


ORIGINAL RESEARCH

Microhabitat selection of meadow and steppe vipers enlightened by digital photography and image processing to describe grassland vegetation structure

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Abstract

Understanding animals' selection of microhabitats is important in both ecology and biodiversity conservation. However, there is no generally accepted methodology for the characterization of microhabitats, especially for vegetation structure. We studied microhabitat selection of three *Vipera* snakes by comparing grassland vegetation structure between viper occurrence points and random points in three grassland ecosystems: *V. graeca* in mountain meadows of Albania, *V. renardi* in loess steppes of Ukraine and *V. ursinii* in sand grasslands in Hungary. We quantified vegetation structure in an objective manner by automated processing of images taken of the vegetation against a vegetation profile board under standardized conditions. We developed an R script for automatic calculation of four vegetation structure variables derived from raster data obtained in the images: leaf area (LA), height of closed vegetation (HCV), maximum height of vegetation (MHV) and foliage height diversity (FHD). Generalized linear mixed models revealed that snake occurrence was positively related to HCV in *V. graeca*, to LA in *V. renardi* and to LA and MHV in *V. ursinii*, and negatively to HCV in *V. ursinii*. Our results demonstrate that vegetation structure variables derived from automated image processing significantly relate to viper microhabitat selection. Our method minimizes the risk of subjectivity in measuring vegetation structure, enables the aggregation of adjacent pixel data and is suitable for comparison of or extrapolation across different vegetation types or ecosystems.

Introduction

Understanding how animals choose microhabitats is a central aim in ecology and is fundamental for evidence-based conservation (Johnson et al., 2004). Microhabitat selection refers to the process by which animals select specific microenvironments of features within their habitat based on various factors, including resource availability, conspecific interactions and interactions with other species. This selection process is a key evolutionary strategy, as it can have a profound influence on individual fitness and the evolution of life-history traits associated with habitat properties (Morris, 2003). On an evolutionary time scale, microhabitat selection can be interpreted as an

adaptive strategy (MacArthur et al., 1962; Pianka, 1973). While intraspecific competition and population density may also play a role in individuals choices (Fretwell & Lucas, 1970; Lawlor & Smith, 1976), the associated costs of these choices are often underexplored (Rosenzweig, 1981).

The characterization of microhabitats, has proven to be difficult and there is no generally accepted methodology applicable across ecosystems, habitat types and animal groups (Stein et al., 2014). More complex habitats, that is, those characterized by higher microhabitat diversity, sustain a higher number of ecological niches and associated species compared to habitats with decreased structural diversity (Loke et al., 2015; MacArthur & MacArthur, 1961). Habitat or microhabitat

diversity is often divided into two components: compositional diversity arises from the identity of different elements, whereas structural diversity arises from the two- or three-dimensional physical arrangement of the elements (Lengyel *et al.*, 2016; Tews *et al.*, 2004). Both aspects can be further subdivided into abiotic components (e.g. composition: soil types, hydrology; structure: elevation, topography) and biotic components (e.g. composition: plant species identity; structure: vegetation complexity). The quantification of abiotic elements and biotic compositional elements is usually straightforward via objective measurements (e.g. for soil types, hydrology: qualitative list of soil types, maps, measurement of groundwater table; for elevation, topography: GPS readings, landform diversity; plant species/association identity: list of species or plant associations). In contrast, a plethora of context-dependent methods have been used to measure vegetation structure (Mushinsky & McCoy, 2016). Several terms have been used for vegetation structure, such as structural complexity/diversity, canopy/foilage height/diversity, vegetation complexity/heterogeneity, architectural complexity (Tews *et al.*, 2004).

In studies of animal habitat selection, vegetation structure is often quantified by measurements of phytomass or by cover estimates. Additional methods include quantifying the presence or cover of structures formed by plants, for example, tussocks, shrubs, dead phytomass such as leaves, height of shoots, leaf area or cover at various heights (Benkobi *et al.*, 2000; Faria & Silva, 2010; Garden *et al.*, 2007; Mizsei, Fejes, *et al.*, 2020; Pringle *et al.*, 2003; Stumpel & van der Werf, 2012; Vermeire & Gillen, 2001). Many of these methods depend on subjective eyeball estimates made in the field confounded by observer bias and measurement error (e.g. on plant cover; Bergstedt *et al.*, 2009; Milberg *et al.*, 2008), or return one value (e.g. vegetation height or phytomass), which, at most, is a proxy for the 3-D physical arrangement or distribution of vegetation elements. These drawbacks prevent generalizations of animal-vegetation structure relationships across habitats, ecosystems and spatial scales. There is thus a clear need for objective methods that provide balanced measurements on variables including both the horizontal and vertical distribution of vegetation elements and the one-value characteristics that succinctly summarize important aspects of vegetation structure.

Reptiles are a group of vertebrates facing significant threats, including habitat loss and degradation, introduced invasive species, environmental pollution, diseases, unsustainable use of natural/seminatural habitats and climate change (Gibbons *et al.*, 2000). By now, one out of five reptile species has become threatened by global extinction, and local extinctions are becoming more common (Cox *et al.*, 2022). To cope with this, conservation priorities should be determined and actions should be implemented to reduce the rate of extinction (Böhm *et al.*, 2013). In Europe, the meadow and steppe vipers (*Vipera ursinii* complex) are among the most threatened reptiles. Lowland populations of this complex (*V. renardi*, *V. u. rakosiensis*, *V. u. moldavica*) lost almost all their habitats due to transformation of grasslands to croplands, and populations in Austria, Bulgaria and Moldova have gone extinct (Krecsák *et al.*, 2003; Mizsei, Zinenko, *et al.*, 2018; Tupikov & Zinenko, 2015). Alpine populations are threatened by overgrazing and climate

change (Mizsei, Szabolcs, *et al.*, 2020). Although habitat restoration and focused habitat management are increasingly used in reptile conservation (e.g. Michael *et al.*, 2018; Péchy *et al.*, 2015; Triska *et al.*, 2016), little is known on the efficiency of these actions due to lack of knowledge on vegetation characteristics preferred by target reptiles or due to lack of proper monitoring (Block *et al.*, 2001; Jellinek *et al.*, 2014; Mizsei, Fejes, *et al.*, 2020; Ruiz-Jaen & Aide, 2005).

Here we evaluate microhabitat selection of grassland specialist *Vipera* snakes by comparing vegetation structure between viper occurrence points ('use' localities) and random points ('availability' localities) in three grassland ecosystems where species of the threatened *V. ursinii* complex of meadow vipers still exist. We studied *V. graeca* in mountain meadows in Albania, *V. renardi* in loess steppes in Ukraine and *V. ursinii* in sand grasslands in Hungary. We applied a rarely used but promising approach to explore animal-vegetation relationships that can objectively describe vegetation structure based on photographs taken under standardized field conditions and analysed by automated, computer-based quantification of relevant structural attributes of the vegetation. We demonstrate the applicability of this approach to address three questions: (1) Which variables describing vegetation structure explain the occurrence of a particular snake species? (2) Are differences between influential vegetation structure variables related to differences in habitat selection between species? (3) Which variables can be recommended for use in studies of snake habitat selection in grasslands? While the standardized photographic approach based on images taken of the vegetation against a vegetation profile board, has been used before (Zehm *et al.*, 2002), to the best of our knowledge, it has not yet been used to study reptiles. We show that the variables derived from this methodology explain a significant part of the variation in snake occurrence and that the approach can thus be an important part of the repertoire of methods to characterize vegetation structure in studies of animal habitat selection.

Materials and methods

Study species

We studied snake-vegetation relationships in habitats of three species representing the three main phylogenetic lineages of the *V. ursinii-renardi* complex (Freitas *et al.*, 2020). *V. graeca* (Nilson & Andrén, 1988) is an endangered species endemic to the Pindos mountains in Albania and Greece, inhabiting dry sub-alpine grasslands above the treeline between 1600 and 2200 m above sea level (Mizsei, Szabolcs, *et al.*, 2018). *V. renardi* (Christoph, 1861) is widely distributed in the steppe biome from Ukraine to China, from lowlands to high mountains and shows high intraspecific genetic diversity (Zinenko *et al.*, 2015). The nominal subspecies *V. r. renardi* sometimes referred to as 'western' or 'lowland' *V. renardi* (Nilson & Andrén, 2001; Zinenko *et al.*, 2015) was involved in our study. *V. ursinii* (Bonaparte, 1835) is a vulnerable species endemic to Europe, consisting of three phylogenetically divergent lineages in sub-alpine meadows and two in lowland grasslands (Ferchoud *et al.*, 2012; Mizsei *et al.*, 2017). *V. ursinii rakosiensis*

(Méhely, 1893), also known as the Hungarian meadow viper, an endangered lowland subspecies (Péchy *et al.*, 2015) was involved in our study. According to the latest phylogenetic studies, the most recent common ancestor of the complex has a Pliocene origin from the Paratethys region, and the major lineages presumably radiated in the Anatolian-Transcaucasian region (*V. renardi*) and on the Balkan Peninsula (*V. ursinii*), while a basal lineage survived in the south Balkans (*V. graeca*) (Ferchaud *et al.*, 2012; Freitas *et al.*, 2020; Mizsei *et al.*, 2017; Zinenko *et al.*, 2015).

Field data collection

Sampling sites were selected in typical viper habitats in Albania (*V. graeca*: Kulmak and Tomorr mountains), Hungary (*V. ursinii*: Peszéradacs meadows) and Ukraine (*V. renardi*: Davydivka steppe) (Fig. 1, Table 1). We selected sites with stable site occupancy and minimal recent disturbances in vegetation structure to minimize potential biases due to seasonal variation. Exact locations are not given due to conservation reasons but are available upon reasonable request from the corresponding author. We searched for vipers by walking slowly in the designated habitat patches in weather conditions suitable for viper activity. When observing a viper, we recorded GPS coordinates, and the time of observation of the individual and the surveyors took photographs of the individual and the observation spot that assisted in future localization of the site. Spatial data on the fine-scale location of recorded vipers (hereinafter: points of known viper presence) were collected for several days at each site (*V. graeca*: 31 July to 12 August 2019; *V. renardi*: 25 September to 2 October 2019; *V. ursinii*: 10 to 15 September 2019 and 19 April to 18 May 2020). The number of days spent with field data collection was adjusted to the density of snakes estimated in previous site visits (Table 1). At the end of each search period, we recorded vegetation structure at all the points of known viper presence. To draw a distinction between the parts of habitats actually used by vipers and the surrounding parts not used by vipers, a minimum convex polygon (MCP) covering the points of known viper presence with a 10 m buffer was drawn. Inside the MCP randomly selected points were placed, at least 10 m far from the closest point of known viper presence (hereinafter: random points) using the Random points in polygons function of QGIS 2.18. The randomly selected points were not in the analyses in case of being placed in areas inaccessible to the surveyor or apparently placed at sites unsuitable for snakes such as rocky cliffs, water bodies or arable fields. The initial number of random points was set at five times the number of presence locations. Effort-corrected density is reported based on the number of observed vipers divided by the multiplier of days spent searching and the number of surveyors.

Quantifying vegetation structure

The idea of using a vegetation profile board to study the density of vegetation can be traced back to Wight (1939) and MacArthur and MacArthur (1961). To our knowledge, Thomson (1975) was the first to take photographs of vegetation

taken against a vegetation profile board for on-screen counting of vegetation elements in cells of a grid laid manually on the projector screen. This technique was deemed tedious and costly and was later developed by Nudds (1977), who quantified vegetation profiles measured as density scores (values from 1 to 5) in 0.5-m strata on a profile board made of plywood and with a surface width of 30.48 cm and height of 2.50 m. After digital photography became widely available, the technique has been standardized (Zehm *et al.*, 2002) and mainly used in studies of vegetation (often extended as ‘parallel photographic method’, e.g. Delai *et al.*, 2018) and arthropods (e.g. Correa & da Silva, 2022) but, to our knowledge, has not yet been used in studies of reptiles.

Vegetation structure was recorded in the field by taking photographs of the vegetation against a whiteboard applying standardized settings. The whiteboard was made of plexiglass of size 0.25 m (width) × 1 m (height), installed in a vertical position on its shorter edge at all viper presence and random locations. The vegetation against the whiteboard was photographed with a Nikon D600 digital SLR camera equipped with a Nikon 55 mm f/2.8 AF MICRO-NIKKOR lens, using spot exposure metering, with an EV + 1.0 exposure compensation. We captured high-quality JPEG images with the camera, and these photographs were taken with a fixed camera height of 0.5 m at a distance of 4 m from the whiteboard (Fig. 2) as in the case of Robel pole use in Volesky *et al.* (1999). We consistently directed the whiteboard towards the sun to maximize brightness and standardize lighting conditions.

The resulting photographs were pre-processed (cropping, white adjustment, retouching) with the GIMP 2.8.18. image editing software. Next, we applied image processing using an automated *for loop* written in the R statistical environment (version 3.6.1., R Core Team, 2019). The script first retrieved the images by the ‘load.image’ function of the ‘imager’ package (Barthelme, 2019), converted them to a black and white image by the ‘grayscale’ function, and then to a binary (0–1) image by the ‘threshold’ function of the ‘imager’ package. The resulting image was converted to a data frame using the ‘as.data.frame’ function and the coordinates of every image pixel covering the whiteboard were calculated (0.25 × 1 m, average resolution: 1–1.5 megapixels image⁻¹). The resulting data frame had three columns for each image, the x and y pixel coordinates (in cm) and the pixel value (0 = white, 1 = black).

We used the data frame to calculate four variables to quantify particular attributes of the vegetation structure. First, leaf area, referred to as LA hereafter, a frequently used quantity in vegetation characterization (Volesky *et al.*, 1999), was calculated as the count of black pixels rescaled to cm² units. Second, we calculated visual obstruction readings (VOR), developed primarily for prairie vegetation based on the Robel pole method (Benkobi *et al.*, 2000; Vermeire & Gillen, 2001). This method takes two readings by eye at a height of 1 m from a distance of 4 m from the pole with height tick-marks: (i) the height at which the pole is first visible, that is, not obstructed by vegetation (low reading) and (ii) the maximum height reached by the vegetation (high reading). The average of the two readings strongly correlates with prairie phytomass (Benkobi *et al.*, 2000; Vermeire & Gillen, 2001). In our study,

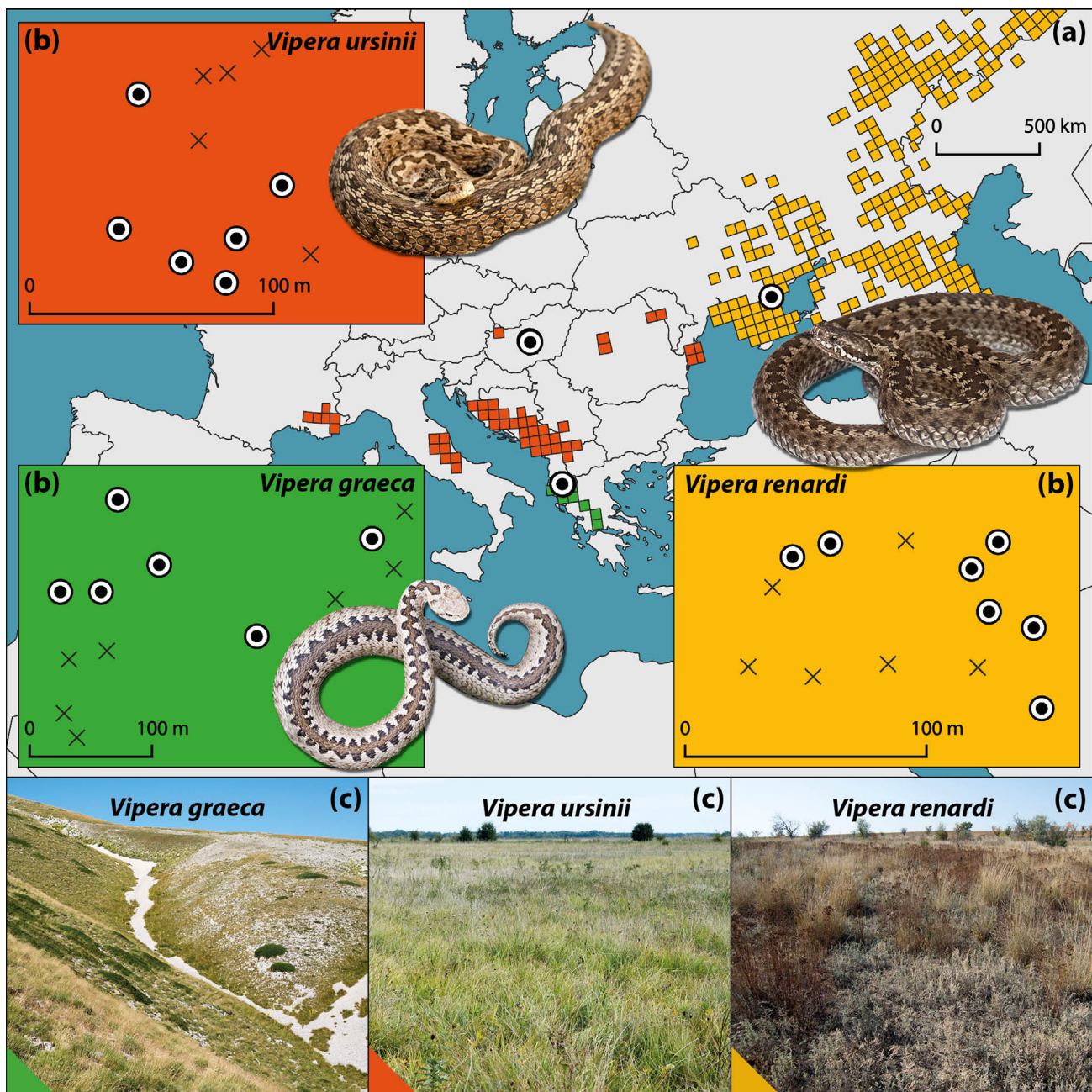


Figure 1 (a) Distribution of the study species in Europe according to Mizsei, Zinenko, et al. (2018) and the location of study sites (dots), (b) example insets for presence (dots) and random (crosses) points at the study sites, (c) grasslands at the study sites.

we modified the lower reading by calculating the maximum height at which 95% of the whiteboard is mantled by the vegetation, and we refer to this as the height of closed vegetation (HCV) (Fig. 2) to avoid confusion with the VOR reading terminology. We chose 95% as a threshold because glinting on some leaves in the image could return white pixels and could thus reduce the true coverage. Third, the high reading was calculated as the maximum height of the vegetation (MHV) regardless of its width, cover or surface area (Fig. 2). Finally,

to characterize the vertical distribution of vegetation, we calculated foliage height diversity (FHD) (Karr & Roth, 1971) as the Shannon diversity of the number of black cells in each pixel row using the 'diversity' function of the 'vegan' package (Oksanen et al., 2019). Calculating FHD from values in each pixel row circumvents the problem of arbitrarily choosing counting intervals, for example, ten 10-cm height intervals in each of which cover is estimated or measured for the calculation of FHD (Karr & Roth, 1971; MacArthur et al., 1962).

Table 1 Number of snakes found (presence locations), search effort, density of snakes and number of random locations studied in the three species

Variable	<i>V. graeca</i>	<i>V. renardi</i>	<i>V. ursinii</i>
Number of presence locations	36	73	32
Search effort (person-days)	125	22	48
Effort-corrected density (individuals/person-day)	0.288	3.318	0.667
Number of random locations	157	249	320
% of random locations in inaccessible areas	47%	17%	0%

Other variables

We applied further variables that may influence the occurrence of snakes at each site. In *V. graeca* habitats, we estimated the cover in proportions of bare rock, grass and shrub surfaces in a circle of 1-m radius around points of viper presence and random points. Visual estimates were recorded by three surveyors independently and mean values were used for each point. In *V. renardi* habitats, we recorded the number of rodent burrows in a circle of 1-m radius around points of viper presence and random points as these snakes often use rodent burrows for hiding. In *V. ursinii* habitats, there were no rocks or shrubs, and rodent burrows were rare and not recorded.

Statistical analyses

We analysed whether and how vegetation structure and other characteristics of grassland habitats affect the fine-scale occurrence of snakes by building generalized linear mixed models

(GLMM) separately for each study species. In GLMMs, presence/absence of vipers was incorporated as a binary dependent variable, while the four variables characterizing vegetation structure were applied as fixed explanatory variables, with grass/rock/shrub surface cover in *V. graeca* GLMMs and number of burrows in *V. renardi* GLMMs as additional fixed variables. The sampling site was incorporated in the GLMMs as a random factor to control for the spatial non-independence of the observations. We fitted the GLMMs specifying binomial error distribution using the ‘lme4’ package (Bates *et al.*, 2014). We then used an information-theoretic framework and a model selection approach (Burnham & Anderson, 2002) to run all possible combinations of fixed effects to identify models with substantial empirical support based on Akaike differences ($\Delta_i = \text{AIC}_i - \text{AIC}_{\text{min}} < 2.0$) and to perform model averaging based on the relative importance of explanatory variables using the ‘MuMIn’ package in R (Bartoń, 2018).

Results

The number of snakes found (presence locations) varied from 32 to 73 across the three species. *V. graeca* and *V. ursinii* were much rarer locally than *V. renardi*, and the search effort-corrected density was an order of magnitude higher in *V. renardi* than in the other two species (Table 1). Vegetation structure was recorded by white-board photography in a total of 141 presence locations and 726 random locations. Almost half of the pre-randomized locations in the alpine habitats of *V. graeca* were in inaccessible cliffs and 17% of pre-randomized locations fell on roads or water bodies in *V. renardi* habitats (Table 1).

Most of the vegetation structure variables followed a normal distribution, except for MHV in *V. ursinii* habitats, where

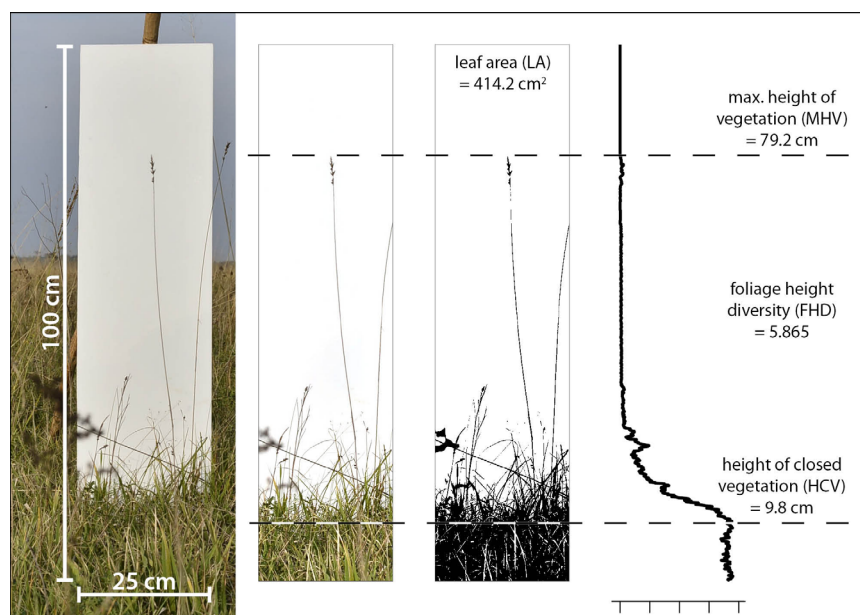


Figure 2 Main steps of processing the whiteboard images and example of the vegetation structure variables.

vegetation was at some sampling points taller than 1 m, that is, the height of the whiteboard (Figs 1c and 3). The correlations between vegetation structure variables were usually not significant, except between LA and HCV in *V. graeca* and in *V. ursinii* habitats and when data were pooled across species, and also between LA and FHD in *V. graeca* habitats (Fig. 3). We observed the highest values of all vegetation structure variables at the *V. ursinii* sites, and experienced the lowest values at *V. graeca* localities, while the *V. renardi* habitat showed the lowest HCV values among the sampled localities (Fig. 3).

In *V. graeca*, the full GLMM returned no significant main effect, whereas HCV was included in all and shrub cover was included in five of the six best models ($\Delta AICc < 2$). The averaged parameter estimate was significant and positive only for HCV (Table 2), indicating a higher chance of occurrence of *V. graeca* in taller and closed vegetation.

In *V. renardi*, LA and the availability of burrows had significant explanatory power in the full model and LA, FHD and

availability of burrows were included in both best models ($\Delta AICc < 2$). The effects of LA and availability of burrows were significantly positive, whereas that of FHD was negative (Table 2), indicating higher chances of *V. renardi* occurrence in microhabitats with more homogeneous cover and more burrows.

In *V. ursinii*, the full model had the lowest AICc value, and in the two best models ($\Delta AICc < 2$), LA and MHV had significant positive effects, whereas HCV had a significant negative effect (Table 2), indicating higher chances of *V. ursinii* occurrence in tall, high-cover but more open vegetation.

Discussion

Our study provided key results in the development of field data collection and data processing methodology for quantifying the role of vegetation structure in studies of animal microhabitat selection and in understanding how vegetation affects

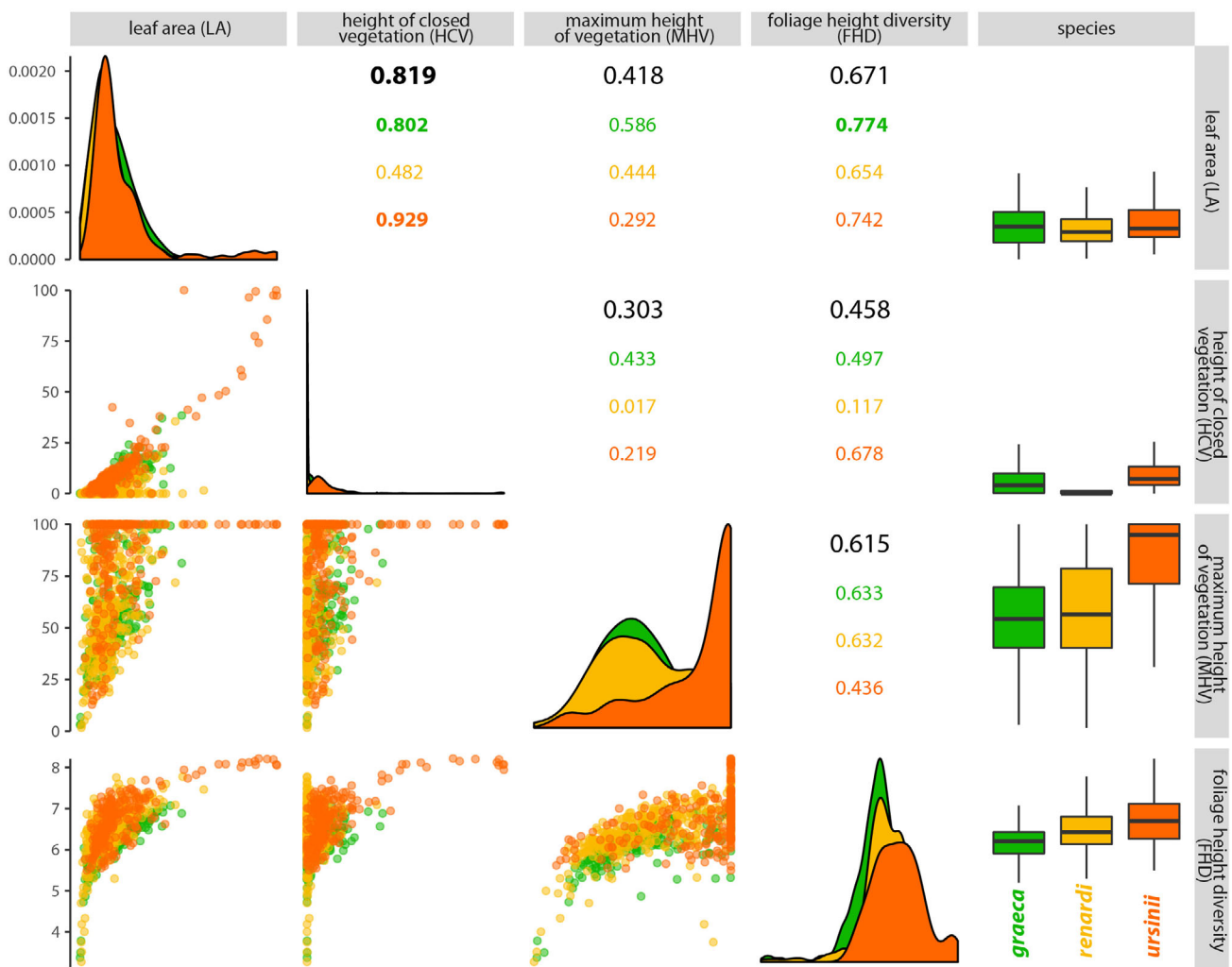


Figure 3 Vegetation structure variables: distributions (histograms in diagonal), correlations (lower left panels), Pearson’s correlation coefficients (upper right panels) and boxplots (right-hand column) of vegetation structure variables by species. Correlation coefficients in black are for data pooled for the three species, and coefficients in boldface type indicate significant correlations ($P < 0.05$).

Table 2 Model selection of generalized linear mixed models testing the effects of vegetation structure and other relevant variables on presence–absence of vipers in grasslands and averaged parameter estimates of the best models ($\Delta < 2.0$)

Species	Model	d.f.	R^2	logLik	AICc	Δ	Term	Estimate	SE	Z	P
<i>Vipera</i>	HCV + shrub cov.	4	0.17	−74.44	157.09	0	HCV	0.073	0.0297	2.4444	0.0145
<i>graeca</i>	HCV + shrub cov. + grass cov.	5	0.18	−73.68	157.68	0.59	shrubs cov.	0.0889	0.1491	0.5944	0.5522
	HCV + shrub cov. + stone cov.	5	0.18	−73.79	157.89	0.8	grass cov.	0.0819	0.1996	0.409	0.6826
	HCV + grass cov. + stone cov.	5	0.18	−74.02	158.36	1.26	stone cov.	0.0582	0.2048	0.2832	0.777
	HCV + grass cov. + shrub cov. + stone cov.	6	0.19	−73.01	158.48	1.38	MHV	−0.0044	0.0108	0.4008	0.6886
	HCV + MHV + shrub cov. + stone cov.	5	0.17	−74.36	159.04	1.94					
<i>Vipera</i>	LA + FHD + burrow av.	5	0.1	−155.02	320.24	0	LA	0.0022	0.0007	2.9799	0.0029
<i>renardi</i>	LA + MHV + FHD + burrow av.	6	0.1	−154.92	322.1	1.86	FHD	−0.4424	0.2393	1.8417	0.0655
							MHV	−0.0036	0.0078	0.458	0.647
							burrow av.	0.1846	0.078	2.3581	0.0184
<i>Vipera</i>	LA + HCV + MHV + FHD	6	0.05	−98.43	209.11	0	LA	0.003	0.0013	2.2664	0.0234
<i>ursinii</i>	LA + HCV + MHV	5	0.04	−99.83	209.84	0.73	MHV	0.0162	0.0081	1.9872	0.0469
							HCV	−0.0743	0.0332	2.2298	0.0258
							FHD	−0.7795	0.4734	1.6409	0.1008

Note: Significant parameter estimates are highlighted in bold letters.

the occurrence of the three snake species in grasslands. Our results demonstrate that variables relevant in describing vegetation structure can be derived from the automated processing of images taken by standardized whiteboard photographs. In addition, at least one of the variables so derived significantly related to the occurrence of snakes in three species in three widely differing grassland ecosystems.

Our method decreases subjectivity in quantifying vegetation structure as it returns exact cover values along with the vertical range in pixel rows and does not rely on estimates by eye, and minimizes observer bias and measurement error. Moreover, it does not require arbitrarily delimited measurement classes to characterize vertical variation in structure. Our method considers image pixels as the unit of analysis, however, larger units can be specified (e.g. 4, 8 or 16 image pixels) allowing analyses at different resolutions (upscaling) and reducing computing time. Repeating the analyses at different unit sizes can provide further insight as it offers the possibility of studying scale-dependence in habitat selection, that is, the identification of the environmental grain size at which animal-vegetation relationships are the strongest (Gunton et al., 2014; Lengyel et al., 2014). The objectivity of the method designed to measure features of vegetation structure near to ground also allows comparisons made in two or more species or ecosystems and also allows local measurements to be extrapolated to larger areas, habitat types or ecosystems.

Our results support the role of vegetation structure in microhabitat selection of snakes. While compositional habitat diversity (plant species composition) and estimated cover of vegetation have been reported to influence the occurrence of reptiles (Nemes et al., 2006; Stumpel & van der Werf, 2012), our study confirmed that vertical aspects of vegetation structure can also be important in the habitat selection of reptiles (Mizsei, Fejes, et al., 2020). For all three viper species studied, HCV or LA were among the most

important variables, indicating that vipers chose microhabitats where the vertical cover of the grass was higher than average, as measured in random locations. In the case of *V. ursinii*, a previous study (Máté & Vidéki, 2007) did not find a relationship between snake occurrence and plant species composition at the same study grassland. Our study thus also exemplifies that considering the structural aspects of vegetation can provide additional explanatory power in predicting the occurrence of snakes in microhabitats.

The role of vegetation structure in the microhabitat selection of snakes may involve a trade-off between the need to hide from predators and the need to thermoregulate (Hansen et al., 2018; Wilgers & Home, 2007), which may be preferred in lower or sparser vegetation (Muri et al., 2015). This trade-off hypothesis is a plausible explanation of the opposite effects of HCV on the occurrence of *V. graeca* (positive) and *V. ursinii* (negative), it is important to recognize that different species may have varying optimum values along the continuum of vegetation height. Considering the negative relationship in the latter species, it has to be noted that tall wetland plants were common in *V. ursinii* habitats, representing more dense cover and shading, which probably reduced the possibility of sunbathing for the vipers (Muri et al., 2015), which can explain why vipers appeared to avoid microhabitats with tall and dense vegetation. Furthermore, prey availability and the quality of the prey community can vary across both time and space, potentially exerting additional influence on microhabitat preferences (Mizsei et al., 2019). Further experimental studies are needed to investigate this trade-off and the potential influence of prey availability on microhabitat selection in snakes. Such knowledge will be fundamental for habitat restoration and conservation management actions for snakes.

Two limitations of this study need to be mentioned for the correct interpretation of our results. First, our study was limited

by the small number of presence locations for *V. ursinii* and the high spatial scatter of *V. graeca* presence locations in the study area. Unfortunately, these rare endangered species have low detectability due to their low abundance, hidden lifestyle and camouflaged body pattern. Data collection requires huge sampling effort that represents significant challenges in logistics and person-power. A potential source of error common in such studies is the assumption of absence in random locations where the species is not found because absence cannot be deduced without uncertainty as the individuals of the study species may actually live at particular random locations (Olivier & Wotherspoon, 2006; Philips *et al.*, 2009). In our study, the possibility of this error was high in *V. renardi*, which species showed extreme abundance in the study habitat and several new presence locations were found during sampling 'random' locations, thus we thoroughly searched the sites before taking the pictures. Another practical limitation of our method is that placing the whiteboard on uneven ground or very dense vegetation may result in changes in the vegetation next to the whiteboard, which may distort the value of vegetation structure variables. To avoid this problem, we recommend a careful trimming of the vegetation in the plane and on the backside of the board to make sure that the board is standing firmly on the ground. Vegetation structure can show dynamic changes during the active season of vipers, and we acknowledge the potential for temporal variation. However, to mitigate this, we selected study sites with consistent site occupancy and minimal recent disturbances in vegetation structure, such as overgrazing, mowing or burning. We recommend to implement vegetation structure sampling in shorter period of time or repeatedly to avoid potential statistical and interpretation issues raised by temporal pattern mixing with spatial pattern.

In conclusion, the supplementation of standardized whiteboard photography with automated image processing allows the calculation of simple measures of vegetation structure that can provide additional insight into animal-vegetation relationships beyond the role of plant species composition. The combined use of field recording and image processing offers several options that broaden the range of cost-effective ecological survey methods and can make a substantial contribution to the design and implementation of evidence-based conservation, including the conservation of endangered, grassland specialist vipers.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

E.M., M.B., G.R., CV., G.D. and S.L. conceived the ideas and designed methodology; E.M., M.B., G.R., B.B., D.R., M.S. and A.M. collected data; E.M. programmed image processing and analysed the data, E.M. and S.L. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data availability statement

This study was conducted via the implementation of an R script consisting automated image processing to measure leaf area (LA), height of closed vegetation (HCV), maximum height of vegetation (MHV) and foliage height diversity (FHD). The R script and data are available at Zenodo (<https://doi.org/10.5281/zenodo.7644154>).

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