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Biodiversity collision blackspots in Poland: Separation causality from stochasticity in roadkills of butterflies

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ABSTRACT

Collisions with cars are an important mortality factor for many wild animals. Measures to mitigate road mortality are costly so should be implemented using cost-effective measures in locations where the road mortality is consistently highest and non-random in different species. It is thus important to identify what features causes these biodiversity collision blackspots. Almost all of the data and literature on collisions refer to vertebrates with little known about invertebrates. We used data on butterfly roadkills in three large landscape plots in Poland to identify sites where the collision rate seems to be routinely high. Biodiversity collision blackspots were identified from occurrence in successive years using spatial hierarchical clustering. Biodiversity collision blackspots comprised just 4% of the total road length, but included 49% of all road-killed butterflies. Habitats within 500 m of each blackspot was compared to random non-blackspot sites using generalized linear mixed models. The occurrence of blackspots was linked with high traffic volume, but only when cover of grassland in a landscape was high and verges had low plant species richness. Similarly, blackspots occurred with high probability when traffic volume was high but especially if grassland cover in the landscape and verge mowing frequency were also high. These blackspots had higher species richness and abundance of butterflies in the surrounding landscape than in random sites. Biodiversity collision blackspots analysis identified road sections of high road mortality for different butterfly species. Moreover, blackspots were also indication of species rich areas of conservation concern that were intersected by roads. Thus, conservation practitioners may direct mitigation measures, such as less frequent mowing and speed limit, in a cost-efficient manner in these spatially-limited locations.

1. Introduction

The increasing and more affluent human population linked to the development of the automotive industry has resulted in both more and wider roads (Selva et al., 2011), while technological change has resulted in faster traffic. Roads are known to be a cause of disturbance for some natural populations. Roads also lead to habitat fragmentation by dividing continuous habitat into separate blocks and impeding both the movement of individuals and gene flow (Forman and Alexander, 1998; Trombulak and Frissell, 2000; Forman et al., 2003; Tanner and Perry, 2007; Jackson and Fahrig, 2011). The presence of roads can change the soil, microclimatic conditions, and pollutant levels (Forman et al., 2003; Moroń et al., 2012). For example, increased nitrate levels may affect both plant and animal populations (Port and Thompson, 1980). The most obvious and direct impacts of roads is probably through mortality linked with vehicle collisions (Malo et al., 2004; Seiler, 2005; Rytwinski and Fahrig, 2012; Cosentino et al., 2014).

Road mortality may be considered as an example of a point process, which is a type of random incident for which any one realisation takes a set of isolated points either in time or geographical area (Diggle, 2003; McDonald, 2013). However, individual incidents may generate a non random spatial pattern of incident densities when these incidents result from some, say environmental, factors (Diggle, 2003; Daley and Vere-Jones, 2008). Road mortality is known to have a considerable impact on the local population viability of many vertebrates, especially amphibians and mammals (Hels and Buchwald, 2001; Falcucci et al., 2009; Rytwinski and Fahrig, 2012; Silva et al., 2012; Teixeira et al., 2013). Little is known

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about its cause, pattern or impacts in insects, despite the fact that insects are among the most commonly recorded roadkills (Rao and Girish, 2007; Ranea et al., 2008; Soluk et al., 2011).

Several measures for alleviating road mortality of other taxa have been proposed, and have been implemented at local or landscape scales, including fencing, speed limits, tunnels, road signs, bridges and carrying individuals across the road (Van Langevelde et al., 2009; Ascensão et al., 2013; Smith and Sutherland, 2014). Road verges have become an important surrogate of semi-natural habitat in modified landscapes (Ruiz-Capillas et al., 2013). In butterflies or bumblebees, road mortality may be mitigated by widening road verges, sowing some flowering plants, less frequent mowing and retaining more grassland in the landscape (Munguira and Thomas, 1992; Ries et al., 2001; Skórka et al., 2013). However, such mitigation actions may be costly and too expensive to implement along all road sections (Beaudry et al., 2008; Litvaitis and Tash, 2008; Polak et al., 2014).

The cost and inconvenience of implementing measures to reduce road mortality implies that they should be positioned at high concentrations of incidents of different species within a limited geographical area, referred to as road mortality blackspot or hot spots (Gomes et al., 2008; Litvaitis and Tash, 2008; Cureton and Deaton, 2012; Iosif, 2012); here we use the term "biodiversity collision blackspots", focusing on multispecies collision incidents. Identifying biodiversity collision blackspots and subsequent comparison of these blackspots with non-blackspot sites is an important exercise since it allows the separation of areas where mortality is linked with specific features of a road or/and landscape (causality) from the areas where mortality is simply accidental with clusters due to stochastic processes during the sampling period. Implementing mitigation measures in the latter areas may be a waste of resources, but most of studies do not recognize this dual nature of road mortality.

Identifying biodiversity collision blackspots requires knowledge of the number and spatial locations of roadkills; identifying these sites is usually based on arbitrary criteria, and is often synonymous with the sites where collision occurred (Litvaitis and Tash, 2008), or it is made by personal judgement, which is likely to be highly subjective. Several statistical methods allow the identification of blackspots basing on objective, statistical criteria (Anderson, 2009). One such method is nearest neighbour spatial hierarchical clustering (Anderson, 2009), which compares spatial location of incidents with a random distribution of points across a landscape. Moreover, there are several interpolation methods (Gattrell et al., 1996; Gomes et al., 2008) that may improve discrimination between biodiversity collision blackspots and areas with low rate of road mortality. However, they are rarely used in published studies on road mortality of animals (Ramp et al., 2006; Gomes et al., 2008).

Having identified the location and number of biodiversity collision blackspots, one may predict sites where number of roadkills of different species would be the largest by comparing local and landscape features of biodiversity collision blackspots and random sites with average or low rate of road mortality. The high road mortality may results from (1) high population sizes of different species living in the vicinity of roads and at road verges, (2) the specific structure of a road and traffic, (3) the landscape composition around the road and (4) interactions between these factors. However, it is unknown how biodiversity collision blackspots differ from road sections with average or low mortality rate in insects.

In this study, we used data on butterfly roadkills in three large agricultural landscapes to predict the number and spatial locations of biodiversity collision blackspots by using the nearest-neighbour hierarchical clustering and spatial interpolation methods (Johnson, 1967; King, 1967; Everitt, 1974). Then, we compared species richness and abundance of butterflies as well as traffic volume, road features and landscape composition between biodiversity collision blackspots and randomly chosen sites along roads. We validated our findings by comparing the number of mortality incidents in blackspots and randomly chosen sites with independent counting of dead butterflies in a different year. We also investigated factors affecting number of roadkills in biodiversity collision blackspots and randomly chosen sites.

2. Methods

2.1. Study area

We conducted our study in three landscape plots in southern Poland (Fig. 1, Table 1, .kmz files in Supplementary Material). The plots represented three distinct agricultural landscapes; their characteristics are given in Table 1. Plot Krakow, was an agricultural landscape near large town, plot Proszowice was located in intensive farmland and plot Tarnow was located in less intensive farmland (with small fields and numerous abandoned fields). The

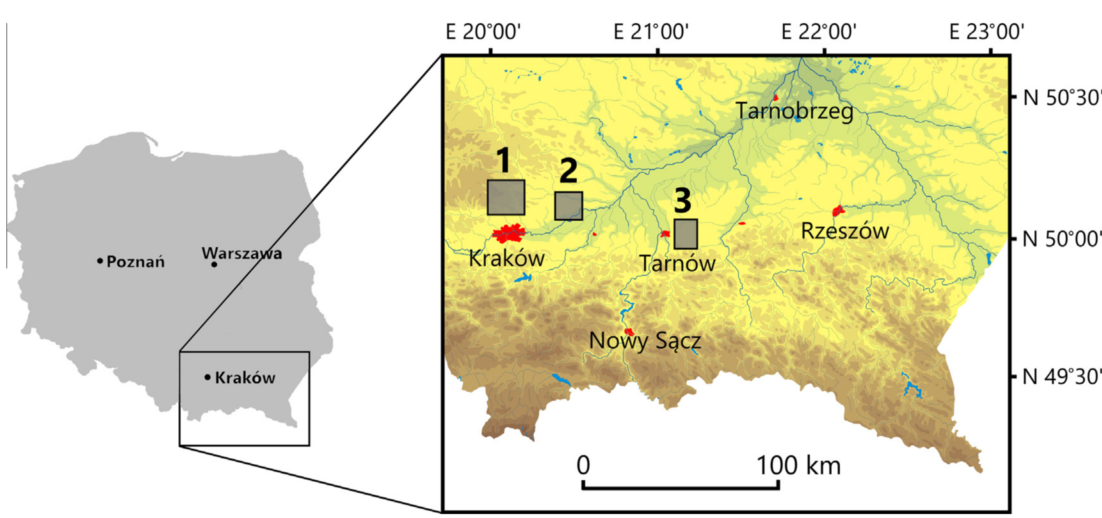

Fig. 1. Map of Poland and localization of the studied landscape plots. Explanations: 1 – plot Kraków, 2 – plot Proszowice, 3 – plot Tarnów. Colors in right panel represent altitude above sea level, from low (green) to high (brown) Larger towns are red patches, rivers and waterbodies are in blue. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Characteristics of the study landscape plots.

	Landscape 1 (Kraków)	Landscape 2 (Proszowice)	Landscape 3 (Tarnów)
Area (km^2)	189.9	114.1	130.8
Road network (km)	349.3	189.8	150.0
Number of roadkills	557	220	204
Number of roadkills $\times km^{-1}$	1.6	1.2	1.4
K-statistic (significance)[a]	0.66 (<0.001)	0.70 (0.006)	0.72 (0.011)
Number of estimated blackspots[b]	19	11	10

[a] K-statistic describes level of clustering in distribution of roadkills. The index values close to zero and one indicate perfect clustering and random distribution, respectively.
[b] Results from the spatial hierarchical clustering analysis.

dominant crops in these areas are wheat (20%), rye (10%) and various vegetables (15%). Grassland cover varied between 8% and 13% of the area, forests constitute a small part of the area (8–10%) and are typically highly fragmented mid-field woodlands. Villages are spread among the fields and cover about 10% of the area.

2.2. General scheme of the study

The study was divided into two elements. The first element was performed during 2010–2011 and aimed to collect records of roadkilled butterflies in the entire road network within three landscape plots in order to identify clusters of roadkills (biodiversity blackspots). Having identified blackspots we performed the second element of the study in 2012. This aimed to identify features of the blackspots and randomly chosen non-blackspot sites and to validate the identification of blackspots through independently counting dead butterflies in both types of site.

2.3. Sampling of roadkills along roads to calculate mortality blackspots

We sampled roadkilled butterflies in 2010 and 2011 along all roads in each landscape. Five surveys were completed each year between middle of June and middle of August. As the landscape plots were large, each survey took from two to six days to walk along all roads within the plot. Two or three teams of two observers each operated to collect roadkilled butterflies. Two persons from one team walked in parallel one on each side of the road at a constant pace of about 5 km per hour and collected all dead butterflies found both on the asphalt and on the 1-m wide part of the verge adjacent to the road. Each roadkilled butterfly was placed in a tube with 75% alcohol for further identification. Geographic location of each roadkilled individual was marked using GPSs (Garmin SX60).

2.4. Sampling features of biodiversity collision blackspots and random locations

Blackspots may differ from non blackspot sites in several features that can be related to road structure and its adjacent verge, landscape composition in the vicinity and local butterfly species richness and abundance. Once the biodiversity collision blackspots were established (based on data from 2010 and 2011; see below) in 2012 we measured several features of the road, landscape composition in its surrounding and species richness and abundance of (both alive and dead) butterflies both in the biodiversity collision blackspots and in an equal sample of randomly selected sites in road sections that were not identified as blackspots. We measured these features within a 500 m radius from the geographical centroid of the blackspot or random site. This distance was chosen because a circle of this radius comprised about two standard deviations of the area of most numerically calculated blackspots and it represents a maximum dispersal distance for the majority of butterfly species. Larger radius would have caused overlap of circles. The following environmental variables were measured in blackspot and control sites: (1) traffic volume, (2) width of the verge on each side of the road, (3) number of plant species on the verges, (4) mowing frequency index and (5) cover of grasslands in a landscape. We also measured other environmental variables: road width, forest cover, human settlement cover and road verge width. However, we did not include them in the analysis because they were highly correlated with other explanatory variables and preliminary analysis indicated their inclusion did not improve our understanding of the blackspot emergence. Namely, road width was strongly correlated with traffic volume ($r_s = 0.593$, $P < 0.001$), forest cover that was negatively correlated with grassland cover ($r_s = -0.407$, <0.001), the cover of human settlements was also negatively correlated with grassland cover ($r_s = -0.271$, $P = 0.015$), width of road verges was highly variable even within one transect (1–3 m). In our analyses we tested up to three-order interactions between variables. Enlarging the list of explanatory variables would exclude the possibility of effectively testing all the main effects along with their interaction terms, we therefore selected what were potentially the most important variables for the biology of butterflies living alongside roads.

We counted dead butterflies in blackspots and random sites to check independently whether they differed in the number of roadkills. Similarly, we counted alive butterflies on road verges and in the surrounding landscape in both types of the site. We used transects to count dead and living butterflies in blackspot and random sites at roads (for more details see: Skórka et al., 2013). In each of the blackspot and randomly selected non-blackspot site we established 200 m-long transects on the roads and in the surrounding landscape (Fig. S1 in Supplementary Material). Each transect comprised two parallel lines, one on either side of the road. Thus, the sampling unit used in our analyses was data from these two lines on either side of the road (400 m in total, Fig. S1). The geographical centre of the blackspot (or randomly chosen site) was always in the middle of each road transect. Transects in a surrounding landscape similarly comprised two lines, located on opposite sides of the road about 200 m from, and parallel to, the road line. Transects in a landscape sometimes crossed a mosaic of habitats (Fig. S1 in Supplementary Material).

When surveying roads, observers collected dead butterflies and placed them in 75% ethanol for subsequent identification. Butterflies were collected from asphalt and 1 m wide part of verge adjacent to the road. In 2012, immediately after collecting dead butterflies, the observer counted those living on the road verges within a 5 m distance from the road's edge, again on each side of the road. We counted the living butterflies after collecting the dead ones in order to not influence the number of roadkills collected during the observer's work at the road verge. The observer walked at a speed of approximately 100 m per 10 min while both collecting dead butterflies and counting living ones at transects in 2012. We made twelve surveys in each transect between the end of April and beginning of September 2012. Butterflies were usually counted every ten days, however this time interval varied depending on weather and was also adjusted to cover the peak of adult flight (Vessby et al., 2002; Heliölä and Kuussaari, 2005). Butterflies were surveyed between 9:00 and 16:00 (Central European Time Zone UTC/GMT + 1 h) during favorable weather conditions, defined as with temperatures of at least 16 °C, a wind of 3 or less on the Beaufort scale (<4 m $\times$ s^{-1}), and cloud cover not exceeding 25%.

To measure traffic volume, we made four, one-hour-long counts of all the passing vehicles in the middle of each road transect. The vehicle counts were made between 11:00 and 17:00. Start times were selected randomly from the time interval 11:00 and 16:00.

Counts were done approximately every 2 weeks in both blackspot and random sites with dates close to butterfly surveys. To estimate plant species richness we selected ten square plots of 1 m^2, five on each side of the road (both on transects at roads and in the surrounding landscape). Within these plots, we counted all the wild plant species and measured their cover. The plant species were counted once during the season, between mid-June and mid-July.

Mowing frequency was recorded during each transect survey using the index proposed by Valtonen et al. (2006), which describes the total effect of mowing on the vegetation over the study period. The advantage of this index is that it allows researchers to cope with the frequently occurring situation of partially mown verges (Valtonen et al., 2006). It may be also used as a continuous variable in analyses. Each survey was given a mowing intensity value (0 = no mowing, 1/2 = partial mowing, 1 = total mowing) and the value was reduced to the lower level, namely from 1 to 1/2 and from 1/2 to 0, seven weeks after mowing, because the vegetation regenerated. We then summed up the values from each survey for a given transect and used the result for our analyses. As in Valtonen et al. (2006), the index for the unmown and partially mown verges was generally the lowest, that of the verges mown in the late summer was intermediate, and that of those mown during the mid-summer period was the highest. Grassland cover was read from aerial photos (available from: http://www.geoportal.gov.pl) digitalized in Quantum GIS 1.7 software and supported by GPS mapping in field.

2.5. Data handling and statistical analyses

2.5.1. Calculation of road blackspots

There are several different statistical techniques designed to identify 'blackspots' (Everitt, 1974). All of the techniques depend on optimizing various statistical criteria, but the techniques differ among themselves in their methodology, as well as in the criteria used for identification. Because 'blackspots' are perceptual constructs, any technique used must approximate how someone would perceive an area. We used nearest neighbour spatial hierarchical clustering technique (Johnson, 1967; King, 1967; Everitt, 1974). It calculates a distance matrix along the road network and compares the distance between pairs of points to the distance expected in a random distribution of points in the study area, and it clusters those groups of pairs that are unusually close together. The first-order clusters may then be grouped into higher order clusters until either all mortality incidents fall into a single cluster or else the grouping criteria fails. Thus, there is a hierarchy of clusters that can be displayed.

We used CrimeStat software (Levine, 2007) to calculate first-order clusters that were regarded as biodiversity collision blackspots. There were two second-order clusters in one landscape, but this sample size was small and they were not considered further (Fig. 2). We allowed for random search radius when seeking for clusters, which is the default and recommended setup of the software. The *K* clustering index (Smith and Bruce, 2008) was used to estimate the level of spatial clustering of mortality incidents in each landscape. This index may take values between 0 (perfect clustering) and 1 (random distribution along roads).

2.5.2. Interpolation technique

We also used a kernel density estimation, which is an appropriate technique for individual point locations (Silverman, 1986; Härdle, 1991; Bailey and Gatrell, 1995; Bowman and Azzalini, 1997). Kernel density estimation involves placing a symmetrical surface over each point, evaluating the distance from the point to a reference location based on a mathematical function, and summing the value of all the surfaces for that reference location. This procedure is repeated for all reference locations. We used CrimeStat (Levine, 2007) for calculation. The triangular method of interpolation (Burt and Barber, 1996) was chosen assuming that if a site at road is a biodiversity collision blackspot its effect would decrease linearly with the distance. We also used adaptive bandwidth (the width of the kernel), which adjusts the bandwidth interval such that it provides constant precision of the estimate over the entire region. Thus, the bandwidth is narrow in areas with a high concentration of points, whereas the bandwidth will be larger where the concentration of points is sparse. Because the density estimate for every point cannot be calculated (as there is an infinite number of points), we overlaid a grid on top of map with mortality incidents and calculated the density estimate for the centre point of each grid cell. Each landscape was divided into 500×500 grid cells. The resulted map is a risk surface map; each cell represent the probability of butterfly deadly collision.

2.5.3. Comparison of features of the biodiversity collision blackspots and randomly chosen sites

To test which features affect the occurrence of blackspots compared with randomly chosen sites we used generalized linear mixed models (GLMM) with binomial error variance and logit-link function. Blackspots were coded as 1 and randomly chosen reference sites were coded as 0; they were a dependent variable in the analysis. The independent explanatory variables were environmental features measured at the transects: traffic volume, mowing frequency, plant species richness on verges, grassland cover in a landscape and butterfly abundance in transects at road verges and in the surrounding landscape (the abundance was highly correlated with species richness and inclusion of the latter in the analysis did not changed results). We also introduced interactions (up to third order) between explanatory variables in first step but the non-significant ones were removed from the final model. Landscape plot identity was assigned as a random factor in GLMM. Depicting interaction terms is not straightforward in continuous variables. Here, to visualize third-order interactions between continuous variables they were divided into categories (e.g. low traffic vs large traffic) and presented in figures using the methodology of Smart et al. (2004). We also used hierarchical partitioning (Chevan and Sutherland, 1991), to calculate, for each explanatory variable separately, an estimate of the independent contribution to the blackspots' occurrence. Hierarchical partitioning involves measuring the increase in the goodness-of-fit of all models with a particular explanatory variable compared to the equivalent model without that variable. We specified log-likelihood as the goodness-of-fit measure of the model. We then calculated the percentage of the total independent contribution (summed across all variables) accounted for by each explanatory variable. Hierarchical partitioning was conducted using 'hier.part' package (Walsh and Mac Nally, 2005), implemented in R version 3.1.2 (R Development Core Team 2014).

We used a GLMM with negative binomial error variance and logarithmic link function to compare mean abundance and mean species richness of alive butterflies in transects at roads and in the surrounding landscape between biodiversity collision blackspots and random sites at roads. The same GLMM was used to check if the mean number of individuals and species found dead at transects on roads in 2012 differ between blackspots and randomly chosen non-blackspot sites. In both analyses, transect pair (transect at road and in the landscape) nested in landscape identity were assigned as random effects.

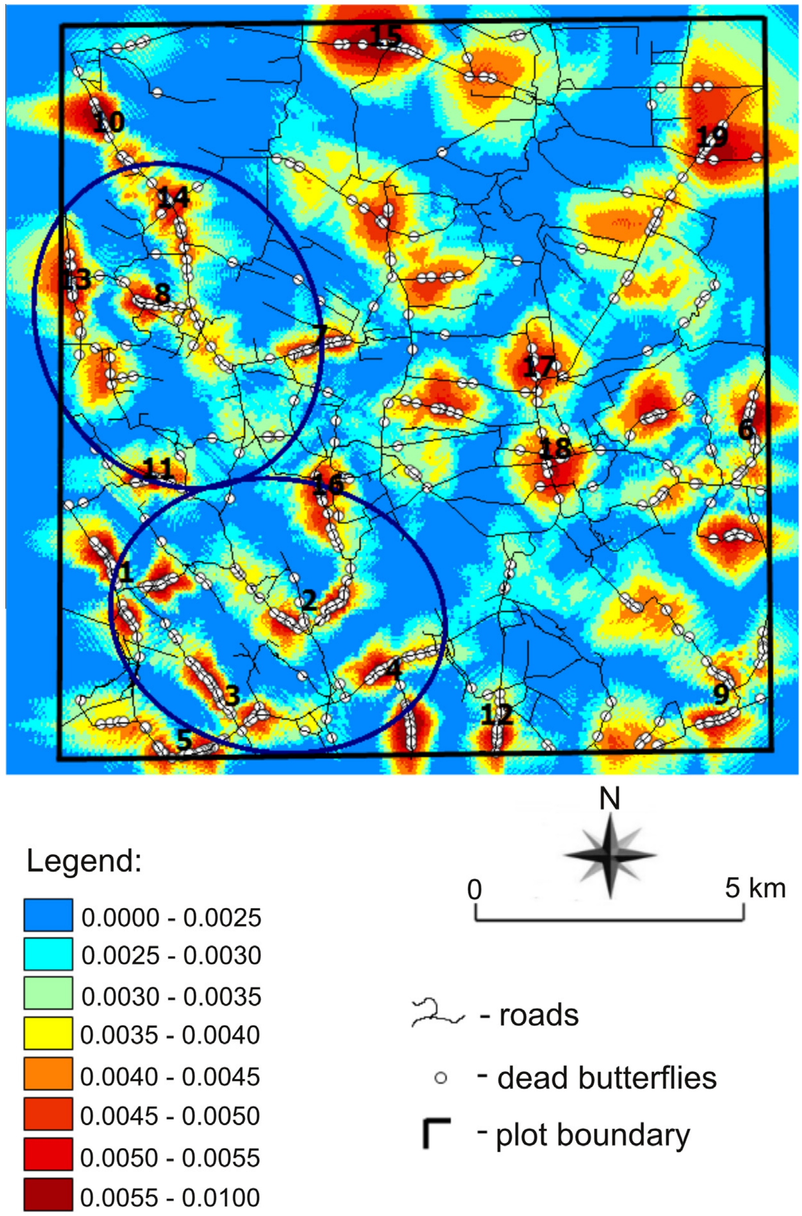

Fig. 2. Butterfly biodiversity blackspots (indicated by numbers) and risk surface interpolation map in the landscape 1 (plot Kraków). Risk surface map indicates the probability of a butterfly's deadly collision. Dark-blue ellipses indicate second-order blackspots. Maps of biodiversity blackspots in other two landscape plots are in Figs. S2 and S3 in Supplementary Material. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

We used a Spearman correlation to relate number of dead species and individuals found in the blackspots in 2010 and 2011 with the number of species and abundance of dead butterflies found there during transects counts in 2012.

Finally, we used GLMMs with negative binomial error and logarithmic link function to relate explanatory variables (the same as in the GLMM explaining blackspot occurrence) with the number of dead butterflies found on transects in both biodiversity blackspots and randomly selected non-blackspot sites in 2012. Models were built separately for biodiversity blackspots and random non-blackspot sites. In addition to these models we used hierarchical partitioning to estimate independent contribution of each explanatory variable to these relationships.

Every explanatory variable was standardized. R^2 in GLMMs was calculated following recommendations by Nakagawa and Schielzeth (2013). All analyses were performed in SPSS 19 (IBM corp.) and R version 3.1.2 (R Development Core Team 2014).

3. Results

3.1. Road mortality in biodiversity blackspots

In 2010 and 2011, we found 981 roadkilled butterflies belonging to 36 species (Table S1 in Supplementary Material). The most common roadkills were *Pieris rapae, Coenonympha pamphilus, Pieris brassicae* and *Erynnis tages* (Table S1 in SI). The mean density of roadkills was 1.37 ± 0.12 SE individuals per 1 km. The mortality incidents showed statistically significant level of spatial clustering in every landscape as indicated by the K-statistic (Table 1). The nearest neighbour spatial clustering indicated that there were 40 road blackspots in total in three landscape plots with probability equalling 0.95 (Table 1, Fig. 2, Figs. S2 and S3 in SI). These blackspots included only 4% of the total length of roads but 476 (49%) of all roadkilled butterflies. Mean number of roadkilled butterflies was 11.9 ± 0.93 SE in a blackspot (range: 6–29). In one landscape there were also two second-order spatial clusters indicating clusters of the blackspots (Fig. 2).

Kernel density interpolation suggested there is higher number of blackspots in all landscapes than the hierarchical clustering (Figs. 2, S2 and S3) but this number was variable depending on classification criteria, thus was considered only as supportive to the clustering method.

3.2. Factors affecting occurrence of biodiversity collision blackspots

GLMM showed that the occurrence of blackspots was affected by the main effects of environmental variables and by both second- and third-order interactions between investigated variables (Table 2). Among main effects the statistically significant effects included mowing frequency, grassland cover in a landscape and abundance of butterflies in a landscape (Table 2). The statistically significant second-order interactions were that between mowing frequency and traffic volume, and between the mowing frequency and grassland cover (Table 2). Two third order interactions affected the identification of blackspots. The first third-order interaction was between traffic volume, grassland cover and plant species richness at road verges (Table 2). The high road traffic had an impact on the occurrence of blackspots but only at roads with few plant species on verges and high cover of grasslands in the surrounding (Fig. 3). Similarly, the second significant third-order interaction term was between traffic volume, grassland cover and

Table 2
Final generalized linear mixed model ($R^2 = 0.51$) explaining the occurrence of biodiversity blackspots.

Effect	Estimate (SE)	*F*	df	*P*
Traffic volume	0.321 (0.287)	1.246	1, 69	0.268
Number of plant species	−0.084 (0.337)	0.061	1, 69	0.806
Mowing frequency	0.563 (0.264)	4.567	1, 68	0.038
Grassland cover in a landscape	0.643 (0.220)	8.695	1, 69	0.005
Abundance of butterflies at verges	0.042 (0.039)	1.127	1, 65	0.298
Abundance of butterflies in a landscape	0.604 (0.261)	5.330	1, 68	0.026
Traffic volume × mowing frequency	0.212 (0.090)	5.435	1, 69	0.023
Mowing frequency × grassland cover	0.327 (0.112)	8.569	1, 69	0.005
Traffic volume × mowing frequency × grassland cover	0.342 (0.115)	8.990	1, 68	0.004
Traffic volume × plant species × grassland cover	0.452 (0.180)	6.302	1, 69	0.014

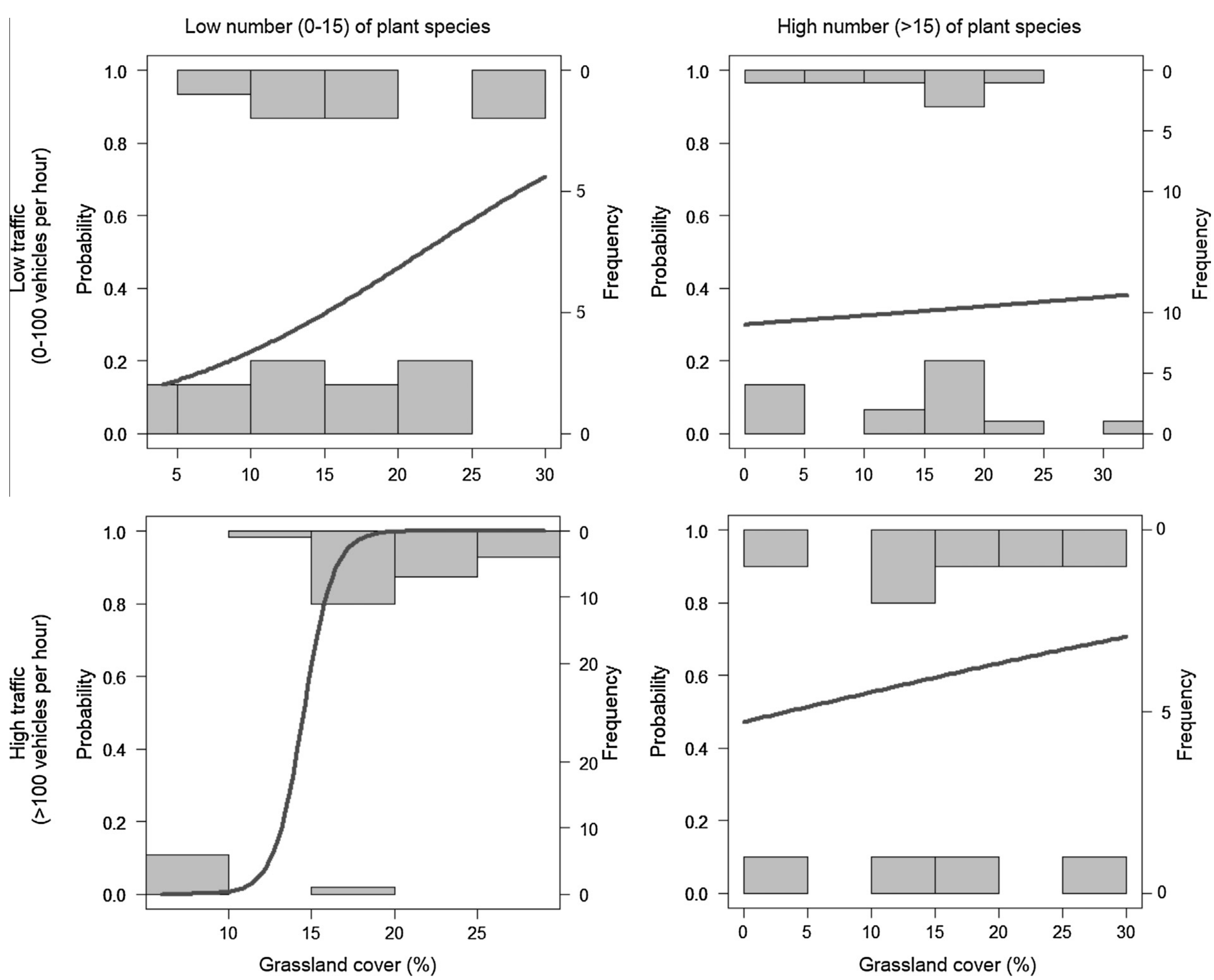

Fig. 3. The graphical illustration of the effect of third-order interaction between traffic volume, plant species richness on verges and grassland cover in a landscape on the occurrence of biodiversity blackspots in butterflies. Traffic volume was classified into two equally sized categories: low (<100 vehicles per hour) and high (>100 vehicles), according to number of passing cars. Similarly, the variable plant species richness was also divided into two categories: with low (0–15) and high (>15) number of plant species.

mowing frequency (Table 2). High mowing frequency of a verge at roads with high traffic volume and with low grassland cover in the landscape was a characteristic occurring in the blackspots. The random effect (landscape identity) was significant (estimate = 9.402, $Z = 7.55$, $P < 0.001$). Hierarchical partitioning analysis indicated that variables that had the highest independent contribution to the biodiversity blackspot emergence were cover of grassland in a landscape, abundance of butterflies in a landscape and mowing frequency (Fig. S4 in SI).

We did not find that blackspots differed from random sites in abundance of butterflies living on road verges (Table 2). However, the occurrence of road blackspots was positively affected by the abundance of butterflies in the surrounding landscape (Table 2). These results were supported by the separate GLMMs for the mean abundance (GLMM $F_{1,\ 76} = 1.878$, $P = 0.175$) and species richness of butterflies living at road verges (GLMM $F_{1,\ 75} = 2.648$, $P = 0.108$) indicating similarity between blackspots and in randomly chosen non-blackspot sites (Fig. 4). Separate GLMMs for abundance and species richness of butterflies living in a landscape indicated that the mean abundance (GLMM $F_{1,\ 78} = 12.017$, $P < 0.001$) and species richness (GLMM $F_{1,\ 78} = 24.731$, $P < 0.001$) were higher in blackspots than in randomly chosen non-blackspot sites (Fig. 4, Table S2 in Supplementary Material).

Validation of the estimated road mortality blackspots was supported by GLMM, where the mean number of roadkilled individuals (GLMM $F_{1,\ 78} = 87.237$, $P < 0.001$) and the number of roadkilled species (GLMM $F_{1,\ 78} = 90.682$, $P < 0.001$) were higher on road transects in the blackspots than in randomly chosen non-blackspots sites (Fig. 5, Table S2 in SI). We also found positive correlation between number of roadkilled species in the blackspots estimated from year 2010 and 2011 and number of roadkilled species found at road transects there in 2012 ($r_s = 0.405$, $P = 0.009$, $n = 40$).

When we analyzed factors affecting number of roadkilled butterflies in transects in biodiversity blackspots in 2012 we found that traffic volume, grassland cover and abundance of butterflies in a landscape all increased the number of roadkills (Table 3). Hierarchical partitioning analysis indicated that grassland cover and butterfly abundance in a landscape had the highest individual contribution to roadkill abundance in biodiversity blackspots (Fig. S5a in SI). In randomly chosen non-blackspot sites the abundance of roadkilled butterflies in transects in 2012 was positively correlated with abundance of butterflies living at verges (Table 3). This variable had also the highest individual contribution as indicated by the hierarchical partitioning analysis (Fig. S5b in SI).

4. Discussion

Our study showed that road mortality of diverse butterflies is common along roads in studied landscapes, however there are certain locations with exceptionally high number of car-induced mortalities. These locations clearly differ in several features from random areas located at roads.

Finding mortality clusters indicates that road mortality is partially a non stochastic process and that certain areas are more prone to road mortality than others. The estimated number of spatial clusters is relatively low; they embraced 4% of the whole road length, but almost half of all roadkilled butterflies. From the practical perspective, this result has considerable consequences because it allows identification of biodiversity blackspots and allows concentrating conservation efforts in geographically limited area and thus may greatly improve efficiency of mitigation actions.

Our results also allowed us to identify second-order clusters that embraced densely clustered first-order blackspots in one of the studied landscapes. Thus, the nearest neighbour spatial hierarchical clustering may be useful tool to find mortality-prone areas also at multiple spatial scales.

4.1. Features of biodiversity collision blackspots

Several features affected differences between blackspots and areas with lower road mortality rate. However, there were

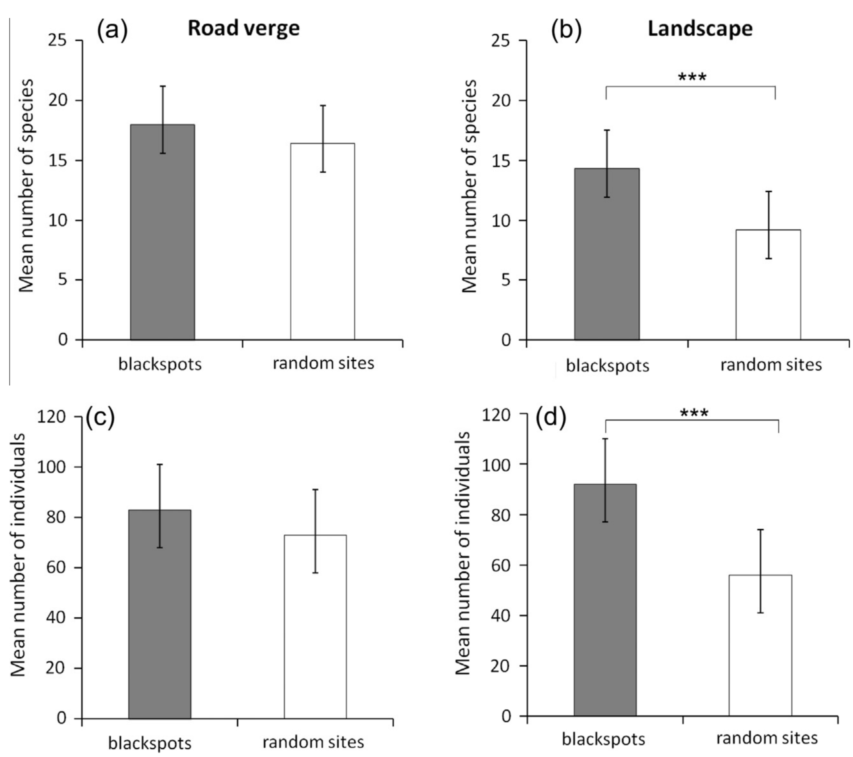

Fig. 4. Mean number of species (a and b) and abundance (c and d) of living butterflies found on road verges (left panel) and in the surrounding landscape (right panel) in biodiversity blackspots (dark bars) and randomly chosen sites (white bars) in 2012. Whiskers indicate 95% confidence intervals, *** – $P < 0.001$.

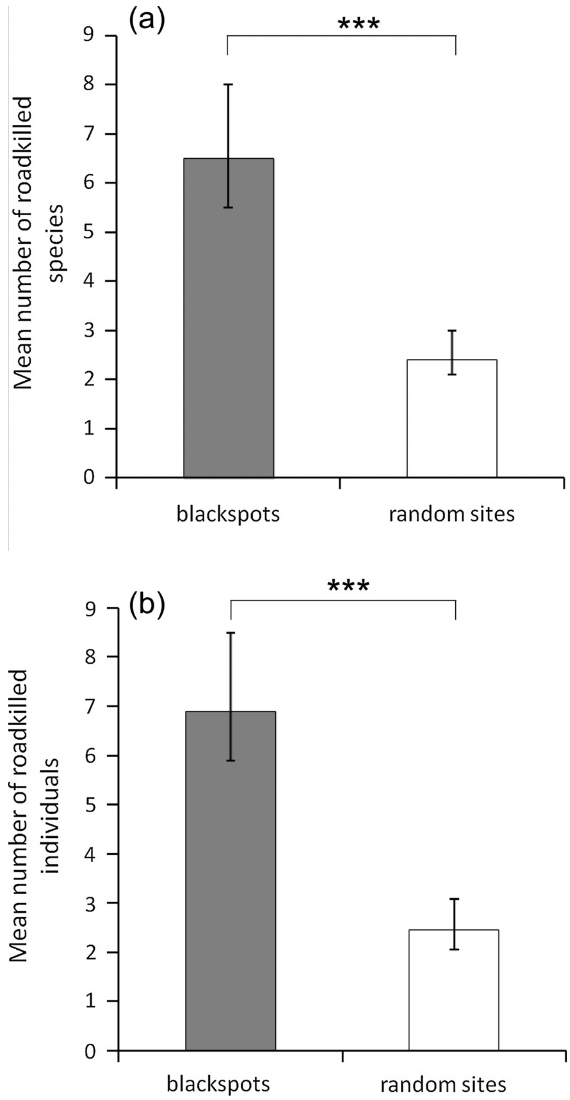

Fig. 5. Mean number of roadkilled species (a) and individuals (b) found at roads in biodiversity blackspots (dark bars) and randomly chosen sites (white bars) in 2012. Whiskers indicate 95% confidence intervals, *** – $P < 0.001$.

Table 3
Generalized linear mixed models explaining the abundance of dead butterflies on transects in biodiversity blackspots and in randomly chosen non-blackspot sites in 2012.

Effect	Estimate (SE)	*F*	df	*P*
Abundance of roadkills in biodiversity blackspots (R^2 = 0.67)				
Traffic volume	0.208 (0.089)	5.389	1, 33	0.027
Number of plant species	−0.028 (0.063)	0.199	1, 33	0.659
Mowing frequency	0.095 (0.052)	3.287	1, 33	0.079
Grassland cover in a landscape	0.221 (0.095)	5.497	1, 33	0.026
Abundance of butterflies at verges	0.148 (0.125)	1.407	1, 33	0.244
Abundance of butterflies in a landscape	0.312 (0.095)	10.770	1, 33	0.002
Abundance of roadkills in random non-blackspot sites (R^2 = 0.10)				
Traffic volume	0.315 (0.559)	0.136	1, 33	0.714
Number of plant species	−0.054 (0.501)	0.035	1, 33	0.853
Mowing frequency	0.858 (0.517)	2.605	1, 33	0.116
Grassland cover in a landscape	−0.836 (0.506)	3.356	1, 33	0.076
Abundance of butterflies at verges	2.398 (0.682)	11.997	1, 33	0.001
Abundance of butterflies in a landscape	0.365 (0.712)	1.055	1, 33	0.312

statistically significant interactions between variables that determined whether a given location is a blackspot. The complex impact of the interacting variables on the occurrence of mortality blackspots indicates that it not easy to determine occurrence of such sites based only, for example, on aerial images. Thus, counting of dead animals should be always recommended to identify blackspots and factors leading to mortality at these locations.

Road traffic had an impact on the occurrence of blackspots at roads with few plant species in verges and high cover of grasslands in the surrounding landscape. Low plant diversity on road verges is an indication of low suitability/quality of a verge for butterflies (Ries et al., 2001; Skórka et al., 2013). However, high grassland cover in a landscape probably causes the influx of individuals into roads intersecting butterfly habitats. Thus, when verges are of low quality for butterflies, and road traffic is high, it may lead to increasing rate of butterfly collisions with cars and emergence of a blackspot. Moreover, high traffic volume increased number of roadkilled butterflies in the blackspots, but not in randomly chosen sites. Thus, planting abundant flowers on road verges may thus act as environmental filters that stop individual butterflies from crossing the road and minimize mortality when the traffic is high (Skórka et al., 2013).

We also found that high mowing frequency, in general, led to emergence of blackspots. The effect of mowing on blackspot occurrence was especially high at roads with low grassland cover in the landscape. Mowing is a disturbance that may increase dispersal (Weber et al., 2008) and thus causing frequent road crossings, exposing butterflies to collisions with vehicles. We cannot exclude the possibility that mowing itself may be a cause of death but, in fact, it is also vehicle-related mortality (mowing is typically executed by tractor mowers). Low grassland cover may be synonymous of low quality habitats in the surrounding of roads and thus butterflies are more willing to cross roads, exposing themselves to collision with vehicles, rather than enter the inhospitable habitats outside the road verge. This effect may be also augmented by the fact that road verges on both sides of one road are usually mown at different times making crossing the asphalt a more favorable option for butterflies when grassland cover is low in surrounding landscape.

Biodiversity blackspots, on average, did not differ in species richness and abundance of butterflies living at road verges from the random sample of locations not classified as blackspots. However, the occurrence of blackspots was determined by high species richness and abundance of butterflies in the landscape surrounding the road. Also, number of roadkills within the blackspot was correlated with abundance of butterflies in a landscape. It is an important result indicating that influx of individuals from the surrounding of a road may affect blackspot occurrence. Thus, it is possible to use data on the occurrence of road mortality to identify the conservation valuable areas in the vicinity of roads.

4.2. Methodological considerations

The techniques to delineate blackspots depend on optimizing various statistical criteria, but the techniques differ among themselves in their methodology as well as in the criteria used for identification (Gomes et al., 2008). The nearest-neighbour hierarchical clustering used in this study was set to use only statistical criteria during estimation of number and location of blackspots. One can use more specific input criteria, for example the minimal number of points in a blackspot in the analysis, which may result in different number of the blackspots found. Therefore, we also used spatial kernel density interpolation to support our finding from the nearest-neighbour spatial hierarchical clustering. The density kernel interpolation technique is appealing in terms of producing attractive risk-surface maps, but it should be considered only as a supportive and explorative method. It weakness lies in that mortality incidents did not occur at all of the locations within the hottest color grid cell. Moreover, the number of classes, and the assignment of cases to them, may affect the look and perception of the map (Snow et al., 2014). The risk surface maps produced by kernel density interpolation suggested larger number of road biodiversity blackspots than the number produced by nearest-neighbour spatial hierarchical clustering. However, the latter were always located within blackspots produced by the interpolation. Thus, the interpolation technique may be useful to get rather general information on car-mortality risky areas.

We found that, indeed, the number of species and abundance of dead butterflies found on transects in 2012 were larger at the earlier identified blackspots than transect at randomly chosen locations. Also, the number of road mortality incidents embraced by the blackspots in 2010 and 2011 was positively correlated with the number of dead butterfly species found in 2012 at transects there. This confirms that the estimated road biodiversity blackspots were real and, probably, at relatively constant locations across years.

In this study, we included fewer explanatory variables than in our former study on butterfly mortality (Skórka et al., 2013). However, as explained in the Methods section, we selected variables that had direct potential impact on butterflies and allow efficient testing of models with interaction terms. Of course, variables omitted in this paper may affect mortality of butterflies. Nevertheless, their effect would be difficult to interpret due to multicollinearity problem. Finally, our former study (Skórka et al., 2013) had a different objective, which was description of general pattern of road mortality and included examining whether species traits correlated with roadkill numbers. In contrast, in the present study, we focused on subsets of the road mortality that were spatially clustered in non-random manner in order to implement mitigation actions in a cost-efficient way.

In this study we did not considered species-specific traits in the emergence of blackspots, as we were interested in multi-species clusters of roadkills. It is possible, however, that some species may be more prone than others to collisions with cars and add more to the occurrence of blackspots. In our earlier study (Skórka et al., 2013) we demonstrated that smaller species were overrepresented in a sample of roadkills, probably due to their slow speed and low flight above the asphalt. However, mobility of species did not affect the mortality (Skórka et al., 2013). Of course, having said that, our approach enables to test the effect of species-specific traits on blackspot occurrence, e.g. one may expect that smaller species should have higher number of blackspots at roads than larger species, after accounting for their abundance and this should be tested in future works.

4.3. Practical recommendations

We think that the nearest-neighbour spatial clustering analysis is a straightforward method, which requires data that are relatively easy to collect in field. Our study design may be commonly applied in landscapes where mitigation actions are planned. These methods may be applied to either a group of species or individual ones, depending on the conservation target. As we have shown, predicting butterfly biodiversity blackspots in the landscape along roads may not be quite easy when it is based, for example, only on aerial/satellite images as interactions between traffic, fine-scale verge characteristics and surrounding landscape may hinder this task. Thus, collecting dead individuals and noting their geographical location may be the most effective means of identifying the areas with high and non-random road mortality, at least in butterflies. To mitigate high number of butterfly mortality incidents in the identified blackspots we recommend using less frequent mowing of the verges. In blackspots with high cover of grassland in a landscape sowing flowering and host-plant species on verges might be appropriate. However, it requires further specific behavioural studies in order to determine how butterflies respond to different plant species richness to avoid the scenario that road verges with high plant species richness are ecological trap for butterflies. Also, in blackspots the speed limit may be introduced as it regarded the effective tool alleviating road mortality in butterflies (Mckenna et al., 2001).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biocon.2015.04.017. These data include Google maps of the most important areas described in this article.

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