen over other commonly used connectivity methods as circuit theory models movement ecology via random walk pathways across all available movement possibilities (McRae et al. 2008). The software Circuitscape v.4.0.5 (McRae et al. 2008) was used, implementing circuit theory principles and assuming that the intensity of current flow between two nodes (locations) is proportional to the number of times an individual goes from one node to another moving through the particular cell under consideration (McRae et al. 2008). Thus, current flow can be used as a measure of the probability of movement between the two nodes to provide continuous estimates of landscape connectivity for the species considered (McRae et al. 2008). To estimate current flow, Circuitscape simultaneously considers all possible pathways connecting nodes (locations) accounting for an underlying resistance map to movement, producing a map where current density varies across pixels (analogous to the probability of use by random walkers; Koen et al. 2014).

Since high current is produced near nodes, using species locations as nodes could lead to a biased estimation of landscape connectivity (Koen et al. 2014) and thus, similarly to Pitman et al. 2016, a landscape-scale permeability map was developed independent from the former source or destination locations. Specifically, 10–300 random nodes were placed at intervals of 10 around the perimeter of the study area and then Circuitscape was run to identify the optimum number of random nodes required to generate an unbiased landscape connectivity map (Koen et al. 2014). After each iteration, the Pearson correlation was calculated to compare the resulting current density to those estimated, placing 1,000 random locations within the study area; a current density map was considered independent of node placement when the curve comparing correlation coefficients to the number of node pairs reached an asymptote (Koen et al. 2014; Pitman et al. 2016). Subsequently, the final current map was rescaled between 0 and 1 (indicating low and high current, respectively).

Results

A total of 222 specimens were found, 167 males and 55 females, in 21 of the 34 sites monitored (Table 2).

Multi-collinearity was not detected amongst the predictors (VIF > 3) and thus they were all considered in further analyses (Table 1). These 18 predictor variables resulted in a total of 153 bivariate predictor combinations. The mean values of AUC and the Boyce index for the ESMs were 0.945 (\pm 0.007 SE) and 0.903 (\pm 0.081 SE), respectively (Suppl. material 1: Table S.2). The cut-off value identified by EP was equal to 20 and thus a total of 34.29% of the study area above this threshold was estimated. These areas corresponded to 351.31km² divided into 935 patches where the stag beetle could potentially occur (Fig. 2).

The probability of occurrence for the stag beetle increased with habitat diversity and native broad-leaved forests, oak forests and grassland. The other forest coverage, such as those with black locust, beech, chestnut and black cherry showed a unimodal relationship peaking approximately at 70%, 8%, 55% and 13% respectively (Fig.3).



Figure 2. a Suitability areas for the stag beetle derived by the Ensemble of Small Models (green and black areas indicate suitable and unsuitable areas, respectively) **b** Resistance surface for the stag beetle (greenred scale indicates lower-higher resistances, respectively) derived by the Ensemble of Small Models, ESMs (1- probability of occurrence) **c** current map for the stag beetle (black-yellow scale indicates lower-higher connectivity, respectively) derived from resistance surface using Circuitscape software.

	Locality	Years									Total	
Site		2012		2013		2014		2015				
		М	F	M	F	Μ	F	Μ	F	M	F	
1	Buguggiate	0	0	0	0	0	0	0	0	0	0	
2	Azzate	0	0	0	0	0	0	0	0	0	0	
3	Galliate lombardo	1	1	2	0	1	0	2	0	6	1	
4	Casale litta	0	0	0	0	0	0	0	0	0	0	
5	Inarzo	0	0	0	0	0	0	0	0	0	0	
6	Inarzo	0	0	0	0	0	0	0	0	0	0	
7	Vergiate	5	1	4	2	0	1	2	1	11	5	
8	Casciago	0	0	1	0	1	0	1	1	3	1	
9	Barasso	1	0	2	0	3	0	3	2	9	2	
10	Biandronno	0	0	0	0	0	0	0	0	0	0	
11	Biandronno	0	0	0	0	0	0	0	0	0	0	
12	Besozzo	0	0	0	0	0	0	0	0	0	0	
13	Malgesso	0	1	1	0	1	0	2	0	4	1	
14	Brebbia	0	0	0	0	0	0	0	0	0	0	
15	Travedona-Monate	2	1	0	1	1	0	3	0	6	2	
16	Cadrezzate	1	0	1	0	2	0	1	0	5	0	
17	Cadrezzate	10	5	4	2	4	1	4	0	22	8	
18	Taino	5	0	5	0	2	0	1	1	13	1	
19	Vergiate	0	0	0	0	0	0	0	0	0	0	
20	Sesto Calende	0	0	0	0	0	0	0	0	0	0	
21	Vergiate	2	0	1	0	1	0	2	0	6	0	
22	Bodio Lomnago	1	0	1	0	1	0	1	0	4	0	
23	Bregano	-	_	0	2	1	0	3	0	4	2	
24	Vergiate	-	_	1	0	1	0	1	0	3	0	
25	Vergiate	-	_	2	0	1	0	2	1	5	1	
26	Arsago Seprio	-	_	0	0	0	0	0	0	0	0	
27	Sesto Calende	-	_	0	0	0	0	0	0	0	0	
28	Sesto Calende	-	_	2	0	1	0	1	0	4	0	
29	Castano Primo	-	_	-	—	11	8	7	3	18	11	
30	Castano Primo	-	_	-	_	2	0	3	1	5	1	
31	Lonate Pozzolo	-	_	-	_	2	0	4	1	6	1	
32	Vizzola ticino	_	_	-	_	7	4	5	3	12	7	
33	Vizzola ticino	-	-	-	-	6	3	3	5	9	8	
34	Vizzola ticino	_	_	_	_	8	2	4	1	12	3	

Table 2. Number of specimens collected within the study area in each sampling site from 2012 to 2015. (F= Female; M= Male).

The probability of occurrence for the stag beetle was unimodal related to distance to watercourses and distance to human settlements (with a peak at 800m and 180m respectively) and negatively related to shrub-lands, croplands, sparse and dense human settlements and increased distance to roads (Fig. 3).



Figure 3. Response curves and 95% confidence intervals (in grey) of the probability of occurrence of the stag beetle derived by the Ensemble of Small Models in relation to predictor variables values.



Figure 4. Response curves and 95% confidence intervals (in grey) of landscape connectivity of the stag beetle estimated through Circuitscape in relation to predictor variables values.

Using the inverse of the probability of occurrence as a resistance surface (Fig. 2), an unbiased landscape connectivity map was developed using ≥ 120 random nodes. In fact, the curve comparing Pearson correlation coefficients to the number of node pairs reached an asymptote at the value of 120 node pairs (Suppl. material 1: Fig. S.1) and thus the current density map was considered independent of the node placement (Fig. 2).

The landscape connectivity showed similar patterns of relationships with the considered predictor variables, except for oak forest coverage which tended to decrease as landscape connectivity increased (Fig. 4).

Discussion

This study took place within projects aimed at preserving the natural habitats in a human-dominated landscape that play a crucial role in connecting the Mediterranean basin to Northern Europe. In this context, suitable areas were identified for stag beetle reproduction and fundamental corridors for this species during dispersal. The most recent and robust species distribution and landscape connectivity modelling techniques were applied. The results confirmed that occurrence and connectivity of the beetles is related to natural habitats instead of anthropogenic habitats. Moreover, the research highlighted a complex puzzle in how managing invasive tree species (such as black locust thicket) may provisionally help in maintaining native animal populations in human-dominated landscapes. The research also identified that ESMs may be suitable for modelling rare species distributions and estimating landscape connectivity, provided that detectability problems are overcome.

Ensemble of small models and landscape connectivity

SDMs and landscape connectivity based on resistance surfaces are not without caveats. On one hand, the predictive accuracy of SDMs decreases considerably when rare species are considered in the models, as few species' occurrences and many predictor variables lead to model overfitting and thus reduced generalisation and applicability of the models (Lomba et al. 2010, Breiner et al. 2015). On the other hand, empirical knowledge about the factors facilitating (or hindering) individuals from moving across landscapes is often limited (Graf et al. 2007) and thus resistance surfaces are often derived from expert opinions. These can be potentially biased by the experience of the expert(s), scarce knowledge about the study area and limited or lacking information and published literature on the ecology of species (Zeller et al. 2012, Stevenson-Holt et al. 2014).

However, ESMs can overcome model-overfitting for rare species and thus provide more accurate predictions compared to standard SDMs (Breiner et al.2015) and it has been shown that ensemble predictions perform better compared to single modelling techniques (Marmion et al. 2009). Moreover, SDMs are increasingly and successfully used to derive resistance surfaces, avoiding subjective expert opinions (Wang et al. 2008, Milanesi et al. 2016a, b) and, recently, attention has shifted from single ecological corridor identification between species locations to independent node-based models (Koen et al. 2014).

Despite their high predictive accuracy, ESMs have not been often used to model rare species distribution and they have never been used to derive resistance surfaces to model landscape connectivity. Thus, in this study, a novel application of ESMs has been provided and it is inferred that they might be valuable tools for estimating unbiased landscape connectivity. Since the presence of rare species and landscape connectivity are amongst the most frequently cited criteria for site selection by conservationists (Tuomisto 2010), by combining these two important aspects of biodiversity, ecologists are strongly encouraged to implement this framework in order to develop successful management policies and conservation actions.

Patterns of distribution and landscape connectivity of the stag beetle

Suitable habitats for the stag beetle in the study area were mostly located in the largest patch of contiguous forest along rivers and lakes within the two natural parks, the Ticino Valley Regional Parkand Campo dei Fiori Park. Less suitable habitats were identified in the south-eastern part of the study area, which includes an intensive agricultural matrix, the Milano Malpensa airport and several cities surrounding Milan.

The relationship between the probability of occurrence predicted by ESMs and the predictor variables considered in this study is consistent with the ecological requirements of the species (Thomaes et al. 2008, Harvey et al. 2011). Specifically, response curves showed that forests and grasslands were the most suitable habitats for the stag beetles, while shrub-lands, croplands and human settlements (both sparse and dense) were largely avoided. On a landscape level, woodlands were the primary habitat type for this saproxylic species, since they are the main sources of high amounts of coarse woody debris, (Ranius and Kindvall 2006, Davies et al. 2008). In fact, in many European countries, the stag beetle is mainly associated with oak woodlands and, secondarily, with other tree species such as beech, chestnut and willow (Harvey et al. 2011). As expected, oak and mixed forests were found with the highest probability of occurrence followed by chestnut forests. Some invasive woodland, such as black locust and lack cherry, also seemed to be suitable for the species. In particular, the black locust forests ensured the maximum probability of occurrence for the stag beetle if they extended to about 70% of the landscape. However, over this threshold the stag beetle occurrence decreased rapidly.

This result is of interest as no previous information on the use of invasive woodlands by the stag beetles has been available.

The idea of a negative impact of invasive tree species on native species and ecosystems is generally supported (Maerz et al. 2005; Powell et al. 2011). However, most of these studies refer to herbaceous and shrub invasive plants, while the effect of the widespread invasive tree species on animal communities is still hotly debated. When considering saproxylic beetles, a general avoidance for some exotic coniferous tree species was recorded for *Pinus brutia* in Israel (Buseet al. 2010) and for *Pseudotsuga menziesii* in Germany (Müller et al. 2015). A recent study, however, carried out in the temperate forests of northern Italy (Della Rocca et al. 2016), demonstrated that the black locust dead wood, as well as that with oak, hosts a wide range of saproxylic beetles and thus, acts as a surrogate for native deadwood for saproxylic beetles. Some studies supported the hypothesis that the use of exotic plants by native insects increases with extension and time since their introduction (Brändleet al. 2008; Branco et al. 2015). Thus, considering the wide distribution of the black locust and its presence over a long period
in the north Italian forests, it is likely that the stag beetle could use this exotic species similarly to other native species, such as oaks.

It was found that the black locust forest can contribute to stag beetle occurrence if its coverage does not exceed 70% of the landscape. This means that the remaining 30% should be represented by other tree species or forest types, especially oak trees and broad-leaved forests. It is possible that, despite the extensive spread of invasive forests, the occurrence of the stag beetle is assured by suitable natives tree species that remain in a small proportion scattered in the invaded area. This occurred in northern Spain, where the stag beetle was found in a Eucalyptus plantation due to the presence of very old chestnut trees remaining within those plantations (Marco Mendez *personal comm*).

These results showed that the stag beetle remains within the proximity of urban settlements and is positively affected by the presence of roads although it does not seem to be as anthropophilic as in other European countries such as Belgium or Great Britain (Thomaes et al. 2008, Harvey et al. 2011). As suggested by Thomaes et al. (2008), the thermophilous character of the species (Napier 2003) could drive it to the warmer microclimate of cities. Thus, since the Italian mean summer temperatures are considerably higher than those of central and northern Europe (5th assessment of the Intergovernmental Panel for Climate Change, IPCC AR5WG1 2013; http://www.ipcc.ch), the species remains distant from urban centres and only uses open human infrastructure, such as roads, to facilitate flight and to warm themselves before flight (Harvey et al. 2011).

Considering landscape connectivity, the stag beetle was directly related to the high coverage of woodlands. However, oak forests did not seem to be as important for the species during movement and dispersion compared to other forest types. This phenomenon is probably due to the difference that naturally exists between the habitats used for dispersion and those used for reproduction, as has already been reported for other species (Blàzquez-Cabrera et al. 2016). Actually, while the role of oaks as primary resources for the reproduction of the stag beetle is widely recognised (Thomaes et al. 2008), it is less critical for its dispersion.

Finally, human settlements (both sparse and dense) were the main barriers for movement and dispersion of the stag beetle, while open habitat, especially grasslands and roads, represented important components for the species movement.

Management implications

A successful management policy for the protection of threatened animal species in human-dominated landscapes should take into account strategies for ensuring the persistence of good-quality habitats and landscape connectivity (Rudnick et al. 2012). In fact, for those forest-dependent species which barely survive in fragmented landscapes dominated by human settlements and intensive agriculture, it is fundamental to plan precise and effective management actions for ensuring suitable breeding sites and minimising costs and efforts (Laycock et al. 2009). This is the case for stag beetles living in the highly urbanised Po Plain of northern Italy. According to a recent report on the distribution and conservation status of species and habitats of Community Interest in Italy (Genovesiet al. 2014), for almost all saproxylic species, including the stag beetle, the main pressure factors are represented by forestry and silvicultural practices, such as removal of dead wood and stumps for economic and civic purposes and felling old-growth trees in both forests and urban parks. Due to the development of ecological corridors for the stag beetles in this study area, sites were identified along the corridors to maintain ancient woods, forest remnants, hedgerows and old deciduous trees, represented by both native and exotic species. In between this network of strict conservation areas, tree retention and islands of leftover parts of trees and snags after harvest (Heikkala et al. 2014; Vandekerkhove et al. 2013) can be implemented. In this way, larval habitat, represented by deadwood on the ground or rotting stumps, is provided and the survival of stag beetle populations is assured (Van der Sluijs et al. 2004).

These findings also showed that black locust deadwood can be considered in forest management operations aimed at restoring habitats for the reproduction of the stag beetles and which can serve as a temporary food source for the larvae. However, in light of a conservation strategy for this saproxylic beetle, it is essential to ensure the presence of other forest types, especially mixed broadleaved forests and to use black locust only in combination with oak deadwood.

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Supplementary material I

Additional information

Authors: Francesca Della Rocca, Giuseppe Bogliani, Pietro Milanesi

- Data type: species data
- Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

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RESEARCH ARTICLE



Monitoring of saproxylic beetles in Croatia: following the path of the stag beetle

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Abstract

As a member of the European Union, Croatia is obliged to report on the conservation status of 220 animal non-bird species listed in the annexes of the Habitats Directive (92/43/EEC), for which purpose a monitoring system is being established. Concerning saproxylic beetles, seven species present in its territory have to be monitored: Lucanus cervus, Cerambyx cerdo, Morimus funereus, Rhysodes sulcatus, Cucujus cinnaberinus, Rosalia alpina and Osmoderma eremita complex. Out of these species, a monitoring programme has only been established for Lucanus cervus, which partially includes participation of non-experts. In 2015 and 2016, a public campaign was organised in order to collect observations of *Lucanus cervus* and two other saproxylic beetles that are easily recognisable by the public: Morimus funereus and Rosalia alpina. Data gathered through this campaign serve as an addition to the mapping activities and monitoring of the species' range. So far, more than 650 citizen observations have been collected, providing data on species presence in 216 10×10 km² grid cells intended for reporting on the species' range. Besides the public campaign, since 2014, public institutions for managing nature protected values have been involved in population monitoring for which they received education through several workshops. Altogether, 21 sites have been included in the monitoring of the stag beetle so far. Data collected for Lucanus cervus on standard transects, by tree and ground pitfall traps and tree trunk surveys at night will be discussed. To the present time, eight public institutions have been involved in stag beetle population monitoring and the number has been continuously increasing.

Keywords

citizen science data (CSD), *Lucanus cervus*, Habitats Directive, habitat suitability model, monitoring, reporting, *Morimus funereus, Rosalia alpina*, species distribution range, observation on transects

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Introduction

As the most recent member of the European Union, the Republic of Croatia, joining in 2013, has an obligation, according to Article 17 of the Habitats Directive (92/43/ EEC) and Article 12 of the Birds Directive (2009/147/EC), to report on the conservation status of the species and habitats listed in the annexes of the directives by 2019. The conservation status assessment has to be undertaken for seven saproxylic beetles, *Lucanus cervus, Cerambyx cerdo, Morimus funereus, Rhysodes sulcatus, Cucujus cinnaberinus, Rosalia alpina* and *Osmoderma eremita* complex which are present in its territory and listed in the annexes of the Habitats Directive.

Of the seven saproxylic beetles, to date a monitoring programme at the national level has only been developed for the stag beetle (Šerić Jelaska 2013). It was compiled during the pre-accession period, through the European Union's Instrument for Pre-Accession Assistance (IPA) Programme "Natura 2000 Management and Monitoring – NATURA MANOMN" (EuropeAid/129747/D/SER/HR) in 2012, following the experiences of other EU countries (Harvey et al. 2011a, b, Alvarez and Alvarez 1995 etc.), especially of neighbouring Slovenia and Italy (Campanaro et al. 2012, Vrezec et al. 2012).

However, Croatia has its own specificities concerning geographical position, geology, forest management practice, as well as socio-economic and political history. The country spreads across three biogeographical regions: Mediterranean, Continental and Alpine and the stag beetle has existed in all three of them (Harvey et al. 2011a, Šerić Jelaska 2013). Forests and woodlands cover around 40% of the land area (Oikon Ltd 2004) and the majority is represented by natural forests. Virgin forests without logging activities are spread over 1400 ha comprising 4.4% of the total forested area. Most of the forests are national property, managed by the state owned company, Croatian Forests Ltd. Two management methods have dominated: selective logging for mixed aged stands in beech-fir mixed forests and regular logging in other forests types (Matić 2009).

Before mapping and monitoring activities connected to the Habitats Directive, no other systematic surveillance for the stag beetle has been conducted in Croatia (Harvey et al. 2011a). Historic data on species presence in Croatia, taken from museum collections in the country and abroad (Croatian Natural History Museums in Zagreb, Split and Rijeka, Hungarian Natural History Museum, Royal Belgian Institute of Natural Sciences, Faculty of Science and Faculty of Forestry at the University of Zagreb), relevant literature (e.g. Depoli 1926–1940, Koča 1900, 1905, Novak 1952, 1970) and also some latest observations made by biologists and foresters, were summarised and published in a paper by Harvey et al. (2011a). These data represented the baseline for formulating the national monitoring programme for the stag beetle.

In the European Union, several non-invasive monitoring methods have been developed and tested over the last 20 years such as trapping of adults (with or without lures), counting living adults during transect walks (flying or on the ground), surveys of tree trunks, mapping adults by citizens, counting road kill individuals and predation remains etc. (e.g. Alvarez and Alvarez 1995, Campanaro et al. 2012 and 2016, Chiari et al. 2014, Harvey et al. 2011a, b, Mason et al. 2015, Vrezec et al. 2012). Most of these methods are suitable for non-experts, relatively inexpensive and non-invasive, but still have some weaknesses when it comes to application and standardisation (Campanaro et al. 2016, Harvey et al. 2011b, Mason et al. 2015, Zapponi et al. 2017). Different practices within EU countries and lack of standardised protocols may impede the conservation actions of this species and also many other threatened species at the European level (Campanaro et al. 2016).

Due to a lack of experts, vis-à-vis the need for a large input when the Habitats Directive requirements are applied, it was decided to gradually include non-experts as far as possible in order to have financially feasible and scientifically relevant long-term surveillance of the stag beetle across three biogeographical regions. For the first time, the results have been presented on a systematic survey by non-experts from public institutions for management of nature protected values (national parks, nature parks, county institutions etc.) in monitoring stag beetle populations following the national monitoring programme; and unsystematic data gathering by citizens as a part of the surveillance that provided additional sources of data for mapping and monitoring of the range of the stag beetles. In addition, data gathering by citizens were organised for *Morimus funereus* and *Rosalia alpine*, that are among largest European saproxylic coleopterans and easily recognisable. After four years of surveillance, the efficiency of the stag beetle collecting methods and the potential of non-experts were discussed, as well as the problems which were faced during monitoring and mapping activities.

Material and methods

Methodology according to the national monitoring programme (Šerić Jelaska, 2013)

According to the Croatian national monitoring programme for the stag beetle, monitoring of the conservation status consists of (i) monitoring of population size and structure on plots, utilising three data sampling methods, counts on evening transects, survey of tree trunks and tree pitfall traps with lures; and of (ii) systematic range mapping, accompanied by non-systematic data gathering on the species distribution. The methodology was suggested as described below.

Monitoring on transects should be conducted along forest roads or walking paths inside the forest or at the forest edge during favourable weather conditions (e.g. without rain) in June/July period when it is the seasonal peak of adults' activity (Harvey et al. 2011a, Vrezec 2008, Vrezec et al. 2009). Transects should be undertaken in the evenings (between ca. 8 pm and 9 pm) by one person slowly and continuously walking along a transect 100 to 1000 m long, counting flying adults and those on the ground. Evening transects should be applied in 10 successive days in two repetitions, between the middle of June and the middle of July. The methodology was adjusted according to Vrezec et al. (2009, 2012).

The night survey of one to ten tree trunks should be conducted during warm and dry evenings, immediately after the evening transect. Tree trunks should be surveyed in

ten successive days in two repetitions, preferably one in the second half of June and one in first half of July. This method was suggested by Vrezec et al. (2012) for monitoring stag beetle populations based on aggregation of stag beetles on live standing tree trunks where they were present mainly for feeding.

The tree pitfall traps should be made of plastic bottles (2 litres) cut off to two thirds of their height, placing the upper part inside the lower part and thus making a plastic pot with a funnel. The opening should be broad enough for the stag beetle to fall into the trap. Three to ten traps should be placed per site, attached to a trunk of live standing tree, at 1.5–2 m of tree height. The traps should be filled with bait, e.g. fruit, rum with sugar or grated fresh ginger root (Harvey et al. 2011b). As with previous methods, traps should be checked every day in 10 successive days in two repetitions, preferably one in June and one in July.

The efficacy of these three sampling methods, proposed by the monitoring programme, was tested in the Continental region at two locations, Mt. Medvednica and Maksimir Park in Zagreb town, during June/July in 2013 and in the Mediterranean region, at Mt. Učka and Krk Island locations, in 2014, where the presence of the stag beetle had already been confirmed.

Involvement of non-experts in monitoring of conservation status

As mentioned above, due to the lack of experts, it was concluded that involvement of non-experts in monitoring the conservation status was required to fulfil the reporting obligations. As a part of the nature protection sector is already directly present in the field, employees of the public institutions (PIs) for management of nature protected values were recognised as prized field researchers who could facilitate the monitoring activities. In order to stimulate and educate these potential associates, in summer 2015, the Croatian Agency for the Environment and Nature Protection (CAEN), within the EU Natura 2000 Integration Project - NIP (IBRD 8021-HR), organised two workshops for the implementation of the monitoring of stag beetle population. The workshops consisted of theoretical and practical parts. During the theoretical part, participants were educated on saproxylic beetle ecological functions, threat status and conservation measures, stag beetle biology and finally on monitoring methodology. The practical part consisted of field visits to stag beetle habitats where all three methods were demonstrated and tested by the participants themselves. In total, 29 employees from 17 PIs participated at the workshops.

Besides population monitoring, all PIs were encouraged every season to record stag beetle observations in order to facilitate species mapping and range monitoring. For the same purpose, in spring 2015 and spring 2016, CAEN initiated a broad citizen science campaign for collecting observations of stag beetle and other two larger and easily recognisable species *Morimus funereus* and *Rosalia alpina*. The campaign was announced via the CAEN website, websites and mailing lists of several biological civil society organisations, most popular social networks and media. As well as data on observation locality and date, photographic evidence was requested for species identity verification. Citizen involvement was encouraged by rewarding every hundredth record and most attractive species photograph with the set of red books of wild species of Croatia. All reported observations were published and credited on the Bioportal - geoportal of the Croatian Nature Protection Information system (CAEN 2017).

Implementation of the monitoring programme

As a result of previous actions aimed at encouraging PIs to participate in monitoring and mapping activities, eight PIs joined the monitoring activities within the next two years and conducted the monitoring on an additional six locations in the Continental biogeographical region.

At each location, one to two sites were selected (Table 1). Study sites were placed in natural and urban forests and forest edges, within protected areas and outside. The principal tree species at each location are listed in Table 1. Study sites were scattered from eastern to western Croatia, including lowland areas, mountains and one island.

Altogether, 21 transects at lengths between 100–700 m were set along forest roads or walking paths inside forests or at forest edges. The survey was conducted in the evenings between 7 pm and 9 pm by one person spending 30 minutes of slow continuous walking along the transect and counting flying adults and those on the ground. Evening transects were applied in successive 9 to 20 days when weather conditions were favourable. The relative abundance in Table 2 represented the number of beetles counted on a transect per 100 metres per day at each location.

In addition, 4 to 12 traps were employed at each site and each trap was attached to the trunk of a live tree. The traps were filled with three different baits: fruit (peach and banana mixture), rum with sugar (only the first year, Vrezec et al. 2012), red wine-with vanilla flavoured sugar and grated fresh ginger root (Harvey et al. 2011b). Traps were set for a period of 10 to 20 days. During the field study, traps were checked every to every second day prior to counting on transects.

Although it was not suggested by the national programme, ground pitfall traps were also used at three sites (Table 2). The ground pitfall traps were formed by 0.5 litre plastic pots, buried in the soil, with an opening at the soil surface level. They were filled with the same bait as tree traps. At each sampling site, traps were set randomly and approximately 10 metres apart. They were exposed for 19 and 20 days. The relative abundance was calculated as average number of individuals caught per one trap per night at each location (one trap night is the catch of one trap in one night).

The survey of tree trunks was carried out between 8 p.m. and 9.30 p.m. in warm and dry nights. Ten to twenty trunks were randomly surveyed per site per day using an electric torch.

The relative abundance was calculated as the average number of individuals observed per surveyed trunk per day.

Monitoring of adult beetles was undertaken from 2013 to 2016 in period between 15 June and 19 July.

Biogeographical	Location	Public	No.	Voor	Habitat	Main tree enosies at location	
region	Location	Institution	of sites	Iear	type	Main tree species at location	
	Mt.	Medvednica	1	2016	Natural	Quercus petraea, Fagus sylvatica,	
	Medvednica	Nature Park	1	2016	forest	Robinia pseudoacacia	
			1	2015	Natural	Quercus petraea, Fagus sylvatica,	
			1	201)	forest	Robinia pseudoacacia	
					Natural	Quercus petraea, Robinia	
			2*	2013	Contraction	pseudoacacia, Fagus sylvatica,	
					Torest	Picea abies	
	Zagrah	Malainin Darla	2	2012	Urban	Quercus petraea, Q. robur,	
Continental	Zagreb	IVIAKSIIIIII FAIK	2	2015	forest	Q. cerrris	
	Nedelišće	Međimurje	1	2016	Forest	Quarrans on Cartinaus hatula	
	INCUCIISCE	Nature	1	2010	edge	Quercus sp., Curpinus beium	
	Radoboi	Krapina-Zagorje	1	2016		Quercus betraed	
	Radoboj	County	1	2010		Quercus perineu	
		Žumberak-				Quercus cerris, Fagus sylvatica,	
	Mt Žumberak	Samoborskogorie	2	2016		Carpinus betula, Populus	
	Witt. Zumberak	Nature Park	2	2010		alba, Acer pseudoplatnaus,	
		I VALUIC I AIK				Betula pendula, Prunus sp.	
	Slavonski Brod	Natura Slavonica,			Forest	Fagus sylvatica, Quercus petraea,	
	Mladavodica	Brod-Posavina	1	2016	edge	Carpinus betula, Corylus avellana,	
	Iviladavõdica	County		euge		Salix alba, Populus sp., Fraxinus sp.	
	Spačara	Vukovar-Srijem	2	2015	Natural	Quercus robur Cartinus betula	
	Spacva	County	2	2017	forest	Quireas iooni, Curpinus betuu	
	Spačya	Vukovar-Srijem	2	2014	Natural	Quercus robur, Carpinus betula	
	opaeva	County	2	2011	forest		
	Mt Papuk	Papuk Nature	2 2015		Natural	Quercus petraea, Fagus sylvatica,	
	- The Pupuk	Park			forest	Prunus sp.	
	Mt. Učka*	Faculty of	2	2014	Forest	Quercus pubescent, Ostrya	
Mediterranean		Science			edge	carpinifolia, Carpinus orientalis	
	Krk Island*	Faculty of	2	2014	Natural	Quercus ilex, Q. pubescens,	
	KIK Island	Science	-		forest	Carpinus orientalis	

Table 1. Characteristics of study areas, PIs that were included and the year when monitoring was undertaken.

*Sites that were used for testing methods proposed by the monitoring programme (Šerić Jelaska 2013)

Data analyses

For comparisons of sampling data and methods efficiency, non-parametric statistical tests, χ^2 and Kruskal-Wallis were used. In addition, cumulative average number of individual species recorded per day at each transect was calculated to reveal how many consecutive days were needed before the average number of observed specimens per day stabilised on the transect (i.e. reduction of variation).

Spatial analyses of the results were made in ArcGIS 10.1. (ESRI 2012).

To estimate the efficiency of the citizen science campaign with respect to spatial coverage of data on the stag beetle, habitat suitability maps have been produced for the stag beetle using Maxent (Phillips et al. 2006). All data collected were geo-referenced with assigned values of their spatial uncertainty. In this analysis, only those data with spatial precision being equal to circle with 2000 metres radius, or smaller, have been

at two locations in Spačva and Slavonski Brod area) in Croatia. Number of observations/individuals (No. obs. and No. ind.) and relative abundance for used methods Table 2. The results of simultaneous observations of stag beetles during 2013-2016 using three methods at 21 sites (and ground pitfall traps as an additional method at each location representing sampling effort are presented.

		F	No. tree	No.	Traps	Evening 1	transects	Ħ	ree traps	Gro	und traps	Nig ¹ of	ıt survey trunks
Location/Year	No. transects/ sites (length, m)	Iransect evenings	traps per site	ground traps per site	exposure days	No. obs	No.obs/ 100 m * day	No. ind	No.ind/ one trap*day	No. ind	No. ind/ one trap*day	No. ind	No.ind/ night*site
Mt. Medvednica/2016	1 (700 m)	6	10	-	19	13	0.21	~	0.037	0		0	0.000
Mt. Medvednica/2015	1 (700 m)	6	10	-	17	19	0.30	54	0.318	0	-	0	0.000
Mt. Medvednica/2013	2 (100 m; 100 m)	20	10	-	20	160	2.00	14	0.018	0	/	4	0.050
Zagreb, Maksimir Park/2013	2 (100 m; 100 m)	20	10	-	20	122	1.53	2	0.003	0	/	4	0.050
Međimurje, Nedelišće/2016	1 (350 m)	20	10	-	20	683	9.76	140	0.700	0	/	15	0.750
Zagorje, Radoboj/2016	1 (340 m)	14	12	-	16	270	5.67	9	0.031	0	/	ŝ	0.214
Mt. Žumberak/2016	2 (390 m; 900 m)	11	4	1	17	39	0.14	0	0.000	0	/	25	0.568
SlavonskiBrod, Mladavodica/2016	1 (100 m)	14	12	12	20	43	3.07	14	0.058	7	0.029	3	0.214
Spačva (Vukovar-Srijem County)/2015	2 (200 m; 200 m)	19	12	12	19	137	1.14	19	0.021	0	0.000	20	0.333
Spačva (Vukovar-Srijem County)/2014	2 (200 m; 200 m)	16	12	12	20	50	0.39	1	0.001	2	0.002	3	0.047
Mt. Papuk/2015	2 (100 m; 100 m)	15	12	1	10	21	0.53	11	0.023	0	/	4	0.100
Mt. Učka/2014	2 (200 m; 200 m)	17	12	1	22	119	0.88	12	0.011	0	/	7	0.103
Krk Island/2014	2 (200 m; 200 m)	10	12	1	15	187	1.56	16	0.022	0	/	26	0.433

used, resulting in 449 input data, out of which as many as 392 had their precision below 1000 metres radius. As descriptors (i.e. environmental variables), two independent sets have been used, one consisting of anthropogenic-related variables (distance from settlements, distance from roads and interpolated human population density), the second representing climatic and topographical conditions (seasonal amount of precipitation and mean temperature, slope and aspect). Due to the circular nature of aspect values (e.g. 1° and 359° representing an almost identical aspect), the sine and cosine of aspect values were used, transforming them into two variables ranging from -1 to 1, representing an inclination from north (cosine) and from east (sine) as in, for example Guisan et al. (1999) and Jelaska et al. (2003). The resulting Maxent maps, with their logistic outputs (i.e. values ranging from 0 to 1) were reclassified to binary (0-non-suitable and 1-suitable habitat) grids using two threshold values. In the first step, a conservative 0.5 value was used as a threshold based on which every pixel of Maxent grid is classified into non-suitable (0) or suitable (1) habitat. Given the fact that the stag beetle is a species of special concern, in the second step a less strict rule was used and a threshold value that contained 80% of input data was selected. The latter resulted in using 0.336 as a threshold for the Maxent model developed using human population oriented variables and 0.414 for the Maxent model based on climatic and topographic variables. Thereafter, the above described two pairs of reclassified Maxent models were spatially overlapped, (one based on 0.5 threshold value and one with customised threshold values that included 80% of input data) and percentages of overlapping and unique areas of suitable habitats for the stag beetle were calculated.

Results

Population monitoring

Overall, there were 2282 records of stag beetle adults after monitoring activities on 21 sites within ten locations, sampled during June/July from 2013 to 2016. Of the 21 sites, four were located within the Mediterranean biogeographical region and the rest in Continental Croatia. There was no systematic surveillance conducted in the Alpine region during this period. Such differences in the number of sites in each region were the consequence of the lower response from the public institutions of Alpine and Mediterranean regions to the workshops and monitoring activities.

Amongst three methods applied on all sites, the evening transect was the most efficient in detecting the species presence (Figure 1) with 1863 observations making 81.6% of the total record. Tree traps had 296 records (13% of the total record) but the lowest detection efficiency (at 16 of 21 sites making 76.2%, Figure 1) and the night survey of tree trunks had 114 records (5%) with detection efficiency at 18 sites. Kruskal-Wallis confirmed significant differences between those three methods (H=19.9, p<0.05). Only eight records (0.04 % of total records) using ground pitfall traps, applied at three locations, were obtained (Table 2).



Figure 1. Stag beetle detection efficiency using three sampling methods presented as a proportion of sites with recorded individuals (N sites = 21). The differences were not significant ($\chi^2 = 0.3658$, p<0.05).



Figure 2. Cumulative average number of individual beetles observed on each transect daily **a** in Eastern Continental Croatia **b** Mediterranean region **c**, **d** in Northern Continental Croatia.

Furthermore, all tested methods were sex biased, with the detection of significantly more males than females ($\chi^2 = 13.0384$, *p*-value =0.004554, *p*< 0.05). The lowest proportion of males, 80.7%, for the survey of trunks, 81.7% at counts on evening transects, 88.9% in ground pitfall traps and 90.2%.in tree pitfall traps was found.

Twenty days of evening transects, in two sequences of 10 days each as suggested by the monitoring programme, were achieved at five sites and observations at the other 16 sites were undertaken within 9 to 17 days. During that period, in eastern continental Croatia,



Figure 3. Observation of three saproxylic beetle species reported to the CEAN during the citizen science campaign.

in Spačva T1 2015, it can be clearly seen that, after 11 days, the average number stabilised at ca. 3.5 observed individuals on the transect and at the Mlade Vodice 2016 transect, the average number of individuals at ca. 3.5 was reached after 10 days of transect walk (Figure 2a). In northern continental Croatia (Figures 2c and d), the average number of observed individuals on most of the transects stabilised between 11 and 14 days and in the Mediterranean region, the peak of activity was caught at Krk T2 2014 transect, where the number of observations stabilised at ca. 2.5 individuals between 11 and 14 days (Figure 2b).

Citizen science campaign

Altogether 640 observations of the three saproxylic beetle species were reported to the CEAN by the public during the citizen science campaign, the majority being of stag



Figure 4. Share of observations per species per biogeographical region in the total number of observations reported to CEAN during the citizen science campaign.



Figure 5. Number of 10×10 km² grid cells with confirmed presence of three saproxylic beetle species, based on observation reported to the CEAN during the citizen science campaign.

beetle (555 observations, 87%), then 45 observation of *Morimus funereus* (7%) and 40 of *Rosalia alpina* (6%).

Observations were reported from all three biogeographical regions in Croatia, with the majority from the Continental biogeographical region (Figure 3 and Figure 4).

In total, citizen observations confirmed the presence of these three species in 216 10×10 km² grid cells, intended for reporting on species' range, out of which the presence of the stag beetle was confirmed in 204 grid cells (Figure 5). Comparing the distribution data of the stag beetle in Croatia given by Harvey et al. (2011a) and the data



Figure 6. Overlap of Maxent habitat suitability derived maps based on human population orientated variables (Human) and those representing climatic and relief condition (TopoClim). **A** maps produced using the 0.5 threshold value for reclassifying Maxent output grids **B** maps produced using the custom threshold value for reclassifying Maxent output grids to include 80% of input data. (For more details, see Methods section).



Figure 7. Venn's diagram of percentages of suitable habitats identified by Maxent derived models based on human population orientated variables (Human) and those describing climatic and relief conditions (ClimTopo). **A** areas obtained using the 0.5 threshold value for reclassifying Maxent output grids **B** areas obtained using the custom threshold value for reclassifying Maxent output grids to include 80% of input data. (For more details, see Methods section).

collected through this citizen science campaign, the stag beetle presence is recorded in 100 new 10×10 km² grid cells. Areas where stag beetle presence was left unconfirmed, when comparing to data from Harvey et al. (2011a), are mostly in the Mediterranean biogeographical region and could be attributed to the lower response from citizens.

Analyses of habitat suitability maps derived by Maxent using two sets of descriptors are shown in Figure 6 where obvious differences can be seen in the spatial arrangement of suitable habitats depending on whether model was derived based on human population oriented variables or those representing climatic and relief conditions.

The percentages of suitable habitats for the stag beetle identified by the Maxent model based on human population oriented variables, based on variables describing climatic and relief conditions and those where both models were congruent, are shown in Figure 7.

Discussion

Stag beetle population monitoring

Of the three methods proposed by the Croatian stag beetle monitoring programme (Šerić Jelaska 2013), the standard transect walk proved to be the most efficient, with the stag beetle recorded at all sites in each year and with the highest number of stag beetle observations. All three methods were biased towards males, but the night survey of trunks yielded the lowest proportion of males. The results on detection efficiency are in line with data in Vrezec et al. (2012) using the same methods. These very first data, collected from 2013 to 2016, during methodology testing activities and by involving PIs in population monitoring, indicated that evening transects could be a trustworthy method, as this is also suggested by the latest results on the application of this method at a European scale (Campanaro et al. 2016) and is easy to conduct by non-experts. Of the 17 PIs who attended the workshops, only eight of them were involved in the mapping and monitoring activities. There is still room therefore for progress in encouraging PIs to join, especially PIs from the Mediterranean region since they have not been involved so far. The evening transect, although not technically demanding, may still appear unattractive to employees of PIs as it may demand time after working hours. As the duration of transects varied from 9 to 20 evenings a year, it may be expected that the number of days on transects will be shorter in practice than the number proposed by the programme (twenty days). As suggested by Campanaro et al. (2016), the three weeks survey, including the period between the middle of June and the first week of July, has a high probability of detection of stag beetles. The same June/July period was included within the conducted monitoring activities in Croatia, resulting in high number of observations at all sites, but the proposed three to five weeks period could be hard to achieve by PI employees. However, the five-week period (Campanaro et al. 2016) should be proposed within the national programme to monitor the distribution range when species absence within the empty grid in certain areas needs to be confirmed. For population monitoring on transects, according to the data presented in Figure 2, it seems that minimum number of nights on transects should not be less than 11 and there is no need to exceed 20 days within the favourable June/July period as proposed by the monitoring programme (Serić Jelaska 2013). Even in the Mediterranean region where the monitoring was started earlier than in other areas (Table 2), the peak of activity was reached and the number of observations were stabilised on Krk

T2 transect after 11 days (Figure 2b), while on Krk T1, it seems that the trend started to stabilise a few days later, with a slightly higher number of observations. On Mt. Učka, monitoring of stag beetles' activity had obviously started too early, as can best be seen from the first eight days without any individual observed on transect Učka T2, while, afterwards, the number increases continuously. In northern continental Croatia (Figure 2d), the average number of observed individuals at two transects (Žumberak T1 and T2) was very low and it is surmised that the research probably missed the seasonal peak of their activity.

The lower detection efficiency of tree pitfall traps and trunk surveys and lower number of recorded individual species might raise the question of justification for their application. Trunk surveys proved to be more sensitive to females than the other two methods and pitfall traps in comparison with evening transects and trunk surveys can be set and checked during the working hours of PIs. Therefore, the pitfall trap method with appropriate bait can still be utilised when night surveys are not an option.

Citizen science campaign

Collecting observations of the saproxylic beetle species through a citizen science campaign in Croatia proved that involvement of the public in mapping of these charismatic and easily recognisable species can make a considerable contribution to the knowledge of their distribution. The same conclusion is given by Zapponi et al. (2017) in their study which analysed the distribution data, amongst others on the same three species, collected by the citizen science programme organised in Italy. The number of citizen observations of *Lucanus cervus, Morimus funereus* and *Rosalia alpina* collected during the campaign in Italy is very similar (607) to the number of observations collected during the campaign in Croatia, but with a higher share of *Morimus funereus* (39%) and *Rosalia alpina* (11%) in the total number of observations. As for this study, Zapponi et al. (2017) found that photographic documentation accompanying the observation data is sufficient to validate species identification and thus should be an obligatory part of the observation report.

Spatial analyses of data collected through citizen science campaign (Figure 6) confirmed that those data could be spatially biased towards settlements and roads, leaving a significant portion of unexplored habitats for the stag beetle, hence without data on the status of species of interest in those areas. Even though only citizen science collected data were used (thus closer to settlements and roads) for developing the Maxent model based on variables describing climatic and relief conditions, significant areas were designated uniquely by this model as suitable for the stag beetle (Figure 7, 28% and 51%). As these areas are further from settlements, the citizen science campaign to ensure data in those areas cannot be expected in the future. Hence additional efforts for monitoring of the stag beetle should be focused in those areas, mostly by experts and PIs. Usage of two threshold values for classification into binary suitable/non suitable areas for stag beetles resulted in differences in absolute areas, as well as those in percentages of unique and shared contributions (Figure 7) of used environmental variables (e.g. Humans vs. TopoClim) to predict suitable habitats. Although it is out of the scope of this paper, the importance of threshold selection has been discussed by a number of authors (e.g. Liu et al. 2005; Phillips et al. 2006; Li and Guo 2013; Liu et al. 2016) hence it should be mentioned here and treated with due attention in the future.

Conclusions

Based on the four years' experience in establishing a monitoring system for saproxylic beetles, it is concluded that, with appropriate education, non-experts can be successfully involved in population monitoring of these species, thus compensating for the lack of experts as well as reducing financial costs for the monitoring system. Although citizen science programmes, with accompanying media campaigns, can also considerably contribute to the mapping activities of easily recognisable and charismatic species, they cannot substitute targeted mapping projects due to the observation bias to urban areas.

Author contributions

LSJ and LK developed the initial concept of the paper. LSJ, LK and SDJ wrote the manuscript together. LSJ was involved in population monitoring activities and data analyses; LK was involved in contacting PIs, providing citizen science observations and data analyses and SDJ performed the spatial analyses of presented data and contributed to the initial concept of this paper.

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RESEARCH ARTICLE



Latitudinal cline in weapon allometry and phenology of the European stag beetle

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Abstract

Animal body size commonly exhibits a remarkable variation in response to environmental conditions. Latitude, when correlated with temperature, rainfall and seasonality, represents one of the main determinants of variation in body size, as well as in allometry. It has long been recognised that populations of larger body size are found in colder environments (Bergmann's Rule), a cornerstone of evolutionary ecology. However, the way in which latitude might influence investment in exaggerated weapons of animals has received little attention. The European stag beetle *Lucanus cervus* (Linnaeus, 1758) is the focus of this study. Males of this species exhibit exaggerated mandibles, mainly used as weapons during intra-sexual conflicts. Five populations ranging from northern Italy to the southern limit of the distribution of *L. cervus* were analysed. Combining morphological and phenological data, latitudinal variation in body size, weapon investment and activity period of the adults were evaluated. The analysis of the allometry of mandibles strongly supported the presence of two male morphs. Large males (major morph) invest significantly more in weapons compared to males of the minor morph. Consistent with Bergmann's Rule, these results confirmed that

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the stag beetle body size increased at higher latitudes (N) and that this increase in size triggers an arms race which leads to further exaggeration of male weapons which is particularly evident in major males. In this morph, the mandible allometric coefficient line was steeper for the northern populations. The activity period also varied with latitude, beginning later at lower latitudes. Characterisation and comparison of adult phenologies provide valuable data to be used in the design of monitoring programmes for this threatened species and are important for modelling the species responses to climate change.

Keywords

morphometry, exaggerated traits, animal weapons, geographic variation, secondary sexual characters

Introduction

Variation in animal body size has always attracted considerable interest from biologists. Populations and species can exhibit remarkable variation in body size, as well as in other traits, in response to different environmental conditions (Stillwell 2010). One of the major reasons for variation in environment and ecology is variation in latitude, with corresponding variation in temperature, rainfall and seasonality. The latitudinal clines in body size, described for endotherms, have interested biologists since it was recognised by Bergmann (1848). Bergmann's Rule states that, within a widespread taxonomic group, populations, species and genera of larger body size are found in colder environments, usually located at higher latitudes, while the opposite is observed in warmer environments, usually situated at lower latitudes. In many organisms, individuals living in colder environments grow more slowly but attain larger size as adults (Angilletta and Dunham 2003). Latitude also correlates with resources availability which influences the development time of ectotherms (Arnett and Gotelli 1999). First recognised and used with endothermic animals, Bergmann's Rule has been investigated widely in ectothermic organisms (e.g., crustaceans (Timofeev 2001); insects (Cushman et al. 1993); fish (Belk and Houston 2002); amphibians (Adams and Church 2008, Ashton 2002); reptiles (Ashton and Feldman 2003)). Much debate has arisen regarding the formulation and definition of Bergmann's Rule and this continues to the present day. Does it deal with inter- or intraspecific differences and is it valid for ectothermic organisms (Shelomi 2012)? As ectotherms rely on external sources for body heat, environmental temperature is particularly important and often correlated with the difference in body size (Atkinson 1994). Bergmann's Rule concerning body size clines in ectotherms has given rise to some doubts about its validity and has led to heated debates (e.g., Van Voorhies 1996, 1997, Mousseau 1997). Indeed, a general consensus has not been reached to support the Bergmann's Rule for ectotherms: some authors report the presence of the converse-Bergmann (e.g., Schutze and Clarke 2008), others have stated that Bergmann's Rule cannot be considered a valid eco-geographical law for insects (Shelomi 2012), or Bergmann and converse-Bergmann could not even be mutually exclusive, being driven by different environmental causes (Blanckenhorn and Demont 2004). Using common garden experiments, several studies on insects (Anderson 1973, Cavicchi et al. 1985, 1989, Partridge et al. 1994) proved that body

size increases in low-temperature conditions and that selection favours larger body size at low temperatures (McCabe and Partridge 1997, Reeve et al. 2000). Furthermore, several studies which compared geographical variation and biometric traits of insects, have recorded a latitudinal correlation with body size (Capy et al. 1993, Cushman et al. 1993, Arnett and Gotelli 1999, Huey et al. 2000, Stillwell et al. 2007). Recently, in studying the exaggerated rostrum of the New Zealand giraffe weevil Lasiorhynchus barbicornis (Fabricius, 1775), Painting et al. (2014) linked the latitudinal cline in body size with allometry (sensu Huxley and Teissier 1936). The European stag beetle, Lucanus cervus (Linnaeus, 1758), like many other species of Lucanidae, shows remarkable sexual dimorphism in mandible size and shape (Franciscolo 1997) and a great variation amongst males for this characteristic (Clark 1977, Harvey et al. 2011, Hardersen et al. 2011, Romiti et al. 2015, 2016). The mandibles of L. cervus males can be considered as secondary sexual characteristics (SSC) that are used as weapons in intra-sexual combats (Darwin 1871, Arrow 1951, Mathieu 1969, Otte and Staymen 1979, Lagarde et al. 2005). Knell et al. (2004) recognised two trajectories for the allometric relationship between mandible length and body size in different species of the genus Lucanus. Species with relatively short mandibles exhibit a steeper allometric coefficient (slope of the regression) as size increases; on the other hand, a limit for the development of such exaggerated structures is evident in species with relatively long mandibles. L. cervus males use their mandibles in male-to-male combat (Clark 1977, Lagarde et al. 2005, Fremlin 2009) and major males are equipped with enlarged and more complex mandibles (Romiti et al. 2015, 2016). The analysis of the geographical variation of allometric trajectories allows researchers to obtain more robust conclusions about the relative investment in these SSC. Size and shape of SSC are likely to be the result of sexual selection (West-Eberhard 1983, Andersson 1994, Jennions et al. 2001, Stuart-Fox and Ord 2004) and typically exhibit high levels of developmental plasticity and condition dependence (Cotton et al. 2004), probably as a result of changes in environmental and individual conditions (Cassidy et al. 2014) within and amongst populations. Consistent with Bergmann's Rule and considering L. cervus as a stag beetle species with relatively short mandibles (sensu Knell et al. 2004), it was expected that increasing the latitude would increase the allometric coefficient, as more resources would likely be allocated to the development of the weaponry of the male stag beetles. Romiti et al. (2016) showed that there was no significant variation in the shape of the SSC of the stag beetle male amongst populations at the same latitude in central Italy. To the best of the authors' knowledge, only Painting et al. (2014) have investigated the latitudinal allometric cline of SSC in a beetle species and its correlation with body size variation.

The ecology of ectotherms is particularly affected by latitude as temperature has a strong influence on their biology (Willmer et al. 2000, Sperry et al. 2010). *L. cervus* is a saproxylic beetle, its larvae feed on and grow in dead decaying wood, while the pupal stage and eclosion occur near to the larval development site (Harvey et al. 2011, Speight 1989, Alexander 2008, Nieto and Alexander 2010). Although insects living within tree trunks, roots or logs are usually less sensitive to the photoperiod, their life

cycle (at least during the wood-boring stage) is governed by temperature fluctuations (thermoperiod) (Riba 1976). The majority of insects show daily and annual cycles of activity (phenology) related to climatic change caused by the earth's movements (Saunders 2002). Understanding the phenology of the adult stag beetle is vital if effective monitoring protocols are to be developed, an urgent requirement especially for species of conservation concern. L. cervus is considered a flagship species for conservation of saproxylic fauna. This species is listed in the European Habitats Directive 92/43/EEC (Appendix II), in the IUCN red list of saproxylic beetles as Near Threatened at European level (Nieto and Alexander 2010) and as Least Concern in Italy (Carpaneto et al. 2015), due to its sensitivity to dead wood management and wood exploitation (Audisio et al. 2014). For this reason, several efforts have been made at European level to understand the phenology of the species (e.g., Vrezec 2008, Chiari et al. 2014) and to correlate the adult peak activity with geographical variables (Campanaro et al. 2016). Thus, morphological and phenological data have been combined (i) to assess whether there was a significant latitudinal variation in body size and weapon allometry in L. cervus males and (ii) to acquire information on adult stag beetle phenology across its Italian distribution.

Materials and methods

Sampling design and study areas

A total of 767 males of L. cervus were sampled during the breeding season between May and August in the summer 2012–2016. Captures were performed with an entomological net (\emptyset = 50 cm, telescopic handle = 2 m), by hand and with emergence traps placed on possible oviposition sites detected by means of radio-telemetry. Sampling activities were undertaken in five localities of Italy (Figure 1). These sites cover a latitudinal range of 2.57° which spans from the northernmost to the southernmost limit of the Italian distribution of the species. A total of 111 males were sampled in Monterosso (hereafter MON: 45.944568N, 8.524431E) from 2nd June to 12th July 2012. During two consecutive years, 2014 and 2015, 83 males were sampled in Bosco Fontana (hereafter BOF: 45.198434N, 10.747084E) from 29th May to 17th July 2014 and from 25th May to 4th July 2015. During three consecutive years, 2014-2016, 141 males were sampled in the Croara forest (hereafter CRO: 44.922222N, 9.575000E), from June to August (Scaccini and Anaclerio 2016). A total of 75 captures were performed in Tocerano (hereafter TOC: 43.440278N, 12.187222E) from 7th July to 4th August 2014. In Felceto (hereafter FEL: 43.376404N, 12.275146E), a total of 357 stag beetles were sampled during two consecutive years, 2014 and 2015, from July to August. Starting from the northernmost site, MON is a hill (690 m a.s.l.) characterised by an old-growth chestnut (Castanea sativa) woodland on the banks of Lake Maggiore; BOF is a relict forest of the Po plain (26m a.s.l.), dominated by common oak (Quercus robur)



Figure 1. Overview of the study sites in five localities in Italy. Square: Monterosso (MON), Piemonte Region; Circle: Bosco Fontana (BOF), Lombardia Region; Triangle: Croara (CRO), Emilia-Romagna Region; Cross: Tocerano (TOC), Umbria Region; Pentagon: Felceto (FEL), Umbria Region.

and hornbeam (*Carpinus betulus*), with some introduced species such as the common red oak (*Quercus rubra*); CRO is a relict floodplain forest along the Trebbia river (153m a.s.l.), dominated by oaks (e.g., *Q. petrea*, *Q. cerris*) and chestnut (*C. sativa*) alongside sub-mediterranean species (e.g., *Fraxinus ornus, Sorbus torminalis, Q. pubescens*); TOC is a wooded hill (660m a.s.l.) characterised by mixed-oak forest and some cultivated fields and FEL is a wide clearing within a wood of Turkey oak (*Q.cerris*) along the Tiber river (332m a.s.l.). Each captured individual was marked, measured and weighed before being released. Linear body measurements were taken with an electronic caliper (precision 0.05 mm) and body mass (BM) was measured using a dynamometer (PE-SOLA AG, Switzerland) (precision 10mg). BM was not measured for beetles sampled in CRO. Mandible length and elytron length were measured as described in Chiari et al. (2014), Romiti et al. (2015) and Scaccini et al. (2016). Mandible length (ML) was measured as the linear distance from clypeus to the apex of the outer tooth of the fork

and this can be considered a good proxy for the actual mandible size (Romiti et al. 2016). The elytron length (EL) was preferred to the most commonly used body length to avoid errors caused by the mobility of segments (Juliano 1986). After removing outliers, a total of 763 male stag beetles (pooled dataset) were obtained, divided as follows: 109 for MON, 82 for BOF, 140 for CRO, 75 for TOC and 357 for FEL.

Climate data

Climate data were obtained for the study sites from WorldClim – Global Climate Data site (http://www.worldclim.org/). Mean (MEA), minimum (MIN) and maximum (MAX) monthly temperatures (C°) and monthly total precipitations (PRE) (mm) of the study sites were downloaded according to their coordinates using the geographic coordinate system WGS84 (not projected) in decimal degrees. The data provided by the web site (section "Current") are interpolations of observed data, representative of fifty years (1950-2000) and thus represent monthly averages of the selected variables. Data were downloaded with the highest spatial resolution available: 30 seconds, which correspond to cells of 0.86 km² at the equator (often referred as 1-Km spatial resolution) (Hijmans et al. 2005).

Data analysis

All the biometric variables were natural log transformed (Ln) and checked for normal distribution, as well as the climate variables and phenology, using Shapiro-Wilk normality test, prior to applying any parametric test.

Climatic characterisation

To analyse the variance in climate variables between sites, ANOVA and Tukey HSD tests were performed. The correlation between latitude and climate variables was investigated with the Pearson correlation test. Two discriminant (or classification) techniques were used to categorise sites into groups taking into account their climate variables as predictor characteristics. Both the principal component analysis (PCA) and the linear discriminant analysis (LDA) were applied to the sites' climate. PCA tries to retain most of the variability in the data, whereas LDA (MASS R package) looks for the combination of the climate variables that give maximum separation between the centres of the site data, minimising the variation within each site. The data were transformed, centred and scaled (caret R package) prior to the application of LDA (Kuhn and Johnson 2013). The results were then compared via scatterplots and inspection of the explained variance by the LDA and PCA axes was undertaken.

Morphometry and allometry

For the allometric analysis of male mandibles, the elytron length was used as an independent variable, mainly for three reasons: (i) its proven high correlation coefficient with mandible length (Romiti et al. 2015); (ii) the comparability with previous morphometric studies which used linear distances for L. cervus (Harvey and Gange 2006, Hardersen et al. 2011, Chiari et al. 2014, Romiti et al. 2015, Scaccini et al. 2016) and (iii) to avoid scale dependence issues in comparing traits with different units (e.g., mm vs g) which could affect the data despite the log transformation (Warton et al. 2006). The pooled dataset was used to analyse the allometric relationship between the mandible length (LnML) and the elytron length (LnEL) of L. cervus males. The scaling relationship between these traits was analysed using the linear and segmented regression models (segmented R package, Muggeo 2003). The Davies test (Davies 1987) was used to check for a change in slope in the fitted linear model. Other non-linear regressions (e.g., sigmoidal, gaussian) were not undertaken according to Romiti et al. (2015) which demonstrated the goodness of fit for a segmented model in describing the allometric relationship between mandible and body size. The same models were used for the analysis of intraspecific allometry. The models were compared on the basis of their "goodness of fit" using Akaike's information criterion (AIC) and ANOVA to determine which model gave the best description of the scaling relationship. Models which differed by <2 in the AIC score were considered to be indistinguishable from each other in their explanatory power (Burnham and Anderson 2002). To test how this scaling relationship varies across populations the "SMA function" (SMATR R package, Warton et al. 2012) was used, considering the population as the variable factor. SMA uses standardised major axis (SMA) and major axis (MA) estimations to evaluate the line of best fit and the confidence intervals for both slope (allometric coefficient) and elevation (proportionality coefficient) of each population and to test for significant differences in these regression parameters. The null hypotheses were, respectively: slopes are equal, no difference in elevation. The allometric coefficients (slope of the regression) of minor males, often close to 1, were tested for isometry (H0: slope not different from 1). To investigate whether stag beetle males exhibit differences in BM (excluding Croara population), ML and EL amongst populations, the biometric rough variables were used and tested with the Kruskal-Wallis test and Dunn's test for multiple comparisons (with Bonferroni correction). A PERMANOVA using Euclidean distance matrices (n. perm. = 999) was performed comparing biometric variables amongst different latitudes.

Phenology

To investigate the phenology of the species in each site, it was considered that the adult activity period lasted from the first day of capture to the last one. For the sampling sites with more than a year's field work (i.e., for BOF, FEL and CRO), the first and last cap-

ture date were taken into account, irrespective of the year. In this way, the phenology was dependent on site, rather than be year-dependent. Dates were then transformed into a number in Microsoft Excel 2010, maintaining a fictitious year to avoid any transformation error. Otherwise, inputting the same date for the different years, for example 25/05/2015 and 25/05/2014, different numbers could have resulted i.e. 42149 and 41784 respectively. To test if the phenology varies with latitude, the Kruskal-Wallis test and the Mann-Whitney post-hoc test for pairwise comparison were used.

Results

Despite the Ln transformation of the biometric variables, some maintained a nonnormal distribution (MON_{N=109}: LnML, w = 0.972, p = 0.020; BOF_{N=82}: LnML, w = 0.958, p = 0.009; TOC_{N=75}: LnBM, w = 0.960, p = 0.019; LnEL, w = 0.964, p = 0.033; FEL_{N=357}: LnBM, w = 0.991, p = 0.021; LnML, w = 0.966, p < 0.01). Climate variables and phenological data were normally distributed (See Suppl. material 1: Table 1).

Climatic characterisation

Amongst the analysed climate variables, the monthly total precipitations showed a positive correlation with latitude (Pearson's test: r = 0.32, p = 0.013). The ANOVA results indicated no significant differences between sites as regard their monthly temperatures (MEA: DF = 4, f = 0.29, p = 0.88; MIN: DF =4, f = 0.32, p = 0.86; MAX: DF = 4, f = 0.290.28, p = 0.89), but a significant variation in the variance of the monthly total precipitations (PRE: DF = 4, f = 6.56, p< 0.01). The Tukey HSD test indicated a clear difference in the total monthly precipitations ($p_{\text{MONvsBOF}} < 0.01, p_{\text{MONvsCRO}} < 0.01, p_{\text{MONvsFEL}} < 0.01$, p_{MONvsTOC} < 0.01), with the highest values recorded in MON (See Suppl. material 2: Figure 1). The first 3 PCs of PCA (Figure 2a) explained the total variance between sites (Table 1a), considering all the climate variables. The first 3 linear discriminants of LDA (Figure 2b) explained the total variance of the sites (Table 1b). Moreover, as a result of the great difference between sites regarding their PRE (See Suppl. material 2: Figure 1, thermo-pluviometric diagrams), the posterior probabilities (pp) of LDA indicated that most interpolated climate data of MON were assigned with high certainty to MON (pp mean %: 68%); whereas some uncertainties remained in the assignment of their own interpolated climate data to the other sites (pp mean %: BOF = 10%; CRO = 11%, FEL = 10%; TOC = 1%).

Morphometry and allometry

The result for the pooled dataset regarding the scaling relationship between mandible length (LnML) and elytron length (LnEL), indicated the presence of a switch point at



Figure 2. Scatterplots for the variation of climate variables amongst the sites: **A** first two principal components of the PCA **B** first two linear discriminants of the LDA. The percentage of variance explained by each axis was reported in brackets.

22.19 mm of elytron length (± 1.01 mm) (Table 2A). Indeed, the best fit model was the segmented regression which statistically differed from the linear model, as confirmed by the Davies test (k = 1000, p < 0.001) and which highlighted a switch point at 3.10 of LnEL by the ANOVA and AIC scores comparison (Table 2A). The scatterplot in Figure3 illustrates the scaling relationship between LnML and LnEL for minor and major males, with the steepest allometric coefficient line for those belonging to the major morph. With regard to the intraspecific allometry of the male mandible, the segmented regression resulted in the top ranked models for four of the five analysed populations. For the population of BOF, the linear and segmented model did not differ significantly, considering both the ANOVA result and the Δ AIC for the two models (Table 2B). In all the populations with segmented regression as the top ranked model, males of the minor morph exhibited a lower value for the allometric coefficient compared to major males. The results of the SMA function revealed a significant variation in the allometric coefficient between weapon and body size for major males (above

Α	PC1	PC2	PC3
Standard deviation	1.7303	0.9998	0.0797
Proportion of Variance	0.7485	0.2499	0.0015
Cumulative Proportion	0.7485	0.9984	1
В	LD1	LD2	LD3
Proportion of trace	0.9197	0.0712	0.0090
Cumulative Proportion	0.9197	0.9909	0.9999

Table 1. Summary of the results of the first 3 principal components (PC) and linear discriminants (LD) of principal component analysis and linear discriminant analysis, respectively.



Figure 3. Scatterplots of the allometric relationship between mandible (LnML) and elytron (LnEL) length, with the top ranked model superimposed on the basis of its AIC score. The pooled dataset illustrates the trend of the scaling relationship for all the analysed populations. The allometric trajectory of each population was reported in subsequent scatterplots.

the estimated switch point) (DF = 4, LR = 66.00, p < 0.01). However, minor morph males did not differ in allometric coefficient between populations (SMA: DF= 4, LR = 5.23, p = 0.26), but exhibited a significant difference from isometry (SMA: DF = 351, r = 0.85, p < 0.01), also indicating the presence of a positive allometry for this morph. The allometric relationship between LnML and LnEL for minor and major males is illustrated in the scatterplots (See Suppl. material 3: Figure 2). The results for differences in elevation between populations indicate an overall significant difference (DF = 4, Wald = 207.2, p < 0.01). A non-significant difference in elevation was detected for populations at the same latitude (i.e., TOC and FEL), as a result of the multiple comparison amongst groups (TestStat = 1.19, p = 0.28) (See Suppl. material 4: Figure 3).
Table 2. Summary of the models results, obtained by fitting segmented and linear regressions to the scaling relationship between mandible length and elytron length for the pooled dataset (\mathbf{A}) and for each population separately (\mathbf{B}) .

	Dataset	Model	AIC	AAIC	Adj. R ²	þ	DF	SP (± SE)	SL Minor (± SE)	SL Major (± SE)	ANOVA between models
V	D l U	Segmented	-680.01	62.09	0.71	<0.01	759	$3.10 (\pm 0.01)$	1.78 (± 0.11)	$3.29 (\pm 0.15)$	DF = 1, F = 34.34, P < 0.01
	rooled	Linear	-617.92		0.69	<0.01	761				
в	NOM	Segmented	-136.94	16.73	0.86	<0.01	105	$3.13 (\pm 0.02)$	1.92 (± 0.21)	$3.64 (\pm 0.31)$	DF = 1, F = 10.99, P < 0.01
	MON	Linear	-120.21		0.83	<0.01	107				
	aOa	Linear	-38.20	1.31	0.68	<0.01	80				DF = 1, F = 1.30, P = 0.28
	DOF	Segmented	-36.88		0.68	<0.01	78	$3.04~(\pm 0.07)$	1.77 (± 0.54)	$2.74(\pm 0.31)$	
	CuC	Segmented	-212.50	7.47	0.75		136	$2.99 (\pm 0.03)$	$0.66(\pm 0.69)$	2.48 (± 0.15)	DF = 1, F = 5.80, P < 0.01
	CKO	Linear	-205.04		0.73		138				
	JOL	Segmented	-107.99	1.07	0.70	<0.01	71	$3.02 (\pm 0.05)$	$1.23 (\pm 0.37)$	$2.26 (\pm 0.30)$	DF = 1, F = 2.48, P = 0.09
	100	Linear	-106.92		0.69	<0.01	73				
	DET	Segmented	-455.77	35.36	0.77	<0.01	353	$3.10 (\pm 0.01)$	$1.80 (\pm 0.14)$	$3.28 (\pm 0.19)$	DF = 1, F = 20.57, P < 0.01
	LEL	Linear	-420.41		0.74	<0.01	355				

AIC = Akaike Information Criterion score

 $\Delta AIC = difference$ in AIC value relative to the top model

Adj. r^2 = adjusted r^2 value

DF = degree of freedom

SP (\pm SE) = estimated switch point value (\pm standard error)

SL = slope value, i.e., allometric coefficient

See FIGURE 1 for explanation of sites abbreviations.



Figure 4. Allometric coefficient (± 95% confidence intervals) between weapon (LnML) and body size (LnEL) in relation to latitude for minor males and major males of *Lucanus cervus*.

The overall latitudinal cline for the allometric coefficients for both male morphs is illustrated in the scatterplots with superimposed trend lines (Figure 4). All the biometric variables exhibited a latitudinal variation, according to the result of the Kruskal-Wallis H test (BM: hc = 49.89, p < 0.01; ML: hc = 117.83, p < 0.01; EL: hc = 25.28, p < 0.01). The Dunn's test for multiple comparison showed that the less variable trait was EL, with a significant variation (p < 0.01) only between the population of TOC and the northernmost location (MON). Both BM and ML exhibited a significant variation amongst sites at the extremes of the examined latitudinal range (Bonferroni corrected p values: $BM_{MONvsFEL} < 0.01$; $BM_{MONvsTOC} < 0.01$; $BM_{BOFvsFEL} < 0.01$; BM_{BOFvs-} $\begin{array}{c} \label{eq:construct} & \mbox{MONVsTOC} \end{array} \\ \mbox{MONVsTOC} < 0.01; \\ \mbox{ML}_{MONvsFEL} < 0.01; \\ \mbox{ML}_{MONvsFEL} < 0.01; \\ \mbox{ML}_{CROvsFCC} < 0.01; \\ \mbox{ML}_{CROvsFEL} < 0.01; \\ \mbox{ML}_{CROvs$ dependence of the biometric variables on latitude (BM: f = 39.38, $r^2 = 0.06$, p < 0.01; EL: f = 14.45, $r^2 = 0.02$, p < 0.01; ML: f = 106.35, $r^2 = 0.11$, p < 0.01). The box-plots in Figure 5 A-C illustrated the variation in biometric rough variables amongst populations. The median value of BM varied from around 1.8g (TOC: 1.7g; FEL: 1.9g) of the southernmost populations to 2.3g (BOF: 2.4g; MON: 2.2g); medians of ML and EL ranged, respectively, from 9mm (FEL) to 11mm (MON) and from 21mm (TOC) to 23mm (MON).



Figure 5. Box-plots for the variation in biometric rough variables, between populations: body mass (BM), elytron length (EL) and mandible length (ML). See Figure 1 for the acronyms of the study sites.

Phenology

The populations exhibited a significant variation in adult activity period (Kruskal-Wallis: hc = 114.3, p < 0.01). In particular, the comparison amongst sites highlighted a significant shift in the phenology between the sites close to the Alps (MON and BOF) and those close to the Apennines (CRO, TOC and FEL) (Mann-Whitney: $p_{\text{MONvs-FEL}} < 0.01$; $p_{\text{MONvsTOC}} < 0.01$; $p_{\text{MONvsCRO}} < 0.01$; $p_{\text{BOFvsFEL}} < 0.01$; $p_{\text{BOFvsTOC}} < 0.01$; $p_{\text{MONvsTOC}} < 0.01$;

Discussion

The results revealed a clear distinction between the studied sites, according to the combination of their climate variables. The northernmost site (MON) was shown to be the rainiest locality, with the lowest monthly temperatures (MIN, MEA and MAX). This is in accordance with the updated climate classification map of Kottek et al. (2006) which reports the Piedmont Prealps straddling two climate categories: snow climate, fully humid with cool summer (Dfc) and warm temperate climate, fully humid (Cf). The remaining sites (BOF, CRO, TOC and FEL), although varying in terms of average temperatures, did not differ significantly and were characterised by a dry summer period. Although BOF exhibited the highest summer temperatures and lowest winter temperatures, both northernmost sites show minimum temperatures below zero during winter. Although the humidity and the average monthly hours of sunshine could play an important role in characterising the study sites, the authors were unable to gather data for these variables. The overall trend in total precipitation indicated a correlation with latitude and, consequently, the dry summer period was more pronounced in the Po plain and along the Tiber valley compared to that recorded in MON.

The analysis of mandible allometry on the pooled dataset strengthens the results obtained by Romiti et al. (2015) clearly identifying two morphological classes in L. cervus males: minor and major. Minor males, although characterised by a positive allometry, presented a shallower allometric coefficient line (i.e., low slope value) compared to major males. Thus, at species level, the allometric trajectory undergoes an increase in slope with increase in body size. It is therefore concluded that *L. cervus* belongs to the species of the genus Lucanus, identified by Knell et al. (2004), which invest relatively more in weaponry compared to other Lucanus spp., characterised by relatively long mandibles, where the mandible length is clearly greater than half of the body length, for example, L. laminifer (Waterhouse, 1890) and L. planeti (Planet, 1899). This suggests that, in L. cervus, weapon lengthening is not affected by any depletion of resources by other body parts. The differential development of weaponry between minor and major males could be explained as a balance between the benefit derived from a given amount of investment and its cost (Gadgil 1972). The advantages of bearing enlarged weapons becomes particularly evident during stag beetles male-male interaction, giving an enhanced capacity for dislodging the opponent, both in interspecific and intraspecific contests (Hongo and Okamoto 2013, Lagarde et al. 2005), thus shortening the battle duration (Goyens et al. 2015a) and finally resulting in increased mating opportunities. However, exaggerated mandibles can be costly in terms of impairing locomotion and stability (Goyens et al. 2015b) and constraining flight ability (Goyens et al. 2015c). Despite the great investment in SSC by major males, Romiti et al. (2016) suggested that functional constraints, (for example, enhanced mechanical stress and reduced flight performance), may have shaped the mandibles of larger L. cervus males. Therefore, while some constraints maintain the mandible shape of major males, their lengthening is driven by increase in body size. The segmented regression is the first ranked model in four of the five analysed populations, probably indicating the existence of a genetically determined threshold size at which individuals switch from one morph to another. As with Painting et al. (2014), a latitudinal increase in overall body size of male stag beetles was found. This cline could be positively selected to reduce cooling rate (i.e., to increase the thermal inertia) at high latitudes, consistent with Bergmann's Rule. Despite investigating a narrow latitudinal range, the results obtained indicate a latitudinal cline in weapon allometry and phenology. Indeed, in studies over larger geographical ranges, latitude/altitude-independent confounding factors grow too numerous to reflect a true clinal trend (Shelomi 2012). Nevertheless, it would be interesting to investigate this phenomenon at European level to validate or refute the trend high-

lighted in the present study (cf. Box 1). In accordance with other studies on stag beetles (Rowland and Emlen 2009, Iguchi 2013, Romiti et al. 2016), the most variable trait is mandible length, both within and amongst populations. It is indeed well known that SSC express a huge phenotypic plasticity in both size and shape (Andersson 1982, Fitzpatrick 1997, Cuervo and Møller 2001). In contrast, elytron length, which could be interpreted as a measure of the post-prothoracic segments (mesothorax, metathorax and abdomen), did not show a great latitudinal variation, as was expected for a non-SSC. The mandible length exhibited the same latitudinal trend as body mass, increasing with latitude. Moreover, contrary to what has been demonstrated by Painting et al. (2014) for *L. barbicornis*, at high latitudes, the allometric slope line for major males increases. This variation in the scaling relationship (LnML vs LnEL) derives from a greater investment in weapon size at high latitude coupled with an equal investment in elytron length across the latitudinal range. These results further confirm that males of the European stag beetle are in an arms race, thus the more resources they gather during larval stage, the more they invest in weaponry. In other words, major males, who have grown larger in terms of body mass, have reduced resources for production and maintenance of SSC compared to minor males which did not attain the minimum body size to develop exaggerated weapons. In accordance with this hypothesis, the elevations analysis indicates that individuals of the same size, when belonging to latitudinally distant populations (i.e., northern vs southern), are equipped with more elongated mandibles as latitude (hence body mass) increases. It is worth noting that both larval growth conditions and feeding resources (Moczek 2002) have an effect on the developmental mechanisms that regulate the elaboration of exaggerated structures in holometabolous insects (Shingleton et al. 2008). Indeed, it has been demonstrated that in stag beetles, the development of SSC is closely associated with larval feeding conditions, the mediatory function of the juvenile hormone (Gotoh et al. 2011) and the expression of transcription factors encoded by double-sex genes (Gotoh et al. 2014). Larvae of the studied populations, described above, have grown in different woodland types (e.g., oak grove, chestnut) and have experienced different nutritional conditions. These differences, together with larval microhabitat characteristics, could be responsible for the variation in allometric slope and elevation which are highlighted amongst these populations. In fact, differences in slope and elevation for the scaling relationship between mandibles and body size were also recorded between sites which appeared to be comparable at a macroclimate scale (BOF, FEL and TOC). This led to the conclusion that variables such as deadwood type, decay trajectory (influenced by the way in which the tree died (Parks 1999)) and soil humidity could play an important role in the resulting allometric trajectories. However, it was not possible to separate the role of genetic variation from the local environmental features which influence larval development, SSC plasticity and physiological constraints. In-depth breeding experiments are required, conducted under different microclimate conditions using individuals from the same clutch and these clutches have to be representative of different latitudes. In fact, it has been demonstrated that different populations respond differently to climate changes (Pelini et al. 2012): they can be adapted to historically different conditions (Gilman et al. 2006,

Pelini et al. 2009, Angert et al. 2011) and have different abilities to cope with local environmental changes (Magnani 2009). However, as L. cervus has a long larval stage (3-6 years) which can vary significantly across its distribution (Harvey et al. 2011), this makes these breeding experiments difficult. A latitudinal shift in adult phenology of stag beetles has been identified, indicating an earlier mating season at high latitude. Saproxylic beetles, whose larvae live in and feed on the decaying wood (for example, dead roots, fallen branches and stumps of both living and dead trees), like L. cervus, could be sensitive during the larval and pupal stage to the temperature cycle (thermoperiod) experienced in their microhabitat. The thermoperiod is also likely to depend on microclimate characteristics such as humidity of the woodland and consequently dead wood, as well as ground temperature, all of which are influenced by canopy density and monthly hours of sunshine. In addition, the thermoperiod will be influenced by macroclimate variables which exhibit a latitudinal gradient. Latitudinal variation could contribute to life-stages shift amongst latitudes, thus directly influencing the larval development and/or setting constraints on the availability of food resources. As many aspects of ectotherm physiology are temperature dependent (Huey 1982), the variation in adult activity is likely correlated with behavioural thermoregulation. Male stag beetles actively search for mates while flying at dusk during the mating season (Campanaro et al. 2011, Chiari et al. 2014). Flying activity is closely correlated to ambient thermal conditions (Adams and Heath 1964, Heinrich 2013). It is suggested that the latitudinal shift in activity period could be positively selected to enhance the dispersal ability of males, making their thermoregulation more effective. Individuals at higher latitudes could emerge earlier to make the most of a short, cool and rainy summer period (MON) or, alternatively, to avoid a very hot summer (BOF) (climate category of the Po plain according to Kottek et al. (2006): Cfa, warm temperate, fully humid with hot summer).

Conclusions

The latitudinal cline in body size has been one of the most widely observed patterns in nature and has interested biologists for over 150 years. Animal body size, according to Bergmann's Rule, increases with latitude. By studying five populations which span the Italian distribution of the species, a geographical variation in mandible and body size in the stag beetle *L. cervus* was identified. These results indicate that males of this species invest relatively more in weaponry size at high latitudes, leading to a further exaggeration of this SSC. On the contrary, the size of the post-prothoracic segments did not exhibit a latitudinal cline. As well as defining the allometric coefficient slope for male weaponry, it was shown that the breeding season varies with latitude, beginning later on (late June) at lower latitudes. Characterisation and comparison of adult phenologies, besides being crucial for modelling the insects' response to climate change, provided valuable data for the conservation and monitoring of a threatened species and are also used as flagship and umbrella species for the conservation of saproxylic fauna in Europe.

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Appendix

Box 1. Stag beetle researchers are invited to send us morphometric data on *L. cervus*, including at least: mandible length, elytron length and body mass, measured as in Romiti et al. (2015). We will analyse and compile the results with the intention of submitting an article in January 2018, with all the contributors who submit more than 30 records accredited as co-authors.

Supplementary material I

Shapiro-Wilk normality test on biometric, phenological and climatic variable

Authors: Federico Romiti, Lara Redolfi De Zan, Sarah Rossi de Gasperis, Massimiliano Tini, Davide Scaccini, Matteo Anaclerio, Giuseppe Maria Carpaneto Data type: statistical data

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Link: https://doi.org/10.3897/natureconservation.19.12681.suppl1

Supplementary material 2

Monthly total precipitations and temperatures

Authors: Federico Romiti, Lara Redolfi De Zan, Sarah Rossi de Gasperis, Massimiliano Tini, Davide Scaccini, Matteo Anaclerio, Giuseppe Maria Carpaneto Data type: environment data

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Supplementary material 3

Allometric relationship between mandible (LnML) and elytron (LnEL) length for minor and major morph

Authors: Federico Romiti, Lara Redolfi De Zan, Sarah Rossi de Gasperis, Massimiliano Tini, Davide Scaccini, Matteo Anaclerio, Giuseppe Maria Carpaneto

Data type: statistical data

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Supplementary material 4

Allometric relationship between mandible (LnML) and elytron (LnEL) length of each population

Authors: Federico Romiti, Lara Redolfi De Zan, Sarah Rossi de Gasperis, Massimiliano Tini, Davide Scaccini, Matteo Anaclerio, Giuseppe Maria Carpaneto Data type: statistical data

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RESEARCH ARTICLE



Detection of stag beetle oviposition sites by combining telemetry and emergence traps

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Abstract

The European stag beetle, *Lucanus cervus*, is a flagship species for biodiversity conservation of old-growth forests and is protected under the Habitats Directive. Although it has been the focus of active research in the last two decades, many aspects of its ecology and habitat requirements for the larvae remain poorly known, particularly to what extent certain factors limit larval development. The objectives of this preliminary work were: (1) to explore the feasibility of a non-invasive method for detecting oviposition sites; (2) to attempt the characterisation of above-ground ecological factors recorded in the oviposition sites and (3) to quantify the number of traps and operators needed for obtaining a number of beetles suitable for statistical analysis. In 2014, twelve females were followed by means of radio-telemetry to detect potential oviposition sites in a relict broadleaf forest of northern Italy. In 2015, emergence traps were set in nine sites selected from the 21 sites where females were recorded digging deeply in the soil near to dead wood during the previous year. Traps were checked during the 2015 and 2016 flight seasons. Overall, 15 stag beetles were detected (8 males and 7 females) from five emergence trap sites which were therefore regarded as real oviposition sites. All oviposition sites were characterised in terms of typology of dead wood, tree species, canopy openness, trunk diameter, dead wood volume, decomposition stage (five classes) and wood

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hardness (four classes). All the detected emergence sites belonged to the genus *Quercus*, two being from the allochthonous *Q. rubra*, but no preferences for a dead wood species nor for a typology were shown and a broad variation was apparent for all the considered variables. The mean values of canopy openness, diameter, dead wood volume, decay status and wood hardness were 2.54%, 51cm, 4.92m³, 3 and 3.4 respectively. These data suggested an important heterogeneity in the oviposition sites selection. Although this method (telemetry + emergence traps) provided substantial aid to finding newly emerged beetles, it required a large amount of fieldwork effort, both in terms of time and man-hours. The advantage of the method is its low degree of invasion while its drawback is the amount of effort needed. Calculations were made to assess the minimum number of operators and traps needed to gather a number of data suitable for statistical analysis. It was found that two full time operators should be able to detect about 50 potential oviposition sites in one flight season, 28 of which were expected to be real oviposition sites.

Keywords

emergence traps, radio-telemetry, saproxylic insects, dead wood, oviposition sites

Introduction

Detecting the breeding sites for a protected species is of great importance for its conservation and monitoring, as the knowledge of these crucial spots is needed to optimise management and surveillance.

The European stag beetle, Lucanus cervus (Linnaeus, 1758) (Coleoptera: Lucanidae), is a flagship species for conservation of forest ecosystems, particularly for the saproxylic community (Pratt 2000, Thomaes et al. 2008, Carpaneto et al. 2015, Campanaro 2016, Bardiani et al. 2017b). The stag beetle is protected at the European level and listed in the Annex II of the Habitats Directive. Its larvae develop underground for three to five years, often up to 1m deep, feeding on decaying wood of stumps, logs or roots of a wide range of broadleaf trees and shrubs (Franciscolo 1997, Percy et al. 2000, Harvey et al. 2011a). A key problem for conservation of the stag beetle is that larvae are hard to detect because they live in the subterranean/saproxylic interface between soil and deadwood where they spend the most part of their life cycle. Sampling methods aimed at directly searching for larvae through excavation under log and tree roots are probably too invasive as they could harm the larva and alter the ecological conditions (Harvey et al. 2011b, Chiari et al. 2014b). For these reasons, stag beetles are usually monitored only during their adult phase (Sprecher-Uebersax and Durrer 2001, Sprecher 2003, Campanaro et al. 2011a, b, 2016, Fremlin and Hendriks 2011, Harvey et al. 2011b, Chiari et al. 2014a, Bardiani et al. 2017a, b).

Three methods have been developed for detecting larvae in a monitoring context: Rink and Sinsch (2008) used radio-telemetry to track females up to oviposition sites in a suburban context of Germany, while Harvey et al. (2011b) developed both a chemical method for detecting compounds produced by the larvae and an acoustic method for recording their stridulations in a suburban context in England. A standardised method for detecting sites of larval development and capturing newly emerged beetles could be crucial for conservation and studies on habitat requirements and dispersal ability of the target species (Rink and Sinsch 2007, Tini et al. in press a). In fact, previous telemetric studies showed that the longest distances covered by a stag beetle were recorded from freshly emerged individuals and highlighted the importance of the first period of adult life for dispersal (Rink and Sinsch 2007, Tini et al. in press a). Furthermore, the characterisation of the larval development sites is a key aspect for their conservation, as this type of habitat is likely to be the most critical and limiting factor for the species development. Another important limiting factor is its modest reproduction rate (for an insect) that ranges from 15 to 36 eggs (24 on average) for a single clutch (Sprecher 2003, Harvey et al. 2011a).

In this preliminary study, for the first time radio-telemetry was used in combination with emergence traps set to detect and describe the oviposition sites. The specific objectives were: (1) to explore the feasibility of a mildly invasive method for detecting oviposition sites in forest habitats, assuming that they were also potential development sites for the larvae; (2) to verify whether the method could be used to characterise the oviposition sites in terms of above-ground ecological factors to be used as a proxy for underground conditions of wood and (3) to quantify the average number of individuals captured by emergence traps in order to assess the minimum quantity of traps and operators needed for obtaining the amount of data suitable for statistical analysis.

Materials and methods

Study area

The study area "Bosco della Fontana" is located in northern Italy (Marmirolo, province of Mantua, Region Lombardy) (45°12'N 10°44'E, altitude: 24–26m a.s.l.). This area is one of the last remains (233ha) of the lowland broadleaf forests in the Po valley, an intensively cultivated area. The forest has been a Biogenetic Nature Reserve since 1977, included in the Nature 2000 network as Special Protected Areas (SPAs IT20B0011) since 1998 and a Site of Community Importance (SCI IT20B0011) since 2004. This State Reserve, formerly managed by the national forestry service (CFS), is currently managed by the Ufficio Territoriale Carabinieri per la Biodiversità di Verona [= Territorial Office of Carabinieri for Biodiversity of Verona]. Since 2007, it has also been part of the Italian Long Term Ecological Research Network (LTER-Italy). Around 85% of the Reserve is covered by broadleaf forests, the remaining part consisting of grassland and a small wetland. The deciduous forest of the study area is dominated by Quercus cerris L., Q. robur L., Carpinus betulus L. and Fraxinus ornus L., with Alnus glutinosa (L.) and Fraxinus oxycarpa Vahl along the main watercourses (Mason 2004). Silvicultural practices officially stopped in 1994 (Mason 2004) and forest management was aimed at increasing deadwood for restoring the natural character of the ecosystem. Actions included favouring the ageing of native trees and converting the alien species (Q. rubra L., Juglan snigra L., Platanus spp.) which have been inappropriately introduced in the past, to coarse woody debris (CWD) (Cavalli and Mason 2003). The increase in deadwood,

in this way, would benefit saproxylic insects and the whole forest community. Currently, the study area hosts many large saproxylic organisms, such as *Lucanus cervus, Morimus asper* (Sulzer, 1776) and *Cerambyx cerdo* Linnaeus, 1758 (Mason et al. 2015).

Data collection

In 2014, from 29th May to 10th July, twelve females were captured, radio-tagged and released for detecting potential oviposition sites. In 2015, before the emergence of the adults (early May), these sites were covered with anti-aphid plastic nets (hereafter: emergence traps) to capture newly emerged individuals and hence demonstrating the role of those dead wood spots as larval development sites. After studying emergence activities during these two years, the traps were removed at the end of the study.

The females for radio-tracking were captured mostly at sunset, by hand while they were crawling on the ground or with a hand net while they were flying. The hand net had a circular frame (50cm diameter) and a telescopic handle (up to 199cm). Each captured adult was weighed, marked ventrally with a permanent marker and with a numbered sticker on the right elytron, then equipped with a battery-powered radio transmitter (LB-2X / 0.31g; Holohil Systems Ltd., Carp, Ontario, Canada) (Figure 1). Transmitters were attached to the pronotum using a cyanoacrylate glue (LOCTITE, Super Attack flex Gel; Henkel, Düsseldorf, Germany), as previously undertaken for O. eremita by Chiari et al. (2013). The nominal lifetime of transmitters was 21 days with a lifespan range of 13-22 days. The antenna of the transmitters was reduced to 5cm and adjusted above the elytral suture to limit disturbance to beetle behaviour (Rink and Sinsch 2007). According to Boiteau and Colpitts (2001), electronic tags should weigh no more than 33% of the beetle's weight in order not to hamper flight; in this study, the transmitter weight was less than 18% of the initial body mass (weight of females ranged between 1.8-4.1g), thus the transmitter load was well below the threshold. Transmitter signals were detected by means of a hand-held antenna (Yagi three elements; Wildlife Materials Inc., Murphysboro, IL, USA) connected to a radio receiver (NEW TRX-1000S W; Wildlife Materials Inc.).

The radio-tagged beetles were released the next evening, at the same location from where they had been captured and at a time when there was no rainfall and the temperature was mild. The 'homing technique' (White and Garrott 1990) was used to detect the location of each beetle (Tini et al. in press a, b). To obtain information on beetle activities during the whole 24 hours of the day, one homing procedure each hour for each individual was performed, within modules of four hours per day moved forward by four hours every day (six shifts per week). In this way, the twenty-four hours of the day were covered in six days. The homing procedure always started at the last known location of the individual and lasted, in most cases, for a few minutes, but sometimes it could also occur over several hours. To avoid interference with the behaviour of tagged individuals, they were approached no closer than 0.5m. Although visual detection was not possible when individuals were underground, in dead wood or in the canopy, the



Figure 1. A female of *Lucanus cervus* marked with a numbered sticker on the right elytron and equipped with a battery-powered radio transmitter (LB-2X / 0.31g; Holohil Systems Ltd., Carp, Ontario, Canada).

location was recorded. A potential oviposition site (hence, a potential larval development site) was assumed to be found when the radio-signal of a female was heard for at least 3 successive days underground (within 2m of suitable dead wood including tree, stump and snag or directly under logs). As the radio-signal became progressively less clear, suggesting an increasing depth day by day, the detection probability of an oviposition site was very high. If the female remained at one place for three days, but not near to dead wood, the place was not considered as a potential oviposition site and at least 5 days passed before a hole was dug for checking (in all these cases, a dead female or a radio-tag detached from the beetle was found). Coordinates of each location were identified using a Garmin GPS (Garmin Ltd., Olathe, KS, USA) (MAP 60 CSX).

In the first half of May 2015, emergence traps were set in nine potential oviposition sites based on data obtained during the previous year. Different typologies of dead wood spots were considered as potential oviposition sites: standing dead trees (SDT), lying dead trees (LDT), logs (portions of a trunk or a large branch), stumps, snags, uprooted LDTs, uprooted stumps and roots. A snag was defined as a standing dead tree without branches, with height >130cm and diameter at breast height (DBH) >10 cm; if branches were present, the snag was considered as an SDT; if the snag was less than 130cm in height, it was considered as a stump. The DBH of SDT, LDT and snags was measured.

Emergence traps were made with anti-aphid plastic nets wrapping a large dead wood spot and fixed with nails to the ground and/or to the deadwood (Figures 2 a and b). The margins of the net were carefully kept attached to the bark or the wood surface to avoid the escape of the beetles from the trap. Each trap was checked twice a week from 15^{th} May until 15^{th} July 2015 and from 3^{rd} June until 4^{th} July 2016. The control of each trap lasted about thirty minutes. When adults of *L. cervus* were found, these were captured by opening the net. Each captured beetle was weighed, measured and marked with a numbered sticker on the right elytron and with a permanent marker ventrally. As it was not known what the best time was for net checking, the nets were checked in the evening up to early night (16:00–22:00h) when stag beetles were mostly active in the study area (Campanaro et al. 2016, Bardiani et al. 2017b, Tini et al. in press b), using a headlamp.

For each potential oviposition site, at the end of the 2014 flight season, the following environmental variables were recorded (Table 1): typology of dead wood, tree species, diameter, percentage of canopy openness, dead wood volume, decay status and wood hardness. As the stag beetle larvae exist underground in the interface between soil and dead wood, it was hard to assess the variables which affect their development and it was possible only with excavation (e.g. Rink and Sinsch 2008). Therefore, it was assumed that some variables measured above-ground, such as decay status and wood hardness, may be good proxies for similar subterranean environmental conditions. The percentage of canopy openness was calculated by means of a Gap Light Analyser (GLA version 2.0), a digital image processing software that allows the estimation of gap light transmission indices from true-colour hemispherical (fisheye) photographs (Frazer et al. 1999). The photographic lens was a Fisheye converter FC-E8 0.21×. Photographs were taken under a uniformly overcast sky or close to sunset; these sky conditions provided a perfect diffuse sky, thus avoiding the interference of direct sunlight which can cause errors up to 50% (Nobis and Hunziker 2005). Dead wood volume was calculated by approximating the shape of trunks to a cylinder and the shape of uprooted stumps to two cylinders. Height of trees and length of fallen trunks was measured by means of a dendrometer Vertex III (produced by Haglöf Sweden); this instrument uses ultrasonic signals to determine heights and distances. Decay status was recorded for the



Figure 2. Emergence traps placed on larval development sites (DS) detected in 2014 by means of radiotelemetry. **A** Emergence trap on *Quercus* sp. SDT (DS01) **B** Emergence trap on *Quercus rubra* Uprooted stump (DS10).

above-ground part of the wood piece, according to Hunter and Malcolm (1990), using a score of five classes: (1) no evidence of decay; (2) solid wood, less than 10% changed structure due to decomposition, the wood being solid at its surface and attacked only to a very small extent by wood decomposing organisms, bark being intact or lost only in part, twigs (diameter < 3cm) being absent, unaltered colour of wood; (3) slightly decayed, 10–25% of the wood having a changed structure due to decomposition, twigs being absent, bark being present only in trace amounts, colour of wood having faded; (4) decomposed wood, 26-75% of the wood being soft to very soft, bark and twigs absent, wood colour being light, from faded brown to yellow and (5) very decomposed wood, 76-100% of the wood being soft, bark and twigs being absent, wood colour fading to light yellow or grey. The wood hardness of each dead wood spot was assigned to four classes (from 1 to 4, in order of decreasing hardness) upon the degree of penetration of a knife blade (Opinel n°8, as in Redolfi De Zan et al. 2014): (1) the knife blade penetrates less than 1cm; (2) the knife blade penetrates about 1cm; (3) the knife blade penetrates more than 1cm; (4) the wood is highly soft throughout its entire thickness, the knife blade penetrates completely into the wood.

Number of traps and operators assessment

To estimate the minimum number of operators and traps needed to obtain an amount of data suitable for statistical analysis, the following values were calculated. The emergence site detection ratio was calculated by dividing the number of emergence sites detected by the number of traps used. To estimate the number of emergence sites which can be expected to yield emergence data, the number of set traps was multiplied by the emergence site detection ratio. The minimum, maximum and mean numbers of capture expected in one season were also estimated.

e 1. Number of beetles captured by emergence traps and dead wood variables. ID number of females radio-tagged in 2014 (eight on the whole) which remained
hree days in one dead wood spot, therefore considered as a potential oviposition site and a potential larval development site (DS); number of emerging males
ales captured by emergence traps in 2015 and 2016 in each DS N° and environmental variables. SDT: standing dead tree; LDT: lying dead tree; Ø: diameter
55, SDT, LDT is considered as DBH); DW: dead wood.

Digging	01 V 10	Emerging	Emerging	Date of	- F		Canopy	Ø	DW volume	Decay	Wood
Female		Males	Females	emergence	rypology	opecies	openness (%)	(cm)	(\mathbf{m}^3)	status	hardness
F061	DS01	6	1	22/06/15	SDT	Quercus sp.	3.06	72.5	12.97	3	4
F061	DS02	0	0		LDT	Q.robur	4.83	50	8.39	2	3
F061	DS03				LDT	Carpinus betulus	0.78	33	1.03	ŝ	3
F038	DS04	2		27/06/15 04/07/15	Log	Quercus sp.	2.94	47	0.90	4	3
F060	DS05		2	9 and 27/06/16	Uprooted LDT	Q. rubra	1.28	36.5	1.93	3	4
F060	DS06				Roots	C. betulus	4.48	8	0.01	1	1
F075	DS07				Stump	Quercus sp.	2.75	68	0.32	5	4
F038	DS08				Snag	C. betulus	1.02	52	0.91	4	4
F075	DS09	0	0		Stump	Q. rubra	0.70	65	0.40	2	4
F075	DS10	1	2	27/05/15 (1M) 1/07/15(1F)	Uprooted stump	Q. rubra	1.28	52	4.40	3	4
				9/06/16 (1F)							
F075	DS11				SDT	Q. rubra	1.86	55	4.03	3	4
F075	DS12				Uprooted LDT	unidentified	1.60	80	4.49	5	4
F072	DS13				SDT	Prunus avium	8.61	60	4.23	2	1
F072	DS14				SDT	C.betulus	4.24	22	0.53	1	1
F020	DS15		1	27/06/16	Uprooted LDT	Quercus sp.	4.14	47	4.98	2	2
F091	DS16				Log	Quercus sp.	5.36	32	4.5	2	2
F091	DS17				Log	Fraxinus ornus	11.38	16	8.14	3	2
F091	DS18				Stump	unidentified	5.28	32	0.06	2	1
F013	DS19	0	0		Stump	unidentified	3.76	45	0.08	4	3
F013	DS20	0	0		Uprooted LDT	C. betulus	12.29	31	2.48	1	1
F013	DS21				LDT	C. betulus	2.50	29	0.77	5	4

As this work was a preliminary study, data on which the estimates are based were few, thus the results of the number of traps and operators assessment have very broad confidence intervals and a solid statistical approach to evaluate the dead wood productivity cannot be performed.

Results

Capture data

At the end of the reproductive season for 2014, the 12 radio-tagged females allowed the detection of 21 dead wood spots as potential oviposition sites and these were covered by emergence traps. In the first half of May 2015, due to logistic constraints, only nine of these spots were chosen, based on their accessibility and feasibility of being covered by emergence traps without damaging the surrounding vegetation. In 2015 (from 27th May to 27th June) and 2016 (from 9th to 27th June), 11 (9 males and 2 females) and 4 individuals (all females) were respectively captured by the emergence traps.

During the radio-telemetry study carried out in 2014 (Tini et al. in press a, b), eight females were recorded digging deeply at the periphery of at least one dead wood spot and to spend at least three days underground. It was assumed that these dead wood spots were potential oviposition sites and hence potential larval development sites (Tini et al. in press a) (Table 1). In 2015, 11 individuals were found inside three of the nine emergence traps in five checking days and, in 2016, four individuals were captured inside three traps in two checking days. Overall, five emergence sites were detected.

Characterisation of the oviposition sites

Approximately half (10/21) of the dead wood spots, identified as potential oviposition sites, could be assigned to the genus *Quercus* with certainty: five *Quercus* sp., four *Q. rubra* and one *Q. robur* (Table 1). The other half were: six *Carpinus betulus*, one *Prunus avium* and one *Fraxinus ornus*. For three sites, it was not possible to identify the genus due to the advanced decaying. The typologies of the five sites that were found to be suitable for larval development were: two uprooted LDT, one uprooted stump, one SDT and one group of logs (Table 1). All these five sites belonged to the genus *Quercus*: three were probably *Q. robur* (uncertainty due to advanced rotting stage) and the other two belonged to the allochthonous *Q. rubra*. Canopy openness of these five sites was 2.54% (SD = ± 1.24) on average. The mean diameter value was 51cm (SD = ± 13.28). Dead wood volume was $4.92m^3$ (SD = ± 4.49) on average and the mean decay status was 3 (SD = ± 0.71). Wood hardness values averaged 3.4 (SD = ± 0.89).

The highest number of beetles (7) was captured within the trap DS01 wrapping an SDT of *Quercus* sp. (Figure 2a; Table 1). This emergence trap contained the larg-

est amount of dead wood and, although the decay status was not extremely advanced (score value: 3), the wood was the least hard (score value: 4). The trunk appeared to be full of wood mould, at least below breast height. All the emergences recorded in this trap were detected during the same check. Only one site (DS10) was found to be suitable for larval development in both checking seasons.

Number of traps and operators assessment

In 2015, 29 stag beetles (20 males and 9 females) were radio-tracked by two operators, thus it was calculated that 30 females can be tracked by the same number of operators during the same period. As the radio-tracking of 12 females led to the detection of 21 potential oviposition sites, it was calculated that, with 30 females, it would be possible to detect about 50 potential oviposition sites (30 * 21 / 12 = 52.5). As five emergence sites were detected by mean of nine traps, an emergence site detection ratio of 0.56 (5 / 9 = 0.56) was calculated. Thus, by setting 50 traps, 28 sites are expected to yield emerging adults (50 * 0.56 = 28). It was calculated that 50 traps would be required for the capture of at least 28 emerging adults. For the highest number of captures, the same value as observed in the present work (seven adults) was used (28 * 7 = 196). The mean number of captures obtained by the emergence sites was three, thus for a total of 50 traps a mean number of captures of 84 stag beetles is expected (28 * 3 = 84). Considering about 30 minutes for checking one trap, one operator should be able to control about 25 traps twice a week, working about 4 hours per day. Therefore 2 operators should be able to check about 50 traps twice a week.

Discussion

This study is only a pioneering approach for the combined use of telemetry and emergence traps (wrapping nets), with the aim of detecting the oviposition sites of the stag beetle. Moreover, it was also a preliminary investigation on the characteristics of dead wood spots suitable for oviposition. Emergence traps have previously been used to capture freshly emerged stag beetles by Rink and Sinsch (Rink 2006), but at spots where adults had already been seen emerging in previous occasions, a quite easily detectable event in urban environments. In this case, the study was developed in a forest with a large amount of dead wood where it was hard to detect the effective emergence sites. As the number of observations was low and cannot be analysed in statistical terms, some preliminary considerations were given on the data obtained, valid for launching a number of working hypotheses. Obviously, it cannot be concluded that the dead wood spots that yielded no captures were not occupied by the larvae of *L. cervus*.

According to these results, *L. cervus* showed a broad heterogeneity in the selection of potential oviposition sites, in agreement with previous literature (Percy et al. 2000, Smith 2003, Rink and Sinsch 2008, Harvey et al. 2011a). In fact, these consisted of a

diverse typology of dead wood spots with a high range of values for all variables considered. However, Quercus was the only tree genus where larvae developed successfully. About half of the trees with positive results were Q. rubra, showing that exotic oaks are also potentially suitable for this beetle. Q. rubra was found to be suitable for larval development in the major part of the cases where traps were placed (2/3: 67%) probably because this alien species, destined for a progressive eradication in the study area, was transformed into dead wood and used by the management authorities for increasing saproxylic biodiversity (Cavalli and Mason 2003). Dead wood of Q. rubra is now very abundant and has the right age and stage of decomposition for larval development. The stag beetle does not seem to have a preference either for this species with respect to native oaks, nor for other deciduous trees, although in a previous study developed in England, oak, apple, ash and cherry were found to be more commonly used, likely because they were commonly available (Percy et al. 2000). Smith (2003) reported 27 species as suspected or confirmed oviposition sites, supporting the idea that stag beetles will utilise a range of tree species as breeding sites. A clear preference for a species was not even shown in the study of Rink and Sinsch (2008), where the suitable trees for larval development were oak, sessile oak, white willow, silver birch, cherry and plum trees. Harvey et al. (2011a) reported that, in Britain, larvae have been recorded from 60 different hosts and that, although oaks were dominant, they formed only 9%-19% of records. In the study developed by Rink and Sinsch (2008), almost all the breeding sites were exposed to sunlight, while, in this study, they were located in rather shaded places with a mean value of canopy openness of 2.54%. Probably, such differences in sun exposure are linked to the different climatic conditions. In fact, average temperatures in Germany are generally lower than in northern Italy and sun exposure may be favourable or dangerous, depending on the local climate. Furthermore, in the study by Rink and Sinsch (2008), dead wood diameters ranged between 24cm and 79cm, similar to the values of this study (36.5cm to 72.5cm). Both studies supported the idea that the stag beetles showed a large variation in the oviposition sites selection. In parallel, the stag beetle was not associated to any typology of the selected oviposition sites, although roots were present in most dead wood spots, except for logs. In fact, oviposition sites, in the proximity of roots, were also observed by previous authors (e.g. Percy et al. 2000, Frem-

lin 2009). These data suggest that oviposition sites of the stag beetles are distributed over several microhabitat types with a wide range of values for each parameter considered. Probably, management actions focused on increasing the heterogeneity and dead wood in a forest, with such uprooting or cutting of alien species trees, without removing the wood, potentially leading to favourable conditions for stag beetle reproduction.

This method could be used for improving the knowledge of dead wood requirements for larval development and for studying the first part of the adult lifespan when individuals are more active, at least concerning their dispersal movements (Rink and Sinsch 2007, Tini et al. in press a). Although the proposed method required a large amount of fieldwork, in terms of time and man-hours and yielded a low number of individuals, it was probably less invasive and more feasible than other methods proposed. Moreover, it may yield a larger amount of data if conducted by several operators collaborating in net setting and checking. It was calculated that, in this study area, two full time personnel were able to radio-track up to 30 females in two consecutive months of the local reproductive period (i.e. June-July). Such a fieldwork could lead to the detection of more than 50 potential oviposition sites, a number suitable for a statistical analysis focused on characterising the above-ground ecological parameters, as a proxy for subterranean environmental conditions. In the successive year, a second phase of fieldwork, also conducted by two operators, could record the emergence of about 80 adult stag beetles i.e. a number suitable for the evaluation of each oviposition site. The limited amount of data presented here was mainly related to the fact that it was not possible to work on a full time basis for this research.

In any case, the monitoring of a high number of oviposition sites, investigated by emergence traps, cannot last for more than five years because the nets hinder the females laying their eggs and the number of emergent individuals will become zero in the fifth year after the trap setting. This hindrance to egg-laying, due to the presence of nets over the oviposition site, may lead to an important impact on the reproduction of *L. cervus* in areas where suitable dead wood is scarce and localised. In this study area, where there is plenty of dead wood, such an impact is probably less important and the application of this method during a long-term study, could also be useful for investigating how long the dead wood is suitable for larval development.

An unsolved issue concerns the selection of the best checking time during the day, a problem which can be addressed with this working protocol, based on controls twice a week. In fact, even with more controls per day at different time slots, this problem is linked to beetle detectability under the net mesh that may vary consistently throughout the day in relation to species behaviour and to the visual acuity of the checking operator. Many studies revealed that the peak of stag beetle activity, at least in northern Italy, is in late afternoon to almost one hour after sunset (Chiari et al. 2014a, Campanaro et al. 2016, Tini et al. in press b). Probably, in the late morning and at noon, beetles are not easily detectable because high temperature values make them stand still in the shadow, under fallen leaves or in cavities where they are not visible to the checking operator, while from late evening to sunset, they are more prone to move and become easier to detect, although human sight is less efficient after sunset and needs the use of artificial light.

Conclusions

The data available with this method, if applied to a long term study, could be of great importance for the conservation of *L. cervus*, as they could give information on the effects of different wood decaying stages on stag beetle larval development. The combined use of radio-telemetry and emergence traps is a useful method for finding oviposition sites and for detecting emerging individuals. Little evidence is available on the length of the larval development of the stag beetle from a single oviposition site and location. In fact, the duration of the life cycle may vary between three to five years de-

pendent on several factors such as quality of food and climatic conditions. A long term monitoring of the emergence of stag beetles from a single site can help to calculate the duration of its suitability as a larval development site. Such knowledge could be very helpful in order to build artificial oviposition sites in a protected area, thus allowing the managing authorities to plan the dates for cutting logs or uprooting trees and could predict how long these would be suitable for larval development thus ensuring a continuing availability of essential resources.

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REVIEW ARTICLE



Monitoring and management of Cerambyx cerdo in the Mediterranean region – a review and the potential role of citizen science

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Abstract

The Great Capricorn beetle, *Cerambyx cerdo*, and Mediterranean oak habitats (*Quercus ilex* – 9340 and *Quercus suber* – 9330) are protected by the Habitats Directive (HD). However, in the Mediterranean basin, these habitats are also traditionally used for animal, wood, and cork productions. *Cerambyx cerdo* feeds into the wood of trees and can be perceived by forest practitioners as an umbrella species or as a pest, depending on the context. Monitoring programmes involving forest practitioners could thus focus on assessing: 1) the conservation status of the Great Capricorn beetle and habitats (distribution and abundance of insects and reproductive sites or colonised trees), 2) pest status, and 3) management options to achieve both conservational and economic benefits. Considering that *Cerambyx cerdo* and Cork and Holm oak forests are not priority species or habitats under the HD, targeted funding is likely to be limited for monitoring. In this context, citizen science could gather important information on the target species useful for the monitoring programmes for *Cerambyx cerdo* monitoring and habitat conservation should be seen not only as citizens collecting good data sets, but also as a deeper collaboration amongst different knowledge bodies and perspectives within a community – based environmental monitoring and learning network.

Keywords

Citizen Science, Habitats Directive, Monitoring protocol, Saproxylic beetle

Introduction

The Great Capricorn beetle, Cerambyx cerdo, plays a key role in decomposition of wood and ecosystem functioning of natural and semi-natural oak forests (Buse et al. 2008a). When attacked by C. cerdo and other saproxylics, a tree may survive over long periods with increasing quantities of dead wood and galleries created by larvae. In this long lasting state, the tree represents habitats for other species and C. cerdo is thus considered an important ecosystem engineer and umbrella species (Buse et al. 2008a). For its key ecological role, the species is strictly protected under the European Union's Council Directive 92/43/EEC (Habitats Directive, HD), which requires mandatory monitoring (Article 11). Although the species is still reasonably widely distributed (Figure 1), the beetle is now considered "Near Threatened" at European level by the IUCN because the population in most of the countries is in significant decline and it is dependent upon veteran trees which are also declining in Europe (Horak et al. 2010). The species is also assessed at the Mediterranean level as "Least Concern" since it has a large geographical range and is abundant in the region, although sub-populations are often scattered and there is a low probability that the habitat would recover if destroyed in the future (Buse et al. 2016). Major threats for the species are the decline in the number of old trees situated in open or semi-open landscapes (Buse et al. 2007, Albert et al. 2013), fragmentation and isolation of sub-populations (Buse et al. 2016, but see Torres-Vila et al. 2017), changes in habitat and landscape structure such as plantations with exotic plants and alteration of grazing regimes (Buse et al. 2016, Oleksa and Klejdysz 2017), and forest sanitary measures (Luce 1997, Horak et al. 2010, Buse et al. 2016). In fact, *Cerambyx* spp. are considered serious pests of oak stands in the Mediterranean basin (Martín et al. 2005, Sallé et al. 2014, Torres-Vila et al. 2017), where seminatural Holm oak (Quercus ilex) and Cork oak (Quercus suber) forests are traditionally exploited for pasture and firewood or cork production respectively (Bergmeier et al. 2010). Considering that the species has this double interest for Mediterranean forest management, mandatory monitoring under the HD perhaps should be aimed at assessing both conservation and pest status of C. cerdo.

Citizen science is the practice of engaging volunteers in a scientific project (Bhattachrjee 2005, Burgess et al. 2017, McKinley et al. 2017). By simultaneously engaging a large number of data collectors, citizen science is providing important information to assess the distribution and abundance of protected species (Silvertown 2009, Kosmala et al. 2016, Zapponi et al. 2017). Such information is fundamental to establish conservation priorities and policies (Hochkirch et al. 2013), with the latter also supporting sustainable development of socio-ecological systems (Keulartz 2009). However, to bridge the gap between knowledge about distribution and abundance of species and practical environmental management, it is very important to involve in monitoring those who are responsible for hands-on management of ecological resources, i.e. local practitioners with their perspectives (Hulme 2011). Indeed, even if citizen scientists are often engaged as mere data collectors for large scale monitoring (Lakshminarayanan 2007), they can contribute to science in several ways, e.g. by developing scientific questions,



Figure 1. Distribution map of *Cerambyx cerdo* based on data from the IUCN (background map from Stamen Design, OpenStreetMap).

analysing data, and evaluating results (Silvertown 2009, McKinley et al. 2017). Within this broader view of citizen science, community - based monitoring can be included, a process where concerned citizens, government agencies, academia, local institutions and other stakeholders collaborate to monitor, track and respond to issues of common environmental concern (Conrad and Hilchey 2010), and where local practitioners can be involved. In fact, when local practitioners face conservation tasks, they search for convincing solutions that can be practically implemented without jeopardising community welfare (Horwich and Lyon 2007). For example, limits to sanitary measures of productive oak forests could be perceived by local stakeholders with interest in cork extraction or wood production as unwarranted regulations that could jeopardise their income. Forest practitioners of local authorities could therefore be rather sceptical about taking any action to strictly protect species that could also be considered as pests, such as C. cerdo. To achieve consensus about conservation goals and their compatibility with local community interests, it is therefore important to successfully involve practitioners in the learning process of evaluating conservation problems and viable management solutions for the socio – ecological system at hand (Nichols and Williams 2006, Conrad and Hilchey 2010, Keith et al. 2011, McKinley et al. 2017).

By applying this perspective, the following sections: 1) review management obligations for *Cerambyx cerdo* and oaks under the HD, 2) call for the application of a citizen science that can strengthen the link between *C. cerdo* monitoring and management, and 3) underline some relevant practitioner's objectives of *C. cerdo* monitoring within adaptive management (Nichols and Williams 2006). Overall, there is a gap between available knowledge about *C. cerdo* and information needed to manage Mediterranean oak habitats. This gap could be filled by involving practitioners in evaluating conservation or pest status of the species, as well as viable management options to achieve conservation and sustainable development goals.

Managing Cerambyx cerdo and Mediterranean oaks under the Habitats Directive

The Cerambyx cerdo is listed as a non priority species in annexes II and IV of the HD. That is, core areas of C. cerdo habitats are designated as Sites of Community Importance (SCIs) and included in the Natura 2000 network which must be managed to maintain or restore favourable conservation status of the species (Epstein et al. 2016) (Annex II). Additionally, a strict protection regime must be applied across the entire natural range of C. cerdo within the European Union, both within and outside Natura 2000 sites (Annex IV). In practice, within the whole territory of the European Union, the beetle cannot be deliberately killed, captured or disturbed, and its breeding or resting sites (trees colonised by C. cerdo) cannot be deteriorated or destroyed (HD, Article 12, see Table 1). This strict protection regime can create conflicts when there is an economic interest in oaks and stakeholders may wish to cut down trees or branches colonised by C. cerdo to protect woodlands (Buse et al. 2016). However, the significance of the pest status of the protected C. cerdo should be carefully assessed and disentangled from that of similar species such as C. scopolii and C. welensii which are not protected under the HD and can be associated with C. cerdo (Buse et al. 2008b, 2016, Torres-Vila et al. 2017, Wang 2017). It is thus very important to assess whether and in which conditions C. cerdo can be a serious pest for oak woodlands. Indeed, Article 2 of the HD states that economic issues and local context should be taken into account, while Article 16 allows derogation to restrictions of Article 12 if a risk of damage to forests is shown.

Holm oak and Cork oak forests are protected habitats (HD, Annex I, habitats 9340 and 9330 respectively and habitat 6310 for dehesas with evergreen *Quercus* species). That is, core areas of habitats are designated as SCIs and included in the Natura 2000 network which must be managed to maintain habitats in favourable conservation status (Epstein et al. 2016). To achieve this, the specific structure and functions necessary for long-term persistence of habitats must be maintained and the conservation status of typical species must be favourable (Article 1e, see Table 1). When necessary, land-use planning and development policies should encourage the management of features of the landscape which are of major importance for biodiversity (Article 10). Therefore, forest management of SCIs should aim at maintaining or restoring the typical biological diversity associated with habitat structure and functions, with appropriate management plans (Article 6). How habitat structure and associated saproxylic beetle communities should be maintained or restored is a challenging question (Vodka et al. 2008, Sebek et al. 2013, 2015), with particular

Article	Text (English version, only relevant parts)	Obligations
1	e) The conservation status of a natural habitat will be taken as	Define favourable
	"favourable" when:	conservation status (FCS)
	- its natural range and areas it covers within that range are stable or	for each listed species and
	increasing and	habitat.
	- the specific structure and functions which are necessary for its	
	long-term maintenance exist and are likely to continue to exist for	
	the foreseeable future and	
	- the conservation status of its typical species is favourable as	
	defined in (i);	
	i) The conservation status will be taken as "favourable" when:	
	- population dynamics data on the species concerned indicate that	
	it is maintaining itself on a long-term basis as a viable component	
	of its natural habitats and	
	- the natural range of the species is neither being reduced nor is	
	likely to be reduced for the foreseeable future and	
	- there is, and will probably continue to be, a sufficiently large	
	habitat to maintain its populations on a long-term basis;	
2	1. The aim of this Directive shall be to contribute towards ensuring	Take appropriate measures
	biodiversity through the conservation of natural habitats and of	to maintain or restore species
	wild fauna and flora in the European territory of the Member States	and habitats at FCS.
	to which the freaty applies.	Consider economic, social
	2. Measures taken pursuant to this Directive shall be designed	and cultural issues, and local
	to maintain or restore, at lavourable conservation status, natural	context.
	3 Measures taken pursuant to this Directive shall take account of	
	economic, social and cultural requirements and regional and local	
	characteristics.	
3	1. A coherent European ecological network of special areas of	Identify a suitable Natura
-	conservation shall be set up under the title Natura 2000. This	2000 network for the
	network, composed of sites hosting the natural habitat types listed	conservation of habitats listed
	in Annex I and habitats of the species listed in Annex II, shall	in annex I and species listed
	enable the natural habitat types and the species' habitats concerned	in annex II.
	to be maintained or, where appropriate, restored at a favourable	
	conservation status in their natural range.	
6	1. For special areas of conservation, Member States shall establish	Develop conservation
	the necessary conservation measures involving, if need be,	measures and, if necessary,
	appropriate management plans specifically designed for the sites or	appropriated management
	integrated into other development plans and appropriate statutory,	plans for species and habitats.
	administrative or contractual measures which correspond to the	
	ecological requirements of the natural habitat types in Annex I and	
	the species in Annex II present on the sites.	
10	Member States shall endeavour, where they consider it necessary, in	It necessary, develop
	their land-use planning and development policies and, in particular,	plans and policies for the
	with a view to improving the ecological coherence of the Natura	conservation of landscape
	2000 network, to encourage the management of features of the	leatures important for species.
	randscape which are of major importance for which fauna and flora.	
	continuous structure (such as rivers with their banks or the	
	traditional systems for marking field boundaries) or their function	
	as stepping stopes (such as ponds or small woods) are essential for	
	the migration, dispersal and genetic exchange of wild species.	

Table 1. Obligations arising from the Habitats Directive for the conservation of animal species and habitats.

Article	Text (English version, only relevant parts)	Obligations
11	Member States shall undertake surveillance of the conservation status of the natural habitats and species referred to in Article 2 with particular regard to priority natural habitat types and priority species.	Do monitoring, particularly on priority species or habitats.
12	 1. Member States shall take the requisite measures to establish a system of strict protection for the animal species listed in Annex IV (a) in their natural range, prohibiting: (a) all forms of deliberate capture or killing of specimens of these species in the wild; (b) deliberate disturbance of these species, particularly during the period of breeding, rearing, hibernation and migration; (c) deliberate destruction or taking of eggs from the wild; (d) deterioration or destruction of breeding sites or resting places. 	Take measures to strictly protect animal species listed in Annex IV.
16	 Provided that there is no satisfactory alternative and the derogation is not detrimental to the maintenance of the populations of the species concerned at a favourable conservation status in their natural range, Member States may derogate from the provisions of Articles 12 : a) in the interest of protecting wild fauna and flora and conserving natural habitats; b) to prevent serious damage, in particular to crops, livestock, forests, fisheries and water and other types of property. 	Ask derogations to protect habitats and avoid serious damage to forests, provided FCS of the species is maintained.

reference to the Mediterranean basin which has high and poorly known levels of diversity and endemism (Baselga 2008).

The long lasting association of *C. cerdo* with old and decaying trees (Buse et al. 2007, 2008a) suggests that trees colonised by C. cerdo represent keystone structures (Tews et al. 2004) to maintain saproxylic diversity and functions associated with Mediterranean protected oak habitats (Sirami et al. 2008). Due to past forest exploitation, old growth forests are rare in the Mediterranean basin (Blondel and Aronson 1999, Scarascia-Mugnozza et al. 2000), suggesting the need of delimiting non-intervention areas within the Natura 2000 network to "re-wilding" landscapes (Schnitzler 2014). However, in central European countries, the C. cerdo and other saproxylic beetles have been found to be associated with sun-exposed wood located near ground (Buse et al. 2007, Albert et al. 2013, Oleksa and Klejdysz 2017) and could benefit from the restoration of traditional management practices such as coppice with standards or woodland pastures (Buse et al. 2007, Vodka et al. 2008). Retention forestry is also emerging as a practical way to harvest forest and maintain or restore old-growth features of landscapes (Fedrowitz et al. 2014, Mason and Zapponi 2015). Management options to protect C. cerdo and related habitat structures and functions range therefore from strict protection of old growth forests, to conservation of habitat trees over managed landscapes, and to forest harvesting coupled with grazing and retention (Sirami et al. 2008, Vodka et al. 2008, Sebek et al. 2013, 2015, Fedrowitz et al. 2014, Mason and Zapponi 2015). Traditional forest management practices are thus seen as potential conservation tools to protect saproxylic communities (Buse et al. 2007, Vodka et al. 2008), as well as sustainable and viable production systems (Scarascia-Mugnozza et al. 2000, Sjölund and Jump 2013).
In practice, forest management or conservation bodies face a mandatory task to monitor through space and time a long and incomplete list of species and habitats (Hochkirch et al. 2013), to assess a rather context-dependent favourable conservation status (Epstein et al. 2016), as well as to test management options available to maintain or restore such favourable conditions and achieve sustainable development (Keulartz 2009). Giving this overwhelming task, conservation institutions are forced to focus on priorities (Hochkirch et al. 2013) which implies that important funding explicitly targeted at non-priority species and habitats such as *C. cerdo*, Holm oak and Cork oak woodlands are rather unlikely (see Article 11, HD). Within these challenges and constraints, citizen science has great potential to do most of the required tasks in conservation science, natural resource management, and environmental protection (McKinley et al. 2017).

Citizen science and Cerambyx cerdo management

It has been shown several times that knowledge coming from non-professionals, either called citizen science (Silvertown 2009, Conrad and Hilchey 2010, Kosmala et al. 2016, Burgess et al. 2017, Casula et al. 2017, McKinley et al. 2017, Zapponi et al. 2017) or local ecological knowledge (Anadón et al. 2009, Irvine et al. 2009, Angelstam et al. 2011, Vignoli et al. 2016) can be a reliable source of information for species conservation and management. Nevertheless, many studies involving non-professionals still emphasise the role of citizens as data collectors (Silvertown 2009, Kosmala et al. 2016, Zapponi et al. 2017), while from the social fields there have been calls to move away from using citizens on unequal terms and towards treating citizens as scientists to create learning networks with real transformative potential (Lakshminarayanan 2007, Feyerabend 2011, Bela et al. 2016) to achieve conservation goals (Conrad and Hilchey 2010, McKinley et al. 2017).

In other words, citizen science programmes to assess distribution and abundance of *C. cerdo* and other saproxylics can certainly take advantage of involving many citizen scientists as data collectors (Zapponi et al. 2017). Nevertheless, if a consensus about what to do to protect or manage the species is sought, more focus should be put on community-based environmental monitoring, with the involvement of practitioners to establish objectives, methods and interpret results. In the long term, such an approach is more likely to affect decisions made by the practitioners themselves who are responsible for the management of local natural resources in the face of multiple objectives and uncertainty (Conrad and Hilchey 2010, Keith et al. 2011, McKinley et al. 2017). Indeed, from a management perspective, monitoring for conservation is viewed as an essential element of the adaptive cycle of informed decision-making which includes objectives, potential management actions, models of system response to management actions, with consequent monitoring of relevant state variables (Nichols and Williams 2006, Keith et al. 2011). That is, counting beetles for conservation can practically affect conservation decisions if relevant information about management options can be gathered. For example, if *C. cerdo* is found to be rare in given management scenarios (e.g. coppices without retention or conversion to high forest), abundant in others (e.g. coppice with retention or unmanaged old growth forests) and at damaging levels in productive landscapes (e.g. dehesas with Cork oak), forest practitioners or policy makers can link monitoring results to the best management options available to maintain the species in favourable conservation status at national level without jeopardising local community welfare. From a practitioner's point of view, it is very important that monitoring objectives for *C. cerdo* are framed within a real management perspective.

Practitioner's objectives for Cerambyx cerdo monitoring

Recent efforts in developing standard monitoring protocols for saproxylic beetles focused on applications of advanced statistical tools to address issues of insect detectability and to provide reliable estimates of distribution and abundance that can be compared across large spatial scales (Chiari et al. 2013b, 2013a, Campanaro et al. 2016, Redolfi De Zan et al. 2017). Several sampling methods have been applied to study C. cerdo populations, including visual censuses of adult exit holes to assess microhabitat requirements (Buse et al. 2007, Regnery et al. 2013, Albert et al. 2013, Oleksa and Klejdysz 2017), comparison of evening transects, night surveys of trunks, pitfall and bait traps for distribution and population monitoring of adult beetles in a Natura 2000 network (Vrezec et al. 2012) and bait traps to estimate dispersal in a Mediterranean woodland pasture (Torres-Vila et al. 2017). Most of the available studies come from central Europe and focus on microhabitat selection in open landscapes, as summarized in Table 2. Although it is very rare to find Mediterranean habitats without some traditional land use system (Blondel and Aronson 1999, Scarascia-Mugnozza et al. 2000, Bergmeier et al. 2010), there is very little information about the association of *C. cerdo* with habitats differing in management history (but see Regnery et al. 2013).

Information about the effect of different management histories on *C. cerdo* populations and habitat structure (e.g. number and quality of colonised trees) is very important as even the most reliable estimates of distribution and abundance of species cannot be translated into action if an explanation about the underlying process (e.g. why a trend is negative) is not available (Nichols and Williams 2006). For example, given the assumed preference of the beetle for lower parts of sun-exposed trunks (Buse et al. 2007, Albert et al. 2013, Oleksa and Klejdysz 2017), is the abandonment of traditional management, based on coppice with standards or open woodland pastures, underlying the decline of *C. cerdo* and associated saproxylic communities (Vodka et al. 2008, Sebek et al. 2015)? However, given that *C. cerdo* has been extensively studied in open woodlands of central Europe (Buse et al. 2007, Albert et al. 2013, Oleksa and Klejdysz 2017), does the strong association with lower parts of sun-exposed trunks hold in other ecological contexts? Indeed, detailed habitat preference in the Mediterranean region is largely unknown and may differ from the preference observed in central Europe (Buse et al. 2016). Additionally, *C. cerdo* colonisations have been observed in northern Italy mostly in the upper

Торіс	Reference	ence Country Main tree /]			
Pest status	Martin et al. 2005	Spain	Quercus suber / Woodland		
Microhabitat selection and spatial distribution	Buse et al. 2007 Germany		Quercus robur / Woodland pasture		
Role as ecosystem engineer	Buse et al. 2008a	Germany	Quercus robur / Woodland pasture		
Microhabitat selection	Albert et al. 2012	Czech Republic	Quercus robur / Woodland pasture		
Comparison of sampling methods for distribution and population monitoring	Vrezec et al. 2012	Slovenia	Unknown		
Microhabitat selection	Regnery et al. 2013	France	Quercus ilex / Woodland		
Dispersal	Torres Vila et al. 2016	Spain	Quercus ilex / Woodland pasture		
Microhabitat selection and exotic plants	Oleksa & Klejdysz 2017	Poland	Quercus robur / Woodland pasture		

Table 2	. Main studies	on biology,	conservation,	and management	t of <i>Ceraml</i>	<i>byx cerdo</i> in Euro	ope.
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part of the canopy of dense forests (Redolfi De Zan et al. 2017). This fact would suggest that *C. cerdo* conservation could be as well achieved by delimiting non-intervention areas to maintain or restore structure and function of forests, as requested by the HD to maintain favourable conservation status of natural habitats and typical species. Last, but not least, are sanitary measures for trees colonised by *C. cerdo* needed in productive Mediterranean landscapes such as woodland pastures and do the species in Annex IV really need listing? In other words, microhabitat association and eventual pest status of the species should be more extensively studied in widespread Mediterranean habitats such as Holm oak or Cork oak woodland pastures, coppice with retention and open or closed old growth forests, so that conservation practices of *C. cerdo* and sustainable use of associated habitats could be based on sound knowledge about the socio-ecological system at hand (Horwich and Lyon 2007, Keulartz 2009).

Conclusion

Scientific questions arising from the practitioner's perspective may differ from those arising from professional scientists or amateur naturalists and these are more related to the need to understand which management decisions will result in societal benefits from the development and conservation perspectives. This is an open question in European oak woodlands dominated by *Quercus* species, where *C. cerdo* is considered by many forest practitioners as a serious pest (Sallé et al. 2014), even if it might be confused with other *Cerambyx* spp. known to attack healthy trees in managed forest systems (Torres-Vila et al. 2017, Wang 2017). To address these issues, the citizen science paradigm for *C. cerdo* monitoring and habitat management should be seen not only as citizens collecting good data sets, but as a deeper collaboration amongst different knowledge bodies and perspectives, within a community based environmental monitoring and learning network.

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RESEARCH ARTICLE



The great capricorn beetle Cerambyx cerdo L. in southwestern Poland – the current state and perspectives of conservation in one of the recent distribution centres in Central Europe

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Abstract

Presence-only models can aid conservation and management of threatened, elusive species. A MaxEnt model has been developed for the great capricorn beetle (*Cerambyx cerdo* L., 1758) in south-western Poland and the variables identified best explaining the species' occurrence on a large scale. Once successfully validated, the model was used to (a) illustrate the expected location of the species' habitats in the region and in existing Natura 2000 sites (SACs) in S-W Poland and (b) assess the efficacy of the regional network of national protected areas (NPAs) *versus* Natura 2000 (SACs). Overall, information was gathered on 1025 localities of *C. cerdo* L., 1758 in Lower Silesia. All the records came from the pedunculate oak *Quercus robur* L., 1753. The occurrence of the great capricorn beetle in the study region is limited mainly to its eastern part, with a marked concentration in the valleys of the rivers Odra, Barycz and Bystrzyca. The kernel density estimation analysis also showed the high concentration of occupied trees in the north-western part of the region, clearly isolated from the above-mentioned main populations. Although a considerable part of the localities in the study region (74.2%) occurred within protected areas (PAs), their contribution to the species' conservation varied between the PAs groups. Natura 2000 SACs are the most important PAs, covering more than 30% of

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the predicted area of suitable habitats in the region and more than 45% of optimal habitats. In total, 384 localities of *C. cerdo* L., 1758 were found within the cities, most of them (n = 356) in the city of Wrocław. Forty three percent (43%) of the urban localities of the species (n = 165) in the study region are protected within the regional network of protected areas (OPAs), while those unprotected are mainly concentrated in the city of Wrocław (n = 207). Wrocław also includes 17.1% of the area of suitable habitats and 29% optimal habitats of the species in the region outside the protected area network. To preserve *C. cerdo* L., 1758, forest corridors should be created or restored to bridge the otherwise impermeable gaps revealed by the authors' model and grant protection to the still largely unprotected area of the Lower Silesian territory. The species conservation programme in the region requires the cooperation of various authorities, not only those dealing with nature conservation, but also local governments, state forest management and flood protection authorities.

Keywords

Conservation, saproxylic beetles, long-horn beetle, NATURA 2000, Quercus, Lower Silesia

Introduction

The great capricorn beetle (Cerambyx cerdo L., 1758) is the largest longhorn (Cerambycidae) beetle in Poland and one of the largest beetle species in Europe. Since fairly recently, the species has been under strict legal protection, not only in Poland, but also in most other European countries. It is included in Annex II of the Bern Convention (Convention on Conservation of Species 1979) and in Annexes II and IV of the EU Habitats Directive (Council of the European Communities 1992). Its high conservation status results from the global shrinking of its range which has been observed for more than 100 years (Buse et al. 2007) and resulted in its placement on the IUCN world's list of endangered species, with VU category (IUCN 2017). It should be emphasised that the populations from the north and centre of the continent are the most endangered. For example, in comparison with southern populations, the species has become extinct in Belgium, the Netherlands, Luxemburg, Great Britain, European part of Russia and in Latvia (Alexander 2002, Hedin 2014, Huijbregts 2003, Alekseev 2007, Barševskis and Avgin 2014, Volkova 2015). In Sweden, one population has survived out of the four that were known in the early 20th century; it is located in the nature reserve of Halltrop on the island of Öland (Lindhe et al. 2010, Hedin 2014). The species' decline has also been observed in the Czech Republic, Slovakia and northern Germany (Sláma 1998, Ellwanger 2009, Drag and Cižek 2015). In Poland, as in the adjacent countries, C. cerdo L., 1758 has markedly declined and today, its occurrence is limited to the areas west of the Vistula River, with the concentration of sites in the western (Rogalin Warta Valley) and south-western (valleys of the Odra, Barycz, Widawa, the city of Wrocław) parts of the country (Pawłowski et al. 2002, Gutowski 2004, Starzyk 2004, Bunalski 2012, Stachowiak 2012). The above authors regard the population from south-western Poland as the strongest and, in terms of the occupied in area, largest in the country and, as shown by the data from the adjacent countries, also in the region. An interesting and, at the same time, disturbing fact is the lack of recent data on the occurrence of the great capricorn beetle in well preserved forest

complexes of the north-eastern part of Poland, for example Białowieża Forest. For this reason, despite the existence of numerous populations in southern Europe and North Africa where locally (Morocco, Algeria and Mallorca, Spain), the great capricorn beetle is reported as a pest of oak woods (e.g. Chakali et al. 2002, González et al. 2010, El Boukhari et al. 2015, Torres-Vila 2017, Torres-Vila et al. 2017), the state of preservation of the species in the Atlantic and continental biogeographical regions is regarded as poor (U2; Ellwanger 2009). This phenomenon results mainly from habitat destruction (natural disappearance or felling of old, ancient deciduous trees in forests, parks, roadsides and on flood banks) with a simultaneous absence of natural or artificial oak regeneration. Another probable reason for the species' disappearance in Poland may also be the decreasing levels of groundwater which affects the condition of trees inhabited by C. cerdo L., 1758 (Jankowski and Świerkosz 1995). Some authors also point to modern forest management as the reason for the species' disappearance in that part of the range which favours thick, fast-growing and shaded forests at the expense of the earlier, light and sparse tree stands. The consequences of the reduced area of adequate habitats include fragmentation and isolation of individual populations, most of them having a patchy character (Stachowiak 2012). In Poland, only populations in the valleys of Barycz and Odra seem to be sufficiently extensive and connected by semi-natural ecological corridors, formed by forests or linear tree stands in open landscape (e.g. tree rows along roads or on flood banks; single oaks on floodplain meadows).

As mentioned above, adults of *C. cerdo* L., 1758 are noticeably large, with body length up to 60 mm. Additionally, the beetle seems to be even larger due to its extremely long antennae which, in males, can be twice as long as the body. Its imposing larvae, which can be longer than adults (up to 100 mm), are xylophagous on different oaks, rarely on other tree species (e.g. Luce 1997, Sláma 1998, Neumann and Malchau 2010, Torres-Vila et al. 2017, Oleksa and Klejdysz 2017). Adults are observed from late spring to summer, chiefly at dusk and in the evening. Considering these facts, the best detection method is searching for fresh signs of larval activity on trunks or branches i.e. wide and long galleries or characteristic holes with a red interior. Due to the beetle size and the poor quality of its food, the larval development takes place over a period from 3 to 5 years. According to recent studies, the species prefers trees with sun-exposed trunk, diameter more than 60 cm, thick bark and presence of sap exudation (Buse et al. 2008, Albert et al. 2012, Oleksa and Klejdysz 2017). Despite the generally small dispersal and sedentary behaviour, some adults cover distances exceeding 1000 m, with maximum rates of even 400 m/day (Torres-Vila et al. 2017).

The EU regulations on creating the ecological network Natura 2000, in force in Poland since 2004, have stimulated the interest in the great capricorn beetle in this part of Europe. The requirements imposed then on Poland and pertaining to designation and establishment of areas Natura 2000 and the consequent natural history inventories, have contributed to a better knowledge of the species in various regions of the country. Most of the records from Poland, including its south-western part, date from the boundary of the 19th and 20th century (for review, see Burakowski et al. 1990). They are thus historic records and, in many cases, imprecise.

The objective of this paper is to update the knowledge on the distribution of the great capricorn beetle in Lower Silesia as one of the last large refuges of the species, not only on a regional and country-wide scale, but also in Central Europe. The data, combined with climate-habitat variables, were used to create a model based on the algorithm MaxEnt in order to specify: 1) factors which affect the occurrence/distribution of the species in the studied area; 2) present state of habitat fragmentation and areas which are crucial for the species' protection, considering the "conflicting" areas where the protection may be rendered difficult, for example, urban areas and to propose a strategy for effective conservation and identification of the most important threats and 3) assess adequacy/efficacy of the previous and present nature conservation systems in the context of the species' preservation in the region.

Regarding the last-mentioned goal, the species is interesting in that, in Poland, it has been under legal protection since 1952 (!) and thus offers a unique opportunity to evaluate the two systems – the one based on nature reserves and landscape or national parks and the one based on habitat protection areas, especially considering that Natura 2000 is subject to criticism not only by that part of the community not interested in nature conservation, but also by naturalists and scientists (Cardoso 2012, D' Amen et al. 2013, Hochkirch et al. 2013).

Methods

Study area

The study region, covering the north-east part of the Lower Silesian province (approx. 9,571 km²), is located in south-western Poland. The altitude ranges from 60 to 703 m above sea level (mean 134 m a.s.l.). The climate is temperate with an average annual rainfall of about 600 mm. According to Corine Land Cover maps (CLC2006; available from: http://www.eea.europa.eu/data-and-maps/data/clc-2006-vector-data-version), the region is dominated by agriculture (~65% of the total area), especially arable land (53.4%). Forests cover 25% of the total area, of which 54% are coniferous forests, 19.4% broad-leaved forests and the remaining 26.6% – mixed forests. Urban areas (class 1.1 in CLC) cover 5% of the total area.

Nationally designated protected areas (NPAs) are represented by 32 nature reserves and 4 landscape parks with a total surface of approx. 81 km² (0.8% of the region) and 1,014 km² (7.3%), respectively. In total, the NPAs cover a surface area of 1,021 km². The Natura 2000 network consists of 39 sites, including 8 Special Protection Areas (SPAs) designated under the Birds Directive (total area of 863 km² in the region) and 31 Special Areas of Conservation (SACs) under the Habitats Directive (total area of 1,213 km²). All the stand-alone SPA sites were excluded from these analyses as they are only aimed at protecting bird species, while the SACs were included, increasing the total protected surface in the study region by 451 km². In total, the overall protected areas (OPAs) cover an area of 1,472 km² (15% of the region).



Figure 1. Study region with the network of nationally protected areas (NPAs) and overall protected areas (OPAs).

Field surveys

The data used on the occurrence of the species was collected in 2001–2013 by the staff, co-workers and volunteers of the Laboratory of Invertebrate Conservation Biology and Protection, Wrocław University. Each tree with signs of occupancy by the beetle's larvae such as holes with a red interior, or on which adults or their remains were found, was regarded as an occupied locality. As there are no other *Cerambyx* spp. of comparable size in Poland, potential mistakes in the recognition were minimised. Other large cerambycid species like *Ergaster faber* (L., 1761) are monophagous on Scotch pine *Pinus silvestris* L., 1753.

The species records were located in the field using a hand-held GPS and then converted into the ESRI shape-file format for later use. The occupied trees were determined at the species level. Each locality was classified into one of the five adopted habitat categories: forests, roadside trees, parks, solitary trees and other. The term 'solitary trees' means a single tree situated away from dense tree stands as well as trees loosely distributed in the agricultural landscape. In the case of forest localities, those within the management of the State Forests were distinguished, in the case of roadside trees – those located on flood banks. Besides, all the localities were grouped according to their territorial-administrative appurtenance (municipalities, forest districts) and location within protected areas. In the last case, three forms of protection were considered: nature reserves, landscape parks and Natura 2000 (SACs).

Data analysis

To estimate the current and potential distribution of *Cerambyx cerdo* L., 1758 in southwestern Poland, the kernel density estimation and the ecological niche modelling were used.

Kernel density estimation. – the kernel density estimation (KDE) in the Geospatial Modelling Environment (GME) programme (Beyer 2012) was used to assess the current spatial distribution of the species. The KDE is a non-parametric method that is used to estimate the probability density function of a random variable, with no specific assumptions about the underlying distribution shape. It was also used previously to characterise the distribution and abundance of species across space (Martins et al. 2013) and delineation conservation areas (O'Brien et al. 2012; Denoël and Ficetola 2015). A Gaussian kernel density function was selected and the optimal bandwidth was estimated using a plug-in method (Wand and Jones 1994), implemented from '*ks*' package in R (Duong 2007). Ten KDE values were considered from 10% to 95%, each of which corresponds to the smallest area protecting 10-95% of the species locations. For KDE calculations, a grid size of 0.01 km² (100 x 100 m cell) was used. The 95% KDE contours were used to present the overall current species distribution range in the region.

Ecological niche modelling. – To predict suitable habitats for *C. cerdo* L., 1758 in the study region, the ecological niche model (ENM) was developed, using the maximum entropy algorithm implemented in MaxEnt, version 3.4.0. (Phillips et al. 2017a, 2017b). MaxEnt software uses presence-only data to predict the distribution of the species by finding the probability distribution of maximum entropy, subject to a set of constraints that represent the incomplete information about the target distribution (Phillips et al. 2006). The result is a 'suitability map' depicting the probability of occurrence of the species at each raster cell of the area covered.

To eliminate redundant or spatially auto-correlated occurrence points, the *spatially rarefied occurrence data* tool in the SDMToolbox in ArcMap (Brown 2014) was used, thus reducing the occurrence localities to a single point within the distance of 1 km. Finally, in the model, 161 localities out of the total 1025 species' occurrences were used.

As environmental variables, 19 bioclimatic layers and an altitude layer from World-Clim (http://www.worldclim.org, Hijmans et al. 2005) and 6 layers related to forest fragmentation and availability of suitable *Quercus*-dominated forest patches were initially selected. Forest fragmentation layers were created based on the raster of Morphological Spatial Pattern Analysis of forest cover map, downloaded as a file MSPA 2006 from http://forest.jrc.ec.europa.eu/download/data/forest-data-download/. MSPA operates in raster images at the pixel level, where the input map is a binary representation of a landscape coded as foreground (habitat patches) or background (non-habitat patches) (Vogt et al. 2007). The source raster (with a 25 m spatial resolution) was converted into a polygon shapefile and then four separate vector layers with the following MSPA classes were created: 'core forests' (interior area of forest patch excluding forest edges), 'islet forests' (patches too small to contain core forest), 'edge forest' (edges at the outside of forest patches) and 'perforated forest' (edges along openings inside larger forest patches). (For a detailed description see: Soille and Vogt 2009). The edge width was 25 m. As the next step, the percentage of the each MSPA class in each grid of the final raster was calculated with a spatial resolution of 30 arc second (0.93 x $0.93 = 0.86 \text{ km}^2$ at the equator). The last two environmental variables, the percentage and maximal age of Quercusdominated forest patches, were based on forestry maps (available at: https://www.bdl. lasy.gov.pl/portal/). Subsequently, the pairwise correlations were checked amongst all environmental variables using SDMToolbox (Brown 2014) and factors with Pearson correlation coefficient values exceeding 0.7 (Dormann et al. 2013) were removed. Due to the high correlation between many variables (Suppl. material 1), 10 environmental variables for the MaxEnt analysis (see Table 1) were finally used.

The model in MaxEnt was built using 50 bootstrap replicate runs with the 'random seed' option. The records were split into 75% for training and 25% for testing for bootstrap replications. To facilitate model convergence, the maximum iterations were increased to 1,000. A jackknife test was then performed with all data to estimate the weight of each environmental variable in the model. The complementary log-log (cloglog) output was used with habitat suitability on a scale of 0-1, with higher values representing more favourable conditions for the species occurrence. The jackknife tests of variable importance were also used to identify those with important individual effects.

The final model was the average of all runs. To evaluate the performance of the final model, the mean area under the curve (AUC) was used for the receiver operating characteristic curve, calculated from 50 bootstrap models. The AUC ranges from 0 to 1, where a score of 1 indicates perfect discrimination, a score of 0.5 implies predictive discrimination that is no better than a random guess and values <0.5 indicate performance worse than random (Elith et al. 2006).

To distinguish between suitable and unsuitable habitat, the 10th percentile training presence (10%TP) threshold was applied. This threshold predicts unsuitable habitat for 10% of the most extreme occurrence records, as these may represent recording errors, ephemeral populations, migrants or the presence of unusual microclimatic conditions within a cell (e.g. Morueta-Holme et al. 2010). A high suitability of grid cell of output map was assumed if it showed presence probability higher than 0.632. Such predicted probability could be called a "typical" location of the species and corresponding to the predicted probability of occurrence of 0.5 for such a location in MaxEnt's logistic output (Phillips et al. 2017a). Finally, the suitability of habitat was categorised, based on the following classification: optimal ≥ 0.632 , 0.632 < moderate $\geq 10\%$ TP, unsuitable < 10%TP.

To assess of efficacy of the protected areas for the conservation of *Cerambyx cerdo* L., 1758 in the region, the contribution of each form of protected areas (PAs) was included to the protection of the known localities, as well as predicting habitats in

Name	Description [unit]	Range	Mean ± S.D.	Data source
bio10	Mean Temperature of Warmest Quarter [°C*10]	140 - 182	174.2 ± 3.6	Worldclim (www.worldclim.org)
bio11	Mean Temperature of Coldest Quarter [°C*10]	- 358	-16.3 ± 4.6	Worldclim (www.worldclim.org)
bio12	Annual Precipitation [mm]	527 - 711	554.4 ± 13.1	Worldclim (www.worldclim.org)
bio19	Precipitation in Coldest Quarter [mm]	78 - 114	87.6 ± 5.2	Worldclim (www.worldclim.org)
Quercus	Quercus-dominated forests* [%]	0–95	3.8 ± 9.8	Forest Data Bank (https://www.bdl.lasy.gov.pl/portal/)
<i>Quercus</i> age	Maximum age of the <i>Quercus</i> - dominated forests* [years]	0–235	34.6 ± 55.1	Forest Data Bank (https://www.bdl.lasy.gov.pl/portal/)
core	Interior area of forest patch excluding forest perimeter [%]	0–100	17.9 ± 28.3	MSPA Pattern Maps (http://forest.jrc.ec.europa.eu)
edge	Edges – Outside perimeter of forest [%]	0-24	3.2 ± 4.1	MSPA Pattern Maps (http://forest.jrc.ec.europa.eu)
islet	Forest islets – disjointed forest patch and too small to contain core [%]	0-19	0.7 ± 1.3	MSPA Pattern Maps (http://forest.jrc.ec.europa.eu)
perf	Perforation – Inside perimeter of forest site [%]	0-25	0.9 ± 2.5	MSPA Pattern Maps (http://forest.jrc.ec.europa.eu)

Table 1. The environmental variables used in ecological niche modelling of *Cerambyx cerdo* L., 1758 in south-western Poland.

* only forest stands with domination of native oaks, *Quercus robur* L., 1753 and *Q. petraea*, (Matt., 1784) were included.

the region. In addition, to assess the independent impact of the establishment of the Natura 2000 network on the species' protection, the proportion of the regional network of national protected areas (NPAs) was compared, such as nature reserves and landscape parks alone, in the current network of overall protected areas (OPAs), including Natura 2000 SACs. In the analysis of the contribution to the conservation of the species' habitats, an assessment was included for both predicted habitats in the whole study region and within the area of probable occurrence of *C. cerdo* L., 1758, delineated by the isopleth of 95% KDE.

Results

Current distribution and ecological preferences

In total, information was gathered from 1025 localities of *C. cerdo* L., 1758 in Lower Silesia; a decided majority (91.4%) were single trees, the remaining cases being groups of 2 to 7 occupied trees. In 67 localities (6.5%), the number of inhabited trees was unknown. All the records came from the pedunculate oak *Quercus robur* L., 1753. Despite the presence of other oak species in the environs of the localities (native *Q. petraea* (Matt., 1784) or introduced *Q. rubra* L., 1753 and *Q. cerris* L., 1753), no cases of other oaks being inhabited by *C. cerdo* L., 1758 were found.



Figure 2. The current distribution of *Cerambyx cerdo* L., 1758 (n = 1025 locations) in south-western Poland and predicted species range delineated by kernel density estimations (KDE) using plug-in bandwidth selection. White lines show the border of 95% KDE. The dashed areas represent the network of overall protected areas (OPAs).

The occurrence of the great capricorn beetle in the study region was limited mainly to its eastern part, with a marked concentration in the valleys of the rivers Odra, Barycz and Bystrzyca (Fig. 2).

The KDE analysis also showed the high concentration of the occupied trees in the north-western part of the region, clearly isolated from the above-mentioned main populations of *C. cerdo* L., 1758 (Fig. 2). Overall, 95% KDE covered an area of 2,015 km², i.e. 21% of the study area. However 50% of the known localities (50% KDE) occurred in an area of just 270 km² which accounts for 2.8% of the study area.

The species' occurrence in the region, delineated by the isopleth of 95% KDE, largely coincides with the distribution of the highly suitable habitats predicted by Max-Ent modelling (Fig. 3). The model showed excellent predictive performance, with average training AUC for the replicate runs of 0.915 (SD = 0.011). The uncertainty of the prediction, expressed by the standard deviation of the 50 fitted models, showed a small increase in uncertainty especially in the south-eastern and north-western parts of



Figure 3. Averaged habitat suitability map for *Cerambyx cerdo* L., 1758 in south-western Poland (right) and standard deviations of predicted probabilities of occurrence (left) from MaxEnt models. Black-bordered areas show the network of existing protected areas (OPAs), white-bordered show the 95% kernel density isopleth.



Figure 4. Violin plots of the habitat suitability within the 10 areas delineated by kernel density estimations (KDE) for *Cerambyx cerdo* L., 1758 in SW Poland. White dots indicate medians, box edges represent the inter-quartile range and the grey region and curve show the probability density function.

the study area as well as in the southern parts of SAC Łęgi Odrzańskie (Fig. 3). Based on the 10th percentile of training presence cloglog threshold (value of 0.2039), suitable habitats cover 23.9% of the surveyed area (2,288 km²) and optimal habitats (threshold value of 0.632) cover only 5.2% of its area (502 km²). Fifty four percent (54%) of the total area of suitable habitats and as much as 74% of optimal habitats were within the 95% KDE (1,241 km² and 383 km², respectively). The predicted habitat suitability decreased with a decreasing KDE value (Fig. 4).

Table 2. The re	elative contributions and	permutation importan	ice of the environ	nmental variables	to the
MaxEnt model.	Values shown are average	es over replicate runs.			

Variable	Percent contribution	Permutation importance
bio11	27.2	43.8
bio19	16.6	18.4
Quercus age	12.7	4.3
Quercus	11.4	2.2
bio12	10.3	8.8
edge	9.3	7.7
islet	6.5	2.5
bio10	3.2	7.5
core	2.0	1.9
perf	0.9	1.8



Figure 5. Results of jackknife test of variable importance using training gain. Values shown are averages over 50 replicate runs for each predictor variable alone (left) and the drop in training gain when the variables are removed from the full model (right). Explanation of variable codes: see Table 1.

Seven variables made more than 5% contribution to the MaxEnt model (Table 2). The jackknife test showed that the environmental variable with the highest gain, when used as the only variable, was the precipitation in the coldest quarter (bio 19), which appeared to convey the most useful information by itself. On the other hand, the predictor with the most information not present in other variables was the mean temperature of the coldest quarter (bio 11) which most reduced the gain when it was omitted (Fig. 5). In addition, some landscape variables, such as the percentage of oak stands, forest edges or forest islets as well as the age of *Quercus*-dominated forest stands, might also influence the species' distribution. Although the probability of the species' occurrence generally increased with increasing temperature and decreasing precipitation, extreme values appear to be avoided (Fig. 6). The probability of occurrence was also higher in places with old-growth *Quercus*-dominated forest stands, with a higher pro-



Figure 6. Response of *Cerambyx cerdo* L., 1758 (cloglog distribution of occurrence probability) to environmental variables used in ecological niche modelling with MaxEnt. Response curves for all 50 bootstrap models are shown with the mean curve in red.

portion of native oak species in the stands. However, it also decreased with the highest values. The response curves also show that the probability of the presence of the species increased with the decline in the proportion of forest interior (core) and the increase in the area of forest edge and forest islets.

Species distribution and protected areas

Although a considerable part of the localities of *C. cerdo* L., 1758 in the study region (74.2%) occurred within protected areas (PAs), the participation of each group of PAs in the species conservation was different (Table 3). Taking into account the surface area of the predicted habitats, Natura 2000 SACs are the most important group of PAs, covering more than 30% of the predicted area of suitable habitats in the region and more than 45% of optimal habitats (Table 3). Establishing the Natura 2000 network has increased the number of protected localities of *C. cerdo* L., 1758 from 129 to 730 (12.6% and 71.2% of the known localities in the study region, respectively) and has also significantly increased the area of suitable habitats of the species under protection. In particular, the area of the optimal habitats covered by PAs has grown considerably, increasing by more than twice (Fig. 7).

Most (69.2%) of the 295 known localities of the species outside the protected areas were located in urban areas. In total, 384 localities of *C. cerdo* L., 1758 were found within the cities, most of them (n = 356) being in the city of Wrocław. Forty three percent (43%) of the urban localities of the species (n = 165) in the study region are protected within the regional network of protected areas (OPAs), while those unprotected are mainly concentrated in the city of Wrocław (n = 207). Wrocław also brings together 17.1% of the area of suitable habitats and 29% of the optimal habitats of the species in the region outside the protected area network. For the probable area of the species' occurrence (95% KDE), these values are 32.8% and 40.2%, respectively. Wrocław, together with the regional protected areas (OPAs), encompasses more than 91% of the known localities of the species and more than 62% of its optimal habitats in the study region.

	Landscape parks (1014.5 km ²)	Nature reserves (80.8 km ²)	Natura 2000 SACs (1213.2 km ²)
Number (percentage) of localities of C. cerdo in PAs	101 (9.9%)	43 (4.2%)	725 (70.7%)
Area (percentage) of predicted suitable habitats in PAs	465.7 km ² (20.4%)	46.9 km ² (2.0%)	712.0 km ² (31.1%)
Area (percentage) of predicted optimal habitats in PAs	110.8 km ² (22.1%)	8.9 km ² (1.8%)	226.6 km ² (45.1%)
Area (percentage) of predicted suitable habitats in PAs within 95% KDE	301.3 km ² (24.3%)	44.2 km ² (3.6%)	466.9 km ² (37.6%)
Area (percentage) of predicted optimal habitats in PAs within 95% KDE	87.9 km² (22.9%)	8.9 km ² (2.3%)	185.6 km² (48.4%)

Table 3. Effectiveness of the systems of protected areas (PAs) in protecting *Cerambyx cerdo* L., 1758 in south-western Poland.



Figure 7. Surface areas (km²) obtained by ENM (MaxEnt) within the study region and the 95% kernel density isopleth for *Cerambyx cerdo* L., 1758 for three different suitabilities covered by national protected areas (NPAs: nature reserves + landscape parks) and overall protected areas (OPAs: NPAs + Natura 200 SACs). Percentages represent the percentage of the total area of the habitat type within the study region and the 95% KDE. Suitability thresholds: optimal \geq 0.632, 0.632 < moderate \geq 0.2039, unsuitable < 0.2039.

Discussion

More than a thousand localities of C. cerdo L., 1758 were recorded confirming that the studied region held numerous localities of the species which is declining in many countries in Central Europe within its north and central range distribution. The fragmentary character of historic data makes it difficult to say with certainty if the results show a satisfactory state of the species' conservation or only a better knowledge of its distribution (Burakowski et al. 1990, Starzyk 2004). Considering the lack of distributional data from the westernmost part of the region, the Silesian-Łużyce Lowland, despite the historic records and the presence of trees with signs of previous occupation (Starzyk 2004), probably the latter is true. Similarly, the complete absence of the great capricorn beetle in the southern part of the region, in the belt of Sudetic foothills and basins, despite historic records of its presence (Burakowski et al. 1990, Starzyk 2004) and the records from lower mountain altitudes in Europe (Sláma 1998), seems to confirm the observed decline of C. cerdo L., 1758 in the region. As in other populations from the northern and central parts of the continent, the pedunculate oak proved to be the only host tree for the larvae (Buse et al. 2007, Albert et al. 2012). At present, there is no satisfactory explanation for this monophagy, especially considering the availability of large trees of other species, including native and alien members of the genus Quercus, for example the northern red oak Q. rubra L., 1753. This North American species was

quite common within the studied area and many of its individuals have achieved a significant size compared to infested specimens of *Q. robur* L., 1753. Recently Oleksa and Klejdysz (2017) hypothesised that differences in the structure of the bark (deeply fissured in *robur*, smooth in *rubra*) and in physicochemical characteristics of the wood and phloem may play important roles in host selection and avoidance of the latter by *C. cerdo* L., 1758.

The results of ENM confirmed the thermophilous character of the species. These results also suggested that there is a relatively higher probability of finding *C. cerdo* L., 1758 in areas with older oak stands, although a negative impact of core forest areas and positive effect on the percentage of forest edges indicates the avoidance of the forest's interior.

The concentration of records of C. cerdo L., 1758 in the valleys of the rivers Odra, Bystrzyca and Barycz, besides the favourable thermal conditions compared to the adjacent areas, can also be explained by the presence of relatively numerous deciduous forests, including some with a high proportion of oak, which have been preserved because of the little use of those areas for intensive agriculture and because of planting oak on dykes of fish ponds as in the Barycz valley. Furthermore, the character of such tree stands, in the form of smaller or larger forest islands or rows, seems to suit the species' requirements. It is noteworthy that the trees in such stands grow far apart, thus enabling a faster growth in thickness and in turn results in thicker bark, both of those parameters being very important for the species (Buse et al. 2007, Albert et al. 2012, Oleksa and Klejdysz 2017). The preference for trees of larger diameter may result both from the large size of the larvae and thus greater food requirements and from the large sun-exposed areas of larger trees. The latter is also closely associated with the situation of the host trees in the landscape; this model and other studies show that, in this case, it is the so-called openness of habitat that is important. In the conditions of Central Europe, a longer feeding period for the larvae may be crucial for completion of the life cycle and maintaining viable populations.

Despite the passing of more than 20 years since the launch of the System Natura 2000, it still causes great controversies in Poland and in other countries of the European Union (Maiorano et al. 2007, 2015, Verovnik et al. 2011, Albuquerque et al. 2013, D'Amen et al. 2013, Hochkirch et al. 2013, Lisón et al. 2013). Paradoxically, the most criticised aspect is the establishment of the areas based on scientific criteria, i.e. the objective value of the area for given habitats and species (listed in the Annexes of the Habitats Directive), without giving priority to consideration of spatial management plans, forest management plans or opinions of local communities (e.g. Charbonneau 1997, Krott 2000, Weber and Christophersen 2002, Paloniemi and Tikka 2008, Zehetmair et al. 2015). Some of the opponents of the system also identify the threat to the development of regional and local communities (Krott 2000, Eben 2007, Makomaska-Juchiewicz 2007).

In the case of invertebrates, including insects, the species lists in Annexes II and IV of the Habitats Directive have also been criticised (Hochkirch et al. 2013). There is no doubt that the critics are right in their views that the lists are dominated by representatives of a few orders, especially butterflies and beetles which are mainly represented by

spectacular and charismatic species (Cardoso 2012). Such critique however disregards the fact that, besides the social awareness which is certainly important, such species are often very important for the preservation of macro- and microhabitats. It should be mentioned that, preserving the population of the great capricorn beetle in good condition, may be of significance for other organisms. The beetle is virtually the only species in this part of Europe which fully deserves the name of environmental engineer and umbrella species. As shown by the studies in northern Germany (Buse et al. 2008) on the fauna of saproxylic beetles associated with the pedunculate oak, the number of species is significantly higher in oaks occupied by the great capricorn beetle. Furthermore, endangered species, according to the relevant red lists, are more abundant in oaks occupied by the beetle. As many as 33 species, 31 of them from the German red list, were recorded only in such trees (Buse et al. 2008). Additionally in the study region, an array of beetle species were recorded, some of them very rare, for example Lacon querceus (Herb., 1784) or Dermestoides sanguinicollis (F., 1787), only in oaks occupied by the great capricorn beetle (Smolis et al. 2016). Recent studies in Spain also showed that hollows in oaks occupied by the capricorn beetles, Cerambyx welensii Küst., 1846, had a greater species richness of beetles, compared to unoccupied trees (Micó et al. 2015). Protection of such species like C. cerdo L., 1758 not only makes it possible for the many accompanying saproxylic organisms to occur, but may also favour conservation of many rare and endangered taxa.

Another argument used by the opponents of Natura 2000 was that it doubled the forms of nature conservation which existed and functioned in all European countries, especially area and species protection. These results do not confirm this objection, but instead show how essential and effective a tool Natura 2000 is in conservation of species such as C. cerdo L., 1758. Within nature reserves and landscape parks which occupy 80.8 km² and 1014.5 km², 43 (4.2%) and 101 (9.9%) of the species' localities, respectively were found. In the network Natura 2000, with its area of 1213.2 km², 725 localities (70.7%) were found. Great differences between the compared systems, in favour of Natura 2000, also pertain to the area (percentage) of predicted suitable habitats, area (percentage) of predicted optimal habitats, area (percentage) of predicted suitable habitats within the 95% KDE and the area (percentage) of predicted optimal habitats within the 95% KDE (Table 3). It should also be added that in 10 Natura 2000 areas in south-western Poland C. cerdo L., 1758 has significant populations and is regarded as a "target species" (Standard Data Forms, 2017). These results showed that, amongst the existing forms of protection, it is Natura 2000 (SACs) and the activities undertaken in such areas that will have the greatest effect on the preservation and effective protection of the capricorn beetle and its related biodiversity. As suggested by data from other countries, the capricorn example is not an exception in the context of insect protection in the decades to come (Jurc et al. 2008). It should be emphasised that a total of 1207 Sites of Community Importance (Natura 2000 sites) have been designated in the EU where the great capricorn beetle is recorded (http://ec.europa.eu). As shown

by Jurc et al. (2008) and Bosso et al. (2012), Natura 2000 areas are important for the preservation of rare and endangered beetle species. It is optimistic to note that most of the recorded localities and also predicted and suitable habitats of the species are, in theory, under legal protection resulting from their location within protected areas (Fig. 7).

The pessimistic aspect is associated with the possibility of preserving the species in urban areas, as the potential threat to public health from the beetle-occupied trees might result in fallen branches (Carpaneto et al. 2010) which are often partially dead or dying. In the studied region, nearly 40% of the localities were situated in densely populated areas which even now causes conflict between beetle protection and the safety of citizens. The concentration of localities in the above areas results from the species' climate and habitat preferences: it chooses the warmer microclimate of cities and selects large and rather exposed pedunculate oaks (Strojny 1985, Sláma 1998, Buse et al. 2007, Albert et al. 2012) which are abundant in urban areas (parks, roadside tree rows), where the trees are not removed once they exceed the so-called felling age and most often live to be ancient.

On the other hand, the occurrence of the species "close to humans" offers a unique opportunity to educate the society in matters of protection of saproxylic organisms; as a result of its size and interesting biology, the great capricorn beetle is an ideal candidate for an educational subject.

Conclusions

The results not only supplement and update the distributional information on the great capricorn beetle in south-western Poland, but they may also contribute to devising an effective strategy for conservation of this endangered species. The presented data clearly suggest that such a strategy would require the cooperation of many authorities: local governments and forest management institutions such as State Forests, City Green Management (with their communal forests and city parks), water management authorities (floodplains and flood banks) and roads management. The results point to a particular responsibility for the authorities of the largest city within the region Wrocław which holds more than 30% of the localities. Additionally, the largest owner and manager of the forests in Poland, the State Forests, should take an active part in formulating and improving such a strategy.

This study confirms that modelling distributions of the saproxylic species can provide an objective means for the identification of potentially optimal and suitable habitats for their conservation. The analyses on protected area efficacy showed that existing national networks of conservation areas alone are not adequate for preserving species such as *C. cerdo* L., 1758. Thus, Natura 2000 is the cornerstone on which protection strategies should be built. However, adaptive management plans need to be compiled and then implemented both in each area and their surroundings.

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Supplementary material I

Table S1. Correlation matrix of all initial environmental layers selected for modelling in MaxEnt

Authors: Marcin Kadej, Krzysztof Zając, Adrian Smolis, Dariusz Tarnawski, Katarzyna Tyszecka, Adam Malkiewicz, Monika Pietraszko, Marcin Warchałowski, Radosław Gil Data type: statistical data

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Supplementary material 2

Distribution data of Cerambyx cerdo

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RESEARCH ARTICLE



Attraction of different types of wood for adults of Morimus asper (Coleoptera, Cerambycidae)

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Abstract

Morimus asper reproduces mainly in freshly dead wood and, as a consequence, populations are affected by modern forestry practices. The taxon *M. funereus*, now incorporated into the species *M. asper*, is protected by the Habitats Directive (Council Directive 92/43/EEC) and its monitoring has received attention in recent years. Larvae of *M. asper* are polyphagous, but some studies indicate that adults prefer the wood of some tree species. Freshly cut log piles, which attract adults, have been proposed as a monitoring tool. For monitoring programmes, it is essential to select the most appropriate wood and therefore the attraction of different types of wood for adults of *M. asper* was investigated in two sites in northern Italian, using freshly cut log piles. The first experiment was carried out in the Nature Reserve Bosco della Fontana (a lowland forest, Mantua province), testing two autochthonous species (*Carpinus betulus* and *Fraxinus ornus*) and two allochthonous species (*Juglans nigra* and *Quercus rubra*). The second study was conducted in the Parco Naturale Regionale delle Prealpi Giulie (a mountain area, Udine province), employing *Fagus sylvatica, Fraxinus excelsior* and *Picea abies* (all autochthonous species). The population of this area belongs to the taxon formerly named *M. funereus*. In both sites, adults clearly preferred the wood of some particular species: *J. nigra* at Bosco della

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Fontana and *F. sylvatica* in the mountain area. This is the first study which compared the attraction of several tree species and it showed that this selection is important for the monitoring of *M. asper*.

Keywords

Longhorn beetles, deadwood, monitoring, biodiversity, conservation, Northern Italy, Bosco della Fontana, Julian Prealps

Introduction

Biodiversity loss is altering the functioning of ecosystems (Cardinale et al. 2012) and also continues to be a concern in Europe (de Heer et al. 2005, Henle et al. 2007, Nieto and Alexander 2010). Modern forestry has substantially changed the species and age composition of forests over the last centuries (Bengtsson et al. 2000, Gossner et al. 2013) and these changes have resulted in loss of dead wood in terms of quantity, quality and dynamics with obvious consequences for forest biodiversity in general and particularly for those species associated with dead wood (Siitonen 2001, Stokland et al. 2012, Seibold et al. 2015). Saproxylic beetles, i.e. species that depend on dead wood material at some stage of their life cycle (Speight 1989, Stokland et al. 2012), are reliable indicators of many aspects of dead-wood ecology (Stokland et al. 2012, Gossner et al. 2013). The presence of dead wood and saproxylic Coleoptera in forests, woodlands and parklands is indicative of high quality mature habitats (Davies et al. 2008). Many saproxylic beetles, occurring in Europe, are threatened (Nieto and Alexander 2010) due to continued habitat loss caused by logging and wood harvesting as well as the decline in veteran trees (Speight 1989, Nieto and Alexander 2010) and these concerns have led to 17 species of saproxylic beetles being listed in the Annexes of the Habitats Directive (Council Directive 92/43/EEC). Article 11 of this Directive specifies that monitoring of the conservation status of these species is obligatory for all member States. For Italy, Trizzino et al. (2013) were the first to propose standard monitoring protocols for all arthropods listed in the Habitats Directive and, recently, a manual for the monitoring of all species and habitats of community interest was published for Italy. This manual provides methods and protocols recommended for the monitoring required by the Habitats Directive (Stoch and Genovesi 2016).

The Cerambycidae is one of the largest families of all Coleoptera and the largest family of saproxylic beetles (Grimaldi and Engel 2005, New 2010); it comprises primary saproxylics, hence initiating the decomposition process and preparing the substrate for colonisation by the secondary and tertiary saproxylics (Hanks 1999, Stokland et al. 2012). Many authors have suggested that Cerambycidae might be particularly suited as indicators in forest ecosystems (Holland 2007, Ohsawa 2010, Hardersen et al. 2014). One species of this family, *Morimus asper* (Sulzer, 1776), has recently received particular attention. In the past the European populations of the genus *Morimus* were divided into five species (Reitter 1894, Dajoz 1976), but a recent study, based on COI and ITS2 gene sequences, found that all European and Turkish populations studied should be referred to as a single species, *M. asper* (Solano et al. 2013). This study confirmed the recent classification by several authors that *M. asper* and *M. fu*-

nereus belong to the same species (e.g., Sama and Löbl 2010, Sama and Rapuzzi 2011, Danilevsky 2015) and here the two taxa *asper* and *funereus* Mulsant, 1863 have been considered as subspecies of *M. asper*. This classification has implications for the conservation of this taxon, as *M. funereus* is listed in Annex II of the Habitats Directive and its status needs to be re-evaluated in the light of the new taxonomic results. For that purpose, a standard monitoring method for *M. asper* is urgently needed.

Probably the first method proposed for the monitoring of *M. asper* has been published by Vrezec et al. (2009), based on pitfall traps, placed in groups around fresh stumps. In contrast, Campanaro et al. (2011) suggested the use of freshly cut log piles as bait for monitoring and this method has also been proposed by Trizzino et al. (2013) and Bologna et al. (2016) for the monitoring of *M. asper*. Chiari et al. (2013) investigated this method in more detail and reported that it is reliable for detecting the presence and abundance of the target species and that occupancy probabilities increased with increasing volume of log piles. Hardersen et al. (2017) found that dead wood with a diameter of more than 12 cm was more attractive. Most authors concur that *M. asper* is a polyphagous species (e.g., Sturani 1981, Luce 1996, Sama 2002, Polak 2012) and this suggests that many tree species would be suitable for the monitoring. However, the data from Hardersen et al. (2017) suggested that the wood of two tree species tested did not have the same attraction for the adults of *M. asper*. Bărbuceanu et al. (2015) also reported that *M. asper* preferred certain tree species over others. As the freshly cut log piles are meant to function as bait for adults of *M. asper*, it was important to investigate the power of attraction of different types of wood and to analyse if the wood from different tree species were equally suitable for monitoring. The aim of this study was to test whether the choice of tree species affects the power of attraction of woodpiles for the adults of *M. asper*, investigating also allochthonous species. Two forests in northern Italy were investigated in two independent case studies.

Materials and methods

Study sites

Both study sites are located in northern Italy (Figure 1). The Nature Reserve "Bosco della Fontana" covers an area of 233 ha and is located in Central Northern Italy, Lombardy region (province of Mantua, municipality of Marmirolo). The reserve (altitude of 25 m a.s.l.) is part of the Natura 2000 network "IT20B0011 – Bosco Fontana". The forest, which covers an area of about 198 ha, is mainly composed of *Carpinus betulus, Quercus robur* and *Q. cerris* and has been classified as belonging to the association *Polygonato multiflori* – *Quercetum roboris* Sartori 1984 (Campanaro et al. 2014). No wood has been extracted from the reserve since 1994 (Mason 2002) and this has led to large amounts dead wood being present (Travaglini et al. 2007). As a consequence, a large population of *M. asper* is present in the reserve (Hardersen et al. 2017) and it belongs to the nominate subspecies. The study sites were situated between 45.19961°N, 10.73476°E and 45.19848°N, 10.74199°E.



Figure 1. The two study areas, located in Bosco della Fontana (**A**) and in the Parco Naturale Regionale delle Prealpi Giulie (**B**) (northern Italy). White dots in A represent single log piles, white dots in B represent blocks of three log piles.

The Parco Naturale Regionale delle Prealpi Giulie (hereafter referred to as: Prealpi Giulie) was set up in 1996 and covers an area of about 10000 ha. It is located in North East Italy, in the Friuli Venezia Giulia region (province of Udine). The research was conducted in a forest of the municipality of Resia, locality Starmiza di Resia (between 46.343490°N, 13.299400°E and 46.341420°N, 13.307800°E) which is part of the Natura 2000 site "IT3320012 - Prealpi Giulie Settentrionali". The study area was situated at a range of altitudes between 750 m and 850 m a.s.l. and was covered by almost pure stands of Fagus sylvatica which belong to the Illyrian Fagus sylvatica forests (Aremonio-Fagion). These were managed by shelterwood cutting and naturally occurring dead wood was removed, resulting in an almost pure stand of beech which was young, even-aged and poor in dead wood. The morphology of the local terrain was often very steep and, as a consequence, small scale landslides locally created mounds of dead wood. Additionally, dead wood accumulated over time in some small and isolated parts and these were not accessible by man. Results, obtained from preliminary research in 2014 and 2015 in this site, showed that the population density of *M. asper* was low, with a clustered structure (unpublished data) and that the expected effects of forest management and the observed population parameters of M. asper were concordant. Here the local population belonged to the subspecies *funereus*.
Building the freshly cut log piles

At Bosco della Fontana, freshly cut log piles (FCLP) were built from 26.01 to 21.03.2016, utilising trunks and branches with diameters from 13 to 45 cm of the following tree species: Carpinus betulus, Fraxinus ornus, Juglans nigra and Quercus rubra. All trees used came from the Nature Reserve. The wood was cut into logs with a length of 60 cm. These were stacked in 28 FCLPs (seven FCLPs for each tree species) and had a volume of approximately 0.3 m³. Each pile contained at least one log with a minimum diameter of 30 cm. The FCLPs were randomly ordered at the sides of forest roads, spaced at intervals of 50 m along these forest roads as indicated in Figure 1. In the Prealpi Giulie, the wood piles were built between 03.05 and 04.05.2016 from three tree species: Fagus sylvatica, Picea abies and Fraxinus excelsior. All trees utilised came from the Parco Naturale Regionale. The trunks used had diameters between 13 and 35 cm and were cut into logs with a length of 60 cm. As it was known that *M. asper* has a clustered distribution in the site investigated (unpublished data), a randomized block design was employed with distances of 85-215 m between the various blocks. The FCLPs were built in seven blocks and each block consisted of three piles built each from one of the three tree species. A total of 21 FCLPs were built along the transect indicated in Figure 1 and each had a volume of approximately 0.3 m³. Each pile contained at least one log with a minimum diameter of 26 cm. The average distance between blocks was approximately 115 m.

Searching for M. asper

The searches for adults of *M. asper*, requiring two people, consisted of a thorough inspection of the surface of each log of the FCLP, without dismantling the wood piles. Each specimen encountered was caught by hand and placed in a plastic holding container. Once the inspection of the FCLP had been concluded, all individuals were counted and sexed. Immediately after sexing, all M. asper were released on the same FCLP where they had been encountered. Phenology and peak activity of adults differed between the two study sites due to different climatic conditions: at Bosco della Fontana, the highest number of adults was present in April (Hardersen et al. 2017) whereas the highest number was observed in the Prealpi Giulie in June (unpublished data). Thus, at Bosco della Fontana, adults of *M. asper* were searched for once a week from 29.03 to 17.05.2016 (i.e. a total of eight samplings) between 20:00h and 22:30h (Table 1). In the Prealpi Giulie, adults of M. asper were searched for from 17.05 to 12.08.2016 between 18:00h and 20:00h. Here it had been planned to search each FCLP once a week, but bad weather (rain and low temperatures) forced the schedule to be modified (Table 1). Here, a total of 13 samplings were carried out.

Bosco de	Bosco della Fontana		Prealpi Giulie		
Date	N. of M. asper	Date	N. of <i>M. asper</i>		
29/03/16	18	17/05/16	0		
05/04/16	45	25/05/16	3		
12/04/16	66	01/06/16	1		
19/04/16	48	10/06/16	5		
26/04/16	36	21/06/16	6		
03/05/16	65	25/06/16	6		
10/05/16	59	30/06/16	6		
17/05/16	48	04/07/16	5		
		15/07/16	6		
		21/07/16	8		
		26/07/16	5		
		04/08/16	0		
		12/08/16	0		

Table 1. Survey dates and numbers of *M. asper* observed for the two study sites.

Statistical analysis

To compare the numbers of males and females over the sampling period, the Wilcoxon matched-pairs signed-ranks test was applied. To assess differences amongst the tree species in their attraction for *M. asper*, the data collected during all sampling dates were pooled for each site and compared with the Friedman's Test. This analysis was carried out separately for all specimens, males and females. Then a post-hoc Dunn's Test for Multiple Comparisons was applied. The tests were carried out with the software GraphPad InStat 3.1a for Macintosh.

Results

At Bosco della Fontana, a total of 385 individuals of *M. asper* were counted and, in the Prealpi Giulie, a total of 51 adults were observed (Table 1). This corresponded to an average of 1.7 *Morimus* adults on each FCLP during one survey for Bosco della Fontana and to an average of 0.19 *Morimus* adults on each FCLP during one survey for the Prealpi Giulie. Over the sampling period, the sex ratio was highly significantly in favour of males in both study areas (Bosco della Fontana: 318 males, 67 females, P=0.008, Wilcoxon matched-pairs signed-ranks test; Prealpi Giulie: 42 males, 9 females, P=0.002, Wilcoxon matched-pairs signed-ranks test).

At Bosco della Fontana, the number of adults, both males and females observed on the different types of wood, was significantly different (Friedman's Test, all adults, Fr=22.1, P <0.0001; males, Fr=21.9, P<0.0001; females, Fr=12.2, P=0.007). The wood of *J. nigra* and *Q. rubra* was significantly more attractive than that of *F. ornus*, with *C. betulus* being intermediary, considering all specimens, males and females, separately (Figure 2).



Figure 2. Bosco della Fontana. Average number (\pm SE) of adults of *M. asper* observed per sampling on the seven wood piles built from wood of different tree species. Different capital and small letters above columns (total adults) and on the right (males and females) indicate significant differences among wood types at the 0.01 and 0.05 levels, respectively (Dunn's Multiple Comparisons Test).

In the Prealpi Giulie, the number of individuals observed on the different types of wood was significantly different for all adults and for males (Friedman's Test: all adults, Fr=10.6, P=0.005); males, Fr=10.2, P<0.006). The number of female individuals observed followed the same pattern as for all adults and for males, but was not significant (Friedman's Test: females, Fr=3.7, P=0.16). The wood of *F. sylvatica* was significantly more attractive than that of *P. abies* with *F. excelsior* being intermediary, considering all adults and males (Figure 3).

Discussion

On the FCLPs of both sites investigated, adults of M. *asper* were observed regularly and permitted the monitoring of the local populations. These data are in line with the findings by Chiari et al. (2013) and Hardersen et al. (2017) that FCLPs are reliable for detecting the presence and abundance of the target species. In both sites, M.



Figure 3. Prealpi Giulie. Average number (±SE) of adults of *M. asper* observed per sampling on the seven wood piles built from wood of different tree species. Different capital letters above columns (total adults) and on the right (males and females) indicate significant differences among wood types at the 0.01 level (Dunn's Multiple Comparisons Test).

asper males were recorded more frequently, as is commonly observed when surveying Cerambycidae (Lopez-Pantoja et al. 2008, Drag et al. 2011) and this also applies to *M. asper* (Chiari et al. 2013, Hardersen et al. 2017).

At Bosco della Fontana, the average number of *M. asper* observed on each wood pile was 1.7 during each sampling whereas, in the Prealpi Giulie, this number was only 0.19. Even though the two studies are not directly comparable, the large difference in the number of observed adults is indicative of different population densities. The high figure observed at Bosco della Fontana revealed a large population, probably as a direct consequence of the management actions which are aimed at the protection of dead wood in all its forms (Campanaro et al. 2014). In contrast, for the Prealpi Giulie, the data gathered indicated the presence of a small and patchy population of *M. asper*, in line with the current management of the forest investigated which is almost devoid of dead wood. The unfavourable conservation status of this protected beetle species is directly linked to the paucity of dead wood in this site of the Natura 2000 network and it would therefore be important to modify the management of these beech forests to

increase the amount of dead wood, as indicated by the plan for the conservation and development of the Park (Piano di conservazione e sviluppo del Parco Naturale delle Prealpi Giulie, Decreto del Presidente della Regione Friuli Venezia Giulia 20 marzo 2015 n. 062/Pres).

This is the first study which compared the power of attraction of several tree species and it showed that the selection of the species is important for the monitoring of *M. as*per. This study is also the first showing that some tree species attracted hardly any adults. These results were also consistent when males and females were considered separately. In the Prealpi Giulie, the results for females followed the same pattern as in all adults and in males, but they were not significant. This may have been caused by the low number of female individuals observed. The results confirmed that the wood of some tree species is more attractive to M. asper (Bărbuceanu et al. 2015, Hardersen et al. 2017). It was surprising that the two allochthonous species (J. nigra, Q. rubra) tested at Bosco della Fontana were most attractive for the adults of *M. asper*, as a preference for these non-native species cannot have evolved by natural selection. In contrast, the wood of the dominant species of Bosco della Fontana, C. betulus, attracted only 53% of adults when compared to *J. nigra* and the wood of the other autochthonous tree species was not attractive at all; F. ornus attracted only 3.7% of adults. These data are in line with the fact that the genus Fraxinus (or the family Oleaceae) is not mentioned as a host plant for this longhorn beetle (e.g., Romero-Samper and Bahillo 1993, Sama 2002, Dojnov et al. 2012).

In the Prealpi Giulie, the dominant tree species, *F. sylvatica*, attracted the largest number of adults of *M. asper* and these findings are in agreement with numerous authors who indicated that beech is one of the main host species (Romero-Samper and Bahillo 1993, Sama 2002, Polak 2012, Bărbuceanu et al. 2015) and with Fusu et al. (2015) who stated that beech is one of the preferred wood types by *M. asper*. In this case study, the second most attractive species was *F. excelsior*, a congener of *F. ornus*, which was the least attractive species at Bosco della Fontana. *Picea abies* was the least attractive species, even though *M. asper* was reported to complete its life-cycle in trunks of this tree species (Sturani 1981).

Adults of *M. asper* must be able to locate freshly dead wood, often a scarce resource, to reproduce. Once a tree has died, the decay process results in an extensive release of volatile organic compounds (Holighaus 2012) and host-plant recognition of insects depends on ratios of plant volatiles (Bruce et al. 2005). This has also been shown for adults of Cerambycidae (Paschen et al. 2012). For example, the composition of volatiles clearly separate hardwood from softwood species and, to some extent, individual species (Holighaus 2012). Even though no specific information is available on host location by *M. asper*, it seems likely that they seek out the preferred woodtypes by means of volatile organic compounds.

The study revealed a further important aspect for the monitoring of *M. asper*, i.e. the weather. When a cold spell arrived in mid-late April in the province of Mantua, the number of adults observed almost halved. Similarly, in the Prealpi Giulie, late May and early June were characterised by particularly cold weather with frequent rain and the number of adults observed was low. These observations are in accordance with

Stanić et al. (1985) and Polak (2012) who reported that, at low temperatures (respectively below 12°C or 17°C), activity of *M. asper* is much reduced.

In summary, this study is the first which compared the attraction of numerous types of wood to adults of *M. asper* and showed that the wood of the tree species tested was not equally attractive. This finding is in line with Bărbuceanu et al. (2015) and Hardersen et al. (2017) who found that certain tree species are more attractive. Thus, when selecting wood for FCLPs for the monitoring of *M. asper*, it is important to choose wood which is attractive. Currently it can be said that the wood of *F. ornus* and P. abies should not be used for building wood piles. In contrast, the wood of the following trees attracted large numbers of adults: J. nigra, Q. rubra, Q. robur and F. sylvatica (Hardersen et al. 2017 and current study). While the wood of C. betulus and F. excelsior was moderately attractive for M. asper, monitoring could still be carried out. (Hardersen et al. 2017 and current study). For long-term monitoring programmes, it is essential to assess the long-term availability of the wood. It is also important that the wood of the selected tree species is freshly dead and has a diameter larger than 12 cm (Hardersen et al. 2017). This study, together with those by Chiari et al. (2013) and Hardersen et al. (2017), demonstrated that by investigating the factors which influence the selection of dead wood by the target species, the monitoring method for this elusive, localised and protected saproxylic species can be optimised.

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RESEARCH ARTICLE



Spatial analysis of the habitat and distribution of Osmoderma eremita (Scop.) in trees outside of woodlands

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Abstract

The endangered and specialised saproxylic beetle Osmoderma eremita occurs in veteran trees, a habitat particularly threatened by changes in agricultural landscapes. Its conservation requires information about populations and key features of host trees. Surveys of 8,014 trees (pollarded or in hedgerows) were carried out and analysed, based on habitat description (tree level) and spatial information (hedgerow length and distance to the nearest inhabited tree). A suitable cavity was present in 61% of the trees and O. eremita was detected in 42 trees, mainly in Salix (30 observations), the most common tree amongst those surveyed. A small or absent crown was a significant factor in explaining the beetle's presence, as was the distance to the nearest inhabited tree. The largest population of O. eremita, 19 inhabited trees, was found in a wide and continuous area formed by trees with suitable cavities, with distances of less than 250m from each another. Seven smaller areas, with 7, 5 or 1 inhabited trees, were also found. When analysing inhabited trees on a 1km² grid, 17km² hosted O. eremita, corresponding to a dense network of 63km of hedges. The presence of O. eremita significantly increased per km² with increasing length of hedges and this variable was thus used to guide forthcoming investigations directed toward Osmoderma. As the hedgerows existing in 1999 had decreased by 6.1% in 2009, it is concluded that the long term survival of O. eremita is under threat. The preservation of trees outside woodlands is urgent and has already started, in connection with Natura 2000 policies. Regeneration and creation of new hedgerows is also ongoing and can be reinforced both by using Salix and by promoting pruning to increase formation of cavities.

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Keywords

Insect conservation, Habitat quality, Osmoderma eremita, Saproxylic beetle, Hollow tree

Introduction

Wood pastures, hedgerows with trees and trees outside woodlands (TOW) (Alexander et al. 2016) are known to host an important biodiversity, including specialists, threatened, rare and protected species (Vallauri et al. 2005, Fayt et al. 2006, Stokland et al. 2012, Ramírez-Hernández et al. 2014), as well as species useful for agriculture (Burel et al. 2004). Specialists are mostly represented by saproxylic species i.e. "organisms involved in or dependent on the process of fungal decay of wood, or on the products of that decay and which are associated with living as well as dead trees" (Alexander 2008). Osmoderma eremita (Scopoli, 1763) (Coleoptera, Scarabaeidae), the hermit beetle, is a member of these saproxylic species, endemic to Western Europe (Audisio et al. 2007). It is listed as Near Threatened in the European IUCN Red List. The species strictly depends on veteran trees, as it inhabits decaying heartwood inside tree hollows, a very specific habitat type which is already highly fragmented and subject to a significant and continuing decline in Europe (Nieto et al. 2010). O. eremita is vulnerable to habitat fragmentation (e.g. Ranius and Hedin 2001) and is considered a relict species of primeval forests (Müller et al. 2005). It is also listed in Appendix II of the Bern Convention and is strictly protected in Europe as a priority species in Annexes II and IV of the Habitat Directive 92/43/EEC. EU member States are thus required to protect, manage and monitor the habitat of O. eremita, in accordance with the ecological needs of the species. In France, this is done via both national and regional strategies for biodiversity (MEDDE 2012). Diverse operational tools are used, such as strict protection by law, delimitation of ecological corridors, regional plans for ecological coherence and the Natura 2000 network. In France, the most recent evaluation of the conservation status for O. eremita is U2: Unfavourable-Bad (Bensettiti and Puissauve 2015). Indeed, important populations continue to be threatened by planning decisions (e.g. Dirksen and Mériguet 2009).

In the northwest of France, a dense hedgerow network persisted over centuries in connection with the traditional system of agriculture. The maximum density of the hedgerow network was reached at the end of the 19th century and decreased from the 1950s onwards, mainly as a result of intensified farming practices (Burel and Baudry 1990, Dubois et al. 2009). In the context of attempts to preserve the remnants of this network, *O. eremita* represents an umbrella species, a species whose conservation is expected to confer protection on a large number of other naturally co-occurring species (Roberge and Angelstam 2004). Protecting *O. eremita* can help to build conservation policies for hedgerows with trees and TOW and can also facilitate the raising of public awareness for relevant conservation issues (Luce 2001).

The Parc Naturel Régional des Boucles de la Seine Normande (PNR-BSN) is partly covered by a dense and extensive network of large trees following the Seine river. This area is also characterised by the presence of two large cities, Rouen and Le Havre. The Seine river, with the major ports of Rouen, Le Havre and Paris, is economically important. This context negatively impacts wetland ecosystems: dredging, drainage and gravel mining are common activities and today, this last activity probably has the most significant negative impact on old trees as it is often carried out inside the hedge-row network. Conflicts between nature protection and economic development are thus a serious problem. Moreover, historic data collected before 1950 suggested that *O. eremita* could be present in the hedgerow network. The PNR-BSN hence decided to use this species as an indicator of the quality of the network. In accordance with the state services (DREAL), this was integrated into the objectives and action plans of Natura 2000 sites, as well as into the PNR-BSN conservation strategy for 2012–2016 (Gaudet 2013). Three years of research and inventories have yielded numerous data, which the present study will analyse.

The specific aims of this study are to:

- a) contribute to the knowledge on the ecology of *O. eremita*;
- b) explore the characteristics of the inhabited trees and their spatial patterns in the hedgerow network in the PNR-BSN;
- c) predict favourable areas where *O. eremita* is likely to occur and contribute to the establishment of guidelines for actions favourable for the protection of the species.

Material and methods

Ethics statement

This study is based on fieldwork carried out with prefecture permits dated 19/08/2008 (fieldwork from summer 2008 and earlier) and 30/07/2013 (fieldwork from 2013 to 2016).

Species studied

Until recently, European *Osmoderma* were considered as a single species, *O. eremita* (Scopoli, 1763), but recent evidence regarding morphology and from sequencing of mtDNA cytochrome C oxidase I gene, leads to a division into two distinct lineages. West European populations belong to *O. eremita* and the eastern ones to *O. barnabita* Motschulsky, 1845, while in Southern European ancient glacial refuges, there are three others species: *O. italicum* Sparacio, 2000, *O. cristinae* Sparacio, 1994 and *O. lassalei* Baraud & Tauzin, 1991, respectively endemic to the Italian peninsula, Sicily and Greece and European Turkey (Sparacio 2000, Ranius et al. 2005, Audisio et al. 2007, 2009, Landvik et al. 2013, Zauli et al. 2016).

Osmoderma species live in the cavities of large and old broad-leaved trees and, very likely, they all have the same biological requirements. Suitable trees can be in

old-growth forests (Luce 1995, Chittaro and Sanchez 2015) or outside woodlands, especially in wooded pastures, hedgerows (Vignon 2008), rural avenues and old parks around and inside cities (Oleksa et al. 2007, Blanc 2012). A female lays an average of 40 eggs. Larvae live in wood mould created by heartwood fungi (red rot formed mostly by *Laetiporus sulphureus* (Bull.) Murrill or *Fistulina hepatica* (Schaeff.) With.). They develop for 2 to 3 years, depending on the conditions in the cavity, e.g. mainly hygrometry and nutriment quality (Tauzin 2005) and the presence of microbial activity (Landvik et al. 2016a). An average mortality during the nymphal stage of approximately 25% has been observed (Tauzin 2005).

In Sweden, Ranius and Hedin (2001), observed only a low proportion (15%) of beetles dispersing from their natal tree. In contrast, in Italy, smaller populations per tree have been found and dispersing adults are exposed to a high predation rate (at least 28%) (Chiari et al. 2013). According to this study, 39% of dispersing individuals reached distances of greater than 250m. Generally dispersal abilities of the hermit beetle are very low, of the order of a few hundred metres. Radio-tracking yielded maximum flight distances of 180m (Hedin et al. 2008), 700m (Dubois and Vignon 2008) and 1,504m (Chiari et al. 2013). Other techniques confirmed this range of dispersal: 190m (Ranius and Hedin 2001) and 2,090m (Valainis et al. 2015) with mark-recapture studies, about 500m calculated with DNA markers (Oleksa et al. 2013) and a maximum single flight distance of 1,454m and a maximum cumulative flight distance of 2,361m obtained with a flight mill (Dubois et al. 2010). Dispersal is probably guided by the (R)-(+)- γ -decalactone, a pheromone emitted by males to attract females and sometimes also males in various other species of *Osmoderma* (Larsson et al. 2003, Svensson et al. 2009, Zauli et al. 2016).

In France, although *O. eremita* is widely distributed, a large proportion of the known localities are old or very old and many local populations are already extinct (Tauzin 2005). Currently, the largest populations are in the western part of the country, the south of the Massif-Central and around the Mediterranean sea, where hedge-row networks formed a continuous habitat before 1950 (Muséum national d'Histoire naturelle 2003, Vignon 2008). In the PNR-BSN, the first and only historical record is prior to 1950 and it concerns the plateau of Barneville-sur-Seine and Caumont, in south-east PNR-BSN. No other local information about the species was known prior to the intensive investigations started in 2005 (Vignon and Dubois 2005, Simont and Dufrêne 2007, ALISE Environnement et al. 2008).

Study area

Fieldwork was carried out in the territory of the PNR-BSN, North-West of Paris, France (49°27'03"N; 00°39'57"E). In this area, hedgerows and orchards still form a dense network in areas such as the Seine and Risle valleys, the Marais Vernier and the southern plateau. The territory of the PNR-BSN has not been studied exhaustively. Based on aerial photographs, study sites were selected that included a dense network

of hedges with trees and pollarded trees. In 2007, the studied area covered the Seine river floodplain at the meanders of Jumièges, Anneville/Ambourville, Roumare and the Roumois' plateau. In 2008, the survey investigated the western part of the PNR-BSN, the Roumois' plateau, the meander of Brotonne, the Marais Vernier and the Risle valley. Selected sites were studied exhaustively: all suitable trees within each site were surveyed (woodlands excluded).

Field search of Osmoderma

The field inventory of trees was undertaken during summer and autumn 2005-2015, with most work being done in 2005 and 2007–2008. Over the period from 2012-2015, trees inhabited by *Osmoderma* or with indices of presence were surveyed (see below) in order to confirm the activity of the species. Altogether, 8,014 trees were investigated (Table 1).

If necessary, ladders (length 4m) were used to reach the trunk cavities. A total of 38 cavities were unreachable. Cavities were explored visually with a lamp and their depth was estimated with a small diameter metal rod. The surface of the wood mould was inspected in order to search for indications of the presence of *Osmoderma*, those indications being live larvae and imagines, cocoons made with wood mould, remains of exoskeleton and/or larval faecal pellets. When no indications were observed on the surface, a small amount (approximately 1 litre) of wood mould was sieved and spread out on a white sheet in the field for careful inspection. Afterwards, the wood mould was returned to the cavity. The characteristic odour of the male pheromone was also considered as indicating the presence of *O. eremita*.

The detection of *Osmoderma* in cavities may be difficult and increasing the sampled volume of wood mould increased the probability of detecting larvae (Chiari et al. 2014). A volume of up to 8 litres of wood mould per tree was retained by Ranius and Jansson (2002). Here a compromise was adopted between the desire to survey a large number of trees and the volume of wood mould investigated per tree. As indices of presence can endure for a long time in a cavity, especially in dry conditions, only direct observation of live larvae, nymphs and imagines are proof of the actual presence of *Osmoderma* in a cavity (Dubois 2009). However, the presence of remains of exoskeletons is strongly correlated with an actual population (Ranius and Jansson 2002). In this study, it was decided to classify a tree as a host of *O. eremita* if live individuals (larvae and/or imagines) were seen or if cocoons, remains of exoskeletons or abundant amounts of faecal pellets were observed.

Tree and environmental variables

During the field work, spatial coordinates of each tree were taken with a GPS. Trees were described and cavities examined. The trees descriptors were: identification to spe-

Year of sampling	Type of survey	Trees surveyed	Trees inhabited by Osmoderma eremita	Reference
2005	Targeted searches (Scale: tree patch)	4,181	8	(Vignon and Dubois 2005)
2007	Full inventory (Presence-Absence data)	4,378	29	(Simont and Dufrêne 2007)
2008	Full inventory (Presence-Absence data)	3,608	2	(ALISE Environnement et al. 2008)
2012	Targeted searches (Scale: tree)	5	4	PNR-BSN
2013	Targeted searches (Scale: tree)	383	10	PNR-BSN
2014	Targeted searches (Scale: tree)	31	22	PNR-BSN
2015	Targeted searches (Scale: tree)	2	2	PNR-BSN
Total		8,014	42	This study

Table 1. Data sources and description for *Osmoderma eremita* in the PNR-BSN. Some trees have been surveyed two times or more.

cies level, evolution stage of hollow defined according to Ranius et al. (2001) and which incorporates an estimate of the volume of the cavity and of the wood mould, date of last pruning, size of the tree crown.

All tree variables were pair-tested to confirm the absence of correlations. Values of large discontinuous variables were then grouped into smaller categories to reinforce the explanatory powers of the models: the information on tree species was reduced to the three most common genera plus a fourth category which contained all other genera; the developmental stage of the cavity was grouped into three categories instead of five; crown development was reduced to three categories instead of five (Table 2).

Hedgerows with trees were measured using the 1:2,000 and 1:5,000 maps of the PNR-BSN. Ortho-photographs from 1973, 1999 and 2009 were digitalised and classified according to hedgerow types, amongst which were selected only hedgerows composed of trees or containing trees with a minimum length of 15m (forest edges excluded). Solitary trees, small series of pollards and orchards were also included. In this article, the terminology tree outside woodland (TOW) was used to refer to a hedgerow with trees together with solitary and/or pollards and orchards.

Connectivity was measured in two ways. First, the distance to the nearest neighbour tree inhabited by *O. eremita* was used. There is evidence that this measure is less powerful (Moilanen and Nieminen 2002, Buse et al. 2016), for it ignores the numerous sources of colonisation in the landscape (Prugh 2009). Second, the length of TOW per 1 km² was used, a buffer measure within a limited neighbourhood of the focal TOW, with no effect on distance. This measure was expected to have more explanatory power (Moilanen and Nieminen 2002, Prugh 2009, Ranius et al. 2010).

The landscape context of TOW was described by considering groups of trees at the 250m spatial scale. Groups were constructed by applying a buffer zone around any tree having a suitable cavity (i.e. cavity of stage 2, 3 or 4) and then by merging the adjacent polygons. The areas of the resulting zones (in hectares) were then calculated, the number of trees counted and the length of hedges contained in these polygons.

Variable	Description	Categories used in models
Tree genera	<i>Salix</i> (n = 3,961). <i>Fraxinus</i> (n = 2,227). <i>Populus</i> (n = 1,286). <i>Carpinus</i> (n = 185). <i>Quercus</i> (n = 74). Other trees + Missing data (n = 281).	<i>Salix</i> (n = 3,961). <i>Fraxinus</i> (n = 2,227). <i>Populus</i> (n = 1,286). Other (n = 540).
Developmental stage of cavity	Stage 0: no cavity (n = 576). Stage 1: beginning of cavity development, not deep, small amount of mould (n = 2,157). Stage 2: Cavity with mould and a small entrance (n = 1,125). Stage 3: Large cavity with an important volume of mould (>5 litres) and a small entrance, optimum stage for <i>Osmoderma</i> (n = 1,067). Stage 4: Large and open cavity, empty of its mould after natural degradation of the trunk. The cavity soon to be uninhabitable by <i>Osmoderma</i> (n = 2,711). Stage 5: Dead or alive tree with emptied cavity (n = 373). Missing data (n = 5).	Suitable = Stage 2+3+4 (n = 4,903). Unsuitable = Stage 1+5 (n = 2,530). Absent = Stage 0 (n = 576).
Date of last pruning	Old (>10 yrs). Medium (5-10 yrs). Recent (< 5 yrs). Missing data (n = 9).	Old (n = 5,369). Medium (n = 1,605). Recent (n = 1,031).
Crown development	Absence of crown either because tree is dead or after a recent, major pruning (n = 351). Small crown (n = 810). Medium crown (n = 1,700). Large crown (n = 1,975). Very large crown (n = 3,170). Missing data (n = 8).	Absent or small (n = 1,161). Medium size = Medium + large (n = 3,675). Very large (n = 3,170).
Presence of Osmoderma eremita	Observation of larvae, adults, exoskeleton remains and/or faecal pellets of larvae of <i>O</i> . <i>eremita</i> .	Presence (n = 42). Absence (n = 7,972).
Distance to the nearest tree inhabited by <i>Osmoderma</i> <i>eremita</i>	Minimum distance, in metres, to the nearest tree with presence of <i>O. eremita</i> , calculated after GPS coordinates of the trees.	Metric.
Length of TOW per 1km ²	Length of hedgerow with trees per square of a 1km ² grid.	Metric: Min.: 0.0; Median: 2,616; Mean: 2,844; Max.: 7,768.

Table 2. Description of Variables.

Ranges of 1km² and 250m were chosen because they corresponded respectively to the average dispersal distances already published for *O. eremita* (see above), as well as to infrequent but longer dispersal distances.

Data analysis

To find the variables that significantly affect the occurrence of *O. eremita*, two techniques were used. First, univariate analyses were used to assess each variable independently against occurrence of *O. eremita*. Second, generalised linear models (GLMs) were run with a binomial error distribution and logit link function. One important weakness of GLMs is that they consider the absence data to be certain. However, it is clear that the detection probability of *O. eremita* in wood mould sampling is well below 100% (Chiari et al. 2014, see discussion). Models were compared using Akaike's information criterion (AIC). The AIC value was calculated as -2*log-likelihood+k*npar, where npar represents the number of parameters in the fitted model and k = 2 (Burnham and Anderson 2004). The best model which minimises the overall AIC, was retained by means of a stepwise model selection by AIC (stepAIC), available in the MASS package of the R software.

Means of the data issued from TOW measurements in the 1km² grid were compared using one-way ANOVA. Correlations were explored with the non-parametric Spearman's rank correlations.

All statistical analyses and models were carried out with the R software version 3.1.0, RStudio version 0.99.896 and PAST version 2.17c (Hammer 2012). Mapping and calculation of spatial data were carried out with the software QGIS version 2.16.2.

Results

Temporal changes in the hedgerow network: 1973–2009

The PNR-BSN comprises 900 squares of 1km², inside of which 70,616 hedgerows and TOW have been mapped, for a total length of 3,898km according to the most recent analysis based on the aerial photographs from 2009. TOW represented 1,258.6km, a number that includes 151.2km of discontinuous hedgerow with trees and at least 5.4km of pollards in rows (pollards were not identified everywhere).

From 1973 to 2009, an increase of the total length of hedge length was found: +30.9% between 1973 and 1999 and +6.2% between 1999 and 2009 (Figure 1). These results might suggest a satisfactory situation but it is also true that 875km of hedges that had existed in 1973 were absent in 2009 (all types). The length of TOW diminished by 6.1% from 1999 to 2009. Hedges created between 1999 and 2009 were mostly low hedges composed of bushes. These were mostly concentrated around newly urbanised areas and are clearly unsuitable for *Osmoderma*. Plantation and tree re-growth represented 1,117km of young trees in 1999, reaching 1,341km in 2009 (+20.1%). These young trees are still mostly without cavities.



Figure 1. Temporal changes of the hedgerow network in the PNR-BSN.

Occurrence of Osmoderma eremita

Of the 8,014 trees studied, 4,903 (61%) presented a cavity suitable for *O. eremita* and 42 were identified as being inhabited by *O. eremita*. Four trees hosted live adults; the others contained adult remains and/or faecal pellets of larvae. The data indicated a hermit beetle population mostly confined along the meander of Jumièges, Anneville/Ambourville and Roumare.

The surveys recorded a majority of trees belonging to *Salix*, *Fraxinus* and *Populus*. *O. eremita* was clearly abundant in *Salix*, with 30 inhabited trees identified. However, the ratio between the numbers of trees surveyed versus trees inhabited by *O. eremita* was highest for *Quercus*, with 4.05% of the trees inhabited. In contrast, this value was lower for *Salix* (0.76%) and even lower for *Fraxinus* (0.22%).

Most of the willows had been pruned and developed a suitable cavity in 74.6% of cases, a ratio which represents a higher percentage of possessing a cavity when compared to ash (51.6% of trees with cavities) and poplars (37.0% of trees with cavities) (Table 3).

Habitat description

The best fitting GLM (AIC = 426.39) identified Tree genera, Developmental stage of the cavity, Crown development and Distance to the nearest tree inhabited by *O. eremita* as the most important variables. Date of last pruning and Length of TOW per 1km² were excluded during the selection process (Table 4).

Trees genera	Number of trees	Trees with cavities in stage 2, 3 and 4	Presence of Osmoderma eremita (% of the number of trees)
Acer	63	40	0
Alnus	63	53	2 (3.17%)
Carpinus	185	147	0
Fraxinus	2,227	1,149	5 (0.22%)
Populus	1,286	476	0
Quercus	74	29	3 (4.05%)
Salix	3,961	2,953	30 (0.76%)
Tilia	73	25	0
<i>Castanea</i> , <i>Crataegus</i> , <i>Malus</i> , <i>Ulmus</i> , etc., plus unidentified	82	31	2 (5.26%)†
Total	8,014	4,903	42

Table 3. Count of inventoried trees according to the presence of suitable cavities and observations of *O. eremita.* †: refers exclusively to unidentified trees species.

Table 4. Coefficients and probabilities associated with the variables of the best fitting GLM predicting the presence of *O. eremita*. Significance thresholds: 0.001 % (***), 0.01 % (**), not-significant (ns). GLM deviances: Null deviance = 524.79 on 8,004 degrees of freedom; Residual deviance = 408.39 on 7,996 degrees of freedom; AIC = 426.39.

Variables	Estimate	Standard error	z	Pr (> z)
All variables	-23.70	1,718	-0.014	0.98899
Tree genera - Other	2.143	0.5943	3.606	0.00031 ***
Tree genera - Populus	-13.71	974.9	-0.014	0.98878
Tree genera - <i>Salix</i>	1.353	0.4915	2.752	0.00592 **
Crown development - Medium	1.094	0.6221	1.759	0.07858
Crown development - Absent or small	2.016	0.6333	3.183	0.00146 **
Evolution stage of hollow - Suitable	17.38	1,718	0.010	0.99193
Evolution stage of hollow - Unsuitable	17.70	1,718	0.010	0.99178
Distance to the nearest tree inhabited by Osmoderma eremita	-0.001163	0.0003581	-3.248	0.00116 **

Spatial analysis: 250m buffer.

Significant values for the variables selected in the GLM involved trees other than *Fraxinus* and *Populus* and underlined the importance of *Salix*, inside of which most of the observations of *O. eremita* were made. A small crown or its absence also played a significant and positive role in the model. Finally, a greater distance to the nearest inhabited tree negatively affected the model.

TOW with a suitable cavity (thereafter named STOW), with a distance of less than 250m from other STOW, were distributed in the PNR-BSN within 75 areas disconnected from each another. Only a few of these areas were wide and included many STOW, whereas all other areas were small and included fewer STOW (Figure 2). As expected, the number of STOW was correlated with the surface of the areas (Spear-



Figure 2. Distribution map of inhabited trees in the PNR-BSN according to the TOW length in each 1km² square. Spatial analysis: 1km² grid.

man's D = 0.874660, p < 0.0001) and ranged from 0.05 STOW/ha to 4.14 STOW/ ha. The largest area (843.68ha) included the highest number of STOW (2,072) but not the highest density (2.46 STOW/ha). In this area, the highest number of trees inhabited by *O. eremita* (n = 19) had been found. Only one inhabited tree was found in each of four areas, five inhabited trees in one area and seven inhabited trees were discovered in two areas (range: 0.05 STOW/ha to 2.22 STOW/ha).

Only 96 squares from a total of 900 were included in the tree inventory. These represented 238km of TOW (19% of the total length). Trees inhabited by *O. eremita* belonged to 17 squares, including 63km of TOW (5% of the total length) (Table 5). Hedgerow lengths reported in the sub-sample of inventoried squares were significantly different from the total (ANOVA: Q = 5.847; p = 0.0001233***). The sampling protocol introduced a bias in favour of squares with the highest length of TOW (on average 177% of the whole average length). However, the squares with presence of *O. eremita* contained significantly more TOW than other squares investigated (ANOVA: Q = 6.574; p < 0.0001***), respectively 3.69km and 2.48km, in comparison with 1.40km for the PNR-BSN. As a consequence, squares inhabited by *Osmoderma* contained 2.5 times more TOW than the average in the PNR-BSN (Figure 3).

With the GIS system, it was possible to identify squares with a total length of TOW equal to or greater than that of the squares with trees inhabited by *O. eremita*.

Data per square	PNR-BSN (n = 900 squares)	Sampled trees (n = 96 squares)	Trees inhabited by O. eremita (n = 17 squares)	
TOW length (km)	1,340.90	238.00	62.66	
Average length (km) of TOW	1.40	2.48	3.69	
Median length (km) of TOW	1.19	2.27	3.86	

Table 5. Lengths of TOW in the 1km² squares of the PNR-BSN. Data from 2009.



Figure 3. Frequencies of 1km^2 squares according to the TOW length. Three sample groups are presented: PNR-BSN (n = 900 squares); Sampled trees (n = 96 squares); Trees inhabited by *O. eremita* (n = 17 squares). All differences between groups are significant at the threshold of 0.001% (ANOVA).



Figure 4. Distribution map of the TOW and hedges in the PNR-BSN indicating the TOW length in each 1km² square.

In this manner, two large areas were delimited which may have been occupied by the hermit beetle i.e. the meander of Roumare, in the west of the city of Canteleu and the lower part of the Risle valley, plus the plateau of Saint-Pierre-du-Val (Figure 4).

Discussion

Habitat characteristics

Osmoderma species are known to live in the cavities of many tree species: *Quercus* (many studies), *Tilia* and *Alnus* (Oleksa et al. 2007), *Castanea* (Macagno et al. 2012, Chittaro and Morin 2013), *Fagus* (Tauzin 2005) and others (for a more complete list, see e.g. Ranius et al. 2005). Landvik et al. (2016b) considered the species to be opportunistic with respect to their host trees. On the other hand, *Fraxinus* and *Acer* seem to be avoided by the hermit beetle even in locations where they are abundant (Oleksa et al. 2007, Dubois et al. 2009, Kadej et al. 2016). The heartwood of those trees is rarely colonised by red-rot fungi and thus cavities suitable for *O. eremita* are not created. In contrast, the data from the study showed that willows can be key species when they are abundant (see also Sebek 2008) and this finding is important from a conservation point of view. However, the rate of occupancy in this study was in line with most of the previous studies in that it was higher for oaks (4.05%) and lower for willows (0.76%) and ash (0.22%).

The GLM model used showed a weakness as it considered absence data to be certain. This is unrealistic with *Osmoderma*, as it is a "hard-to-find" species in many situations (Chiari et al. 2014). A solution to minimise the impact of "false absences" in the occupancy modelling is to repeat surveys at a site, or in multiple sub-sites, in order to estimate species detection probabilities. This detection might fit the 'closure' (*i.e.* no changes in occupancy) assumption between surveys (Rota et al. 2009, Kendall et al. 2013). Violations of closure typically lead to overestimates of the probability of occurrence (Rota et al. 2009). After multiples surveys, the obtained probability of species presence can be added as a simple probabilistic argument in the occupancy models (MacKenzie et al. 2002, Tyre et al. 2003, MacKenzie 2005). Another solution is to consider background data. Background data establish the environmental domain where the species can be more likely present than on average. They can include or reflect the sampling bias (Phillips et al. 2009) which can be added in regression-based modelling methods such as MaxEnt (Dudík et al. 2005).

In this study, the used model indicated that the date of last pruning was irrelevant but showed that a small or absent crown was a significant factor. As, in most cases, pruning is the main cause for a reduced size or an absence of canopy, this result seems incongruous at first. Moreover, in an agricultural landscape, willows had generally been pollarded to avoid the growth of large branches subject to breakage over the medium term (Juillerat and Vögeli 2004). The significance of small crowns in the model may therefore have resulted from the predominant presence of *Salix* in our data, though it is also clearly obvious that tree species and crown size are not correlated. Consequently, the three categories used to assess the date of last pruning, based on markers at 5 and 10 years, may ultimately be too narrow to provide reliable information about the development of suitable conditions for the hermit beetle. In a way, crown size better correlates with this parameter, as it integrates old pruning and the resulting long process of cavity formation. The time scale of 5 and 10 years had been chosen for practical reasons, to prioritise trees in need of pruning.

TOW density and O. eremita presence

Inhabited trees were found to be more abundant in the larger areas established by applying a 250m buffer zone around any TOW with a suitable cavity. No link with the density of hollow trees per ha was however found. The areas with the highest densities of STOW were searched without success. The observed densities in areas with O. eremita were at most 2.46 STOW/ha. Ranius et al. (2011) suggested a density of 0.69 hollow oaks per ha within a radius of 192m to ensure the presence of two cavityspecialist arthropods. In this study, densities of hollow trees were higher in most areas, corresponding better to the situation studied by Hedin et al. (2008) (1.4 to 4.3 hollow tree/ha) or indicated by Tauzin (2005) in Fontainebleau (1.6 hollow tree/ha) and Vignon and Orabi (2003) in the Mayenne and Sarthe departments (1.4 hollow tree/ ha). Dubois et al. (2009) also found a better response by O. eremita to lower densities, close to 1 hollow tree/ha, than to higher ones (1.4 hollow tree/ha). Open landscapes thus seem to be crucial for O. eremita in northern Europe (Ranius and Jansson 2000, Landvik et al. 2016b), whereas it is less so in a Mediterranean climate (Chiari et al. 2012). Several other factors may also be important to explain the observed patterns: low detectability (false absences), environmental factors (unsuitable cavities, dispersal difficulties in dense canopy conditions (e.g. Dubois and Vignon 2008)) and historical factors (ancient local extinction without re-establishment).

Conclusion and implication for the conservation of O. eremita

This study confirmed the presence in the PNR-BSN of the endangered and highly specialised saproxylic beetle *O. eremita*. This confirmation was often based only on traces of its presence, but also on live specimens. It seems likely that *O. eremita* survived because the landscape of the studied area had not dramatically changed. Moreover, this area benefited from a favourable situation with about 10 times more suitable hollow trees than observed in a nearby site which comprised similar numbers of trees surveyed and trees inhabited by *O. eremita* (Dubois et al. 2009).

This study also showed that the length of TOW existing in 1999 had decreased by 6.1% in 2009. Thus, if no protection measures are undertaken, the long term survival of *O. eremita* in the PNR-BSN is under threat. In the upper Elbe valley, Lorenz (2013) described a decrease of suitable trees of approximately 3% to 5% per year. This decline is higher than the annual mortality rates given by Drobyshev et al. (2008) of about 1% for oak populations located outside dense forests. At a rate of 3%, it would take only 10 to 15 years to lose a quantity of TOW comparable to that which was observed between 1973 and 2009. If the decline continues, Lorenz estimated that *O. eremita* may seriously decline and eventually become extinct in the next 40 years (Lorenz 2013). Although the situation appeared more favourable in the PNR-BSN, it will take decades before young trees develop cavities to replace the existing ones. Therefore, policies and actions must be decided rapidly and with the long term perspective in mind (>150 years).

An action plan for the preservation of old trees is already in progress in the PNR-BSN. It includes several approaches: financial help for pruning, a dedicated commission, an awareness campaign for the public and schools, a reflection on the economic role of pollarded trees in relation to firewood and, wherever possible, an integration of measures for the conservation of pollarded trees into urban planning documents. This is in agreement with the proposal of Stegner (2002), for the conservation of the hermit beetle in Saxony, to include monitoring, direct protection and publicity efforts. Regeneration and creations of new TOW are priorities and are ongoing in the PNR-BSN, mainly inside protected areas and in the Natura 2000 network. Between 2005 and 2013, 2,174 trees have been planted and about 2,000 have been pruned with the help of the PNR-BSN (Coppée et al. 2016). Management of existing trees by pollarding is to be favoured to enhance the formation of cavities (Messerschmidt and Schmidl 2015). Willows can play a key role in this respect as they grow fast and are quickly colonised by heart-rot fungi, even when young. As their life-spans are generally short, ten times less than oaks (Otto 1998), they must be pruned in order to survive for adequate periods of time (Juillerat and Vögeli 2004). After pruning, they develop cavities faster than hardwood trees. With regards to oaks, Ranius et al. (2009) described a very slow process, with about 200-300 years required for 50% of trees to develop cavities and 400 years are required for all oaks to develop cavities. In a study on Salix alba, Sebek et al. (2013) found a probability of cavity formation of approximately 80% in pollards with a diameter of 30-60cm, but only approximately 30% of non-pollards of the same diameter class developed cavities. On the landscape scale, Kirby (2015 suggested a model of regeneration as a series of episodic events, rather than a continuous recruitment of young trees. This strategy would lead to cohorts of different ages that will neither compete with existing trees nor modify the character of the site. This makes it possible to focus work on small areas with greater efficiency.

In the medium term, it is important to work to establish *O. eremita* as a flagship species for the public, even if this status will not necessarily confer protection to all other taxa associated with the flagship species (Roberge and Angelstam 2004). Moreover, Sebek et al. (2012) were unable to find clear evidence that saproxylic beetles are positively related to the presence of *O. barnabita*. This association would appear to occur rather as a function of the cavity parameters (Micó et al. 2015) or at the tree and stand levels, especially in relation to the sunlight conditions (Horák et al. 2014, Sebek et al. 2016, Parmain and Bouget 2017). Many indicators strongly suggest that veteran trees hosting *Osmoderma* are also inhabited by remarkable and highly diverse saproxylic beetle populations (Ranius 2002, Chittaro and Sanchez 2015). With the help of *O. eremita* as a flagship species, it appears possible to reconnect landowners to their veteran trees which are also witnesses of ancient practices and cultural heritage (Blanc 2012, Hartel and Plieninger 2014).

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RESEARCH ARTICLE



Evidence for geographic substructuring of mtDNA variation in the East European Hermit beetle (Osmoderma barnabita)

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Abstract

The genus *Osmoderma* is a flagship taxon of invertebrate conservation in Europe and encompasses a complex of four accepted species. While species limits amongst *Osmoderma* have been intensively studied, patterns of intraspecific variation are poorly known. In this paper, the authors focus on clarifying the phylogeographic structure of the East European *Osmoderma barnabita* using samples from Croatia to Finland. Samples of hind legs were collected from populations in Latvia and Finland (n=186) and combined with previously-published sequences from GenBank and museum specimens (n=10). In a partial sequence of the mitochondrial COI gene (759 bp), 26 closely related haplotypes were found. Beetle samples from different parts of Europe were distinct and showed no overlap in haplotype composition. The solitary population of Finland proved to be monomorphic and all 97 individuals sampled here belonged to a single haplotype unique to this region. The results suggest the Northern parts of Eastern Europe to be dominated

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by a single COI haplotype to which most of the other haplotypes are linked by one or two mutations. The pattern seems to reflect a founder effect or a strong bottleneck event. While *O. barnabita* is widely distributed over Eastern Europe, current patterns of mitochondrial genetic diversity appear influenced by population history and little homogenisation by ongoing gene flow. From a conservation perspective, the patterns suggest that regional populations might need to be managed as subunits and that the population of Finland may be affected by low genetic diversity.

Keywords

Osmoderma, population expansion, demographic history, phylogeography, sub-populations, threatened species

Introduction

Genetic diversity within a single species is a fundamental aspect of biodiversity and can be used in species conservation and management (Woodcock et al. 2007, Frankham et al. 2009, Todisco et al. 2010, Solano et al. 2013). Analyses of DNA polymorphisms offer high resolution and can resolve intraspecific patterning even within morphologically similar cryptic species (Hebert et al. 2003b, Bickford et al. 2007, Murray et al. 2008, Huemer and Hebert 2011, Jusoh et al. 2014, Solano et al. 2016). For efficient species conservation, resolving such patterns is necessary to recognise genetic issues amongst sub-populations, e.g. genetic erosion, genetic distance or potential ESUs (evolutionarily significant units) (Crandall et al. 2000, Fraser and Bernatchez 2001, Abellán et al. 2007, Frankham et al. 2009, Zauli et al. 2016).

A wealth of molecular methods is now available for detecting the level of intraspecific diversity or divergence amongst sub-populations (Todisco et al. 2012, Theissinger et al. 2013, Drag et al. 2015), with sequences of the mtDNA COI gene (mitochondrial DNA cytochrome *c* oxidase *subunit* 1 gene) forming a popular target (Williams et al. 2006, Hajibabaei et al. 2007, Painter et al. 2007, Knopp et al. 2011, Čandek and Kuntner 2015). Despite distinct constraints on sequence variation in the COI locus (e.g. Moritz and Cicero 2004, Galtier et al. 2009, Smith et al. 2012, Pentinsaari et al. 2014b, 2016), mitochondrial DNA offers several advantages for molecular population studies: first, the target DNA is available in high copy numbers and thus easily extractable. Second, the effective population size of such maternally inherited markers is only half that of nuclear genes and mtDNA is hence particularly sensitive to founder and bottleneck effects (Avise 2004). Finally, the COI gene, in particular, offers a convenient level of variation to address patterns and processes at intermediate time scales, with a high level of variation amongst beetle species specifically (Wirta et al. 2010, Pentinsaari et al. 2014a, Pentinsaari et al. 2016).

In Europe, the migration of organisms after the Pleistocene glacial period has significantly influenced patterns of genetic variation within species (Hewitt 1996, 2000; Donner 2005). Following the retreat of the ice sheet, the predominant expansion routes were from Southern Europe towards the North (Hewitt 1999, 2004) or from some cryptic refugia in Central and Eastern Europe (Stewart and Lister 2001, Schmitt and Varga 2012). Factors in the demographic history of sub-populations (e.g. isolation, gene flow and gene drift) may explain present-day ranges, differences in ecology and ultimately resource use (Knopp et al. 2011, Miraldo and Hanski 2014, Solano et al. 2016, Zauli et al. 2016). They may also influence current levels of genetic variation, affecting the evolutionary potential of a given (sub)-population (Avise 2000, Weber et al. 2000, Dalén et al. 2007, Allendorf et al. 2013). Thus, information on the demographic history of the species can be applied as a valuable tool in conservation actions of threatened species (Audisio et al. 2009, Todisco et al. 2010, Solano et al. 2013, Drag et al. 2015).

In Europe, one example of a taxon presumptively expanding from South to North after the glacial period is the genus *Osmoderma* LePeletier & Audinet-Serville, 1828 (Audisio et al. 2007, 2009). These 'hermit beetles' comprise a flagship taxon for arthropod conservation and are included in Annexes II and IV of the Habitats Directive of the European Union as a priority species for conservation (Anonymous 1992, European Commission 2007). The genus *Osmoderma* is particularly vulnerable to the loss of veteran trees, as its larvae requires tree cavities (Ranius and Nilsson 1997, Landvik et al. 2016a) where it occurs in nutritious wood mould substrate (Landvik et al. 2016b). Such large trees and such cavities have become rare in modern forests – a development now threatening the diversity of saproxylic species in Europe (Nieto and Alexander 2010, Stokland et al. 2012, Carpaneto et al. 2015).

The hermit beetles were previously thought to be a single species, *Osmoderma eremita*. However, following the revisions by Gusakov (2002) and Audisio et al. (2007, 2009), it is currently divided into two main clades (with a primarily West- and an East-European distribution, respectively), encompassing a total of four confirmed species (Audisio et al. 2007, 2009). The exact taxonomy of these species has been debated and the updated nomenclature of Audisio et al. (2007) has henceforth been adopted by the authors. The West European cluster comprises widely distributed *O. eremita*, with *O. cristinae* and *O. italicum*. Whether the latter form 'good species' is nonetheless debated (Audisio et al. 2007, Audisio et al. 2009). The East-European clade encompasses two species; the widely distributed *O. barnabita* and the Greek *O. lassallei* (Audisio et al. 2007).

In this paper, the authors focus on *Osmoderma barnabita* within the Eastern clade of the hermit beetle, occurring from Northern Greece across Eastern parts of Europe and Western Russia to South West Finland (see Audisio et al. 2007, 2009). Focusing on a partial sequence of the mtDNA COI gene from individuals across Europe, the aim is to (i) test whether Hewitt's paradigm (Hewitt 1996, 1999, 2000, 2004) applies to this species, (ii) examine the partitioning of mtDNA haplotype diversity amongst regions, (iii) propose possible processes based on the patterns found and (iv) make an inference to the implications of these patterns and processes for conservation.

Material and methods

Sample collection

To obtain comprehensive material from the full range of *O. barnabita*, three sources were used: samples of hind leg tarsus or tibia from live beetles sampled in Latvia and Finland (n=186), previously published sequences from GenBank (n=3) and new sequences from dry-mounted and ethanol-stored museum specimens (n=7). In total, material was obtained from 196 individuals collected in 9 countries (Figure 1; Table 1; Suppl. material 1).

Non-destructive sampling was achieved by pheromone trapping (in Finland and Latvia); for a trap description see Landvik et al. (2016a). As pheromone, +-gamma-decalactone (W236012-25G-K, Sigma-Aldrich^{*}, SAFC^{*}, St. Louis, USA) was applied, this being a genus-specific pheromone released by the males (Larsson et al. 2003, Svensson et al. 2009, Zauli et al. 2016). Samples consisting of a hind leg fragment were taken from live beetles which were released after sampling. Leg fragments were preserved in 96 vol-% purified ethanol and stored at a temperature of -18 °C ±2 °C before DNA extraction. The majority of all specimens (n=186) was collected in two areas: Finland (n=97) and Latvia (n=89). The Finnish samples were collected in summer 2011 and 2012 in the Turku city region, within a single sampling area of 8.3km² encompassing Ruissalo, Artukainen, Jänessaari, Muhkuri, Pansio and Runeberg Park. The Latvian samples were collected in an area of 4.1km² in the Pededze Valley (see Suppl. material 1: Table A1).

Museum specimens (total n=7), from single locations, were obtained on request from Central and Eastern European museums (Figure 1, Table 1; data from Estonia, Hungary, Romania and Russia). Data obtained from GenBank included three previously published sequences from Croatia, Germany and Slovakia (see Suppl. material 1: Table A1; Audisio et al. 2009). A specimen collected from Greece (Audisio et al. 2009) was removed from the final analysis due to doubts regarding its species identity. A set of fifteen previously published sequences from Poland (n=8) and Finland (n=7) were not used in the final dataset, as they were clearly shorter (255 bp lesser) than the rest of the sequences used here (cf. Svensson et al. 2009), or offered shorter reads of clean sequence than the rest of the sequences used (cf. Landvik et al. 2013).

DNA extraction, amplification and sequencing

Total DNA was extracted from leg samples using the Macherey-Nagel NucleoSpin Tissue kit following the manufacturer's instructions. To amplify a more diverse fragment (approx. 800 base pairs) than the most commonly used 'barcoding region' (Hebert et al. 2003a) of the mitochondrial COI gene, a primer pair (COI-Ob2 f: TGATTATTTTCGACAAACCACAAA and COI-Ob2 r: TTGCATAGATTATTC-CTAATGTGC) was designed by using previous *Osmoderma barnabita* mtDNA COI


Figure 1. Sampling sites of *Osmoderma barnabita* and of CO1 haplotypes within regions. The regional sub-populations defined in the text are indicated by hatched areas. Sampling sites are identified by open circles.

sequences from GenBank (see Audisio et al. 2009, Landvik et al. 2013). For PCR, KAPA2G Robust HotStart ReadyMix was used with a reaction volume of 12.5µl reaction, wherein the concentration of primers was 0.2µM and DNA 3µl. The thermal profile of PCR was: first 95°C for 3min, then 95°C for 15s, 52°C for 15s, 72°C for 30s for 40 cycles and finally 72°C for 5min and 10°C for 2min. Successful amplicons were Sanger-sequenced by Macrogen Europe, Amsterdam.

Table 1. Origin of sequence data used in this study (*reared individual). Sequenced specimens were collected from Central, Eastern and Northern Europe. The main part of the dataset (*N*=193) consists of new sequences (GenBank accession codes: KY362552–KY362744), with additional material obtained from Audisio et al. (2009). Extended information of sampled individuals (e.g. GenBank accession codes) is provided in Suppl. material 1: Table A1.

Country	Location	Collecting dates	Coordinates	No. individuals	Reference
Croatia	Plitvice Lakes Nat. Park	30.7.2002	44°52'N, 15°34'E	1	Audisio et al. 2009
Estonia	Koiva woodland	n.a.	57°40'N, 26°15'E	1	current study
Finland	Turku region	18.7–1.8.2011 and 4–30.7.2012	60°25'N, 22°09'E	97	current study
Germany	Saxony, Hagberg	12.6.2005	51°32'N, 14°38'E	1	Audisio et al. 2009
Hungary	Győr, Győr-Moson-Sopron	20.1.2011*	47°42'N, 17°36'E	1*	current study
Hungary	Sárvár, Vas county	28.7.2008	47°17'N, 16°57'E	1	current study
Latvia	Pededze Valley	5-26.7.2011	57°30'N, 26°53'E	89	current study
Slovakia	Zvolen, Dobrá Niva	7.2006	48°28'N, 19°06'E	1	Audisio et al. 2009
Romania	Roades, Brasov	12.7.2012	46°04'N, 25°03'E	1	current study
Russia	Tolmachevo	23.6.2011	58°51'N, 29°52'E	3	current study

Genetic data analysis

Estimation of haplotype relationships and genetic population structure

MtDNA sequences were edited with Geneious v8.1.7 (Kearse et al. 2012) to a length of 759 base pairs and aligned for analyses using MUSCLE (Edgar 2004). A minimum spanning network (MSN) of mtDNA COI haplotypes was constructed using package *pegas* (Paradis 2012) in R statistics version 3.1.2 (R Development Core Team 2015). For analyses, Europe was split into three main regions from which samples were available: i) the Baltic region including Western Russia (BRR=Latvia, Estonia and Western Russia); ii) Central and Eastern Europe (CEE=Croatia, Germany, Hungary, Slovakia and Romania) and iii) South-West Finland (FIN=Turku region). Genetic diversity within these regional populations was assessed based on estimates of haplotype number (*hn*, the total count of different haplotypes), haplotype diversity (*h*), mean pairwise difference of nucleotides (*i*) and nucleotide diversity (π) as calculated in DnaSP version 5 (Librado and Rozas 2009). High values of these indices are directly proportional to high levels of genetic diversity and, for example, high *h* coupled with low p is indicative of a high number of unique haplotypes, often resulting from a recent expansion (Rogers 1995).

Changes in historical population size

Historical signatures of population growth were assessed for the entire dataset by comparing the observed distribution of pairwise differences between haplotypes and the expected results under a constant population size model, a sudden-demographic expansion model and a spatial-demographic expansion model. Statistically significant differences between observed and simulated expected distributions were evaluated with the sum of the square deviations (*SSD*) and Harpending's raggedness index (*hg*) (Rogers and Harpending 1992, Harpending 1994). The constant population size model was run in DnaSP version 5 (Librado and Rozas 2009) and the expansion models were run in Arlequin version 3.5 (Excoffier and Lischer 2010). Additional evidence of historical population expansion was obtained from neutrality tests sensitive to population fluctuations; Tajima's *D* (Tajima 1989), Fu's F_s (Fu 1997), Ramos-Onsis & Rozas' R_2 (Ramos-Onsis and Rozas 2002) and comparison of diversity indices (*h*, π). All statistics were calculated using DnaSP version 5 (Librado and Rozas 2009). The significance of all statistics was assessed with 10000 coalescent simulations. Tajima's *D* and Ramon-Onsis & Rozas' R_2 are able to detect population expansions from a small sample size, while Fu's F_s can be more effective when analysing large samples with a rapid coalescent time (Fahey et al. 2014). Significantly negative departures from zero for Tajima's *D* and Fu's F_s values may indicate population expansions (Tajima 1989, Fu 1997, Drummond and Rambaut 2007).

Results

COI diversity and substructuring

This dataset of 196 mtDNA COI sequences included a total of 26 unique haplotypes. All haplotypes were closely related to each other and separated by only one to four mutations from the central COI haplotype (H5; Figure 2). Despite this overall similarity, haplotypes from different geographic regions occupied different and non-overlapping parts of the minimum-spanning network (Figure 2), suggesting strong phylogeographic structuring amongst regions. Haplotype diversity also differed substantially amongst regions. In the northernmost population (Finland), only a single haplotype was detected despite an extensive sampling effort (N=97), whereas in the region South of it, 19 haplotypes were detected in a similar-sized sample (N=93; Figure 2). Overall, haplotype diversity (h) increases southwards, with values for sub-populations ranging from 0 in Finland to $1 \pm SD 0.0093$ for haplotypes from different regions (CEE; Table 2). Correspondingly, the mean pairwise difference amongst mtDNA sequences from a given region (Figure 1) was highest in Central and Eastern Europe but naturally zero in the Finnish population (Table 2). Variation in nucleotide diversity (π) reflected this overall trend, again ranging from zero in the Finnish site to maximum values in the region of Central and Eastern Europe (CEE; Table 2).

Changes in historical population size

All tests applied suggested that either population expansion or pronounced selection had occurred. Values of both Tajima's D (D=-1.9575, P=0.002) and Fu's F_s (F_s =-22.2775,



Figure 2. Minimum spanning networks presenting 26 haplotypes in *O. barnabita*. The size of each circle corresponds to its relative frequency in the total sample. The number in each circle offers a unique identifier for each haplotype (H1-H26). Haplotype samples collected from the Baltic region and Russia (BRR: Estonia, N=1, H5; Latvia, N= 89, H5, H10-26; Russia, N= 3, H5, H8) are coloured in orange, from Central and Eastern Europe (CEE: Croatia, N=1, H1; Germany, N=1, H2; Hungary, N= 2, H6, H7; Slovakia, N= 1, H3; Romania, N= 1, H9) in light green and Finnish samples from the Turku region (FIN, H4: Artukainen, N= 3; Jänessaari, N= 4; Muhkuri, N= 7; Pansio, N= 1; Ruissalo, N= 81; Runeberg Park, N= 1) in dark blue, with haplotype numbers in white. Smaller black dots on lines between individual haplotypes indicate the number of mutation steps separating them.

Table 2. Mitochondrial DNA CO1 sequences variation in *Osmoderma barnabita*. Estimates of genetic diversity amongst regions, with the following metrics identified: N= regional sample size (number of individuals), hn= number of distinct haplotypes, h= haplotype diversity, P= polymorphic sites, i= mean pairwise genetic differences (uncorrected p distances), π = nucleotide diversity. Calculations are based on a sequence length of 759 bp.

Area	N	bn	b (mean \pm s.d.)	P	i (mean ± s.d.)	π (mean ± s.d.)
FIN (Turku region, Finland)	97	1	0	0	0	0
BRR (Baltic region and Russia)	93	19	0.771 ± 0.0019	17	1.3478 ± 0.8428	0.0018 ± 0.0012
CEE (Central and Eastern Europe)	6	6	1.000 ± 0.0093	10	3.3300 ± 1.9861	0.0044 ± 0.0030
BRR and CEE combined	99	25	0.798 ± 0.0400	25	1.5811 ± 0.9490	0.0021 ± 0.0014
Pooled sample	196	26	0.705 ± 0.0300	26	1.3201 ± 0.8262	0.0017 ± 0.0012

P>0.001) were negative and statistically significant, thus indicating either an expansion or strong selection within the overall population of *O. barnabita*. Population expansion was further confirmed by significant values of Ramos-Onsis' and Rozas' statistics

 $(R_2=0.0257, P=0.029)$. This analysis of mismatch distributions revealed significant Harpending's raggedness (hg=0.0275, P=0.025), again implying that the null hypothesis of constant population size can be rejected. Turning to the two models of sudden demographic expansion versus spatial expansion model, neither could be rejected (SDD=0.0011, P=0.770; hg=0.02747, P=0.79; SDD=0.0006, P=0.820; hg=0.02747, P=0.90). Thus, the patterns seem compatible with either model.

Discussion

The current distribution of haplotype diversity in *O. barnabita* seems consistent with a recent expansion through Eastern Europe. As a result of these historic processes, haplotype diversity decreased from the South northwards to a single haplotype present in the northernmost population. Distinct haplotype clades were found within different parts of Europe, suggesting strong phylogeographic structuring amongst regions. Each of these findings has implications for the conservation and management of this flagship species, as will be further discussed below.

Historic population expansion in O. barnabita

The current distribution of mtDNA haplotypes in *O. barnabita* seems highly indicative of demographic expansion. Overall, the haplotype network showed a star-shaped topology as characteristic of population expansion after a bottleneck, wherein newer mutations form groups of (mostly) lower-frequency haplotypes budding from a central haplotype (Figure 2; see Slatkin and Hudson 1991, Avise 2000, Charlesworth and Charlesworth 2012, Fahey et al. 2014). All tests of expansion proved significant and attested to a rapid growth of the European population. Different regions within Europe were characterised by different and non-overlapping haplotypes (cf. Figures 1 and 2), which could have been partly caused by low sampling intensity in more southern regions. Nevertheless, the main pattern of expansion seems more compatible with colonisation followed by diversification *in situ* than with the expansion of an already diverse population with gradual decrease of extant diversity along the expansion route. The overall network is compatible with colonisation from a founder population, where ancestral haplotype diversity has decreased due to repeated bottleneck effects along the colonisation route. Regional diversity has later recovered with rapid population growth, producing variants surrounding the central haplotype (cf. Figures 1 and 2).

Given the geological history of Europe (Donner 2005), the expansion to more northern areas has clearly taken place after the Pleistocene ice ages – since the species now occupies regions previously covered by ice (Hewitt 1999, 2000, 2004). During the maximum extent of the ice, the species was likely confined to a refugium in the Balkan region (Audisio et al. 2007, 2009) or to some unknown cryptic refugium or refugia in Central or Eastern Europe (e.g. Stewart and Lister 2001, Schmitt and Varga 2012). Thus, in the case of *O. barnabita*, these results cannot fully rule out the existence of unknown cryptic refugia. Even so, the current phylogeographic structure detected within the species *O. barnabita* is compatible with general patterns envisaged across the genus *Osmoderma* (Audisio et al. 2007, 2009) and congruent with general post-glacial migration patterns in other animals and plants (e.g. Hewitt 2000, Hewitt 2001, Schmitt and Seitz 2002, Gratton et al. 2008, Dapporto 2010). Given the specific habitat of *O. barnabita* in old, hollow trees, the species' expansion route has probably followed the northward expansion of old-growth deciduous forests (Ferris et al. 1998, Hewitt 1999, Drag et al. 2015).

Current haplotype diversity in O. barnabita

Given the imprints of post-glacial history described above, the distribution of mtDNA variation within the current range of *O. barnabita* is characterised by two patterns: distinct differences in the haplotype composition of different regions and a marked decrease of genetic diversity towards the North. Both patterns attest to the fact that, following the colonisation of regions, later gene flow may have been much too weak to homogenise genetic composition.

In terms of genetic diversity, all metrics of diversity decreased northwards. The highest levels of mtDNA COI diversity were encountered in Central and Eastern Europe (Figure 1). Here, every sampling site revealed some locality-specific haplo-type (Figures 1 and 2), suggesting high diversity even at a small scale within regions. However, many of these samples were too small to merit firm conclusions and more extensive sampling could reveal not only more shared haplotypes, but also currently unknown central haplotypes.

Beetles in the genus *Osmoderma* specialise in old deciduous trees (Ranius and Nilsson 1997, Ranius et al. 2005), a resource which is very patchily distributed across Europe. *Osmoderma* beetles appear relatively weak dispersers both amongst trees within sites and even more so amongst sites (Ranius and Hedin 2001, Chiari et al. 2013, Oleksa et al. 2013), further emphasising the isolation of extant populations. Overall, these patterns suggest that the current European population of *O. barnabita* is composed of rather distinct subunits. Importantly, the genetic marker used here was selected for the high resolution of mitochondrial loci, linked to their small effective population size and high sensitivity to sampling effects (Avise 2000, Avise 2004). Further studies will be needed to reveal how these patterns are reflected in nuclear or other genetic markers (for a valuable recent resource, see Goossens 2015).

Conservation and management implications

From an applied perspective, two key implications of the reported patterns for the conservation and management of *O. barnabita* have been proposed. First, there might be a need to manage some isolated populations as independent genetic units in different parts of the species range, as they may be on different demographic and evolutionary trajectories. Second, the solitary northernmost population seems genetically impoverished, suggesting a possible risk of limited evolutionary potential.

With regard to local management units, it has been suggested that regionally adapted small populations are sensitive to changes in the environment (Ciofi et al. 2009, Frankham et al. 2009, Allendorf et al. 2013). Each unit should thus be treated as potentially unique – and also any reintroductions or transfers amongst populations should be carefully considered (cf. Frankham et al. 2009, Allendorf et al. 2013). Yet, it is stressed that variation at the single mitochondrial marker locus, used here, may be poorly reflective of variation at more selectively relevant loci and that the current patterns should be supplemented by studies of variation at other loci (cf. Goossens 2015) – and of variation in phenotypic traits.

When it comes to the genetic diversity of the northernmost population, the current low level of diversity suggests that increased attention should be given to genetic aspects in managing this unit. Persistent isolation, lack of gene flow, increased rate of inbreeding and influence of genetic drift may result in detrimental genetic changes which can elevate the regional extinction risk (Frankham et al. 2009, Allendorf et al. 2013). On the other hand, the purging of deleterious alleles may counter-balance such negative impacts (Lande 1988, Haikola et al. 2001, Keller and Waller 2002).

What creates a particular challenge to *O. barnabita* is the combination of longerterm, climatically-driven forces with current anthropogenic impact on the environment. The former process has eroded diversity over time, during the expansion phase and the latter is now causing additional isolation for the remaining populations: given its specialisation for old deciduous trees, the *Osmoderma* species complex is currently faced with a highly fragmented landscape all over its European range (cf. Ranius et al. 2005, Audisio et al. 2007). Many populations of hermit beetle species are currently confined to small tree patches in agricultural landscapes and urban areas, causing conflict between conservation and human interest (Flåten and Fjellberg 2008, Oleksa 2009, Carpaneto et al. 2010, Stokland et al. 2012, Siitonen and Ranius 2016). Local gene pools can only be safeguarded by securing the habitat at a local level. Conservation and management should focus on improving the quality and amount of the existing habitat, as the primary needs of efficient species-based conservation (cf. Jansson et al. 2009, Manning et al. 2013, Carlsson et al. 2016, Landvik et al. 2016b).

Importantly, the situation of *O. barnabita* is likely shared by many saproxylic species associated with scarce, diminishing and fragmented habitats (Nieto and Alexander 2010, Stokland et al. 2012). While less explored, other species may suffer from similar genetic threats – due to similar, historic population processes (cf. Painter et al. 2007, Trizzino et al. 2014, Gouix et al. 2015) worsened by current, anthropogenic habitat loss. Insights into the phylogeographic structure of *O. barnabita* may thus help guide future efforts to safeguard the saproxylic fauna of veteran trees. As further steps for assessing the conservation status of the *Osmoderma* species complex in Europe (Nieto and Alexander 2010), clear-cut assays of genetic diversity and inbreeding depression amongst *Osmoderma* populations and comparative studies using complementary genetic markers are suggested. This information may guide efficient recommendations on how best to manage these charismatic invertebrate species.

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Supplementary material I

Details of the Osmoderma barnabita specimens included in the data set

Authors: Matti Landvik, Andreia Miraldo, Pekka Niemelä, Uldis Valainis, Raimonds Cibuļskis, Tomas Roslin

Data type: specimens data

Explanation note: Details of the *Osmoderma barnabita* specimens included in the data set. Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the

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RESEARCH ARTICLE



Cucujus cinnaberinus (Scopoli, 1763) at its terra typica in Slovenia: historical overview, distribution patterns and habitat selection

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Abstract

The saproxylic beetle, Cucujus cinnaberinus, has received increasing research attention in Europe since the adoption of the Habitats Directive and establishment of the Natura 2000 network. The history of the species has been investigated as well as the influence of abiotic and biotic variables on the distribution of C. cinnaberinus in Slovenia which is at the limit of its range and also terra typica for the species. The species was first described in 1763 by Joannes A. Scopoli in Carniola, a duchy of the Habsburg Monarchy. Today, most of the territory of Carniola is situated within Slovenia. C. cinnaberinus is particularly common in the eastern part of the country, but very scarce in the mountainous western part. According to historical and recent distribution patterns of C. cinnaberinus in the former Carniolan territory, the region of Ribnica-Kočevje in southern Slovenia is proposed as the most probable type locality of the species. Although the bulk of the C. cinnaberinus population in Slovenia is confined to the lowlands, the species has been found up to 1095 m a.s.l., albeit at a much lower abundance due to the influence of climate and forest structure. Although C. *cinnaberinus* is a quite an opportunistic species regarding host tree selection, it has been shown to exhibit a preference for Tilia, Populus and Robinia. It is suspected that the high abundance of C. cinnaberinus in lowland floodplain forests is due to the recent human-induced increase in preferred fast-growing and shortlived host trees, i.e. the planting of poplar trees and spread of invasive Black Locust (Robinia pseudoacacia) after the 1960s. In contrast, in montane forests, preferred host trees (e.g. Tilia) represent < 1 % of all growing stock. Although montane C. cinnaberinus populations are rare, they could still be important for the conservation of the species, since montane habitats cover the largest area within the species' distribution range.

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Keywords

saproxylic beetle, Natura 2000, type locality, Carniola, altitudinal distribution, host tree selection, macrohabitat, large-scale survey

Introduction

The Directive on the Conservation of Natural Habitats and of Wild Fauna and Flora or the Habitats Directive (92/43/EEC) was adopted in 1992 and subsequently spurred intensive research activity on species of conservation concern in Europe. This is particularly evident with respect to the saproxylic beetles which is amongst the most threatened beetle groups in Europe (Nieto and Alexander 2010) and is reflected in the number of published papers listed in the Web of Science on 21 saproxylic beetle species of conservation concern, with 87 % of all papers published after the year 1992 and, in particular after 2010, when more than 50 % of all papers were published. However, research activity has been skewed towards more widespread and charismatic species (e.g. Ranius et al. 2005, Harvey et al. 2011), at least that concerning species ecology, monitoring, distribution and conservation biology (see Appendix: Table S1).

There are only four well studied species in this sense, namely *Osmoderma eremita*, *Morimus funereus, Lucanus cervus* and *Rosalia alpina* and three moderately studied species (*Cucujus cinnaberinus, Cerambyx cerdo, Limoniscus violaceus*), while the majority of species have been poorly studied or not studied at all. While this deficiency in research greatly hampers the conservation management of Natura 2000 sites, on the other hand, it stimulates applied research on species survey methods, monitoring and conservation management (e.g. Bussler and Müller 2009, Carpaneto et al. 2010, Harvey et al. 2011, Bergman et al. 2012, Gouix and Brustel 2012, Chiari et al. 2013, Walentowski et al. 2013, Gough et al. 2014, Le Gouar et al. 2015, Campanaro et al. 2016, Carlsson et al. 2016, Hopkins and Thacker 2016, Larsson 2016).

Until recently, *Cucujus cinnaberinus* was considered as a poorly known species (Horák 2011). It is confined to Europe and is distributed from the Mediterranean to Scandinavia and from Russia to Spain, but with a scattered distribution pattern, probably due to past population decline and local extinctions (Horák and Chobot 2009, Horák et al. 2010). The species has however an extremely cryptic lifestyle, as the larvae and adults live under the bark of dead tree trunks (Horák et al. 2008) and this might also be a reason for the low detection rate of the species in the past (Horák et al. 2010).

Recently, field survey techniques have shifted from focusing on adult beetles to the detection of larvae under the bark which appears to be a much more efficient detection method (Bussler 2002, Straka 2006, Vavra and Drozd 2006, Horák and Chobot 2011, Vrezec et al. 2012, Gutowski et al. 2014). By applying the larval survey technique, new discoveries of the species have accelerated, as have rediscoveries in regions where the species was previously thought to be extinct (Mazzei et al. 2011, Kovács et al. 2012, Fuchs et al. 2014, Holly 2014, Mainda 2014, Hörren and Tolkiehn 2016, Šag et al.



Figure I. The Duchy of Carniola (dark grey) in the period of Joannes A. Scopoli's research activity between 1754 and 1769 with Carniolan provinces marked with their original Latin names. Recent state borders in the region are marked in the background. The map was redrawn after Florjančič de Grienfeld (1744).

2016, Tovar and Baena 2016). This intensified research activity uncovered new records of other *Cucujus* species and led to the discovery of a new endemic species in Europe (Horák et al. 2009, 2011, Bonacci et al. 2012, Gutowski et al. 2014). Horák et al. (2008, 2010) claimed that the *C. cinnaberinus* population stronghold is situated in central European countries, although this might change due to increasing knowledge on species distribution and abundance in southern Europe (Mazzei et al. 2011, Vrezec et al. 2011, Kovács et al. 2012, Šag et al. 2016).

In 1763, Joannes A. Scopoli described the species as *Meloe Cinnaberinus* from Carniola (also Carniolia, Krain, Kranjska), a duchy of the Habsburg Monarchy. Today, most of the Carniolan territory is situated within Slovenia (Figure 1), where the type specimen originated (Scopoli 1763). This fact has however been largely neglected in recent papers (e.g. Horák and Chobot 2009, Horák et al. 2010, Bonacci et al. 2012) partly as a result of the lack of knowledge about the type locality of *C. cinnaberinus* (i.e. the Duchy Carniola was frequently misinterpreted in the past; Baker 1999) which was not specifically mentioned in the original description of Scopoli (1763) and partly as a result of lack of knowledge on the recent status of *C. cinnaberinus* in the former Carniolan territory and the current Slovenian territory (Pirnat and Drovenik 2004, Horák et al. 2010, Vrezec et al. 2011). Therefore, the aim of this paper was to review historical and recent knowledge about the distribution of *C. cinnaberinus* in its *terra*

typica in Slovenia, with special emphasis on J. A. Scopoli's research activity in the region (Petkovšek 1977, Baker 1999, Štih et al. 2008), in order to identify the most probable type locality of the species. As *C. cinnaberinus* is highly genetically polymorphic (Røed et al. 2014), it is essential to define the type locality as a population reference for further taxonomic and phylogenetic studies of this and related species.

Taking into account recent distribution maps of *C. cinnaberinus* (Horák et al. 2008, Horák and Chobot 2009), it seems that Slovenia represents the limit of the species' distribution since there is no population known in the western neighbourhood of Northern Italy (Brandmayr et al. 2016). This situation presents the opportunity to study the species' micro- and macrohabitat selection patterns at the edge of its distribution, where it is rarer and less abundant. So far, the species' habitat parameter associations have been intensively studied only in central Europe, in its population stronghold where it is very abundant and its population is even expanding (Horák et al. 2010, 2011). This study therefore aimed: (1) to assess horizontal and vertical distribution patterns of *C. cinnaberinus* in Slovenia at its distribution edge, based on large-scale field sampling guided by a potential distribution model; (2) to review historical data of species occurrence in Slovenia from 1763 onwards and (3) to determine species macro- and microhabitat selection patterns to assess the limitation parameters of the species' distribution at the edge of its range.

Methods

Study area

The study was conducted over the whole territory of Slovenia (20,273 km²) which is a predominantly montane country with more than one-third of the surface lying above an elevation of 600 m a.s.l. (Perko and Orožen Adamič 1998). With forests covering 58 % of its area, Slovenia is one of the most forested European countries (Slovenia Forest Service 2015). Despite forest exploitation being an important economic activity, Slovenia has largely managed to preserve the original forest communities and populations of indigenous trees, even in some areas with a large amount of deadwood (Slovenia Forest Service 2015). The prescribed amount of deadwood biomass left in the forest varies up to 3 % of the total wood stock in the stands (Uradni list RS 2009, 2016).

The dominant tree species is European Beech (*Fagus sylvatica*), forming the most frequent forest associations of *Fagetum*, *Abieti-Fagetum* and *Querco-Fagetum* in Slovenia (70 %; Slovenia Forest Service 2015). Most of the western part of the country is montane with prevailing tree species of European Beech, Silver Fir (*Abies alba*), Norway Spruce (*Picea abies*), European Larch (*Larix decidua*), Sweet Chestnut (*Castanea sativa*) and Sessile Oak (*Quercus petraea*) (Perko and Orožen Adamič 1998). In eastern Slovenia, the forest has been mainly preserved where land is less suitable for agriculture and in floodplains along major river banks (Mura, Drava, Sava and Krka). They are characterised by a large volume of deadwood; for example, in the floodplain forest along the Mura River, it ranges from 15 to 24 m³/ha (Ferreira and Planinšek 2016). The prevailing species in floodplain forests are Common Alder (*Alnus glutinosa*), Pedunculate Oak (*Quercus robur*), Willow (*Salix* sp.), Poplar (*Populus* sp.; including hybrid Poplars) and European Hornbeam (*Carpinus betulus*) (Perko and Orožen Adamič 1998). Intensive plantations of hybrid poplars were established in the period from 1960–1980 (Božič and Krajnc 2012). Plantations were established on alluvial sites along the main rivers in Slovenia (Drava, Krka, Mura, Sava, Savinja, Soča) with primarily native poplar and willow stands. Additionally, Black Locust (*Robinia pseudoacacia*) is the most widespread alien tree species in Slovenia and accounts for almost two-thirds of the growing stock of non-native tree species (Kutnar and Kobler 2013).

Only part of Slovenia belonged to the former Duchy of Carniola which existed in the former Holy Roman Empire and later in the Habsburg Monarchy between 1364 and 1918 (Kos 1929, Štih et al. 2008). According to recent state borders in the region, the territory of Carniola is located in Slovenia, except for a small part that is included within the current borders of Italy and Croatia (Figure 1). In the period of J.A. Scopoli's research activity in Carniola between 1754 and 1769 (Petkovšek 1977), the duchy was divided into five regions: northern Carniola Superior (today, Gorenjska, including the mountainous area of the Julian Alps), western Carniola Interior (today, Notranjska and part of Primorska, including the northern part of the Dinaric karst region), eastern Carniola Inferior and Media (today, Dolenjska, extending to the river banks of the Sava and Krka and including the current major forest complexes in southern Slovenia) and Istria Austriaca (NE part of Istria and mainly within the current territory of Croatia) (Florjančič de Grienfeld 1744, Brelih et al. 2006).

According to *Flora Carniolica* (Scopoli 1760, 1772) and *Entomologia Carniolica* (Scopoli 1763), J. A. Scopoli began researching the flora and fauna of Carniola shortly after his arrival in Idrija with the first surveys in the year 1755 (Petkovšek 1977). To demonstrate the study area of Scopoli in the territory of the former Duchy of Carniola, we took into account all known localities visited by Scopoli and mentioned in his major works (Scopoli 1760, 1763, 1772, Petkovšek 1977, Baker 1999, Štih et al. 2008) with a circular area of a 10 km radius around every locality. The overlap of all historical and recent records of *C. cinnaberinus* within Scopoli's study area was used as a guideline in defining the possible type locality from which the Scopoli (1763) type specimen originated. Scopoli's collection with his type specimens is most probably lost (Brelih et al. 2006).

Historical Cucujus cinnaberinus range assessment

Data on the past distribution of *C. cinnaberinus* in the territory of Slovenia were obtained from literature reviews (Brelih 2001, Drovenik and Pirnat 2003) and by examination of historical entomological collections of coll. Ferdinand Jožef Schmidt (period between 1819 and 1878; stored in Slovenian Museum of Natural History in Ljubljana-PMSL), coll. Josip Stussiner (end of the 19th and beginning of the 20th

century; stored in PMSL), the Central Collection of Slovenian beetles (collection curated by Savo Brelih with material originated from coll. A. Bianchi, S. Brelih, E. Jaeger and J. Peyer from the period between the end of the 19th century to 2012; stored in PMSL), coll. Josef (Giuseppe) Müller (first half of the 20th century; stored in Museo Civico di Storia Naturale, Trieste), coll. Jože Staudacher (period between 1918 and 1940; stored in PMSL), coll. Alfonz Gspan (period between 1918 and 1940; stored in PMSL) and coll. Egon Pretner and Božidar Drovenik (period between 1920 and 2010; stored at Research Centre of the Slovenian Academy of Sciences and Arts, Ljubljana). All historical records were accidental findings since no systematic surveys of the species were known before the year 2008 (Vrezec et al. 2009). Historical records were geolocated at a scale of 10 kilometres and were used only for the purpose of horizontal distribution analysis in this study.

Recent assessment of Cucujus cinnaberinus distribution patterns

In Slovenia, the first large-scale surveys of *C. cinnaberinus* started in 2008 (Vrezec et al. 2009) by searching for larvae under the bark of dead trees (Straka 2006, Vavra and Drozd 2006, Vrezec et al. 2012). From the sites from where no adult beetles were obtained, the larvae were taken to the laboratory and reared to verify species identification. The procedure of rearing larvae in the laboratory until they reached the adulthood was the same as described in Gutowski et al. (2014). From most of the sites, voucher specimens were preserved. Surveys were directed towards the areas from which historical data existed (Brelih 2001, Drovenik and Pirnat 2003) and towards the floodplain forests which were reported as the main *C. cinnaberinus* habitat in central Europe (Straka 2006, Horák et al. 2008, Schlaghamersky et al. 2008).

In the period 2008–2011, 944 geolocated data items (deadwood inspections and occasional findings) were collected. In 53 of those, *C. cinnaberinus* presence was confirmed, including lowland as well as montane populations. However, assessing the distribution range of rare and elusive species is difficult, especially when historical data are scarce, this usually being the case for species with a cryptic lifestyle due to past methodological and knowledge limitations. In saproxylic beetles, potential distribution models have proved to be an essential tool for species distribution assessments and for designing targeted field surveys (Thomaes et al. 2008, Ranius et al. 2011, Bosso et al. 2013).

In a previous study, this approach was applied to assess the potential distribution of *C. cinnaberinus* in the territory of Slovenia based on available data until 2011 as a guideline for a further large-scale field study (Vrezec et al. 2014). The data were used to construct spatial models by a machine learning process resulting in the assembly of a model based on 100 regression trees. From the model of potential habitat suitability, the theoretical distribution model for 0.70 and 0.50 probability of species occurrence (Figure 2) was extracted. On this basis, further surveys were designed focusing on the most promising areas for the species in the country between 2012 and 2016, taking



Figure 2. The theoretical distribution model of *Cucujus cinnaberinus* in Slovenia based on the sites with 0.70 (black areas) and 0.50 (grey areas) probability of species occurrence according to the potential habitat suitability model based on the data set collected in the period 2009–2011 (Vrezec et al. 2014). The model was used as a guideline for a large-scale species survey in the period 2012–2016.

into account the whole territory of Slovenia with lowland and montane habitats. The technique of searching for larvae under the bark of dead trees was applied in all areas. The abundance index of the species was estimated as a percentage of inspected trees with confirmed *C. cinnaberinus* presence.

Microhabitat analysis

During field surveys, microhabitat characteristics of inspected dead tree trunks were recorded (i.e. tree species, diameter and length of the trunk). Although dead tree trunks were investigated throughout the whole of Slovenia, only the locations where *C. cinnaberinus* presence was confirmed were included in further microhabitat analysis. To describe *C. cinnaberinus* host tree preferences, a modified version of Ivelev's electivity index D (Jacobs 1974) was used. In further analysis, only trees with 25 or more observations were included (*Alnus, Fraxinus, Populus, Quercus, Robinia, Salix* and *Tilia*). For analysis of microhabitat data, a generalised linear model (GLM) with binomial error distribution was used (McCullagh and Nelder 1989). The independent variable was the absence and presence of the larvae or imago of *C. cinnaberinus* under the bark of sampled trunks. The independent variables were tree species and the diameter and length of the inspected tree trunk. With the variable diameter of the sampled dead tree trunk, a nonlinear relationship was detected. Therefore, the quadratic variable of the diameter of the tree trunk was added to the model (Zuur et al. 2010). Furthermore, the variable was log + 1 transformed, because outliers were detected. Records with incomplete data for any of the variables were deleted. Model selection was performed on the basis of stepwise backwards selection using the χ^2 test (McCullagh and Nelder 1989). When the variable did not significantly add to the model, the variable was dropped.

Macrohabitat analysis

For analysis of the macrohabitat of *C. cinnaberinus*, all collected geolocated field data in the period 2009–2016 were used and data points were described with variables of deadwood stock, altitude, amount of deciduous tree wood stock, canopy cover and solar radiation. The deadwood was sampled on 724 and 746 plots dispersed in a $4 \times 4 \text{ km}^2$ grid over the whole of Slovenia by the Slovenian Forestry Institute in 2007 and 2012, respectively (Slovenian Forestry Institute 2016).

To assess the variables at each *C. cinnaberinus* survey point, all the plots in a radius of 10 km around the survey point were taken into account. The deadwood stock (m³/ha) per survey point was averaged over time and space within a 10 km radius. With this approach, a robust dataset for a longer period within 10 km of the location of the sampled tree trunk for *C. cinnaberinus* was created. The wood stock of deciduous trees (m³/ha) and the canopy cover for every sampling site were assessed at the stand level and obtained from the Slovenian Forest Service database (Slovenia Forest Service 2015).

The canopy cover was divided into four classes: dense canopy closure (canopy very dense, branches deformed), normal canopy closure (branches meet each other, branches not deformed), sparse canopy closure (canopy very open, branches of neighbouring trees do not meet when windy) and patchy canopy closure (the gap may be one or more tree crowns) (Slovenia Forest Service 2015). The altitude and slope were extracted from the digital elevation model of Slovenia (Surveying and Mapping Authority of the Republic of Slovenia 2010). The annual solar radiation was obtained from GIS layers of annual solar radiation for the whole Slovenia (Zakšek et al. 2005, Kastelec et al. 2007).

For the analysis of macrohabitat selection, a GLM with binomial error distribution was used. The dependent variable was the presence and absence data of the larvae of *C. cinnaberinus*. The independent variables were slope, altitude, wood stock of deciduous trees, canopy cover, solar radiation and deadwood stock. The wood stock of coniferous trees was not included since it was the inverse of the wood stock of deciduous trees (Vrezec et al. 2014). The deadwood was transformed with a log + 1 transformation. The altitude and the amount of deciduous trees were transformed with a square root transformation. This was undertaken because outliers were detected. Model selection

was performed in a similar way to the microhabitat analysis. The analysis was done in the programme R (R Development Core Team 2011).

Results

Cucujus cinnaberinus distribution in Slovenia: the historical and recent situation

In total, 365 records of *C. cinnaberinus* were collected in Slovenia in the period from 1763 to 2016. The majority of the records (96 %) were however found after the year 2008, when a systematic survey involving the larval search method was initiated (Figure 3; Vrezec et al. 2009). All larvae reared in the laboratory to adult beetles were found to belong only to the *C. cinnaberinus* species. Recent surveys in higher-elevation montane and low-elevation floodplain forests revealed that the abundance of the species was much larger in the latter ($\chi^2 = 32.6$, df = 1, p < 0.001), with the bulk of the population confined to lower elevations, although the species was found to be distributed in a large altitudinal span from 140–1095 m a.s.l. (Table 1, Figure 4).

In contrast, before 2008, the majority of the records were from high-elevation montane forests which is opposite to the recent situation ($\chi^2 = 456.1$, df = 1, p < 0.001; Table 1). Records before 2008 were all occasional findings of single adult beetles scattered around Slovenia (Figure 5), but none of them originated from the recently identified large *C. cinnaberinus* population in floodplain forests alongside the large Slovenian rivers of the Mura, Drava and Sava (Figure 6). The species' populations showed a maximum concentration and abundance of individuals in the eastern part of the country in



Figure 3. Number of records of *Cucujus cinnaberinus* per period in Slovenia from 1763 to 2016 (N=365). The last century is subdivided into decades.

Table 1. Proportion of records of *Cucujus cinnaberinus* in Slovenia found in two forest types in two time periods and occupancy rate of dead host trees in each forest type (only locations with confirmed species presence were included in the calculation of the occupancy rate).

Forest type	1916–2007 (N=6 records)	2008–2016 (N=354 records)	Occupancy rate: MIN–MAX (Median) (N=904 inspected trees, 9 locations)
Higher-elevation montane forests	88 %	7 %	2.0–10.2 % (3.8 %)
Lower-elevation floodplain forests	12 %	93 %	9.6-45.5 % (12.6 %)



Figure 4. Altitudinal distribution of *Cucujus cinnaberinus* in Slovenia (N = 2132 inspected trees).

the lowlands, while its abundance and number of sites gradually decreased towards the western and mountainous part of Slovenia (Figure 6).

The oldest record for *C. cinnaberinus* in Slovenia was obtained in the period from 1755 to 1763; the specific collection site was not reported (Scopoli 1763). According to historical and recent data of *C. cinnaberinus* within the territory of the former Duchy of Carniola, four regions of species occurrence can be identified (from west to east; Figure 7): the Branica River Valley, Mt. Snežnik, the Ribnica-Kočevje region and the lower Sava River by Brežice. Although Scopoli obtained zoological and botanical material from many parts of the former Duchy of Carniola, only a few sites overlap with the known records of *C. cinnaberinus*: the region of former Carniola Media, today the Ribnica-Kočevje region in southern Slovenia and Carniola Interior and the Branica River Valley in the western part of Slovenia (Figure 7). A review of historical and recent data showed that abundance was low in both areas, but much higher in the Ribnica-Kočevje region on the mountains of Velika Gora, Stojna, Kočevski Rog and Stružnica. From here, the species was reported at least twice in the 20th and 21st century and also



Figure 5. Distribution of historical records of *Cucujus cinnaberinus* in the period 1916–2002 in Slovenia before the introduction of the larval search method in 2008.



Figure 6. Results of a large-scale survey of *Cucujus cinnaberinus* in Slovenia conducted in the period 2008–2016. Black dots are species occurrence records (N=354) and white dots are sites of inspected dead tree trunks without confirmation of the species (N=2013).



Figure 7. The Duchy of Carniola with the estimated study area of J. A. Scopoli in the period 1755–1763 (dark grey area) with all known historical and recent records of *Cucujus cinnaberinus* (black dots).

confirmed at seven sites in the large-scale survey in the period 2008-2016 in 10 % of all inspected trees in the region (Figure 6). In contrast, at the western location in the Branica River Valley, only one report from 2002 is known (Drovenik and Pirnat 2003) and the species was subsequently not found despite intensive surveys (N=239 inspected trees) in the period 2008–2016 (Figure 6). Mt. Snežnik produced a similar pattern, where C. cinnaberinus was only recently found in extremely low abundance in only 2 % of inspected trees. In contrast, the species was recently found to be very abundant in poplar plantations around the lower Sava River near Brežice, with 45 % of inspected trees being occupied by C. cinnaberinus, there being no evidence that Scopoli obtained material from the easternmost part of Carniola. From the 19th century, only three specimens of *C. cinnaberinus* were known to have been found in Slovenia and are preserved in the collection of F. J. Schmidt (PMSL), dating between 1819 and 1878, with no exact date and location specified. The first accurate records are from 1916 and 1918 and were found in a montane forest near Planina pri Sevnici by Dr Arthur Hoschek von Mühlheim (coll. A. Gspan in PMSL). Until 2008, before the large-scale survey of the species, C. cinnaberinus was known from a total of eight locations and, in at least three of these locations, the species was not reconfirmed despite systematic inspection of dead tree trunks for larvae (Figure 5 and 6).

of occupied trees and modified Ivelev's electivity index (D). D>0 signifies host trees preferred by <i>C. cin</i>	Table 2. Host tree preferences of <i>Cucujus cinnaberinus</i> with the proportion of available trees, proportion
$I (\mathbf{N}, \mathbf{O}, \mathbf{V}, \mathbf{O}, \mathbf{V})$	of occupied trees and modified Ivelev's electivity index (D). D>0 signifies host trees preferred by C. cin-
naberinus. (IN=854 trees)	naberinus. (N=834 trees)

Host tree	Proportion of available trees in the sample	Proportion of occupied trees in the sample	D
Tilia	0.030	0.076	0.452
Robinia	0.073	0.118	0.256
Populus	0.237	0.328	0.220
Acer	0.013	0.017	0.122
Ulmus	0.023	0.025	0.052
Quercus	0.067	0.067	0.001
Salix	0.337	0.286	-0.119
Prunus	0.025	0.017	-0.203
Fraxinus	0.056	0.025	-0.396
Abies	0.020	0.008	-0.421
Alnus	0.097	0.034	-0.511
Fagus	0.011	0.000	-1.000
Betula	0.004	0.000	-1.000
Picea	0.004	0.000	-1.000
Pinus	0.002	0.000	-1.000

Table 3. Generalized Linear Model statistics of the best model for the microhabitat of *Cucujus cinnaberinus*.

Variables	Estimate	Std. Error	z value	Pr (> z)
(Intercept)	-5.57	0.86	-6.47	9.78E-11
Fraxinus	0.01	0.80	0.02	0.986
Populus	1.25	0.55	2.26	0.024
Quercus	0.54	0.68	0.80	0.424
Robinia	1.66	0.61	2.72	0.007
Salix	0.69	0.56	1.24	0.213
Tilia	2.00	0.68	2.95	0.003
Trunk length < 2 m	-0.57	0.50	-1.15	0.250
Trunk length > 5 m	1.06	0.28	3.77	0.000
Diameter (cm)	0.69	0.22	3.19	0.001

Microhabitat selection patterns

C. cinnaberinus was collected in 11 host tree genera, with *Salix* being the most frequently occupied host tree species and which was also the most frequent in the samples of all inspected trees. However, *Tilia, Robinia* and *Populus* were largely preferred and selected in a much larger proportion than were actually represented (Table 2). The model revealed that the host tree and the length and diameter of the inspected tree trunk were the most important variables explaining the probability of *C. cinnaberinus* presence (Table 3, Fig-



Figure 8. Predicted probability of occurrence of *Cucujus cinnaberinus* depending on the host tree and the diameter and length of the tree trunk. The solid line represents a tree length below 2 m, the dashed line represents trees between 2 and 5 meters long, and the stippled line represents trees longer than 5 m. The empty circles show the presence (1.0) and absence (0.0) of *C. cinnaberinus* in relation to diameter.

Variables	Estimate	Std. Error	z value	Pr (> z)
(Intercept)	-3.36	0.82	-4.11	3.88E-05
Altitude	-0.09	0.03	-3.51	0.00
Amount of deciduous trees	0.07	0.03	2.46	0.01
Amount of deadwood	0.28	0.13	2.16	0.03
Normal canopy closure	-0.12	0.52	-0.24	0.81
Sparse canopy closure	0.73	0.50	1.47	0.14
Patchy canopy closure	0.92	0.53	1.72	0.09

Table 4. Generalized Linear Model statistics of the best model for the macrohabitat of *Cucujus cinnaberinus*.

 Canopy closure classes were compared to the "dense canopy closure" class.

ure 8). The AUC of the model was 0.74 and, therefore, the model explained a fair share of the gathered data. The host tree largely influenced *C. cinnaberinus* colonisation and was of high importance (Δ AIC to model without variables = 12.9). Longer dead tree trunks had a higher probability of being colonised by *C. cinnaberinus* and were of high importance (Δ AIC to model without variables = 31.4). Additionally, *C. cinnaberinus* showed a higher preference for trunks with a larger diameter.

Macrohabitat selection patterns

For the macrohabitat, the best model included altitude, the amount of deadwood and the openness of the canopy (Table 4, Figure 9). Elevation showed a significant negative relationship with the probability of *C. cinnaberinus* colonising a tree. Both the amount of deciduous trees and amount of deadwood had a positive effect on colonisation probability. Canopy openness in general showed an increasing probability from closed canopy to open canopy. The model had an AUC of 0.72 which showed that the model predicts a fair share of the observed data.

Discussion

This study revealed that, within the former Duchy of Carniola, where *C. cinnaberinus* was described for the first time (Scopoli 1763), there are at least four regions that may represent the species' type locality. It is known however that J. A. Scopoli obtained zoological and botanical material for his studies from only two of these regions (Petkovšek 1977, Baker 1999): the Branica River Valley near Vipava and the montane forests of the Ribnica-Kočevje region. The review of *C. cinnaberinus* records showed that the beetle was present in moderate abundance only in the Ribnica-Kočevje region in the past and recent records show a similar pattern. It is known that J. A. Scopoli himself visited the region around Ribnica several times, especially in the autumn when he collected mushrooms (Petkovšek 1977). Additionally, he obtained material,



Figure 9. Predicted probability of occurrence of *Cucujus cinnaberinus* depending on altitude, amount of deciduous trees, amount of deadwood and openness of canopy cover. The solid line shows dense canopy closure; the dashed line shows normal canopy closure; the stippled line shows sparse canopy closure; the dashed-stippled line shows patchy canopy closure. Particular values were taken from altitude (median and maximum values) and amount of deadwood (minimum, median and maximum values) to emphasize the influence of different variables on each other and on the probability of colonization of tree trunks by *C. cinnaberinus*. The empty circles show the presence (1.0) and absence (0.0) of *C. cinnaberinus* in relation to the amount of deciduous trees.

predominantly plants, from the vicinity of Kočevje from a colleague Franz Xaver von Wulfen (Scopoli 1772, Petkovšek 1977). In his description of *C. cinnaberinus*, Scopoli (1763) noted "Octobri M. in *Brassica oleracea* capitata repertus & adlatus",

meaning that "The species was found on a cabbage head and brought to him in mid October". Since C. cinnaberinus is a strict saproxylic species living under the bark of dead trees and with a limited flight season in spring and also in autumn (Horák and Chobot 2011), it is argued that the discovery of the specimen on a cabbage plant suggests only an occasional finding of a single specimen. It is very unlikely that such a finding would be recorded where the species is extremely rare such as in the Branica River Valley where only one record exists from 2002 and where the species was not confirmed again in later intensive surveys. The area is apparently the westernmost limit of the species distribution in SE Europe since there is no record known for the species further to the west in northern Italy (Horák and Chobot 2009, Brandmayr et al. 2016). Conversely, in the Ribnica-Kočevje region, C. cinnaberinus was reported in historical surveys (in 1930s and 1970s) as well as in recent surveys after 2008 at a moderate trunk occupancy rate of 10 %. Therefore, it is proposed that the Ribnica-Kočevje region in southern Slovenia with Mts. Velika gora, Stojna, Kočevski Rog and Stružnica are the most probable type locality for C. cinnaberinus. Despite intensive field studies, no other Cucujus species has been recorded in Slovenia neither recently nor in the past and this may decrease the possibility of misinterpretation of Scopoli's specimen as an identity of C. cinnaberinus.

Throughout the 19th and 20th century, C. cinnaberinus was considered as an extremely rare species in Slovenia with few known records and a similar pattern was suspected in other parts of Europe (see literature review in Horák et al. 2010). However, even large populations could have gone undetected due to the species' elusive behaviour and sparse distribution or due to ineffective survey procedures (McDonald 2004). The latter was particularly evident in C. cinnaberinus, with a dramatic recent increase in records after application of the larval search method and the pattern over the last century is remarkably similar in Slovenia as well as in other countries (Horák et al. 2010, Eckelt et al. 2014). Moreover, this type of data increase is expected in most of the beetle species listed in the Habitats Directive due to an increase in research after the Directive's adoption (see Introduction for literature review). The results from this study and reports of new discoveries of C. cinnaberinus populations in many countries across Europe (see Introduction for literature review) are not in accordance with the interpretation of Horák et al. (2010) that rarity and species absence, especially in southern European countries, is a result of population extinctions or decline in the past, but rather it is a consequence of research deficiency and overlooked populations. A Europe-wide study evaluating the population status of *C. cinnaberinus* and taking into account abundance estimates and research efforts to reveal species distribution and its population strongholds is thus needed. It was confirmed that the observations of Horák et al. (2010) about species population strongholds being limited to lignicultures and riparian forest stands including stands of fast growing invasive alien trees such as Black Locust (Robinia pseudoacacia) also hold true in southern Europe. Surprisingly, there were no historical records for C. cinnaberinus known from these sites in Slovenia which might support the hypothesis that the recent population expansion is due to a change in the structure of riparian forests due to intensifying poplar lignicultures and

spread of invasive non-native tree species (Horák et al. 2010, Eckelt et al. 2014, Fuchs et al. 2014, Hörren and Tolkiehn 2016). In Slovenia, this was particularly true in lowland floodplain forests, in which there was intensive poplar planting in the period from 1960–1980 (Božič and Krajnc 2012) and rapid expansion of the Black Locust which regionally had already reached more than 6 % of the growing stock in the forests (Kutnar and Kobler 2013). Nevertheless, *C. cinnaberinus* was found in a wide altitudinal range from the lowlands up to 1095 m a.s.l. in Slovenia, up to 1400 m a.s.l. in Austria (Eckelt et al. 2014) and even above 1500 m a.s.l. in Albania (Kovács et al. 2012). The field study based, on a preliminary species distribution model, revealed that higher elevation montane forest stands could represent a significant part of the species' distribution range by surface area, although, in these habitats, the species was much less abundant than in the lignicultures and riparian forests of the lowlands.

Due to low abundance, there have been few studies actually reporting the species from montane habitats (e.g. Holzer and Friess 2001, Bussler 2002, Mazzei et al. 2011, Kovács et al. 2012, Eckelt et al. 2014) and, therefore, a major part of the species' distribution range has not been considered in conservation plans. More studies and conservation efforts for *C. cinnaberinus* are thus needed in montane and traditional forest habitats. Focusing only on current population strongholds found in poplar lignicultures and even in invasive tree species stands in the lowlands might be a Trojan horse in *C. cinnaberinus* conservation efforts (Horák et al. 2010). The development of more efficient detection methods based on species pheromones or other semiochemicals that attract species would be helpful in further conservation studies (Larsson 2016), especially in sub-optimal but widespread species habitats.

In this study, low as well as high elevation habitats were included and, in both habitats, the amount of deadwood, amount of deciduous trees and degree of canopy openness was positively associated with C. cinnaberinus probability of occurrence, a fact which was in agreement with other studies on species habitat preference (Horák et al. 2010, Horák et al. 2011). Altitude had a negative effect on species distribution, meaning that, in stands with the same amount of deadwood, the species would be less abundant at higher elevations. A possible explanation could simply be macrophysiological. In ectotherm species, a restricted optimal temperature window at higher altitudes in comparison with lower altitudes might play a decisive role in the species' overall metabolic performance which is reflected in its life-history traits, including its reproduction and development rate (Gaston et al. 2009). Although the development cycle is still not well studied in C. cinnaberinus, Palm (1951) reported that larval development should take at least two years. Nevertheless, the duration of larval development could differ greatly between different climates, as shown for example in the stag beetle (Lucanus cervus), in which the duration of the larval stage can vary from 3 to 6 years and even the number of larval instars can differ regionally (Harvey et al. 2011). C. cinnaberinus is a widespread species, ranging from boreal to warm temperate climates (Horák and Chobot 2009) and also has a wide altitudinal distribution as shown in this study. Its metabolic performance and life history traits might however change with latitude and altitude. Overall, it seems that the species' optimal conditions are in

middle European lowlands in a warm temperate climate with hot and humid summers (Kottek et al. 2006, Horák et al. 2010). In Slovenia, such conditions are found only in the eastern part of the country, while the rest of the country is mountainous, which might be the reason for the lower *C. cinnaberinus* abundance. A second explanation for the effect of altitude could however be ecological, i.e. differences in habitat quality in lowland vs. montane forests. In future studies, the effects of climate conditions on *C. cinnaberinus* distribution patterns should be further examined, especially the effects of temperature and precipitation, to reveal their direct impacts on species life cycles and possible indirect impacts due to habitat structure alterations.

The size of the deadwood has been shown in this and other studies to be important for saproxylic beetles which prefer longer tree trunks with a larger diameter (Jonsson et al. 2005, Lindhe et al. 2005, Jonsell et al. 2007, Jonsell 2008). Although *C. cinnaberinus* was found in a number of host tree species and tree species was not found to be an important predictor in species microhabitat selection in lowland forests (Horák et al. 2011), it was found that tree host species might be important in the overall microhabitat performance of *C. cinnaberinus*. In such opportunistic species, the availability of host tree species should play a crucial role in host selection, where the most abundant tree species in a particular forest stand is also the most frequently occupied. That was the case for *Salix* in our sample of inspected trees and for *Pinus* in Southern Italy (Mazzei et al. 2011).

On the other hand, preferred or optimal tree hosts that are selected in a larger proportion than that available, could facilitate species establishment, population growth or even spread. *Tilia, Populus* and *Robinia* in particular, as tree species, were significantly preferred by *C. cinnaberinus*. Various poplar species were frequently reported as host trees of *C. cinnaberinus* in lowlands as well as in higher elevation forests (Horák et al. 2010, Eckelt et al. 2014, Marczak 2016). In this study, in addition to poplars, invasive Black Locust was found to be the preferred tree host in lowland forest. In addition to *Robinia*, other alien tree species, such as *Aesculus* and *Ailanthus*, were also reported as host trees in Austria, although at low frequencies (Eckelt et al. 2014).

The significant shift from native to invasive tree hosts revealed in this study indicates the great adaptive potential of *C. cinnaberinus*, particularly for rapid growing and short-lived species which could produce larger quantities of deadwood mass over shorter periods. The increase in growing stock and consequently in the deadwood of preferred host tree species in lowland forest stands in Slovenia, especially of *Populus* and *Robinia*, started in the period from the 1960s onwards (Božič and Krajnc 2012, Kutnar and Kobler 2013) and probably created optimal habitat conditions for *C. cinnaberinus*, resulting in an increase in its population and its eventual spread (Horák et al. 2010).

In contrast, there are no such conditions in montane forests where trees such as *Fagus, Abies* and *Picea* predominate in the growing stock (Marinček 1987) but were not found amongst the preferred species in this study or in those of others (Eckelt et al. 2014, Marczak 2016). On the other hand, *Tilia*, which is a preferred host tree in montane forests, has a low growing stock (<1 %) in the montane forests of Slovenia (Puncer 1980) and, therefore, the abundance and population size of *C. cinnaberinus* is

constrained in these stands. Montane *C. cinnaberinus* populations thus remain low and scattered despite the beetle also being found in other dominant tree species in these environments, including *Fagus*, *Abies* and *Picea* (Eckelt et al. 2014, this study).

Conclusions

In conclusion, this study presents new findings regarding the ecology of *C. cinnaberinus* at the limit of its distribution in two types of species habitat that greatly differ in species abundance and overall ecological performance: lowland and montane forests. The study revealed the importance of montane forests for *C. cinnaberinus* conservation, although recent population strongholds are located in lowland riparian forests and lignicultures, these being a consequence of human-induced changes in forest structure due to poplar plantings and expansion of fast growing alien tree species.

To evaluate the future potential of *C. cinnaberinus* expansion and to define conservation management of existing populations, it is necessary to explore the impact of environmental factors that limit the species' distribution, in particular climate (effects of environmental temperature and precipitation) and tree species structure in forest stands. Future environmental change scenarios (climate change, invasion of alien tree species) may cause *C. cinnaberinus* population decline in some regions as well as an increase and expansion in other regions of Europe due to the apparently high dispersal and colonisation capacity of the species (Horák et al. 2010).

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Appendix

Table S1. Overview of the number of published research papers considering at least one of the 21 saproxylic beetle species of European conservation concern and listed on the Web of Science up to the year 2017. Specifically, the number of papers targeting certain species and the number of papers dealing with ecological, monitoring and conservation aspects of the species is given.

Species	Total no. papers	Species targeted papers	Ecology, Monitoring, Conservation	Ecology, Monitoring, Conservation – species targeted
Osmoderma eremita	68	36	61	32
Morimus funereus	50	46	5	3
Lucanus cervus	32	21	19	13
Rosalia alpina	17	14	14	11
Cucujus cinnaberinus	13	7	10	5
Cerambyx cerdo	12	9	7	4
Limoniscus violaceus	9	3	9	3
Pytho kolwensis	5	1	3	1
Boros schneideri	4	3	4	3
Mesosa myops	4	3	2	1
Stephanopachys substriatus	2	0	2	0
Rhysodes sulcatus	2	0	1	0
Agathidium pulchellum	1	1	1	1
Buprestis splendens	1	0	1	0
Phryganophilus ruficollis	1	0	1	0
Stephanopachys linearis	1	0	1	0
Xyletinus tremulicola	1	1	1	1
Propomacrus cypriacus	1	0	0	0
Corticaria planula	0	0	0	0
Oxyporus mannerheimii	0	0	0	0
Pseudogaurotina excellens	0	0	0	0

RESEARCH ARTICLE



New Italian records of Lichenophanes varius (Illiger, 1801) (Coleoptera, Bostrichidae)

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Abstract

Lichenophanes varius (Illiger, 1801) is a Turanic-European-Mediterranean species. In most of European countries, this species is protected at different levels and it is classified as "NT" (Near Threatened) in the IUCN European Red List of Saproxylic Beetles. In Italy, it is classified as "EN" (Endangered). Its larvae are saproxylophagous and develop in branches and rotting trunks of many broadleaved tree genera. Nevertheless, this beetle seems to attack only wood which is already invaded by the mycelia of *Biscogniauxia* spp. (Pyrenomycetes, Xylariaceae). The Italian distribution and ecology of *L. varius* are updated on the basis of recent records; the species is recorded for the first time from Calabria, where it was reared from *Quercus frainetto* Ten. which represent a new host-plant record for this beetle. Finally, the authors discuss the possibility that global warming can promote a resurgence of attacks from the above mentioned phytopathogenic fungi in Italian forests and, therefore, this climatic change can also favour the populations of this red-listed beetle.

Keywords

Bostrichidae, Lichenophanes, protected areas, conservation, faunistic, host plants, Xylariaceae

Introduction

As *Lichenophanes varius* (Illiger, 1801) (Fig. 1) is classified as "EN" (Endangered) in the Italian Red List of Saproxylic Beetles (Nardi et al. 2014, 2015b), hereunder, new Italian records ("New records") are provided for a better knowledge of its chronogeonemy and ecology. Moreover, considering the rarity of this species throughout Europe (cf. Nieto and Alexander 2010, Przewoźny 2011, Muscarella et al. 2013, Geis 2016), some records from other countries ("Other material examined") are also provided.

Materials and methods

Taxonomy and nomenclature

The beetles were identified following the work by Lesne (1899, 1901) and Muscarella et al. (2013).

The systematics and nomenclature of plants and fungi follow, respectively, The Plant List (2013) and Index Fungorum (2017); families and, within each family, species are listed alphabetically.



Figure 1. Adult of *Lichenophanes varius* from the Nature Reserve Bosco della Fontana (Italy) (photo by G. Scaglioni, 2014).

Data collection and phenology

The information on *L. varius* provided by Muscarella et al. (2013), was hereunder updated with unpublished and literature records. The former were obtained after examination of museum collections where large number of saproxylic beetles collected during recent forest monitoring programmes were present (Audisio and Biscaccianti 2008, Persiani et al. 2010, Della Rocca et al. 2014, Di Santo and Biscaccianti 2014, Hardersen et al. 2014, Parisi 2014, Redolfi De Zan et al. 2014, Faccoli and Montecchio 2015, Macagno et al. 2015, Nardi et al. 2015a, Biscaccianti et al. 2016, Matteucci 2016).

For each record, the following information, when available, is provided: administrative region, commune (province), locality, altitude (metres a.s.l.), geographic coordinates (lat/lon DMS, datum WGS84), biotope, date, collector, additional information on the finding, number of specimens and collection. Possible interpolations are given in square brackets. The labels of the examined specimens are written in Italian; hereunder, the regions, the biotopes and the collecting methods were translated into English. The mainland Italian regions are listed from North to South and from West to East, all toponyms being listed alphabetically.

The monthly Italian phenology of this species (Fig. 2) is based upon all available records from 1867 to 2016 (Muscarella et al. 2013, Nardi and Biscaccianti this paper). As these records are not quantitative, the value attributed to each one was standardised according to the following formula: 1/number of months. For instance, the value of the record for 3.6.2014, is 1/1 = 1, while the value of the record dated 18.5-12.7.2010, is 1/3 = 0.333, and this value was attributed to each of the involved months (May, June and July). In the Fig. 2, the value of a single month is given by the arithmetic sum of the above standardised values of the single month.

Unpublished works according to ICZN (2012) are also listed in the references, but their effective status is reported in square brackets.

Acronyms

Specimen depositories: CAB = A.B. Biscaccianti private collection, Roma; CFA = F. Angelini collection c/o Museo di Storia Naturale dell'Università degli Studi di Firenze, Sezione di Zoologia "La Specola", Firenze; CNBFVR = Centro Nazionale per lo Studio e la Conservazione della Biodiversità Forestale "Bosco Fontana" Carabinieri, Marmirolo (Mantova); CGN = G. Nardi private collection, Cisterna di Latina (Latina); CVV = V. Vomero private collection, Roma; MZAC = Museo di Zoologia ed Anatomia Comparata, Roma Tre Università degli Studi, Roma.

Other abbreviations and recurrent terms used in the text: BCWFT = black cross windows flight trap on a beech tree (*Fagus sylvatica*); Bosco = Wood; es. = specimen/s; ex = emerged from; Monte = Mount; P.N. = Parco Nazionale = National Park; sdb = same data but; TCWFT= transparent cross windows flight trap on a beech tree (*F. sylvatica*).

Results

Lichenophanes varius (Illiger, 1801)

Lichenophanes varius (Illiger, 1801): Evangelista and Cristiano 2013: 250, Muscarella et al. 2013: 455, Redolfi de Zan et al. 2016: 237.

New records. PIEDMONT (Evangelista and Cristiano 2013): Caramagna (Cuneo), Bosco del Merlino, relict deciduous plain forest, between 1996 and 2012, ex branches of Quercus robur, many es. LOMBARDY: Marmirolo (Mantova), Nature Reserve Bosco della Fontana, 25 m, 45°12.030'N; 10°44.085'E, Querco-Carpinetum boreoitalicum relict forest, 3.6.2014, M. Bardiani and I. Toni leg., unbaited aerial trap on a Quercus cerris tree at a height of 13 m from the soil, 1 es. (CNBFVR). LAZIO (cf. Redolfi de Zan et al. 2016): Allumiere (Roma), 573 m, 42°09.112'N; 11°54.560'E, relict forest dominated by beech trees (Fagus sylvatica), 19.6.-1.7.2011, G.M. Carpaneto et al. leg., BCWFT, 1 es. (CGN); sdb 612 m, 42°09'05.9"N; 11°54'36.0"E, 2–15.7.2011, TCWFT, 1 es. (CGN); Monti Cimini (Viterbo), Monte Venere, 714 m, 42°20'55.1"N; 12°11'05.4"E, relict forest dominated by beech trees (F. sylvatica), 2-15.7.2011, G.M. Carpaneto et al. leg., TCWFT, 1 es (CGN); sdb 829 m, 42°20'43.7"N; 12°10'52.8"E, 1 es. (CGN); Oriolo Romano (Viterbo), 463 m, 42°09'41.8"N; 12°09'14.0"E, relict forest dominated by beech trees (F. sylvatica), 19.6.-1.7.2011, G.M. Carpaneto et al. leg., BCWFT, 1 es. (CGN); sdb 2-15.7.2011, 1 es. (MZAC). CAMPANIA (cf. Audisio and Biscaccianti 2008): P.N. Cilento e Vallo di Diano, Montesano sulla Marcellana (Salerno), Bosco Cerreta, 510 m, 40°15'33"N; 15°39'30"E, old-growth forest dominated by Q. cerris, 22.7.2008, A.B. Biscaccianti and E. Colonnelli leg., ex branches of Q. cerris (collected 22.5.2008) colonized by Biscogniauxia sp. [possibly B. nummularia (Bull.) Kuntze], 2 es. (CAB). CALABRIA: P.N. Aspromonte, San Luca (Reggio Calabria), Pietra Lunga, Bosco Ferullà, 649 m, 38°10'07'N; 16°02'05'E, old-growth mixed forest dominated by Q. frainetto and Q. ilex, 14.7.2016, A.B. Biscaccianti, F. Manti and E. Castiglione leg., ex branches of Q. frainetto (collected 15–18.3.2016) apparently free of fungi, 1 es. (CAB).

Other material examined. CZECH REPUBLIC: Bohemia, Adamov, 6.[19]69, J. Hladil leg., 1 es. (CFA). FRANCE: Corsica, Corte, 500 m, 23.11.2010, [W.] Pagliacci leg., 8 es. (CVV).

Italian regional distribution. Piedmont (Evangelista and Cristiano 2013), Lombardy, Veneto, Emilia-Romagna, Tuscany, Lazio, Molise, Campania, Apulia, Basilicata, Calabria, Sicily and Sardinia (cf. Audisio and Biscaccianti 2008, Muscarella et al. 2013, Redolfi de Zan et al. 2014, Nardi and Biscaccianti this paper).

Chorotype and distribution. Turanic-European-Mediterranean (cf. Vigna Taglianti et al. 1999, Muscarella et al. 2013). This species is recorded from: Europe (Albania, Armenia, Austria, Azerbaijan, Bosnia-Herzegovina, Bulgaria, Croatia, Czech Republic, Estonia, France (mainland, Corsica), Georgia, Germany, Greece (mainland), Hungary, Italy (mainland, Sardinia and Sicily), Macedonia, Moldavia, Poland, Portugal, Romania, Slovakia, Slovenia, Spain (mainland), Switzerland, Russia (Central Territory and Southern Territory), Turkey, Ukraine), North Africa (Algeria, Egypt, Libya, Morocco, Tunisia) and Asia (Cyprus, Iran, Syria, Turkmenistan, Turkey) (cf. Borowski 2007, Muscarella et al. 2013, Audisio et al. 2015).

Ecology. Lichenophanes varius lives in old-growth or at least in well preserved, broadleaved forests, where its saproxylophagous larvae develop in the dead wood of various genera of trees (e.g. Alnus, Carpinus, Castanea, Fagus, Populus, Quercus, Tilia) (cf. Muscarella et al. 2013). In Italy, this species was reared from the following plants: Carpinus betulus L. (Betulaceae), Quercus sp., Q. cerris L., Q. frainetto Ten., Q. pubescens Willd., Q. robur L. and Q. suber L. (Fagaceae) (cf. Evangelista and Cristiano 2013, Muscarella et al. 2013, Nardi and Biscaccianti this paper). Moreover, Q. ilex L., F. sylvatica L. (Fagaceae), Glycyrrhiza glabra L. (Leguminosae), Populus sp. (Salicaceae), Acer monspessulanum L. (Sapindaceae) and Ulmus sp. (Ulmaceae), are very probably host plants of L. varius in Italy, since adults were also collected or trapped on these botanic species (cf. Muscarella et al. 2013, Redolfi de Zan et al. 2014) and, in other countries, this beetle was also reared from most of these plants (cf. Muscarella et al. 2013).

Lichenophanes varius seems to attack only wood invaded by the mycelia of some Xylariaceae fungi (Pyrenomycetes): *Biscogniauxia* sp. on *Quercus cerris* (Nardi and Biscaccianti this paper), *B. mediterranea* (De Not.) Kuntze on *Quercus* sp., and *B. nummularia* on *Fagus sylvatica* (cf. Muscarella et al. 2013). Nevertheless, no mycological data are available for the other host plants. This peculiar larval ecology, as observed for other saproxylophagous beetles (cf. Rejzek and Vlásak 2000, Persiani et al. 2010, Borowski and Pietka 2014), is, probably, one of the causes of the rarity of this Bostrichid.

In Italy, *L. varius* occurs from sea level to the montane belt (1100–1300 m a.s.l.) (cf. Muscarella et al. 2013, Nardi and Biscaccianti this paper); in this country, the decrease of old-growth deciduous plain forests (cf. Mason 2002, Pignatti et al. 2009) is surely another cause of its rarity.

Figure 2 summarises, according to the month of capture, the phenology of all Italian records (cf. Muscarella et al. 2013, Nardi and Biscaccianti this paper). This figure clearly shows that the species is active in May–July, while in Hungary, the adults are active in mid-summer (Németh et al. 2015), and, in the Forest of Fontainebleau (France), they are active in June–September (Cantonnet et al. 1995).

Discussion

A sole specimen of *L. varius* was previously (year 2000) collected in the above-mentioned locality of Lombardy (Nardi and Zahradník 2004), despite this site, since 1995, was being the subject of accurate entomological research (cf. Mason et al. 2001, 2002, Cerretti et al. 2004, Mason et al. 2006, Stireman et al. 2011, Birtele and Hardersen 2012, Chiari et al. 2013, D'Amen et al. 2013, Hardersen et al. 2014). The latest specimen (year 2014) from this locality was collected during the LIFE Project MIPP



Figure 2. Italian phenology of Lichenophanes varius.

(http://www.wsmipp2017.eu/) by an aerial trap placed on a *Q. cerris* tree (cf. Bardiani et al. 2017). No *Biscogniauxia* species is recorded from this locality, where other fungi of the same family occur (Consiglio et al. 2007, E. Minari pers. comm.). *Biscogniauxia nummularia* occurs in the above site of Campania (cf. Persiani et al. 2010) and, in this place, *L. varius* was reared from branches of *Q. cerris* colonized by *Biscogniauxia* sp. (Nardi and Biscaccianti this paper). Previously, *L. varius* was known from Campania only on the basis of very old records (cf. Muscarella et al. 2013) and most of the woods of these sites appear now unsuitable for the development of this species (cf. Massa and Massa 1980, Cona and Di Pasquale 2007). The record from Calabria is the first for this region, where a single specimen was reared from branches of *Q. frainetto* apparently free of fungi. In Italy, this *Quercus* species was previously unrecorded as host-plant of *L. varius* (cf. Muscarella et al. 2013).

The above records from Lazio have been already published (Redolfi de Zan et al. 2014), but without the label's data. These specimens were obtained using two kinds of aerial traps: transparent cross windows flight trap and black cross windows flight trap (cf. Redolfi de Zan et al. 2014).

The global warming, can favour an expansion of the range of alien species (cf. Walther et al. 2009, Tobin et al. 2014, Rassati et al. 2016), but also those of some autoctonous species such as *L. varius* (Geis 2016). In fact, in recent years, in response to climate change, there has been a resurgence of attacks from the above phytopathogenic fungi in Italian forest stands (Franceschini et al. 2009) and, this resurgence can probably favour the populations of this red-listed species (cf. Muscarella et al. 2013).

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The role of monumental trees for the preservation of saproxylic biodiversity: re-thinking their management in cultural landscapes

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Abstract

Ancient trees present structural and functional characteristics fundamental for sustaining complex and unique assemblages of species. They are a resource globally threatened by both intensive land uses and lack of recruitment. Their disappearance would involve not only the loss of majestic organisms with high intrinsic value, but may also result in the disappearance of rare and endangered species. Italy is currently implementing a new list of noteworthy ancient trees (i.e. monumental trees) and the preliminary results of this new inventory have been analysed as a case study of a national initiative. The provisional list included 950 complete records, corresponding to 65 genera and 118 species. The most abundant species was *Quercus pubescens* Willd while the most common genera were *Quercus, Larix, Cedrus, Fagus* and *Platanus*. Age and size were the most used criteria for inclusion of trees in the census. The fundamental novelty of the new inventory is that it is based on a set of well-defined criteria of monumentality and that it clearly

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recognised the ecological value of ancient trees. Preserving a tree for its ecological role requires a profound cultural shift. The value of microhabitats, structures that have historically been considered defects, should be recognised and managed accordingly. Ancient trees are often part of disappearing cultural landscapes: to preserve the richness and diversity of these habitats, new policies and regulations are needed. The preservation of landscapes, where there is still a high density of ancient trees, should be a priority for all European countries in order to conserve their unique associated fauna and for their irreplaceable functional value for biodiversity conservation.

Keywords

Ancient tree, deadwood, microhabitat, saproxylic, veteran tree

Introduction

Ancient trees, historically preserved for their aesthetic value, are nowadays recognised as key resources that sustain broad and unique assemblages of species. Several names have been used to identify them, such as champion or heritage trees (Orłowski and Nowak 2007), large old trees (Lindenmayer et al. 2012), ancient trees (Hall and Bunce 2011) and veteran trees (Read 2000). With time, these trees may reach a biomass and complexity not achieved by any other living organism (Blicharska and Mikusiński 2014). Having long passed their silvicultural maturity, they tend to present special features that contribute to increasing their ecological value, such as cavities, decaying wood and bark losses (Siitonen and Ranius 2015). It is their age and size, together with the environmental conditions that occur where they live, which determine the occurrence of complex decay processes (Lindenmayer et al. 2014) and which lead to the development of a diversified array of microhabitats. Tree microhabitats are small distinctive substrates, used by several species for forage and shelter (Vuidot et al. 2011) and they are gaining increasing attention as indicators of sustainable forest management (e.g. FOREST EUROPE 2015). The contribution of ancient trees for the conservation of saproxylic species is unmatched since they present an exceptional diversity of microhabitats, some of which may last for centuries (Siitonen and Ranius 2015). The decline of old and hollow trees threatens the conservation of numerous endangered species (Sebek et al. 2013).

Even if, over time, ancient trees tend to accumulate decayed wood, it is important to stress that they "are not necessarily moribund" (Siitonen and Ranius 2015). As time passes, their anatomy tends to change to accommodate these structural alterations: with a process called retrenchment, the canopy becomes smaller and lower (Alexander 2001) and other self-optimisation mechanisms (see Mattheck and Kubler 1997) contribute to their stability and longevity.

They represent a charismatic element, appreciated for their majestic aesthetics and as a cultural heritage, which supports plentiful organisms of fungi, lichens, vertebrates and invertebrates (Alexander 2001; Alexander 2008). The biodiversity they host is unique: in landscapes where they are still well represented, they provide the habitat for rare and threatened species (Butler et al. 2001). Large old trees have been compared to islands, as they also show a species-area relationship, with larger trees hosting assemblages significantly richer and more numerous than smaller trees (Le Roux et al. 2015). In Europe, they tend to survive as part of different cultural landscapes, playing a key ecological role (Alexander, 1999). Old hunting forests, parks of manors and wood-pastures are amongst the places where ancient trees have managed to survive in Europe (Alexander 2001). Ancient trees are a component of silvopastoral systems, which encompass habitats including woodland and grassland at the same time, and show distinctive structures and species composition not generally found elsewhere (Bergmeier et al. 2010). Manning et al. (2006) argued that, considering the unequal effect large old scattered trees have on ecosystems and landscapes, irreplaceable by any other resource, the concept of "functional uniqueness" should be extended to these structures.

The first Italian law that has extended protection to ancient trees dates back to 1939 (Law n. 1497, 29/06/1939), but back then, they were included as "immovable things that have remarkable characteristics of natural beauty". A pioneering census was carried out in 1982: on the initiative of Italian Forest Service, more than 22,000 trees were recorded which resulted in the identification of 1,253 trees of noteworthy interest (Bortolotti 1989). A tree was included in this first census if it presented an exceptional size for its species, a peculiar shape, noteworthy aesthetic features or for its historical-cultural value (Lisa 2011). Numerous regional laws were issued afterwards to preserve monumental trees, but, to be effective, their management and conservation required unified regulations. To upscale the process from regional to national scale, Law n.10 14/01/2013 and Ministerial Decree 23/10/2014 were enacted to require that Municipalities undertake surveys and assess the status of monumental trees, according to predefined protocols. The purpose of the present study was to analyse the preliminary results of the new Italian inventory of monumental trees. To assess its progress, the abundance and distribution of the available records were compared with the data collected during the 1982 census. The obtained results, together with International literature, were used to make recommendations for the management of this rare and threatened resource.

Methods

Drafting of the Italian Inventory

The targets for the new Italian inventory of monumental trees were single trees, tree lines and shrubs with a remarkable development, belonging to both native and nonnative species. Ministerial Decree 23/10/2014 identified seven criteria that should be met, jointly or alternatively (one criterion is sufficient), for the tree to be listed as monumental. These criteria are: 1. significant age and size (with species-specific trunk circumference thresholds); 2. peculiar shape (due to a tree living within its optimum ecological conditions, produced by climatic factors or subjected to human interventions); 3. ecological value (when the tree is the habitat of rare and/or endangered animal species, included in the Habitats Directive (92/43/EEC) and/or Red Lists); 4. botanic rarity (considering both exotic and locally rare species); 5. plant architecture (single trees or small groups that are an integral part of architectural elements); 6. landscape value (trees that are distinctive elements, with toponymy value or historic continuity) and 7. historic-cultural-religious aspects (trees that contribute to the sense of belonging and recognisability of a place, memory of historic events, traditions, religious reference, legends etc.).

Law n.10 14/01/2013 established that municipalities should assess monumental trees present in their territory, with the support of the Forest Service. Specific training was organised for the personnel involved in this assessment. The field surveys were performed by 1-2 people, in urban areas, forests and agricultural areas as well. During the surveys, a specifically designed identification sheet was completed with data on the location, taxonomy, structure, status, required interventions and a brief description of the reasons why the tree or shrub should be considered monumental and according to which criteria. All this information, together with the photographs of the tree or shrub, was passed to the regional authority for approval or rejection. The approved records were then transferred to the Ministry of Agricultural, Food and Forestry Policies for their inclusion in the national list of monumental trees.

Data analysis

To evaluate the progress of the new inventory, it was compared to the data gathered in the 1982 survey by the Forest Service. The 1982 database is available on the webpage of Italian Monumental Trees (http://www.corpoforestale.it/Alberi_Monumentali) while the preliminary results of the new inventory are stored in a specifically designed geoportal (Geoalberimonumentali) with restricted access. For the analysis, data were downloaded from the geoportal on 17/02/2017 and included trees and shrubs recorded from 11/06/2014 to 15/02/2017. Only records with no missing fields were included in the analyses. If a species was included in the database using several synonyms, its taxonomy was rationalised using the freely available working list "The Plant List" (http://www.theplantlist.org/).

Results

The provisional list of the new Italian census of monumental trees included 950 complete records. Compared to the 1982 inventory, the former comprised a higher number of trees (1,253) which were more uniformly distributed across the country (Figure 1). During the 1982 survey, a total of 143 species belonging to 75 genera were recorded while the provisional 2017 list included 118 species belonging to 66 genera (Suppl. material 1). In both inventories, the large majority of the species were Angiosperms (65% and 71% respectively).

The most recorded genera changed geographically and through time: while the 60% of the trees included in the 1982 list belonged to *Quercus, Fagus, Cedrus, Castanea* and



Figure 1. Number of trees recorded during the two inventories (1982 and 2017) in the different Italian regions.

Pinus, the vast majority of the trees in the 2017 list trees belonged to *Quercus*, *Larix*, *Cedrus*, *Fagus* and *Platanus* (Figure 2). The most abundant species was *Quercus pubescens* Willd in both lists, followed by *Fagus sylvatica* L. in 1982 and by *Larix decidua* Mill. in 2017. Although the highest recorded species were trees, the percentage of included shrubs significantly increased from 1982 to 2017 from 4% to 6% ($X^2 = 13.369$, df = 1, p-value = 0.0003). Both lists included invasive alien species: *Ailanthus altissima* (Mill.), Swingle (in the 1982 list) and *Robinia pseudoacacia* L. (in the 2017 list).



Figure 2. Distribution of the most abundant genera included in the a) 1982 inventory and b) 2017 inventory.



Figure 3. Number of criteria associated with each record in the 2017 inventory.

The highest number of records (49%) was included in the 2017 provisional list by applying just one criterion (Figure 3) and for 94%, the criterion for inclusion was the age and size of the tree. Alone or together with other criteria, age and size were associated with 95% of the records. The ecological value was assigned to 9% of the records, in association with age and size (always) and other criteria. Since the definition of the criteria is only part of the 2017 inventory, the corresponding data for the 1982 list was not available.

Discussion

The new inventory compared with the previous one showed two fundamental improvements. First, it is based on a set of well-defined criteria of monumentality. Second, the ecological value of ancient trees for preserving rare associated saproxylic species and other animals which are strictly dependent on the availability of tree microhabitats, is explicitly stated. Compared to the other criteria, preserving a tree for its ecological role requires a profound cultural shift. The value of microhabitats, structures that have historically been considered defects, should be recognised and managed accordingly. Even if the percentage of trees listed as monumental for this criterion was low in the provisional list, it represents an encouraging starting point.

The provisional list showed a very heterogeneous distribution of the records across the country, suggesting that, compared with the previous census, there were factors affecting data gathering. In particular, considering that, according to the new law, the survey must be implemented by municipalities, the process may be suffering from the varied enthusiasm and participation of local authorities. The central coordination of the 1982 census by the Forest Service has probably played a key role in standardising the geographic reach of the survey at the national scale. Nevertheless, considering that the analysed data are part of an ongoing process, the number of records from less active regions will hopefully increase with time.

Several exotic trees were part of the preliminary list of monumental trees, such as *Liriodendron tulipifera* L.(n=8), *Sequoiadendron giganteum* (Lindl.) J.Buchholz (n=40), *Styphnolobium japonicum* (L.) Schott (n=6). However, the inclusion of invasive alien species amongst non-native species should be carefully evaluated: both surveys included species considered to be amongst the 100 most invasive alien species recorded in Europe (DAISIE 2009). These species are controlled or removed according to other frameworks and their preservation as monuments may be counterproductive (e.g. protecting a tree whose seedlings should be actively eliminated). International approaches (e.g. DAISE, 2009) could be used to guide the inclusion/exclusion of problematic species.

The records included in both lists and the current management suggest that the situation it Italy is similar to that which has been observed elsewhere: ancient trees are too often the remnants of long-abandoned traditions. Two of the main threats responsible for their loss in agricultural landscapes are land use intensification and the disappearance of traditional management (Siitonen and Ranius 2015). Actions that should be taken to reverse this process are summarised below.

Restore traditional management to conserve and increasancient trees

Pollarding is one of the three pathways identified by Read (2000) for the creation of ancient trees. The slowed growth of the trunk due to pollarding, together with the small resulting canopy, protects these trees from wind damage, contributing to their longevity (Hartel et al. 2015). Moreover, pollarding greatly increases the probability

of hollow formation (Sebek et al. 2013), providing a microhabitat that hosts complex and specialised communities of species. The interruption of the traditional pruning of pollards compromises not only recruitment but also the survival of the trees that have managed to outlive abandonment. Without intervention, the rate of loss of pollard trees may be dramatic, since the uncut branches tend to become large and compromise tree stability (Dagley et al. 2001, Cantero et al. 2014).

Wood-pastures are facing similar abandonment issues and inaction cannot be a successful strategy to retain and restore large old trees in agricultural landscapes. Several factors threaten the perpetuation of wood-pastures: structural simplification, the disappearance of large old trees and land-use policies that do not recognise their multifunctional nature (Hartel et al. 2015). To preserve and restore the biodiversity of these environments, the relationship between large herbivores and vegetation must be reinstated (Vera 2000). As these cultural landscapes rely on haymaking and livestock grazing to free veteran trees from competitors, the re-establishment of the traditional style of management represents the way forward (Siitonen and Ranius 2015). Even sanctuaries such as Fontainebleau and Białowieża are suffering from a minimum intervention regime: the lack of grazing has contributed to a shift towards a high forest structure with canopies too close to allow the replacement of the large old oak trees (Butler et al. 2001).

It is through both abandonment and intensification, changing from multifunctional to mono-functional land-uses (Hartel et al. 2015), that the loss of cultural landscapes, fundamental for the provision of monumental trees can be observed. In northern Italy, it was once common to pollard mulberry trees (*Morus alba* L., *M. nigra* L.) for silkworm breeding and, at the beginning of the 20th century, this farming system covered 289,000 hectares (Sereni, 1976). Remaining lines of old pollarded mulberry trees along canals and field borders may suggest the presence of these old plantations (Figure 4). Their rarefaction, fragmentation and lack of regeneration threaten the survival of endangered saproxylic beetles, such as Osmoderma eremita (Scopoli, 1763) which managed to survive in agricultural areas due to the microhabitats present in these trees (Corezzola et al. 2012). Even if ancient trees should be preserved as the habitat for endangered saproxylic species included in the Annexes II and IV of the Habitats Directive, specific regulations for the heterogeneous environments where these trees occur are currently lacking. Wood pastures and, in general, mosaic habitats, tend to be neglected in both the Habitats Directive and the Natura 2000 network (Alexander, 2016). Annex I of the Habitats Directive (1992) includes only a few typologies of wooded pastures present in Europe (e.g. 9070 Fennoscandian wooded pastures) and as forest habitats. Cultural landscapes, not listed amongst these few (such as pollarded mulberry fields), are generally managed as agricultural areas and land owners are allowed to cut trees without facing the strict regulations of forest habitats. To conserve the diversity of European wooded pastures sensu lato, it is thus essential to design specific categories, currently missing from Annex I, evaluating their status and extent (Bergmeier et al. 2010).



Figure 4. Lines of pollarded mulberry trees (*Morus* spp.) in the Po river plane, Italy. The pollarded trees present evident large stem cavities due to the pruning of the upper branches. Photo by F. Mason.

Simulations by Gibbons et al. (2008) suggest that recruitment events, taking place every 30–90 years, would ensure the temporal continuity of ancient trees in agricultural landscapes. The long time span proposed by the authors allows adjusting recruitment to the most cost-effective timing. Every intervention that alters the environmental conditions of ancient trees should be carried out cautiously. For example, Dagley et al. (2001) indicated that clearing the vegetation around an ancient tree should be a gradual process, not to increase wind-throw susceptibility. Precise guidelines of good management should be provided to tree owners and managers (see Read 2000 and Lonsdale 2013).

From single trees to landscapes

Conserving veteran trees through time and space may represent the basis for establishing new networks, diffusing genetically valuable saplings originated from local large old trees and restoring natural corridors such as riparian areas (Orłowski and Nowak 2007). For instance, pollarding trees growing along linear elements could support the creation of stepping stones re-connecting refugia of saproxylic species (Sebek et al. 2013).

Coordinating national initiatives: a call for a common European platform

The provisional results suggest the importance of centralised rather than localised (i.e. relying on regional authorities rather than on municipalities) management systems for

the success of the process. The Italian census of monumental trees is just one of the initiatives developed in Europe to monitor and preserve ancient trees. While surveying methods for ancient trees are similar across European countries, the definition of what constitutes a monumental tree varies (Lisa 2011). A common European platform could represent the basis for more effective recognition of the importance of cultural habitats and their associated ancient trees. The preservation of open-grown trees and the re-establishment of traditional management (e.g. through the creation of new pollards) should not just be target of single management plans (e.g. Dagley et al. 2001) but be part of an international strategy to preserve European cultural landscapes and their key features.

Conclusions

Ancient trees are declining in forests worldwide (Lindenmayer et al. 2012) and their disappearance will affect ecosystem processes along with the persistence of numerous species (Lindenmayer et al. 2014). The consumption of natural resources has been threatening the survival of these living monuments (Rigoni Stern 1990) for decades. To prevent the disappearance of ancient trees in cultural landscapes, new policies are needed, with unique temporal (centuries) and spatial (landscapes) reach (Lindenmayer et al. 2014) which require the implementation of traditional management (Butler et al. 2001). Considering the time-lag for their development, these new policies are needed now, otherwise we will have to face an unprecedented temporal discontinuity of a resource fundamental for biodiversity conservation. The preservation of landscapes, where there is still a high density of ancient trees, should be a priority (Siitonen and Ranius 2015) for all countries, for the unique associated fauna and for their intrinsic value.

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Supplementary material I

List of recorded species

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Data type: Adobe PDF file

- Explanation note: The supplementary material contains the list of species recorded during the 1982 and 2017 inventories, showing for each species: number of individuals, average circunference and average height.
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