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## Genetic population structure defines wild boar as an urban exploiter species in Barcelona, Spain



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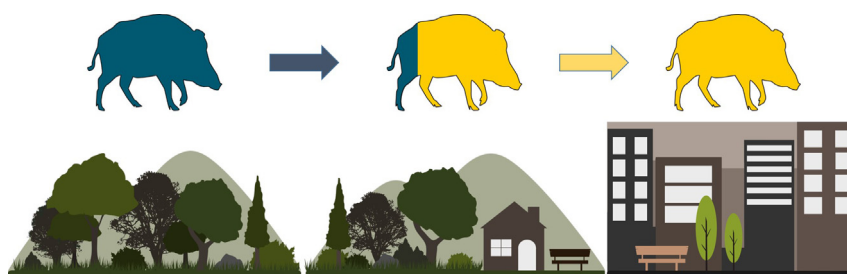
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## HIGHLIGHTS

- Synurbic wild boar populations are genetically different from rural wild boars.
- Conflicting synurbic wild boars establish as urban island populations.
- We define wild boar for the first time as an urban exploiter species.
- Whether synurbic phenotypic changes are genetic or plastic remains unknown.
- Management of urban wild boars should prevent adaptation to urban environments.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Urban wildlife ecology is gaining relevance as metropolitan areas grow throughout the world, reducing natural habitats and creating new ecological niches. However, knowledge is still scarce about the colonisation processes of such urban niches, the establishment of new communities, populations and/or species, and the related changes in behaviour and life histories of urban wildlife. Wild boar (*Sus scrofa*) has successfully colonised urban niches throughout Europe. The aim of this study is to unveil the processes driving the establishment and maintenance of an urban wild boar population by analysing its genetic structure. A set of 19 microsatellite loci was used to test whether urban wild boars in Barcelona, Spain, are an isolated population or if gene flow prevents genetic differentiation between rural and urban wild boars. This knowledge will contribute to the understanding of the effects of synurbisation and the associated management measures on the genetic change of large mammals in urban ecosystems.

Despite the unidirectional gene flow from rural to urban areas, the urban wild boars in Barcelona form an island population genotypically differentiated from the surrounding rural ones. The comparison with previous genetic studies of urban wild boar populations suggests that forest patches act as suitable islands for wild boar genetic differentiation. Previous results and the genetic structure of the urban wild boar population in Barcelona classify wild boar as an urban exploiter species.

These wild boar peri-urban island populations are responsible for conflict with humans and thus should be managed by reducing the attractiveness of urban areas. The management of peri-urban wild boar populations should aim at reducing migration into urban areas and preventing phenotypic changes (either genetic or plastic) causing habituation of wild boars to humans and urban environments.

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## 1. Introduction

Urbanisation, defined as the shift from rural to urban human residency, is a global and exponentially increasing process (United Nations, 2017). Synurbic wildlife can thrive in urban environments benefiting from anthropogenic and natural resources (Castillo-Contreras et al., 2021; Fischer et al., 2015; Kotulski and Koenig, 2008; Stillfried et al., 2017a), reaching even higher population densities than the surrounding countryside (Šálek et al., 2015) and increasing human-wildlife encounters (Soulsbury and White, 2015). Cities may represent ecological niches for wildlife with low (if any) human hunting pressure and predation, high habitat diversity, and anthropogenic food sources (Shochat et al., 2006). Thus, these niches may increase fitness for urban dwellers, compared with their rural conspecifics (Kotulski and Koenig, 2008).

Moreover, human-induced habitat changes and novel biotic interactions produce divergent landscapes that promote city-specific phenotypic traits (Alberti et al., 2017; Ouyang et al., 2018). Management techniques, mostly removal of individuals to solve conflict and control population size, may also act as a selective force in both urban and nonurban systems. Conflict frequency and severity determine the intensity of such management actions, ranging from no intervention in low-conflict situations to extirpation in high-conflict scenarios. In turn, management drives the phenotypic population changes, which are therefore more likely to appear in moderately conflictive urban wildlife. These phenotypic changes can be either responses to urbanisation itself or responses to management measures (Schell et al., 2020). The removal of individuals to control population size may also exacerbate genetic drift and decrease genetic diversity of urban populations after the initial bottleneck related to the founder effect (Combs et al., 2018; Edelhoff et al., 2020; Miles et al., 2019; Nei et al., 1975).

Wild boar (*Sus scrofa*) is an omnivorous generalist species occupying ecosystems from semi-arid deserts to tropical rain forests, and successfully exploits urban habitats (Massei et al., 1996). Across Europe, wild boar populations have increased and sprawled, even colonizing urban habitats (Geisser and Reyer, 2005; Snow et al., 2017; Castillo-Contreras et al., 2018; Zsolnai et al., 2022). Compared with rural wild boar populations, urban and peri-urban areas of European cities such as Barcelona, Berlin, Budapest, Genova, Warsaw and others host populations with larger sizes and higher densities (Cahill et al., 2012; Castillo-Contreras et al., 2018; Frantz et al., 2012; Podgórski et al., 2013; Stillfried et al., 2017b). The presence of wild boars in urban habitats generates new conflicts, such as general nuisance, damage to private and public property (gardens, cemeteries, sport pitches, public parks, etc.), higher risk of disease transmission, increased risk of traffic accidents, and increased public fear of attacks on pets and even on humans (Conejero et al., 2019; Fernández-Aguilar et al., 2018; Wang et al., 2019; Castillo-Contreras et al., 2022).

The recent colonisation of the urban environment by wild boar and the heterogeneity of the urban environments colonised have so far precluded the ecological classification of this species as either urban exploiter (able to exploit but not dependent on anthropogenic resources in urban areas) or urban tolerant (able to use anthropogenic resources and residing in urban landscapes but not generally exploiting urban areas to reach greater densities) (Rodewald and Gehrt, 2014). Although urban wildlife populations can reach higher densities than the rural ones (Luniak, 2004), density may be a misleading measure for this classification. Urban populations may be sinks, with relatively high mortality and/or low reproduction and receiving a constant gene flow from the rural source population (Pulliam, 1988;

Delibes et al., 2001), as defined by the ‘Population Pressure Hypothesis’ (PPH). Alternatively, the ‘Urban Island Hypothesis’ (UIH) predicts a spread of urban founder populations after an initial bottleneck, assuming gene-flow barriers between urban and rural populations leading to genetic differentiation over time (Gloor, 2002; Wandeler et al., 2003). Thus, knowledge about the genetic composition of populations provides a much better measure to understand the colonisation process and allows the ecological classification of urban wildlife and particularly wild boar populations.

Similar to the overall European trend, wild boar populations in Catalonia (northeastern Spain) are consistently growing and they will continue increasing in the future (Cahill and Llimona, 2004; González-Crespo et al., 2018), both in rural areas such as the Natural Park of Sant Llorenç del Munt i l'Obac (SLMO) as well as in peri-urban locations, such as the Collserola Natural Park (CNP) (Minuartia, 2020), which is located within the Metropolitan Area of Barcelona (MAB), bordering the city of Barcelona (BCN; Fig. 1). Concurrently, the presence of wild boar in the urban area of Barcelona has also risen, leading to an increased number of human-wildlife conflicts (Castillo-Contreras et al., 2018 and 2021; Fernández-Aguilar et al., 2018).

This study aims at investigating the effects of urbanisation and associated management measures as drivers of change in genetic diversity in urban wild boar populations, by investigating the genetic structure of both the urban and peri-urban wild boar populations of the MAB and its neighbouring rural areas. The results will contribute to define wild boar as being an urban tolerant or an urban exploiter species, as previously defined (Rodewald and Gehrt, 2014).

## 2. Material and methods

### 2.1. Sampling areas

Wild boar samples were collected in three focal areas and in three additional areas connected to the focal areas (Fig. 1). The focal areas were: i) the peri-urban CNP (41°25'52"N, 2°4'45"E) as part of the MAB; ii) the urban area of BCN (41°23'N, 2°11'E); and iii) the rural SLMO outside the MAB (41°38'29"N, 02°01'05"E).

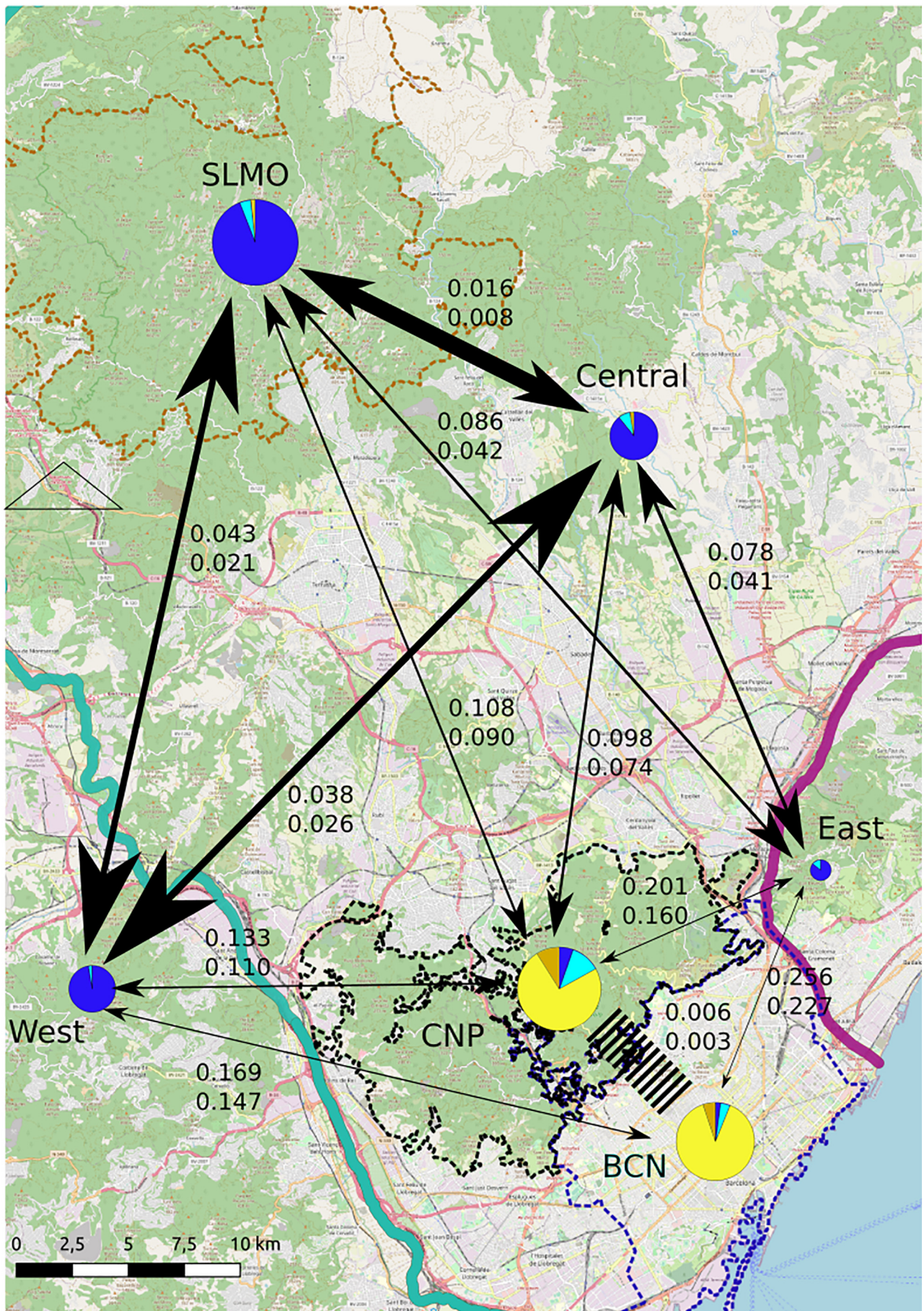
CNP is part of the Catalan Coastal Mountain Range and is located between the rivers Llobregat in the West and Besòs in the East. Being part of the MAB, it is a ‘Natura 2000’ site, surrounded by urban infrastructures (Cahill and Llimona, 2004). CNP location, natural heritage, vicinity to Barcelona and human disturbance of annually up to three million visitors make CNP a peri-urban habitat (Moll et al., 2019; Parc de Collserola, 2020). The south-eastern boundary of CNP borders the second focal area, the urban area of BCN, a city inhabited by ~1.6 million people with a density of 16.149 inhabitants/km<sup>2</sup> (Statistical Institute of Catalonia, 2019). The third focal area, SLMO, is a ~13,700 ha large rocky mountain massif located ~40 km north-west of BCN and CNP (Fig. 1).

The three additional sampling areas were i) the area between SLMO and BCN/CNP (henceforth ‘Central’), ii) the area south-west of river Llobregat (henceforth ‘West’) and iii) the area north-east of river Besòs (henceforth ‘East’).

### 2.2. Sampling

Muscle tissue ( $N = 218$ ), blood ( $N = 127$ ) and spleen tissue ( $N = 62$ ) from 407 wild boars were collected from 2005 to 2018 in the six sampling

**Fig. 1.** Map of sampling areas and sample locations. Focal areas are encircled by dotted colour lines, brown = Natural Park of Sant Llorenç del Munt i l'Obac (SLMO), black = Collserola (CNP), blue = Barcelona (BCN). The three smaller peripheral sampling areas are labelled ‘West’ (west of river Llobregat), ‘Central’ (between SLMO and CNP), and ‘East’ (east of river Besòs). Bold turquoise line = river Llobregat; bold purple line = river Besòs. The groups of sampled wild boars are presented as pie charts within their sampling location. Pie chart size indicates sample size (SMLO  $N = 118$ ; CNP  $N = 112$ ; BCN  $N = 98$ ; West  $N = 35$ ; Central  $N = 37$ ; East  $N = 7$ ). The colour of the pie chart pieces indicates cluster assignment by STRUCTURE (yellow = urban, dark yellow = urban admixed, dark blue = rural, light blue = rural admixed). The arrows display genetic exchange between neighbouring sampling areas (boldness of arrow reflects number of migrants per generation ( $Nm_{FST}$ )); numbers beside arrows indicate population pairwise distance (upper =  $F_{ST}$ ; lower =  $R_{ST}$ ). The barred line between CNP and BCN indicates nearly unhindered gene flow (see also Tables 3 and 4).



areas (BCN  $N = 98$ , CNP  $N = 112$ , SLMO  $N = 118$ , area 'Central'  $N = 35$ , area 'West'  $N = 37$ , and area 'East'  $N = 7$ ). The dataset contained 18 (4.4%) piglets (wild boar younger than six months). In all the areas but BCN, samples were collected from hunted wild boars after drive hunts carried out during the regular hunting season. In BCN, the urban wild boars were captured using teleanaesthesia, drop-nets or corral-traps (Torres-Blas et al., 2020), and euthanized as part of the management plan of the species to reduce human-wildlife conflicts in the urban area of Barcelona (Castillo-Contreras et al., 2016, authorization AC/190 from the Generalitat de Catalunya). After collection, the samples were stored at  $-20^{\circ}\text{C}$  until analysis. UTM coordinates were recorded for each sampled individual in BCN, while the centroid of the drive hunt area was assigned as position for the wild boar samples collected at drive hunts outside BCN (Fig. 1).

### 2.3. Molecular analyses

DNA from tissue samples (muscle and spleen) was extracted using the First-DNA all tissue kit (GEN-IAL GmbH, Troisdorf, Germany), following the manufacturer's extraction protocol with cell lysis carried out overnight at  $65^{\circ}\text{C}$ . DNA from blood was isolated using the Quick-gDNA™ Blood MiniPrep Kit (Zymo Research Corp, Irvine, CA, USA), according to the manufacturer's protocol. Once extracted, DNA was eluted with ddH<sub>2</sub>O and its concentration was measured with a Nanodrop ND1000 (PEQLAB Biotechnologie GmbH, Erlangen, Germany). Samples with DNA concentrations  $>100\text{ ng}/\mu\text{l}$  were diluted to  $100\text{ ng}/\mu\text{l}$ . Seventeen unlinked polymorphic microsatellite loci (Rohrer et al., 1994; Stillfried et al., 2017b) were amplified in six different multiplex PCRs (Table SM1) following the same protocol and using the Type-it Microsatellite PCR Kit (QIAGEN GmbH, Hilden, Germany). Each multiplex PCR was optimized in terms of combinations of loci, primer sequences, and fluorescence dyes (Table SM1). Prior to the genotyping PCRs, amplification conditions were optimized by performing gradient PCRs on a peqSTAR96 universal gradient thermocycler (PEQLAB Biotechnologie GmbH, Erlangen, Germany). Initial denaturation was then set at  $95^{\circ}\text{C}$  for 5 min, followed by 35 cycles of denaturation at  $94^{\circ}\text{C}$  for 30s, annealing at  $63^{\circ}\text{C}$ – $55^{\circ}\text{C}$  ( $1 \times 63^{\circ}\text{C}$ ;  $1 \times 61^{\circ}\text{C}$ ;  $1 \times 59^{\circ}\text{C}$ ;  $1 \times 57^{\circ}\text{C}$ ;  $31 \times 55^{\circ}\text{C}$ ) for 30s and elongation at  $72^{\circ}\text{C}$  for 30s. Final elongation was carried out for 30 min at  $60^{\circ}\text{C}$ . The PCR products were then separated by electrophoresis on a capillary automated sequencer (A3130xl; ABI Applied Biosystems, Foster, CA, USA). Allele sizes were determined using GENEMAPPER v.3.7 based on the GeneScan™ 500 ROX™ size standard (both ABI). To detect potential genotyping errors in the dataset, the software program MICRO-CHECKER v.2.2.3 (Van Oosterhout et al., 2004) was applied, using a significance threshold of  $\alpha = 0.05$ . The software package CERVUS v.3.0.7 (Marshall et al., 1998; Kalinowski et al., 2007, 2010) was used to determine number of alleles per locus ( $N_A$ ) and total number of alleles per population ( $N_A^*$ ), as well as to test for the presence of Hardy-Weinberg-equilibrium (HWE) across loci and for the presence of matching genotypes among the 407 wild boars genotyped. Allelic richness  $A_R$  and inbreeding coefficient  $F_{IS}$  were estimated using the software package FSTAT v.2.9.4 (Goudet, 1995, 2001). The potential presence of linkage disequilibrium (LD) among pairs of loci was determined using the likelihood ratio test (LRT) implemented in the software package ARLEQUIN v.3.5.2.2 (Excoffier and Lischer, 2010), applying a Bonferroni correction for multiple comparisons (Bonferroni, 1936). ARLEQUIN was also used to estimate the number of within population differences (PIX), pairwise population genetic distances, based both on different alleles ( $F_{ST}$ ) and on the sum of squared size differences of alleles ( $R_{ST}$ ), and to determine the number of migrants ( $N_m$ ) among populations (Wright, 1950; Reynolds et al., 1983; Weir and Cockerham, 1984; Slatkin, 1995).

### 2.4. Analysis of population differentiation and structure

To assess potential gene flow among the wild boar populations from different sampling areas, all the individuals were grouped according to their sampling area (henceforth called 'populations') and pairwise population genetic distances were estimated using  $F_{ST}$  and  $R_{ST}$  statistics.

A Bayesian genetic clustering method implemented in the software STRUCTURE v.2.2.4 (Pritchard et al., 2000; Falush et al., 2003, 2007; Hubisz et al., 2009) was applied to assess whether the wild boar metapopulation was at a genotypic equilibrium or descended from more than one ancestral population. The 'admixture model' was used as prior for the ancestry model with  $\alpha$  inferred from the data ( $\alpha_{\text{initial}} = 1.0$ ) for all the populations. The allele frequency model was the 'correlated allele frequencies model' with the following priors: different  $F_{ST}$  values for different populations, prior mean  $F_{ST} = 0.01$  (sd = 0.05), allele frequency parameter  $\lambda = 1.0$ . Ten independent runs were carried out for each  $K$  from  $K = 1$  to  $K = 7$  with a final run length of 300,000 Markov-Chain-Monte-Carlo (MCMC) iterations after a burn-in period of 300,000 iterations. Applying the web-tool STRUCTURE HARVESTER (Earl and von Holdt, 2012), the best fitting  $K$  was estimated using the  $\Delta K$ -method (Evanno et al., 2005), based on the log-likelihood values and their convergence associated with each  $K$ . After deciding on the most probable  $K$ , the average proportional membership ( $q$ ) in percent per cluster was calculated for each individual by averaging  $q$  over all ten runs. The program was applied to four scenarios (Sc): Sc1) including all individuals ( $N = 407$ ); Sc2) including all individuals but piglets ( $N = 389$ ); Sc3) as a sequential run to Sc1: if  $\geq 2$  clusters will be identified under Sc1, each of these clusters will then be analysed separately to test for the presence of an underlying population substructure not detected under Sc1; Sc4) for each of the populations from the three focal sampling areas. The individuals were assigned to a cluster based on their proportional membership ( $q$ ) for one cluster using a threshold of  $q \geq 90\%$ . Following the same strict rule, admixed individuals were defined as individuals with a proportional membership in one cluster of  $50\% < q < 90\%$ .

A Fisher's exact test using the *fisher.test* function within the R software environment (v.3.5.3.2; R Core Team, 2020) was performed to assess differences in the proportion of admixed wild boars and in the proportion of wild boars sampled from the area corresponding to their genetic cluster. These values were compared between the four rural populations (SLMO and all three peripheral areas) vs. the two peri-urban and urban populations (BCN and CNP), as well as between the peri-urban and urban populations (BCN vs. CNP) (Table 1).

## 3. Results

Out of all 407 wild boars, 401 were genotyped at all 17 loci, while six had missing data at one locus. All the genotypes were unique. Thus, all the wild boars were submitted to subsequent analyses. For the full data set ( $N = 407$ ), potential presence of null-alleles was detected at one locus only (Sw936). When applied to the six sampling populations separately, potential presence of null-alleles was detected at several loci: at Sw857 and Sw936 in CNP, at Sw856 in SLMO, at Sw936 in BCN, at Sw155 in 'West', at Sw911 and Sw66 in 'Central', and at Sw936 in 'East' (Table SM1). The LRT indicated potential LDs for four pairs of loci in CNP, four in SLMO, six in BCN, and none in the three smaller areas (Table SM2). As no apparent distribution pattern emerged for presence of null-alleles or LD between pairs of loci, all the 17 loci were kept for downstream analyses.

The number of alleles per locus ranged from  $N_A = 3$  (Sw72) to  $N_A = 16$  (Sw856), with a mean number of  $N_{A(\text{mean})} = 7.06$  (Table SM3). The number of loci deviating from HWE in each sampled population ranged from none to three (Table 2), accounting for ten out of the 17 loci deviating from HWE for the whole of the sample ( $N = 407$ ) (Table SM3). This indicated a Wahlund effect, with a population substructure preventing free mating and leading to a reduction of heterozygosity (Wahlund, 1928), suggesting that the whole population ( $N = 407$ ) was a structured metapopulation. Inbreeding was not detected in any of the six populations sampled (Table 2).

Averaged over all the 17 loci, all six wild boar populations sampled were at HWE (Fig. 2). The 407 wild boars shared  $N_A^* = 120$  alleles across all the loci. Out of those 120 alleles, the largest sampling population (SLMO,  $N = 118$ ) had also the highest total number of alleles ( $N_A^* = 103$ ), while the smallest sampling population ('East',  $N = 7$ ) had the lowest

**Table 1**

Admixed wild boars and assignment for each genetic cluster defined by the STRUCTURE results according to the sampling location. Admixed individuals are those with average membership ( $q$ ) of the other cluster  $50\% < q < 90\%$ .

Location	Admixed/Sampled (%)	Urban cluster			Rural cluster		
		Total (%)	95% CI	Admixed (%)	Total	95% CI	Admixed (%)
BCN	9/98 (9.2) <sup>x</sup>	92/98 (93.9) <sup>x</sup>	89.1–98.6	5/92 (5.4)	6/98 (6.1) <sup>x</sup>	1.4–10.9	4/6 (66.7)
CNP	23/112 (20.5) <sup>y</sup>	93/112 (83.0) <sup>y</sup>	76.1–90.0	10/93 (10.8)	19/112 (17.0) <sup>y</sup>	10.0–23.9	13/19 (68.4)
Subtotal urban locations	32/210 (15.2) <sup>a</sup>	185/210 (88.1) <sup>a</sup>	83.7–92.5	15/185 (8.1)	25/210 (11.9) <sup>a,b*</sup>	7.5–16.3	17/25 (68.0)
SLMO	7/118 (5.9)	2/118 (1.7)	0.0–4.0	2/2 (100.0)	116/118 (98.3)	96.0–100.0	5/116 (4.3)
West	1/35 (2.9)	0/35 (0.0)	–	0/0	35/35 (100.0)	–	1/35 (2.9)
Central	4/37 (10.8)	1/37 (2.7)	0.0–7.9	1/1 (100.0)	36/37 (97.3)	92.1–100.0	3/36 (8.3)
East	1/7 (14.3)	0/7 (0.0)	–	0/0	7/7 (100.0)	–	1/7 (14.3)
Subtotal rural locations	13/197 (6.6) <sup>b</sup>	3/197 (1.5) <sup>b,*</sup>	0.0–3.2	3/3 (100.0)	194/197 (98.5) <sup>b</sup>	96.8–100.0	10/194 (5.2)
Total	45/407 (11.1)	188/407 (46.2)	41.3–51.0	18/188 (9.6)	219/407 (53.8)	49.0–58.7	27/219 (12.3)

BCN = City of Barcelona; CNP = Collserola Natural Park; SLMO = Natural Park of Sant Llorenç del Munt i l'Obac; West = additional sampling area southwest of Llobregat river; Central = additional sampling area between SLMO and BCN/CNP; East = additional sampling area Northeast of Besòs river; CI = confidence interval.

a,b: values with different superscript were significantly ( $p < 0.05$ ) different between urban and rural locations.

x,y: values with different superscript were significantly ( $p < 0.05$ ) different between both urban locations (Barcelona and Collserola).

\* The proportion of wild boars from the rural cluster in urban locations was significantly ( $p < 0.05$ ) higher than the proportion of wild boars from the urban cluster in rural locations.

number ( $N_A^* = 54$ , Table 2). However, when considering the ratio  $R$  between the total number of alleles and the number of individuals [ $R = N_A^*/N$ ] as a measure of genetic diversity (Fickel et al., 2005), 'East' had the highest value ( $R = 7.714$ ), whereas CNP had the lowest value ( $R = 0.678$ ; Table 2). Allelic richness across all 17 loci was highest in 'West' ( $A_R = 3.78$ ; based on a mean number of alleles per locus of  $N_A/L = 5.18$ ) and lowest in BCN ( $A_R = 2.65$ ; based on  $N_A/L = 4.06$ ). Even the smallest sampling population ('East') had a higher allelic richness ( $A_R = 3.29$ ) than BCN. As this was based on  $N_A/L = 3.6$  only, alleles were more equally distributed in 'East' than in BCN (Table 2, Fig. 2). Regarding the differentiation among individuals within a population, we observed the highest number of within-population differences (PiX; Table SM4) in 'Central' ( $PiX_{(F_{ST}/R_{ST})} = 9.97/1700.1$ ), while they were lowest in BCN ( $PiX_{(F_{ST}/R_{ST})} = 7.41/1072.7$ ).

### 3.1. Population differentiation and structure

Genetic population pairwise distances ( $F_{ST}$  and  $R_{ST}$ ; Table 3) indicated the highest differentiation between urban BCN and rural 'East' ( $F_{ST}/R_{ST} = 0.256/0.227$ ), which also had the lowest number of migrants per generation ( $N_m$ ) exchanged between any two sampling populations ( $N_{m(F_{ST}/R_{ST})} = 0.72/0.85$ ; Table SM5, Fig. 1). The second highest differentiation was observed between peri-urban CNP and 'East' ( $F_{ST}/R_{ST} = 0.202/0.16$ ). Both findings indicated that the Besòs river diffculted gene flow between the rural 'East' and the urban/peri-urban BCN and CNP. The smallest

**Table 2**

Population indices across 17 loci and sampling areas analysed in 407 wild boars from the Metropolitan area of Barcelona (MAB) and its rural surroundings.

Pop	N	$N_A^*$	$R [N_A^*/N]$	$N_A/L$	$F_{IS}$
CNP	112	76	0.678	4.471	0.004
SLMO	118	103	0.873	6.059	0.024
BCN	98	69	0.704	4.059	-0.001
'West'	35	88	2.514	5.176	0.020
'Central'	37	92	2.486	5.412	0.062
'East'	7	54	7.714	3.6	0.025
all	407	120	0.295	7.059	0.022

Pop: population, N: population size,  $N_A^*$ : total number of alleles across all loci, R: ratio between total number of alleles and number of individuals,  $N_A/L$ : mean number of alleles per locus,  $F_{IS}$ : inbreeding coefficient (none was significant). CNP = Collserola Natural Park; SLMO = Natural Park of Sant Llorenç del Munt i l'Obac; BCN = Barcelona; 'West' = additional sampling area southwest of Llobregat river; 'Central' = additional sampling area between SLMO and BCN/CNP; 'East' = additional sampling area Northeast of Besòs river.

differentiation was detected between BCN and CNP ( $F_{ST}/R_{ST} = 0.006/0.003$ ; Fig. 1, Table S3). This low differentiation indicated strong gene flow, with the highest number of migrants per generation ( $N_m$ ) exchanged between both subpopulations ( $N_{m(F_{ST}/R_{ST})} \sim 41/82$ ; Table SM5, Fig. 1). These urban BCN and peri-urban CNP sampling populations were also genetically different from rural 'West' (separated by the Llobregat river;  $F_{ST}/R_{ST} = 0.169/0.147$  and  $0.133/0.110$ , respectively; Fig. 1, Table 3). Differentiation between CNP and 'Central' ( $F_{ST}/R_{ST} = 0.098/0.074$ ) and between CNP and SLMO ( $0.108/0.090$ ) were similar to the differentiation of CNP with 'West' ( $F_{ST}/R_{ST} = 0.133/0.110$ ) and between the lower differentiation values of CNP to BCN ( $F_{ST}/R_{ST} = 0.006/0.003$ ) and the higher differentiation of CNP to 'East' ( $F_{ST}/R_{ST} = 0.256/0.227$ ). Both CNP and BCN differed from all the rural sampling populations, including 'Central', more than urban and rural sampling populations differed among them. Pairwise population differentiations among rural sampling populations were generally low (Fig. 1, Table 3).

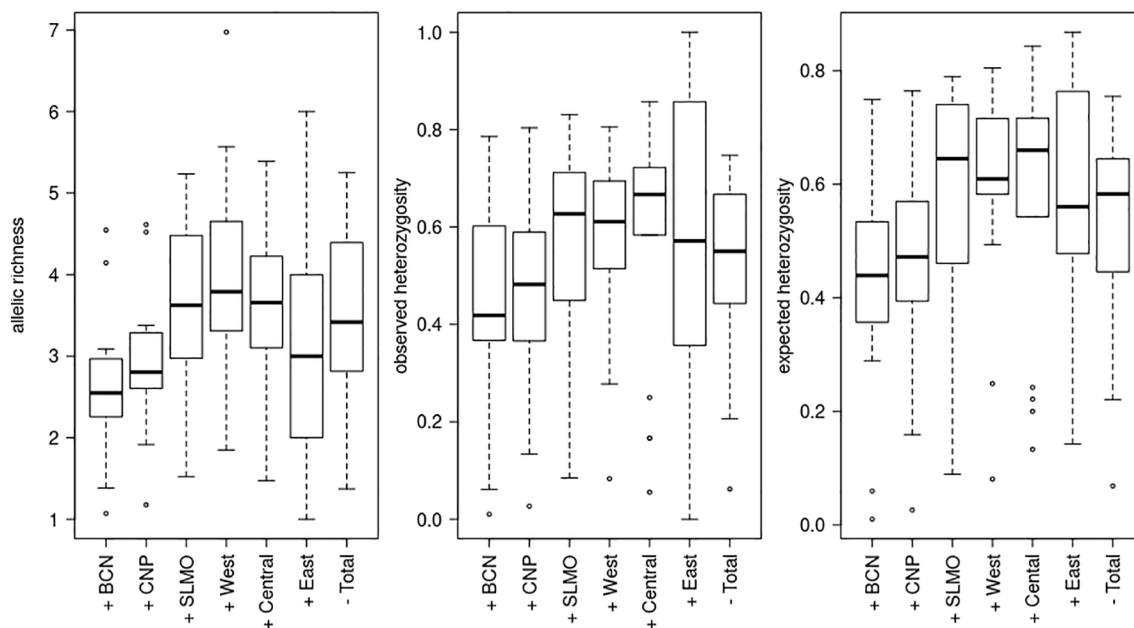
In STRUCTURE run scenario Sc1 ( $N = 407$ ), the  $\Delta K$  estimation approach (Earl and von Holdt, 2012) indicated  $K = 2$  ( $\Delta K = 992.3$ ) as the most likely number of ancestral genotypic clusters for the sampled wild boars (Table SM6). One of the two clusters ('rural') consisted mainly of wild boars sampled in the four rural areas ( $N = 219$ , including 27 admixed individuals), while the other one ('urban') was formed mostly by wild boars from the two urban/peri-urban locations (BCN and CNP  $N = 188$ , including 18 admixed individuals; Fig. 1, Tables 1 and 4, Fig. SM1). The analysis without piglets (STRUCTURE run scenario Sc2) did not change the outcome (Table SM6), with the same two ancestral genotypic clusters ( $K = 2$ ,  $\Delta K = 645.02$ ) and the remaining 389 wild boars still assigned to the same cluster as in scenario Sc1.

The mean values for the genetic diversity indices  $A_R$ ,  $H_{obs}$ , and  $H_{exp}$  (6.82/ 0.56/0.59) and the mean number of alleles per locus  $N_{A(mean)}$  (6.82) were higher in the rural cluster than in the urban one ( $A_R = 3.65$ ;  $H_{obs} = 0.451$ ;  $H_{exp} = 0.451$ ;  $N_{A(mean)} = 3.56$ ; Table 4, Fig. 3. Out of the 120 alleles detected among all the wild boars ( $N = 407$ ; Table 2), 116 were detected in the rural cluster (including admixed individuals:  $N_A = 119$ ) and 61 in the urban one (including admixed individuals:  $N_A = 67$ ; Table 4).

### 3.2. Wild boar origin and admixture

Most of the wild boars belonged to the cluster assigned to their areas, i.e., 88.1% (185/210) of the wild boars sampled in BCN and CNP belonged to the urban cluster and 98.5% (194/197) of the wild boars sampled in rural locations were assigned to the rural cluster (both including admixed individuals). The percentage was significantly ( $p < 0.05$ ) higher for rural locations, meaning that detecting rural wild boars in urban locations (11.9%)

## Genetic diversity of sampling areas



**Fig. 2.** Population indices across 17 loci and sampling areas analysed in 407 wild boars from the Metropolitan area of Barcelona (MAB) and its rural surroundings. Allelic richness across loci based on populations size of area, observed heterozygosity across all loci, expected heterozygosity across all loci. Hardy-Weinberg equilibrium + in front of names: population is at HWE, - : population is not at HWE, *p* values averaged over 17 loci were: *p*(CNP) = 0.398, *p*(SLMO) = 0.354, *p*(BCN) = 0.555, *p*(West) = 0.346, *p*(Central) = 0.509, *p*(East) = 0.662, *p*(Total) = 0.0046. CNP = Collserola Natural Park; SLMO = Natural Park of Sant Llorenç del Munt i l'Obac; BCN = Barcelona; 'West' = additional sampling area southwest of Llobregat river; 'Central' = additional sampling area between SLMO and BCN/CNP; 'East' = additional sampling area Northeast of Besòs river.; "Total" = all samples combined.

was more likely than finding urban wild boars in rural locations (1.5%) (Table 1). The numbers of admixed individuals ( $0.5 < q < 0.9$ ) were significantly higher in the two urban locations ( $32/210 = 15.2\%$ ) than in the four rural ones ( $13/197 = 6.6\%$ ; Table 1). Moreover, the proportion of admixed wild boars was ten-fold and significantly ( $p < 0.05$ ) higher in wild boars sampled outside of their corresponding area (dispersers,  $18/28, 64.3\%$ ) than in those wild boars where sampling location corresponded to cluster assignment (residents,  $25/379, 6.6\%$ ). All urban dispersers in rural locations were admixed ( $3/3, 100\%$ ), whereas in the urban locations (BCN and CNP) 32% of the dispersers ( $8/25$ ) belonged to the rural cluster with  $q \geq 0.9$  (Table 1). Within the urban locations, the proportion of wild boars assigned to the urban cluster was significantly ( $p < 0.05$ ) higher in BCN ( $92/98, 93.9\%$ ) than in CNP ( $93/112, 83.0\%$ ). Conversely, the admixed wild boars were significantly ( $p < 0.05$ ) more frequent in CNP

( $23/112, 20.5\%$ ) than in BCN ( $9/98, 9.2\%$ ; Table 1). The STRUCTURE analyses for the urban and rural clusters (STRUCTURE run scenario S3), as well as for the three main sampling populations (CNP, BCN, and SLMO) (STRUCTURE run scenario S4), revealed weak indication ( $\Delta K = 95.44$ ) for an underlying structure only in CNP ( $N = 112$ ). This further supports the previously detected structure separating wild boars in the rural and urban clusters (Table SM6).

### 4. Discussion

This study sheds light on the colonisation process of the urban environment by wild boar and the effects of both synurbanisation and the associated management measures on the genetic composition of urban wild boar populations, defining the ecological relationship of wild boar with urban areas. The urban genetic cluster originated from dispersers from the neighbouring rural areas, as evidenced by the unidirectional gene flow from rural to urban areas, the lower genetic diversity of the urban populations, the higher number of rural dispersers in urban areas than urban dispersers in rural areas, and the higher proportion of admixed individuals in urban than in rural areas (Table 1). Although the admixed individuals in CNP and BCN indicated that hybridization and gene flow between both clusters occurs, such gene flow was not enough to prevent genetic differentiation between the urban and rural populations. Fragmentation of natural environments is a recognised cause of genetic differentiation. However, founder effect, source-sink dynamics, and/or lack of suitable habitat for dispersers related to urbanisation make synurbanisation a more powerful driver of genetic differentiation than habitat fragmentation (Wood and Pullin, 2002; Wandeler et al., 2003; Schell et al., 2020). The genetic differentiation of an urban and a rural cluster, the lower genetic diversity of the urban cluster, and the lower proportion of admixed wild boars and wild boars from the rural cluster in BCN indicate gene-flow barriers and genetic isolation. Altogether, they suggest that the urban wild boar population in the MAB was

**Table 3**  
Population pairwise distances ( $F_{ST}$ ,  $R_{ST}$ ) among the six wild boar populations sampled in the Metropolitan area of Barcelona (MAB) and its rural surroundings.

Area	CNP	SLMO	BCN	'West'	'Central'	'East'
CNP	-	<u>0.108</u>	<b>0.006</b>	0.133	0.098	0.201
SLMO	0.09	-	0.145	0.043	0.016	0.086
BCN	<b>0.003</b>	0.124	-	<u>0.169</u>	0.138	<b>0.256</b>
'West'	0.11	0.021	0.147	-	0.038	0.089
'Central'	0.074	0.008	0.106	0.026	-	0.078
'East'	0.16	0.042	<b>0.227</b>	0.031	0.041	-

Above diagonal:  $F_{ST}$  (based on number of different alleles), below diagonal:  $R_{ST}$  (based on sum of squared size differences of alleles). Underlined values indicate significant differentiation ( $p < 0.05$ ). Bold: highest and lowest value in the corresponding comparison. CNP = Collserola Natural Park; SLMO = Natural Park of Sant Llorenç del Munt i l'Obac; BCN = City of Barcelona; 'West' = additional sampling area southwest of Llobregat river; 'Central' = additional sampling area between SLMO and BCN/CNP; 'East' = additional sampling area Northeast of Besòs river.

**Table 4**  
Molecular indices per cluster (rural, urban) with and without admixed individuals for the wild boar populations sampled in the Metropolitan area of Barcelona (MAB) and its rural surroundings.

Cluster	N	N <sub>A</sub> <sup>*</sup>	N <sub>A</sub>	A <sub>R</sub>	H <sub>obs</sub> (SD)	H <sub>exp</sub> (SD)	HWE (p)	F <sub>IS</sub>
Rural	192	116	6.82	6.82	0.560 (0.202)	0.590 (0.203)	+(0.225)	0.051
Rural (with admixed <sup>†</sup> )	219	119	7.00	6.99	0.564 (0.200)	0.590 (0.200)	+(0.248)	0.043
Urban	170	61	3.81	3.65	0.451 (0.179)	0.451 (0.177)	+(0.421)	-0.001
Urban (with admixed <sup>†</sup> )	188	67	4.12	3.99	0.462 (0.175)	0.459 (0.172)	+(0.418)	-0.006

<sup>†</sup>: admixed individuals with proportional membership in the indicated cluster of 0.90 > q > 0.50; N: number of individuals; N<sub>A</sub><sup>\*</sup>: total number of alleles; N<sub>A</sub>: average number of alleles across loci; A<sub>R</sub>: allelic richness; H<sub>obs</sub>: observed heterozygosity; H<sub>exp</sub>: expected heterozygosity; SD: standard deviation, HWE: Hardy-Weinberg equilibrium; + : individuals in cluster are at HWE; p: values were calculated across all 17 loci. F<sub>IS</sub>: inbreeding coefficient.

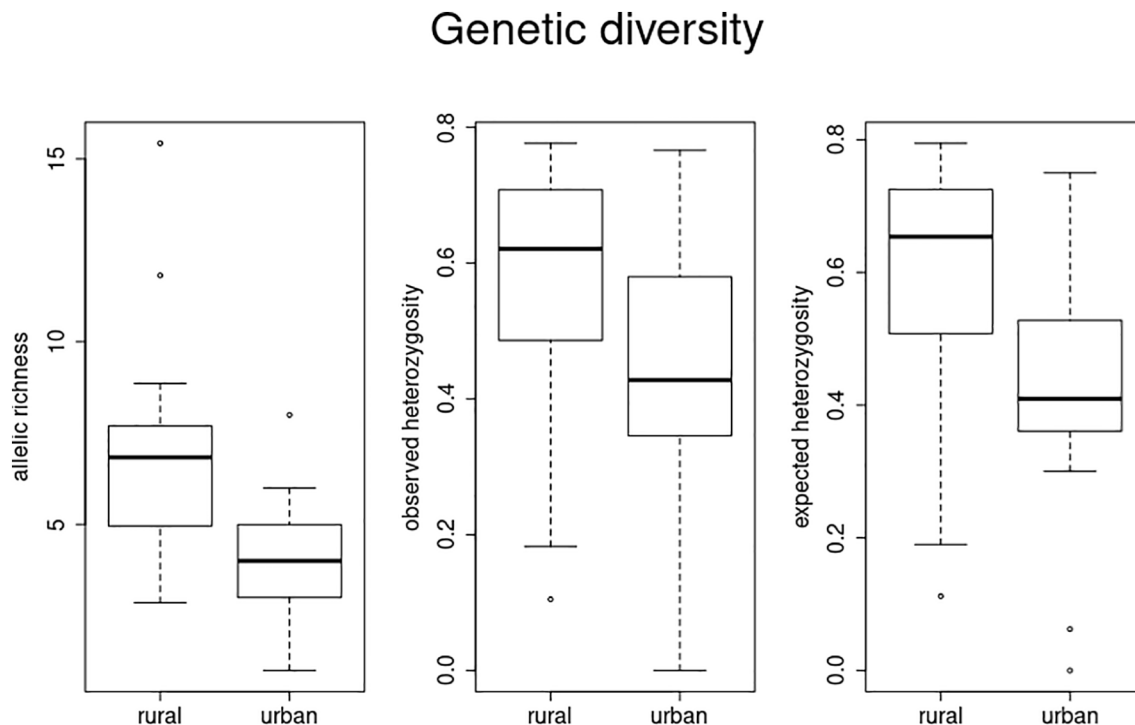
established through a founder effect, supporting the UIH (Gloor, 2002; Wandeler et al., 2003), as previously reported for other urban wild boar populations (Stillfried et al., 2017b; Zsolnai et al., 2022).

Once established in the urban and peri-urban areas, both urbanisation and the associated management measures shape the phenotypic changes and genetic structure of synurbic populations (Rodewald and Gehrt, 2014; Schell et al., 2020). The higher proportions of admixed (20.5%) and rural (17.0%) wild boars in CNP as compared to BCN (9.2% and 6.1%, respectively; Table 1) suggest that admixture between clusters likely occurs in CNP rather than in BCN, with bolder individuals from the peri-urban CNP daring to explore the urban area of BCN (Castillo-Contreras et al., 2018), as reported for urban wild boar and deer (Stillfried et al., 2017c; Honda et al., 2018; Zsolnai et al., 2022). This corresponds to a source-to-sink dynamics from peri-urban CNP to urban BCN as described by the PPH (Pulliam, 1988; Delibes et al., 2001).

Little ( $F_{ST} = 0.006$ ,  $p < 0.05$ ; number of migrants based on  $F_{ST} \sim 41$ ) to no genetic differentiation ( $R_{ST} = 0.003$ ,  $p > 0.05$ ; number of migrants based on  $R_{ST} \sim 82$ ), as previously defined (Hartl and Clark, 1997), was found between the urban wild boars in CNP and BCN (Fig. 1, Tables 3 and SM5), although wild boars from BCN have been reported to grow faster and to be heavier and bigger than their conspecifics from CNP (Castillo-Contreras et al., 2021).

This phenotypic changes could be the result of transgenerational plasticity (TGP), whereby the phenotype of a generation (in this case, bolder behaviour, consumption of anthropogenic food resources, and faster growth and weight gain) is influenced by the environment experienced by former generations (Bell and Hellmann, 2019). Although a genetic basis has been proposed for the changes observed in urban wild boar populations (Zsolnai et al., 2022), plasticity can overcome the constraints of adaptive genetic change by decoupling the genotype from phenotype (Bonduriansky and Day, 2009), and TGP can promote population persistence over generations in changing environments (Jablonka et al., 1995; Pal, 1998; West-Eberhard, 2003; Weyrich et al., 2019). Therefore, disentangling whether changes in phenotypic traits are due to phenotypic plasticity or genetic changes is essential in urban evolutionary ecology (Alberti et al., 2017; Ouyang et al., 2018; Schell, 2018) and to devise the most effective management strategy (Lambert and Donihue, 2020). If these urban phenotypic traits have a genetic basis, the management actions against them will be more efficient than if they are explained by TGP. The genetic structure of peri-urban and urban wild boar populations in this study and the phenotypic changes previously reported (Castillo-Contreras et al., 2021), do not only allow to understand the synurbisation process of wild boar, but emphasize the need of future epigenetic studies to further clarify the relationship between phenotypic changes, genetic changes and potential TGP.

The phenotypic changes observed in the urban wild boars from BCN (Castillo-Contreras et al., 2021) without genetic differentiation from adjacent populations correspond to a situation of frequent conflict with moderate management measures, which exert a selection pressure on bolder phenotypes but do not eradicate the species from the area (Stillfried et al.,



**Fig. 3.** Allelic richness, expected heterozygosity and observed heterozygosity of the wild boars from the rural and urban clusters sampled in the Metropolitan Area of Barcelona and its rural surroundings.

2017c; Honda et al., 2018; Schell et al., 2020). In Barcelona, conflictive urban wild boars are individually removed (Castillo-Contreras et al., 2016), which may act as a management selection driver (Honda et al., 2018; Schell et al., 2020). However, culled individuals might be replaced by the next boldest individuals from the CNP population (Found and St. Clair, 2019), where removal pressure is lower than in all other rural and urban areas due to legal and practical limitations, respectively (Minuartia, 2020). Nevertheless, if the bolder behaviour of BCN wild boars is a phenotypic trait derived from TGP (Bell and Hellmann, 2019), the removal of conflictive individuals could break up such TGP, taking some time for the plastic behaviour to reappear and spread among the wild boar population.

Altogether, the assignment of peri-urban and urban wild boars in a common genetic cluster with a source-to-sink dynamics between both locations, and the behavioural adaptations of urban wild boars to the urban environment, selecting for anthropogenic food resources (Castillo-Contreras et al., 2021) and with a bolder behaviour (Stillfried et al., 2017c), classify wild boar as an urban exploiter species (Rodewald and Gehrt, 2014).

Wildlife responses to urbanisation are associated to landscape-scale differences among cities, causing within-city and inter-city differences. Thus, city structure (including the density of human households and the proportion and distribution of green areas within the urban landscape) conditions both wildlife occupancy and population demography (Fidino et al., 2021). Forested areas (e.g. CNP) allow the establishment of island populations (UIH) as a transition from rural habitats, whereas purely built-up areas act as an attractive sink, mainly because of anthropogenic food resources (Castillo-Contreras et al., 2018 and 2021; Delibes et al., 2001; Stillfried et al., 2017b) and probably a higher mortality rate. Connectivity among forested areas determines gene flow and genetic structure (Munshi-South, 2012). Thus, the distribution and connectivity of green areas in cities determine the existence of different genetic clusters in urban wild boar populations, which evolve by spatial isolation and genetic drift (Stillfried et al., 2017b; Wandeler et al., 2003; Zsolnai et al., 2022). In BCN the single urban wild boar cluster corresponds to the single green area concentrated in CNP. The connectivity among the rural areas surrounding the MAB, as indicated by the little genetic differentiation among them (Fig. 1), suggests that rivers do not pose a barrier for gene flow among wild boar populations, agreeing with previous reports (Frantz et al., 2012; Leaper et al., 1999). However, the urban structures around both rivers could limit gene flow between the rural areas beyond them and the urban locations in the MAB, closer to the city (Fig. 1, Tables 3 and SM5), as suggested by the genetic differentiation between the areas beyond the rivers and the urban areas, as previously reported (Zsolnai et al., 2022). When disentangling the effects of urban areas and rivers in preventing connectivity among urban and peri-urban wild boar populations, the type of waterflow (i.e., Mediterranean stream or Central European river) and the urban structure (presence of forested patches and connectivity among them) must be considered. Further monitoring of the wild boar populations in the MAB (even non-invasively; Fickel and Hohmann, 2006) should contribute to monitor the colonisation of the urban area and potential genetic drift or clustering in other forested patches.

## 5. Conclusion

The wild boar population of Barcelona originated from founder individuals from the surrounding rural areas. The limited gene flow between the founders and their descendants, on the one hand, and the source population, on the other, led to the differentiation of the wild boar population in two genetically distinct clusters, a rural and an urban one. As the degree of admixture between both clusters was higher in the peri-urban area than in the urban area, wild boar can be classified as an urban exploiter species. The presence of suitable habitat patches in the urban area and the connectivity both among them and with the source rural population determines the gene flow among urban and rural populations and the future genetic differentiation in one or more urban wild boar clusters.

Urban environment and population management act as phenotypic and genetic evolution drivers on urban wild boar populations, which have reduced genetic variability due to the bottleneck related to the founder effect. As a consequence, urban wild boar populations differentiate quickly both phenotypically and genetically from rural populations, with lower genetic variability and reduced allelic richness. The intensity of such differentiation depends on the strength of genetic drift combined with the strength of selection pressure posed by the urban environment and the associated management measures. The relation between the species phenotypic plasticity (including TGP) and the selection pressure exerted by the urban environment and the management measures will determine the responses of the urban wildlife population, ranging from the establishment of urban populations phenotypically and/or genetically different from rural populations to the eradication of the population through high management pressure.

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## CRediT authorship contribution statement

**Justus Hagemann:** Formal analysis, Investigation, Methodology, Software, Writing – original draft.

**Carles Conejero:** Data curation, Investigation, Methodology, Writing – review & editing.

**Milena Stillfried:** Conceptualization, Formal analysis, Methodology, Writing – review & editing.

**Gregorio Mentaberre:** Data curation, Funding acquisition, Investigation, Methodology, Writing – review & editing.

**Raquel Castillo-Contreras:** Data curation, Investigation, Methodology, Writing – review & editing.

**Jörns Fickel:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing – review & editing.

**Jorge Ramón López-Olvera:** Conceptualization, Data curation, Funding acquisition, Investigation, Project administration, Resources, Supervision, Validation, Visualization, Roles/Writing – original draft.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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