Research Article

Can attitude toward humans cause isolation?
Marked genetic distinction of urban wild boar population

Maciej K. Konopiński, Grzegorz Baś, Katarzyna Bojarska*

Institute of Nature Conservation Polish Academy of Sciences

Abstract

Large mammals have been colonising urban areas throughout the world. This process is often accompanied by genetic and behavioural changes, and as a result, urban populations may form distinct entities within continuous range of the species. In this paper, we present the results of an analysis of the spatial distribution of genetic variation in urban/suburban populations of wild boars *Sus scrofa*. We used a genetic variation of 12 microsatellite markers to analyse the population structure of wild boars inhabiting a large city (Kraków, Poland) and its rural surroundings. We discovered a profound differentiation between urban and rural areas, with urban individuals forming a distinct genetic group within an otherwise more continuous range of the species. The genetic distinctiveness of the urban wild boar population seems to be maintained not only by physical barriers but plausibly by behavioural differences. Although the chronology of the highway bypass construction may partly explain some of the genetic relatedness between wild boar populations, our results suggest attitudes towards humans may be an important factor influencing immigration to the areas of increased human presence. We discuss possible implications for the management of the wild boar in the city.

Introduction

In the urban-sprawl era, it becomes increasingly important to understand how the process affects wildlife. Human development influences wildlife in a number of ways, e.g. through habitat fragmentation and modification, anthropogenic subsidies and human presence (Marzluff et al., 2001; McKinney, 2002). As a result, some species suffer from habitat degradation and human disturbance, while others adapt to the novel conditions and successfully exploit anthropogenic resources (reviewed by Lowry et al., 2013). Within the latter group, more ecologically flexible species prevail (Chace and Walsh, 2006; Duduś et al., 2014; Lowry et al., 2013). City-dwelling animals often display behaviours that differ from those of their rural conspecifics (e.g. Ditchkoff et al., 2006; Evans et al., 2010; Kitchen and Price, 2010; Šálek et al., 2015). It has been suggested that genetic differences may partially shape this behavioural variation between urban and rural wildlife populations (Partecke et al., 2004, 2005). Moreover, behavioural changes in urban populations may enhance genetic divergence. For instance, modifications to breeding behaviour may facilitate reproductive isolation, and some ethological adaptations to urban habitats are advantageous when competing with new immigrants from the neighbouring, less altered environments (Slabbekoorn and Ripmeester, 2008). As a result, city-dwelling animals may form behaviourally and genetically distinct urban subpopulations (Ditchkoff et al., 2006; Šálek et al., 2015; Stillfried et al., 2017a,b).

Another process that may enhance the diversification of urban wildlife subpopulations is their physical isolation from the surrounding rural subpopulations. Non-favourable habitats and human infrastructure, such as roads, fences and settlements, constitute obstacles for animal movement (Tucker et al., 2018). Urban areas are often surrounded by such anthropogenic infrastructure (e.g., major roads) that prevents animals moving into and out of the cities. Patches of suitable habitats within the cities may form isolated islands for wildlife subpopulations (Stillfried et al., 2017a,b), which may have profound consequences for their demography, health, and genetics (Bradley and Altizer, 2007; Mossman and Waser, 2011; Noël et al., 2007).

Wild boar *Sus scrofa* is one of the most ecologically flexible ungulate species in the world. It inhabits a huge variety of environments, from vast forests to large cities (Cahill et al., 2012; Podgórski et al., 2013). Due to its fast reproduction and non-specialised habitat requirements, it became one of the most widespread wild ungulates in the northern hemisphere (Apollonio et al., 2010; Bieber and Ruf, 2005). In human-dominated landscapes, wild boars living in anthropogenic environments often adjust their spatio-temporal patterns of behaviour to avoid encounters with people while maximising the use of anthropogenic food sources (Podgórski et al., 2013; Thurfjell et al., 2009). Wild boars can successfully exploit any locally abundant food source, which often creates conflicts with humans (e.g., Schley et al., 2008). In addition, the species harbours several wildlife diseases that pose a threat to humans and domestic animals, including the African Swine Fever (Acevedo et al., 2007; De la Torre et al., 2015). This creates a potential for human-wild boar conflicts, particularly in agricultural and urban areas (Frank et al., 2015; Geisser and Reyer, 2004). Long-term management plans based on knowledge of colonisation processes and connectivity of urban populations are necessary to effectively minimise these conflicts (Gamelon et al., 2012; Massei et al., 2015).

*Corresponding author
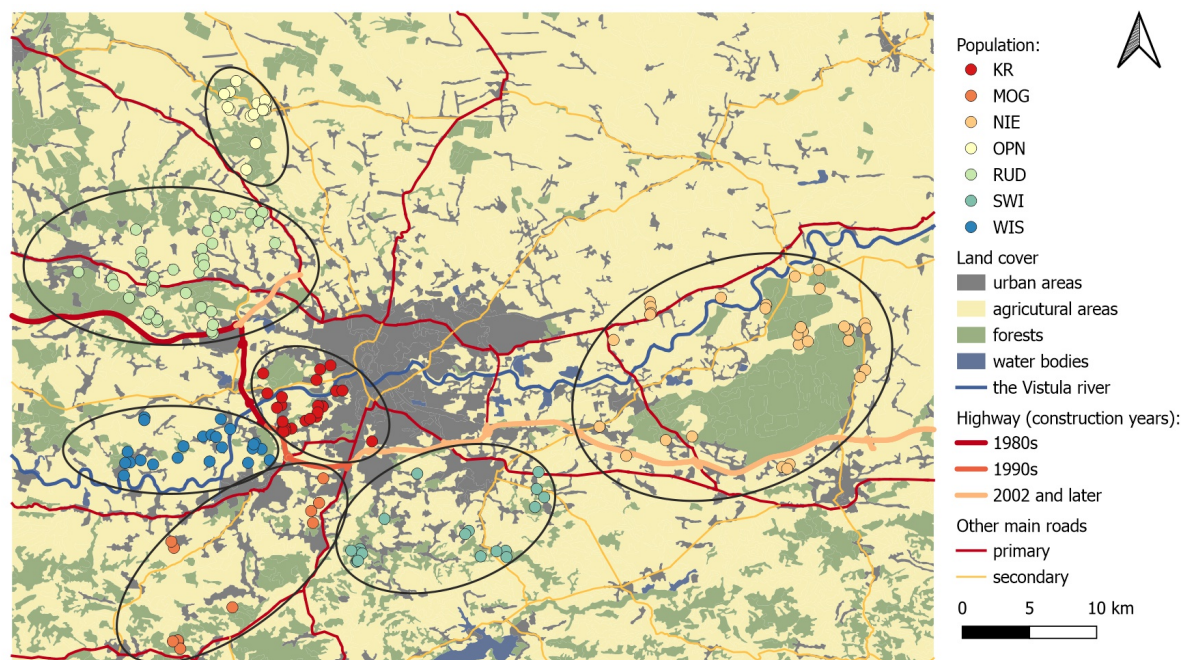Email address:* bojarska@iop.krakow.pl (Katarzyna Bojarska)

Figure 1 – Sampling locations and population delineation of the wild boars in the area of Kraków city and its surroundings (Poland).

Two possible scenarios are commonly considered for urban wildlife populations. The first scenario assumes that animals in the cities comprise descendants of individuals that initally colonised the urban areas. Such a group is now isolated from their conspecifics inhabiting areas outside the city or forms a source of individuals dispersing outside the city. The alternative scenario states that city-dwelling individuals constantly disperse from adjacent rural areas due to urban habitats acting as attractive sinks (Wandeler et al., 2003). Analyses of genetic variation offer an efficient tool to understand both colonisation history and the present connectivity of urban wildlife populations. They may help to develop management plans for the city-dwelling populations (Stillfried et al., 2017a).

The aim of this study was to investigate the history, current status and mechanisms shaping the genetic diversity of an urban population of wild boar in a large city. By comparing population genetic structure and diversity of city-dwelling individuals and their rural conspecifics, we aimed to assess the degree of isolation of the urban population from the neighbouring areas. We also discuss plausible mechanisms and consequences of isolation between wild boar populations.

Materials and methods

Study area

Kraków (50°3′ N, 19°56′ E), with its ca. 800000 inhabitants and 327 km², is the second-largest city in Poland. The westernmost part of the city comprises a large share of meadows, marshes, and deciduous forests, protected as Bielańsko-Tyniecki Landscape Park. Kraków is surrounded mainly by a mosaic of the agricultural landscape, small built-up areas and only a few larger towns. The city's western, southern and in part eastern borders are delineated by a highway bypass built between 1988 and 2016 (Fig. 1). Areas to the northeast of Kraków are covered by agricultural landscape with a very small fraction of forest cover. The city is intersected by a major river, the Vistula. A small national park (Ojców National Park, 21.5 km²) north of Kraków is covered by deciduous forests and surrounded by agricultural landscape.

The wild boar has been observed in Kraków continuously since 1993 (Tomek, 2003). The population is managed by hunting conducted within hunting clubs of the Polish Hunting Association. Genetic samples of hunted individuals were collected within the administrative borders of Kraków (two hunting clubs) and 16 hunting clubs (total area ca. 1600 km²) in the adjacent suburban areas up to 20 km from the city borders (Fig. 1). During the study period, the numbers of hunted

individuals within the city increased from 50 in the hunting season of 2009–2010 to 181 in 2014–2015. In the suburban hunting clubs, the respective numbers were 217 and 445 individuals (data of Polish Hunting Association).

The individuals were grouped into seven populations based on the location of the hunted animals. A network of main roads and stretches of built-up areas (mostly along the main roads), as well as uninterrupted areas of typical wild boar habitat were also accounted for when grouping individuals (forests, meadows, marshes and agricultural landscape; Fig. 1).

Laboratory procedures

Tissue samples were obtained in 2011–2015 from hunted animals (muscle, tongue) and individuals captured for radio-collaring (buccal epidermis collected on cotton swabs, permit of 1st Local Animal Research Ethics Committee no 88/2009). The samples were stored in an ethanol:isopropanol (4:1) mixture. Once brought to the laboratory, the samples were stored at $-20\,°C$ until isolation. Before DNA isolation, the samples were desiccated at $60\,°C$ overnight. The genetic material was extracted using the NucleoSpin Tissue Kit (Macherey & Nagel, Germany) according to the manufacturer's protocol. The quantity and purity of DNA were controlled in Nanodrop 1000 Spectrophotometer (Nanodrop, USA), and the concentration of DNA was equilibrated to $10\,ng\,\mu L^{-1}$.

Twelve microsatellite loci (S005, S0068, S0226, Sw0155, Sw122, Sw24, Sw240, Sw2410, Sw632, Sw72, Sw857 and Sw936) were amplified in two multiplex reactions as described by Costa et al. (2012). Two loci (S0101 and Sw 2008) from the original panel were rejected due to either ambiguous banding pattern or poor amplification. The reaction mixture compositions and thermal profiles were as reported in the original paper. Amplification products were separated in the automatic genetic analyser Applied Biosystems 3130XL (Applied Biosystems, USA) along with GenScan 600 LIZ dye Size Standard (ThermoFisher Scientific, USA). The lengths of the amplified fragments were estimated in GeneMapper 4.0 (Applied Biosystems).

Statistical analyses

All the statistical analyses were performed using R computer environment ver. 4.0.4 (R Core Team, 2009). The script used in the analysis is available as Supplementary Material. The analysed loci were tested for the presence of null alleles using package `PopGenReport` ver. 3.0.4

Table 1 – Summary of the genetic diversity estimates and their 95% bootstrap confidence intervals (CI) in seven wild boar populations in Kraków city and its surroundings, southern Poland. N: number of samples, H_O: observed heterozygosity, H_S: Nei's gene diversity, R_S: allelic richness, H: Shannon diversity index, F_{IS} inbreeding coefficient. The population names are explained in Fig. 1.

Population	N	H_O	95% CI	H_S	95% CI	R_S	95% CI	H_Z	95% CI	F_{IS}	95% CI
KR[1]	26	0.655	0.577–0.728	0.672	0.612–0.728	4.278	3.448–5.135	1.310	1.095–1.524	0.025	−0.046–0.100
OPN[2]	19	0.701	0.614–0.781	0.662	0.608–0.711	4.358	3.548–5.226	1.304	1.114–1.498	−0.056	−0.139–0.028
RUD	41	0.664	0.581–0.749	0.714	0.635–0.789	5.315	4116–6.610	1.538	1.241–1.843	0.073	0.030–0.107
WIS	23	0.666	0.590–0.738	0.690	0.618–0.755	4.841	3.748–5.982	1.425	1.162–1.688	0.037	0.000–0.071
MOG	11	0.749	0.627–0.855	0.698	0.597–0.785	4.990	4.037–6.017	1.467	1.202–1.731	−0.070	−0.162–0.012
SWI	19	0.713	0.633–0.790	0.722	0.661–0.783	5.305	4.332–6.415	1.549	1.309–1.810	0.008	−0.074–0.098
NIE	30	0.661	0.572–0.744	0.692	0.601–0.769	5.380	4.296–6.566	1.510	1.233–1.791	0.037	−0.008–0.095

[1] wild boars in Kraków city
[2] wild boars in Ojców National Park

(Adamack and Gruber, 2014). Departures from Hardy-Weinberg equilibrium were calculated using `hw.test` function from package `pegas` ver. 1.0.1 (Paradis, 2010) with 9999 permutations. Basic diversity estimates such as observed heterozygosity (H_O), gene diversity (H_S; Nei, 1978), and allelic richness (R_S; El Mousadik and Petit, 1996) were calculated using package `hierfstat` ver. 0.5.7 (Goudet, 2005). Unbiased Shannon diversity index estimator (H_Z, Zahl, 1977) was calculated using `ShannonGen` function from `ShannonGen` package (Konopiński, 2020). Confidence intervals of the diversity indices were estimated from 9999 bootstrap replicates over loci. Inbreeding coefficient, F_{IS}, was estimated using `basic.stats` function from the `hierfstat` package. Significance of F_{IS} departure from zero was established with `boot.ppfis` function from `hierfstat` with 9999 bootstrap replicates. Pariwise F_{ST} values (Weir and Cockerham, 1984) were estimated with `pairwise.WCfst` function from `hierfstat`. Confidence intervals were estimated by bootstrapping over loci (9999 replicates) as implemented in `boot.fst` function (`hierfstat`), while statistical significance of each estimate was established by comparing the actual values to the simulated null distribution of F_{ST} values obtained by 9999 random reassignments of samples to populations for each population pair. Components of genetic variation were tested with the analysis of molecular variance (AMOVA, Excoffier et al., 1992) as implemented in `poppr.amova` function from package `poppr` ver. 2.9.2 (Kamvar et al., 2014).

The genetic structure of the population was analysed using three methods based on different principles. First, individual clustering was performed using a Bayesian algorithm that minimises deviation from Hardy-Weinberg and linkage-disequilibrium as implemented in `Structure` ver. 2.3.4 (Pritchard et al., 2000). `Structure` was run assuming correlated allele frequencies and allowing for admixture between populations. Because the diversity among the analysed populations was small (see Results), the program was run with LOCPRIOR option (Hubisz et al., 2009). To estimate the optimal number of clusters, K=1–12 populations were assumed, with 20 repeats for each

K. The runs consisted of 106 Monte-Carlo Markov Chain iterations preceded by 105 iterations as a burn-in period. The results were analysed using the Evanno et al. (2005) procedure as implemented in the R package `pophelper` ver. 2.3.0 (Francis, 2017); the runs with the most likely number of clusters (K) were concatenated using `mergeQ` function.

Second, `Geneland` package ver. 4.9.2 (Guillot et al., 2005) was used for clustering the georeferenced data. The program is based on similar principles as `Structure` but also takes the spatial distribution of the samples as a prior. The program was run in two modes: estimating the likely number of clusters with npopmax=12 and the number of clusters set to the optimal number of groups resulting from the Evanno et al. procedure for Structure runs. The analysis consisted of 107 iterations, with the chain sampled every 105 iterations. The initial 10% of sampled iterations were discarded as a burn-in period. The results of the both assignment tests were plotted on maps using QGIS Desktop ver. 3.2 (Quantum GIS Development Team, 2018).

Third, discriminant analysis of principal components (DAPC) was performed using `adegenet` package ver. 2.1.3 (Jombart, 2008) with the original populations used as a group factor. The optimal number of principal components was established using cross-validation procedure as implemented in the function `xval`, with 1000 replicates and 80% of observations used for the training set. The results of DAPC were presented as a scatter plot using `scatter` function from `ade4` package ver. 1.7.16 (Dray and Dufour, 2007). The individual assignments to the populations were plotted using `compoplot` function from `adegenet`.

Results

A total of 169 samples were collected (Tab. 1, Fig. 1). No null alleles were found in the analysed loci and populations. Only four out of 72 tests (locus/population combinations) for deviation from Hardy-Weinberg expectations returned *p*-values smaller than 0.05, affecting three different loci in three different populations. The lowest *p*-value was 0.005 at locus S0005 in SWI population, and after Bonferroni cor-

Table 2 – Pairwise F_{ST} values with 95% bootstrap confidence intervals (above diagonal) and their *p*-values (below diagonal).

|	KR	OPN	RUD	WIS	MOG	SWI	NIE
KR		0.080 (0.0441±0.1201)	0.034 (0.0173±0.0543)	0.044 (0.0156±0.0773)	0.053 (0.0311±0.0773)	0.075 (0.0356±0.1203)	0.076 (0.0393±0.1152)
OPN	0.001		0.047 (0.0223±0.0755)	0.064 (0.0267±0.1025)	0.062 (0.0267±0.1005)	0.056 (0.0286±0.0832)	0.073 (0.0364±0.1182)
RUD	0.001	0.001		0.018 (0.0016±0.0386)	0.040 (0.0101±0.0826)	0.034 (0.0169±0.0519)	0.044 (0.142±0.0868)
WIS	0.001	0.001	0.002		0.021 (0±0.0468)	0.021 (0.0011±0.0399)	0.004 (−0.004±0.0129)
MOG	0.001	0.001	0.001	0.015		0.031 (0.0025±0.0603)	0.032 (0.0085±0.0592)
SWI	0.001	0.001	0.001	0.004	0.007		0.020 (0.0037±0.0415)
NIE	0.001	0.001	0.001	0.198	0.002	0.004	
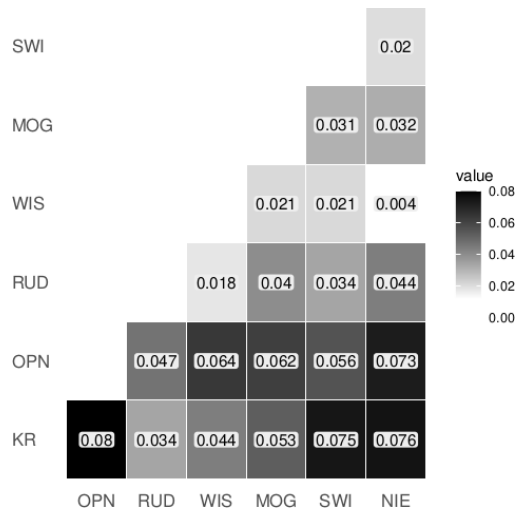
Figure 2 – Heatmap of F_{ST} values between all population pairs.

rection was applied, no deviations from Hardy-Weinberg equilibrium were found to be significant. The F_{IS} values did not depart significantly from zero, apart from the RUD population, where F_{IS} was biased towards positive values (Tab. 1).

The overall variation was relatively high, with mean gene diversity ranging from 0.66 to 0.72, mean allelic richness ranging from 4.28 to 5.38 and mean Shannon's H_Z ranging from 1.30 to 1.55 (Tab. 1). The lowest estimates of the genetic diversity were found in individuals sampled within the city of Kraków (KR population, H_O and R_S) and in Ojców National Park (OPN population, H_S and H_Z); however, the confidence intervals overlapped between each pair of populations

According to the analysis of molecular variance, only 4.45% of the overall diversity can be attributed to the inter-population differentiation, while most of the variation results from intra-individual diversity (93.56%). Wild boar populations from Kraków and OPN were most genetically distinct from others in terms of mean pairwise F_{ST}, while the remaining populations were less divergent from each other (Fig. 2). Although F_{ST} values were often close to zero, of the 21 comparisons only one pairwise F_{ST} (WIS/NIE) did not differ significantly from the null expectations under full panmixia (Tab. 2). This was also the only population pair for which the bootstrap confidence intervals spanned zero.

The results from Structure showed genetic distinctiveness of the KR population. According to the Evanno et al. procedure the most likely number of genetic clusters was K=2 for the analyses without population prior and K=3 in the analyses with population prior included. Both tests grouped wild boars from Kraków in a separate cluster, while the remaining populations consisted only of individuals from outside the city or contained a fraction of the Kraków cluster (Fig. 3). One of the individuals hunted in Kraków was unambiguously assigned to the rural population. Also, the OPN population appeared distinct from the surrounding populations when the number of clusters was set to K=3. The highest proportion of assignment to Kraków cluster was observed in RUD population.

The results of Geneland clustering corroborated those from Structure in terms of the distinct character of KR population (Fig. 4). When set to group the genotypes into K=2 and K=3 clusters, Geneland generated similar results to the outcome of Structure: the division between urban and rural areas remained both for K=2 and K=3, while a separate cluster for individuals from OPN was predicted for K=3. When run without the prior assumption on the number of populations, Geneland grouped genotypes into K=10 different clusters. Besides the KR population, the strongest genetic coherence was observed in populations from RUD and the OPN. RUD population contained three genotypes belonging to KR cluster (no. 8). On the other hand, the populations from south and east of Kraków (WIS, MOG, SWI and NIE) seemed to be intermixed

with three widespread clusters (clusters 3–5) and a few smaller clusters appearing within their range (Fig. 4).

The discriminant analysis of principal components (DAPC) performed in adegenet also showed the genetic distinction of Kraków and the ONP wild boar populations, although the ranges of all the clusters overlapped considerably (Fig. 5). Reassignment of individuals to their predefined populations using DAPC analysis was stronger in KR (0.92), RUD (0.83), and OPN (0.79), and weaker in SWI (0.68), NIE (0.60), WIS (0.30) and MOG (0.09).

Discussion

Our study indicates that the urban population of wild boars inhabiting the city of Kraków is genetically separated from wild boars living in the adjacent, rural or forested areas. The distinctiveness of the urban population is supported by both frequency-based statistics (F_{ST}, DAPC) and the probabilistic assignment methods (Geneland, Structure). The overall diversity between populations was small. The F_{ST} values were close to zero; however, for all but one of the pairwise comparisons they were higher than expected under single panmictic population. Although we cannot rule out the possibility that the most optimal number of clusters in Structure would be K=1, (a result that could not be achieved due to Evanno et al. procedure limitations), the distinctiveness of the urban wild boars indicated by the results of all statistical tests performed in this study, does allow to classify this group as a separate population. This isolation seems to be a consequence of both physical barriers, mostly the highway bypass, and factors related to wild boar behaviour.
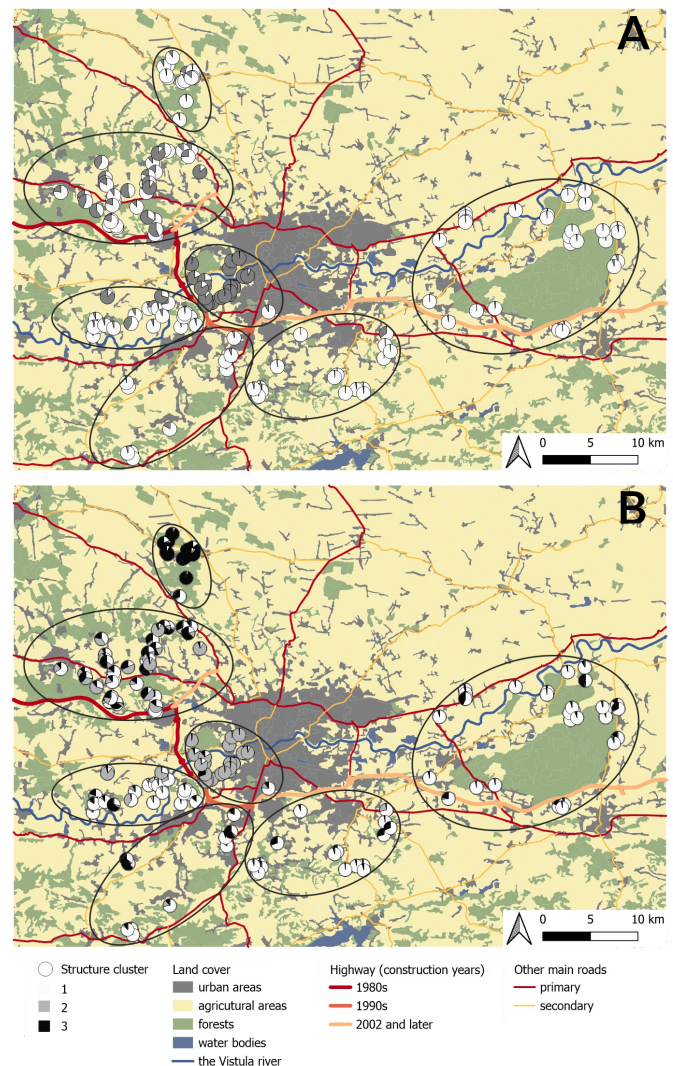

Figure 3 – Structure assignment of the individuals' genotypes to K=2 (A) or K=3 (B) clusters.
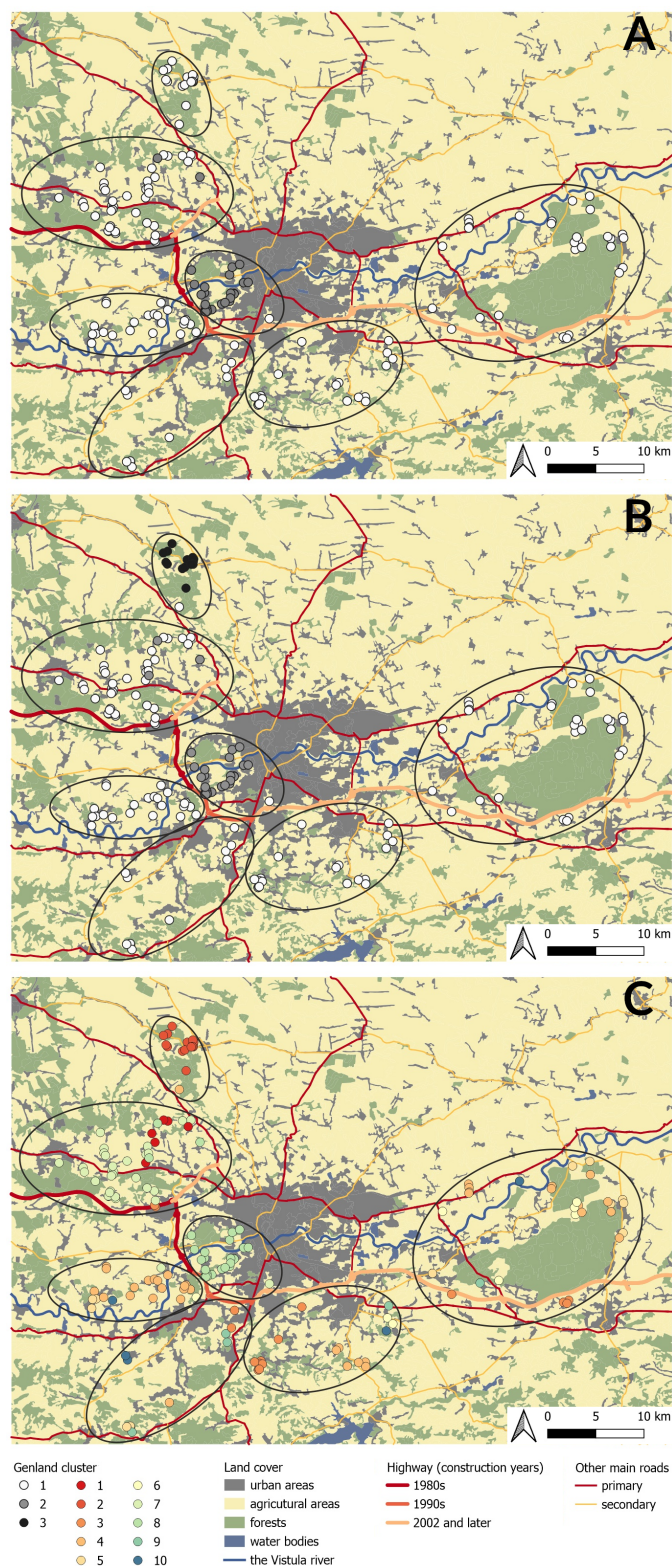
Figure 4 – Geneland assignment of the individuals' genotypes to *K*=2 (A), *K*=3 (B) and *K*=10 (C) clusters.

the highway bypass was built along the western and south-western border of Kraków. This part of the road was joined with an older highway fragment leading to Kraków from the west that had intersected the area occupied by the RUD and WIS wild boar populations since the 1980s. As a consequence, the first individuals colonising Kraków in the early 1990s most likely originated from the RUD population that remained connected with urban areas by patches of forested and undeveloped habitats until the late 90s. In 2003, the northern fragment of the bypass was built across that connection; however, some potential passages remained within the most likely ecological corridors between KR and RUD population.

The highway bypass along the western and southern borders of the city constitutes a movement obstacle for wild boars and therefore contributes to the genetic distinctiveness of the urban population. However, a few genotypes from suburban populations were assigned to the urban cluster, while one of the individuals hunted in southern Kraków was unambiguously assigned to the suburban cluster. This suggests that movement of the individuals across the highway bypass, both into and out of the city, still occurs. Probably, wild boars cross the bypass while moving along the rivers under the highway bridges or on the road overpasses, which has been confirmed by visual observations (Grzegorz Baś, unpublished). Although D'Amico et al. (2016) provided evidence that wild boars avoid proximity of the roads and concentrate their activity in more remote areas, Frantz et al. (2012) found that the populations in Belgium were not effectively isolated by a highway, where such isolation was detectable in red deer in the same area. It is possible that wild boars' willingness to cross the roads change depending on the intensity of human pressure (Podgórski et al., 2013), hence, the isolating effect of the major roads or highways may be even smaller in urban and suburban areas.

Based on our results, the Vistula river, which is ca 100 m wide in Kraków, does not form an effective barrier to wild boar movement. The urban population of the wild boar is consequently assigned to a single cluster by all methods, regardless of which side of the river the individual was sampled. Similarly, individuals from the rural WIS population are grouped into a single cluster despite being divided by the river. The urban population's genetic uniformity could be attributed to the low overall level of the genetic variation in Kraków. Still, the results of a telemetry study showed that some individuals used home ranges located on both sides of the river (Baś et al., 2017), which corroborates the genetic results of our study.
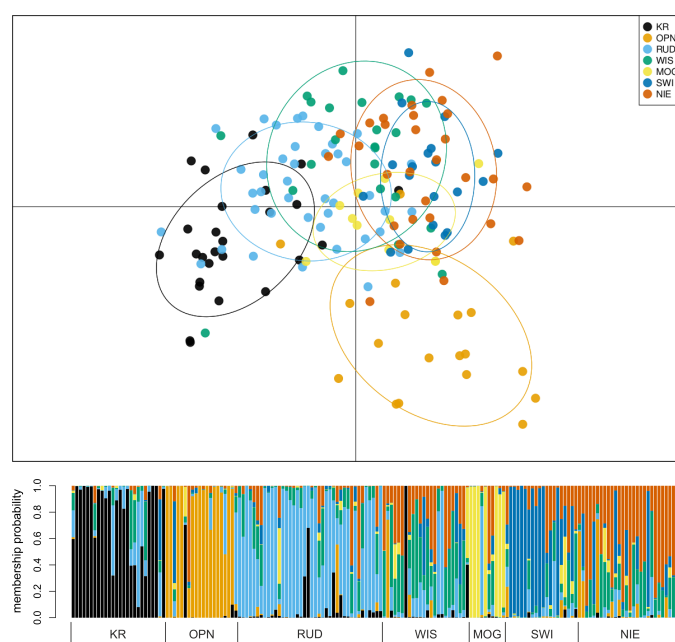

Figure 5 – Results of the discriminant analysis of principal components computed in adegenet. (A) Scatter plot of the first two discriminant functions explaining 34.8% and 24.4% of variance. (B) assignment of the genotypes to the population groups.

Non-physical factors may also contribute to the genetic isolation of the second most distinctive population, i.e. the wild boars inhabiting Ojców National Park (OPN).

The chronology of the highway construction progress may explain some of the genetic relatedness between wild boar populations in the area. The genetic similarity between KR wild boars and the RUD population inhabiting the forested areas northwest of the city, coupled with low similarity of KR population with its closely neighbouring WIS population, are consistent with the timeframe of building the highway bypass of Kraków. In the 1980s and early 90s, the first fragment of

The distinction of the KR population contrasts sharply with the uniformity of wild boar populations inhabiting vast areas south and east of Kraków. This region is relatively densely populated, with a few major roads and long stretches of built-up areas. Wild boars are known to adapt easily to human-induced changes in the environment (Keuling et al., 2008, 2009; Podgórski et al., 2013). Thus, populations outside the city are likely to remain interconnected despite fragmenting factors such as roads or human settlements, if no other isolation mechanisms come into play. In the case of the suburban population, distance does not seem to shape their genetic structure. Except for RUD and OPN populations, reassignment to the population of origin is very weak in suburban populations (0.09–0.68), while F_{ST} values between the most distant WIS and NIE populations is at similar level as between other populations between them.

Despite the large rural wild boar populations being connected with the urban population through movement corridors (e.g. KR-WIS and KR-RUD populations), our results show that urban and rural wild boars are genetically distinct. Therefore, it seems plausible that the urban population's effective genetic isolation is not only a result of physical barriers. The mechanisms causing isolation may include, for instance, reduced fear towards humans, specific adaptation to local food resources, and different circadian activity patterns or spatial use (Podgórski et al., 2013; Stillfried et al., 2017b). Stillfried et al. (2017a) found marked genetic isolation of the wild boar population in Berlin potentially caused by behavioural adaptations to urban conditions.

The isolation of the second most distinct population, OPN, may shed a light on the most important factors influencing the isolation of the urban population. Assignment tests with the higher number of clusters (K=3 or more) and DAPC analysis indicate marked isolation of the wild boars inhabiting Ojców National Park. The distinctiveness of KR and OPN populations can hardly be explained by reduced hunting pressure. Wild boars are intensively hunted both in Kraków (181 individuals in 2014/2015) and Ojców National Park (88 individuals in 2014). Probably the OPN population is capable of exchanging individuals with RUD population, which is only separated from OPN population by a single-lane road. One of the individuals killed on the east side of the road does not assign to an otherwise homogenous OPN cluster, suggesting it did not originate from this population. Moreover, unlike in the case of Kraków city, contrasting with its rural surroundings, no apparent differences in habitat exist in OPN and RUD populations. Hence, we hypothesise that other mechanisms are involved in the limited genetic exchange between OPN and RUD populations. One potentially significant difference between the RUD and OPN areas is the level of human disturbance. Ojców National Park, despite its small size, is a popular recreational spot, covered by a dense network of walking trails (65 km), visited by ca 350000–420000 tourists yearly. In the case of OPN and KR wild boar populations, the genetic isolation may be maintained by adaptation to intense human presence, which prevents immigration of individuals from less disturbed areas (Cook et al., 2017; Schell et al., 2018). This ability may be obtained via social learning and the characteristics of the environment where the young are raised (Donaldson et al., 2012; Mazur and Seher, 2008; Slagsvold and Wiebe, 2011). Wild boars live in matrilineal family groups, and piglets stay with their mothers for at least the first year of their life (Boitani et al., 1994). Therefore, similar to other social vertebrates, young individuals may learn from their groupmates to proficiently exploit resources in urban environments (review: Galef and Giraldeau, 2001). Finally, fast reproduction allows wild boars to compensate for individuals lost due to hunting and other mortality factors (Bieber and Ruf, 2005). Both the OPN and KR populations are the least variable populations among all populations studied. The reduced genetic variation within KR population might indicate a limited number of founder individuals, genetic drift and/or reduced immigration to the urban habitat. The same may hold true in OPN, which despite the lack of strong barriers to immigration, remained less variable than the neighbouring RUD population. Such results could have been expected if the young wild boars learn their attitude towards humans from their relatives within the group (Podgórski et al., 2014).

Conclusions and implications for management of the wild boar population

Our results indicate that the wild boar population in Kraków is self-sustaining despite the relatively high hunting pressure. This is corroborated by an increase in wild boar numbers along with the hunting quotas. Despite being a large, busy city, Kraków offers abundant habitats that provide wild boars with shelter and food, including corn fields, deciduous forests rich in beech and oak trees, grasslands and anthropogenic waste (Baś et al., 2017). City-dwelling wild boars tend to select habitats and foods of natural character and efficiently exploit them despite human disturbance (Stillfried et al., 2017b,b).

The self-sustainability of the urban wild boar population may deteriorate as a consequence of the Ministry of Environment's recent decision to substantially reduce wild boar densities to hinder the expansion of the African Swine Fever virus. However, the results of our study show that prevention of the spread of this disease would not legitimise an increased hunting pressure on the urban population. Due to their isolation, urban wild boar are less likely to be infected by the virus, and could serve as a source population in case the ASF outbreak reduces the population in the neighbouring areas.

Another threat for the urban wild boar population in the future may relate to land-use changes within and around the city. Built-up areas of Kraków are still expanding, occupying former grasslands, marshes and fields, which constitute the most important wild boar habitats within the city borders (Baś et al., 2017). As a consequence, we can expect further fragmentation of the urban wild boar population and a decrease in the availability of natural food sources. This process may negatively affect the reproduction of wild boar and its genetic diversity and reduce the population's growth. It will likely contribute to an increase in human-wildlife conflicts in the city. ✍

References

Acevedo P., Vicente J., Höfle U., Cassinello J., Ruiz-Fons F., Gortazar C., 2007. Estimation of European wild boar relative abundance and aggregation: a novel method in epidemiological risk assessment. Epidemiology & Infection 135(3): 519–527. doi:10.1017/S0950268806007059.

Adamack A.T., Gruber B., 2014. PopGenReport: simplifying basic population genetic analyses in R. Methods in Ecology and Evolution 5(4): 384–387. doi:https://doi.org/10.1111/2041-210X.12158

Apollonio M., Andersen R., Putman R., 2010. European Ungulates and their Management in the 21st Century. Cambridge University Press, Cambridge.

Baś G., Bojarska K., Śnieżko S., Król W., Kurek K., Okarma H., 2017. Wykorzystanie siedlisk przez dziki *Sus scrofa* na terenie Krakowa. Chrońmy Przyrodę Ojczystą 73(5): 354–362. [in Polish]

Bieber C., Ruf T., 2005. Population dynamics in wild boar *Sus scrofa*: ecology, elasticity of growth rate and implications for the management of pulsed resource consumers. Journal of Applied Ecology 42(6): 1203–1213. doi:10.1111/j.1365-2664.2005.01094.x

Boitani L., Mattei L., Nonis D., Corsi F., 1994. Spatial and Activity Patterns of Wild Boars in Tuscany, Italy. Journal of Mammalogy 75(3): 600–612. doi:10.2307/1382507

Bradley C.A., Altizer S. 2007. Urbanization and the ecology of wildlife diseases. Trends in Ecology & Evolution 22(2): 95–102. doi:10.1016/j.tree.2006.11.001

Cahill S., Llimona F., Cabañeros L., Calomardo F. 2012. Characteristics of wild boar (*Sus scrofa*) habituation to urban areas in the Collserola Natural Park (Barcelona) and comparison with other locations. Animal Biodiversity and Conservation 35: 221–233.

Chace J.F., Walsh J.J., 2006. Urban effects on native avifauna: a review. Landscape and Urban Planning 74(1): 46–69. doi:10.1016/j.landurbplan.2004.08.007

Cook M.O., Weaver M.J., Hutton P., McGraw K.J., 2017. The effects of urbanization and human disturbance on problem solving in juvenile house finches (*Haemorhous mexicanus*). Behav Ecol Sociobiol 71(5): 85. doi:10.1007/s00265-017-2304-6

Costa V., Pérez-González J., Santos P., Fernández-Llario P., Carranza J., Zsolnai A., Anton I., Buzgó J., Varga G., Monteiro N., Beja-Pereira A., 2012. Microsatellite markers for identification and parentage analysis in the European wild boar (*Sus scrofa*). BMC Research Notes 5(1): 479. doi:10.1186/1756-0500-5-479

D'Amico M., Périquet S., Román J., Revilla E., 2016. Road avoidance responses determine the impact of heterogeneous road networks at a regional scale. Journal of Applied Ecology 53(1): 181–190. doi:10.1111/1365-2664.12572

De la Torre A., Bosch J., Iglesias I., Muñoz M.J., Mur L., Martínez-López B., Martínez M., Sánchez-Vizcaíno J.M., 2015. Assessing the Risk of African Swine Fever Introduction into the European Union by Wild Boar. Transboundary and Emerging Diseases 62(3): 272–279. doi:10.1111/tbed.12129

Ditchkoff S.S., Saalfeld S.T., Gibson C.J., 2006. Animal behavior in urban ecosystems: Modifications due to human-induced stress. Urban Ecosyst 9(1): 5–12. doi:10.1007/s11252-006-3262-3

Donaldson R., Finn H., Bejder L., Lusseau D., Calver M., 2012. The social side of human–wildlife interaction: wildlife can learn harmful behaviours from each other. Animal Conservation 15(5): 427–435. doi:10.1111/j.1469-1795.2012.00548.x

Dray S., Dufour A.-B., 2007. The ade4 Package: Implementing the Duality Diagram for Ecologists. Journal of Statistical Software 22(1): 1–20. doi:10.18637/jss.v022.i04

Duduś L., Zalewski A., Kozioł O., Jakubiec Z., Król N., 2014. Habitat selection by two predators in an urban area: The stone marten and red fox in Wrocław (SW Poland). Mamm Biol 79(1): 71–76. doi:10.1016/j.mambio.2013.08.001

El Mousadik A., Petit R.J., 1996. High level of genetic differentiation for allelic richness among populations of the argan tree [*Argania spinosa* (L.) Skeels] endemic to Morocco. Theoret. Appl. Genetics 92(7): 832–839. doi:10.1007/BF00221895

Evanno G., Regnaut S., Goudet J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14(8): 2611–2620. doi:10.1111/j.1365-294X.2005.02553.x

Evans J., Boudreau K., Hyman J., 2010. Behavioural Syndromes in Urban and Rural Populations of Song Sparrows. Ethology 116(7): 588–595. doi:10.1111/j.1439-0310.2010.01771.x

Excoffier L., Smouse P.E., Quattro J.M., 1992. Analysis of Molecular Variance Inferred from Metric Distances among DNA Haplotypes: Application to Human Mitochondrial DNA Restriction Data. Genetics 131(2): 479–491.

Francis R.M., 2017. `pophelper`: an R package and web app to analyse and visualize population structure. Molecular Ecology Resources 17(1): 27–32. doi:10.1111/1755-0998.12509

Frank B., Monaco A., Bath A.J., 2015. Beyond standard wildlife management: a pathway to encompass human dimension findings in wild boar management. Eur J Wildl Res 61(5): 723–730. doi:10.1007/s10344-015-0948-y

Frantz A.C., Bertouille S., Eloy M.C., Licoppe A., Chaumont F., Flamand M.C., 2012. Comparative landscape genetic analyses show a Belgian motorway to be a gene flow barrier for red deer (*Cervus elaphus*), but not wild boars (*Sus scrofa*). Molecular Ecology 21(14): 3445–3457. doi:10.1111/j.1365-294X.2012.05623.x

Galef B.G., Giraldeau L.-A., 2001. Social influences on foraging in vertebrates: causal mechanisms and adaptive functions. Animal Behaviour 61(1): 3–15. doi:10.1006/anbe.2000.1557

Gamelon M., Gaillard J.-M., Servanty S., Gimenez O., Toïgo C., Baubet E., Klein F., Lebreton J.-D., 2012. Making use of harvest information to examine alternative management scenarios: a body weight-structured model for wild boar. Journal of Applied Ecology 49(4): 833–841. doi:10.1111/j.1365-2664.2012.02160.x

Geisser H., Reyer H.-U., 2004. Efficacy of hunting, feeding, and fencing to reduce crop damage by wild boars. Wild 68(4): 939–946. doi:10.2193/0022-541X(2004)068[0939:EOHFAF]2.0.CO;2

Goudet J., 2005. `hierfstat`, a package for R to compute and test hierarchical F-statistics. Molecular Ecology Notes 5(1): 184–186. doi:10.1111/j.1471-8286.2004.00828.x

Guillot G., Mortier F., Estoup A., 2005. Geneland: a computer package for landscape genetics. Molecular Ecology Notes 5(3): 712–715. doi:https://doi.org/10.1111/j.1471-8286.2005.01031.x

Hubisz M.J., Falush D., Stephens M., Pritchard J.K., 2009. Inferring weak population structure with the assistance of sample group information. Mol. Ecol. Resour. 9(5): 1322–1332. doi:10.1111/j.1755-0998.2009.02591.x

Jombart T. 2008. `adegenet`: a R package for the multivariate analysis of genetic markers. Bioinformatics 24(11): 1403-–1405. doi:10.1093/bioinformatics/btn129

Kamvar Z.N., Tabima J.F., Grünwald N.J., 2014. `Poppr`: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2: e281. doi:10.7717/peerj.281

Keuling O., Stier N., Roth M., 2008. How does hunting influence activity and spatial usage in wild boar *Sus scrofa* L.? Eur J Wildl Res 54(4): 729. doi:10.1007/s10344-008-0204-9

Keuling O., Stier N., Roth M., 2009. Commuting, shifting or remaining? Different spatial utilisation patterns of wild boar *Sus scrofa* L. in forest and field crops during summer. Mamm Biol 74(2): 145–152. doi:10.1016/j.mambio.2008.05.007

Kitchen K.I.M., Price M., 2010. Tolerance of human disturbance by urban Magpie-larks. Australian Field Ornithology 27: 1–9.

Konopiński M.K., 2020. Shannon diversity index: a call to replace the original Shannon's formula with unbiased estimator in the population genetics studies. PeerJ 8: e9391. doi:10.7717/peerj.9391

Lowry H., Lill A., Wong B.B.M., 2013. Behavioural responses of wildlife to urban environments. Biological Reviews 88(3): 537–549. doi:https://doi.org/10.1111/brv.12012

Marzluff J., Bowman R., Donnelly R., (Eds.) 2001. Avian Ecology and Conservation in an Urbanizing World. Springer US.

Massei G., Kindberg J., Licoppe A., Gačić D., Šprem N., Kamler J., Baubet E., Hohmann U., Monaco A., Ozoliņš J., Cellina S., Podgórski T., Fonseca C., Markov N., Pokorny B., Rosell C., Náhlik A., 2015. Wild boar populations up, numbers of hunters down? A review of trends and implications for Europe. Pest Management Science 71(4): 492–500. doi:https://doi.org/10.1002/ps.3965

Mazur R., Seher V., 2008. Socially learned foraging behaviour in wild black bears, *Ursus americanus*. Animal Behaviour 75(4): 1503–1508. doi:10.1016/j.anbehav.2007.10.027

McKinney M.L., 2002. Urbanization, Biodiversity, and Conservation: The impacts of urbanization on native species are poorly studied, but educating a highly urbanized human population about these impacts can greatly improve species conservation in all ecosystems. BioScience 52(10): 883–890. doi:10.1641/0006-3568(2002)052[0883:UBAC]2.0.CO;2

Mossman C.A., Waser P.M., 2011. Effects of habitat fragmentation on population genetic structure in the white-footed mouse (*Peromyscus leucopus*). Canadian Journal of Zoology 79: 285–295. doi:10.1139/z00-201

Nei M., 1978. Estimation of Average Heterozygosity and Genetic Distance from a Small Number of Individuals. Genetics 89(3): 583–590.

Noël S., Ouellet M., Galois P., Lapointe F.-J., 2007. Impact of urban fragmentation on the genetic structure of the eastern red-backed salamander. Conserv Genet 8(3): 599–606. doi:10.1007/s10592-006-9202-1

Paradis E., 2010. `pegas`: an R package for population genetics with an integrated–modular approach. Bioinformatics 26(3): 419–420. doi:10.1093/bioinformatics/btp696

Partecke J., Hof T.J.V., Gwinner E., 2005. Underlying physiological control of reproduction in urban and forest-dwelling European blackbirds *Turdus merula*. Journal of Avian Biology 36(4): 295–305. doi:10.1111/j.0908-8857.2005.03344.x

Partecke J., Van't Hof T., Gwinner E., 2004. Differences in the timing of reproduction between urban and forest European blackbirds (*Turdus merula*): result of phenotypic flexibility or genetic differences? Proceedings of the Royal Society of London. Series B: Biological Sciences 271(1552): 1995–2001. doi:10.1098/rspb.2004.2821

Podgórski T., Baś G., Jędrzejewska B., Sönnichsen L., Śnieżko S., Jędrzejewski W., Okarma H., 2013. Spatiotemporal behavioral plasticity of wild boar (*Sus scrofa*) under contrasting conditions of human pressure: primeval forest and metropolitan area. Journal of Mammalogy 94(1): 109–119. doi:10.1644/12-MAMM-A-038.1

Podgórski T., Lusseau D., Scandura M., Sönnichsen L., Jędrzejewska B., 2014. Long-Lasting, Kin-Directed Female Interactions in a Spatially Structured Wild Boar Social Network. PLoS One 9(6). doi:10.1371/journal.pone.0099875

Pritchard J.K., Stephens M., Donnelly P. 2000. Inference of Population Structure Using Multilocus Genotype Data. Genetics 155(2): 945–959.

QGIS.org, 2018. Quantum GIS Geographic Information System. QGIS Association. http://www.qgis.org

R Core Team. 2009. R: A Language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Šálek M., Drahníková L., Tkadlec E., 2015. Changes in home range sizes and population densities of carnivore species along the natural to urban habitat gradient. Mammal Review 45(1): 1–14. doi:10.1111/mam.12027

Schell C.J., Young J.K., Lonsdorf E.V., Santymire R.M., Mateo J.M., 2018. Parental habituation to human disturbance over time reduces fear of humans in coyote offspring. Ecology and Evolution 8(24): 12965–12980. doi:10.1002/ece3.4741

Schley L., Dufrêne M., Krier A., Frantz A.C., 2008. Patterns of crop damage by wild boar (*Sus scrofa*) in Luxembourg over a 10-year period. Eur J Wildl Res 54(4): 589. doi:10.1007/s10344-008-0183-x

Slabbekoorn H., Ripmeester E.a.P., 2008. Birdsong and anthropogenic noise: implications and applications for conservation. Molecular Ecology 17(1): 72–83. doi:10.1111/j.1365-294X.2007.03487.x

Slagsvold T., Wiebe K.L., 2011. Social learning in birds and its role in shaping a foraging niche. Philos Trans R Soc Lond B Biol Sci 366(1567): 969–977. doi:10.1098/rstb.2010.0343

Stillfried M., Fickel J., Börner K., Wittstatt U., Heddergott M., Ortmann S., Kramer-Schadt S. Frantz A.C., 2017a. Do cities represent sources, sinks or isolated islands for urban wild boar population structure? Journal of Applied Ecology 54(1): 272–281. doi:10.1111/1365-2664.12756

Stillfried M., Gras P., Börner K., Göritz F., Painer J., Röllig K., Wenzler M., Hofer H., Ortmann S., Kramer-Schadt S., 2017b. Secrets of Success in a Landscape of Fear: Urban Wild Boar Adjust Risk Perception and Tolerate Disturbance. Front. Ecol. Evol. 5. doi:10.3389/fevo.2017.00157

Stillfried M., Gras P., Busch M., Börner K., Kramer-Schadt S., Ortmann S., 2017c. Wild inside: Urban wild boar select natural, not anthropogenic food resources. PLOS ONE 12(4): e0175127. doi:10.1371/journal.pone.0175127

Thurfjell H., Ball J.P., Åhlén P.-A., Kornacher P., Dettki H., Sjöberg K., 2009. Habitat use and spatial patterns of wild boar *Sus scrofa* (L.): agricultural fields and edges. Eur J Wildl Res 55(5): 517–523. doi:10.1007/s10344-009-0268-1

Tomek A., 2003. Określenie możliwości gospodarki łowieckiej populacją dzików oraz określenie ewentualnej pojemności łowisk w rejonie Bodzowa Kostrza, Pychowic i Skotnik, a także Lasu Wolskiego w świetle możliwych zagrożeń i szkód powodowanych przez te zwierzęta. Unpublished report for the City Hall of Cracow. [in Polish]

Tucker M.A., Böhning-Gaese K., Fagan W.F., Fryxell J.M., Van Moorter B., Alberts S.C., Ali A.H., Allen A.M., Attias N., Avgar T., Bartlam-Brooks H., Bayarbaatar B., Belant J.L., Bertassoni A., Beyer D., Bidner L., van Beest F.M., Blake S., Blaum N., Bracis C., Brown D., de Bruyn P.J.N., Cagnacci F., Calabrese J.M., Camilo-Alves C., Chamaillé-Jammes S., Chiaradia A., Davidson S.C., Dennis T., DeStefano S., Diefenbach D., Douglas-Hamilton I., Fennessy J., Fichtel C., Fiedler W., Fischer C., Fischhoff I., Fleming C.H., Ford A.T., Fritz S.A., Gehr B., Goheen J.R., Gurarie E., Hebblewhite M., Heurich M., Hewison A.J.M., Hof C., Hurme E., Isbell L.A., Janssen R., Jeltsch F., Kaczensky P., Kane A., Kappeler P.M., Kauffman M., Kays R., Kimuyu D., Koch F., Kranstauber B., LaPoint S., Leimgruber P., Linnell J.D.C., López-López P., Markham A.C., Mattisson J., Medici E.P., Mellone U., Merrill E., de Miranda Mourão G., Morato R.G., Morellet N., Morrison T.A., Díaz-Muñoz S.L., Mysterud A., Nandintsetseg D., Nathan R., Niamir A., Odden J., O'Hara R.B., Oliveira-Santos L.G.R., Olson, K.A. Patterson, B.D. Cunha de Paula R., Pedrotti L., Reineking B., Rimmler M., Rogers T.L., Rolandsen C.M., Rosenberry C.S., Rubenstein D.I., Safi K., Saïd S., Sapir N., Sawyer H., Schmidt N.M., Selva N., Sergiel A., Shiilegdamba E., Silva J.P., Singh N., Solberg E.J., Spiegel O., Strand O., Sundaresan S., Ullmann W., Voigt U., Wall J., Wattles D., Wikelski M., Wilmers C.C., Wilson J.W., Wittemyer G., Zięba F., Zwijacz-Kozica T., Mueller T., 2018. Moving in the Anthropocene: Global reductions in terrestrial mammalian movements. Science 359(6374): 466–469. doi:10.1126/science.aam9712

Wandeler P., Funk S.M., Largiadèr C.R., Gloor S., Breitenmoser U., 2003. The city-fox phenomenon: genetic consequences of a recent colonization of urban habitat. Molecular Ecology 12(3): 647–656. doi:10.1046/j.1365-294X.2003.01768.x

Weir B., Cockerham C., 1984. Estimating F-Statistics for the Analysis of Population-Structure. Evolution 38(6): 1358–1370. doi:10.2307/2408641

Zahl S., 1977. Jackknifing An Index of Diversity. Ecology 58(4): 907–913. doi:10.2307/1936227

Supplemental information

Additional Supplemental Information may be found in the online version of this article:

`Main_script.R` R script used in the statistical analysis.

`coords.txt` Coordinates of samples used in the analysis.

`Last_N_pops.stru` Structure data file used in the analysis.

`Geneland.R` R script used for Geneland analysis.