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Short Note

Genetic relatedness affects socio-spatial organization in a solitary carnivore, the European pine marten

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Abstract

Genetic relatedness among individuals can play a major role in the socio-spatial organization of mammals. However, only a few studies have documented kin-related spatial pattern in solitary medium-sized carnivores. Using radiotelemetry and genetic data from 14 individuals (six males and eight females), we investigated the relationship between kinship and spatial organization (distance and overlap between home ranges) in the European pine marten *Martes martes* in a wooded area of central Italy. Data analysis included 36 pairs (9 individuals) tracked in spring and summer and 55 pairs (11 individuals) tracked in autumn and winter. Kinship, distance between home ranges and proportion of home range overlap were also compared among inter- and intra-sexual pairs. Spatial distribution of pine martens was affected by genetic relatedness, so that individuals living in close proximity or occupying the same area were also more likely to be closely related. Female pairs had a higher proportion of overlap of their home and core ranges than male pairs. Male pairs were also less related than female and inter-sexual pairs, showing a pattern of space use consistent with male-biased dispersal and female philopatry. Most of the females that overlapped their home and core ranges were genetically related, while only a third of the few male pairs occupying the same territories were kin. However, our results seem to indicate that positive association between home range overlap and relatedness was mainly a consequence of common female philopatry rather than kin-biased tolerance. Male-female pairs had the highest percentage of related individuals overlapping their home and core ranges, suggesting that spatial segregation is unlikely to play a role in preventing encounters between kin.

Identification of factors that affect the socio-spatial structure of natural populations is fundamental for a better understanding of the ecology and evolutionary biology of a species. In mammals, spatial distribution of individuals depends on habitat quality and features, population density as well as kin-related factors including social organization, patterns of dispersal and philopatry (Waser, 1988; Gompper and Wayne, 1996). Genetic relatedness among individuals can therefore play a major role in the socio-spatial organization of populations.

Kin-related spatial structure has been documented in several species of mammals. In particular, relationships between kinship and space use patterns have been described mainly for group-living species (e.g. Gompper and Wayne, 1996; Armitage et al., 2011). Solitary mammals are expected to be less prone to interact with related individuals, however few data are available on the role that kinship may play in shaping the spatial pattern in populations of non-gregarious mammals (Schmidt et al., 2016). Population genetics can provide estimates of relatedness and allow the investigation of patterns of socio-spatial organization that would be otherwise difficult to study, because of the elusive nature of solitary species. Molecular genetic techniques have been used to study the influence of genetic relatedness on spatial organization in solitary large carnivores (Støen et al., 2005; Moyer et al., 2006; Janečka et al., 2007; Schmidt et al., 2016). However, only a few studies have documented kin-related spatial pattern in medium-sized carnivores (Ratnayeke et al., 2002).

The European pine marten *Martes martes* is a medium-size solitary carnivore with a socio-spatial organization structured around the intra-sexual exclusivity and inter-sexual overlap of home ranges, such that

female ranges are determined by resources availability whereas male ranges by female availability (Powell, 1994). However, such spatial organization may in turn depend on resource abundance, habitat conditions and population density (Powell, 1994). Although several studies investigated the effects of environmental and demographic factors on pine marten spatial distribution, to our knowledge there are no published data on the relationships between kinship and spacing pattern in *M. martes*. The aim of our work was to test the hypothesis that genetic relatedness affects the socio-spatial distribution of pine martens in a wooded area of central Italy.

The study was conducted in La Selva Forest (43°13' N, 11°4' E), a deciduous woodland of the sub-Mediterranean zone (see Bartolommei et al., 2016 for details). The study area covers 22 km². Estimated pine marten population density in 2009 was 3.4 individuals per 10 km² (Manzo et al., 2012). A total of 14 adult individuals (six males and eight females) were radio-tracked from January 2008 to March 2013. Pine martens were live-trapped, marked and tracked as described in Bartolommei et al. (2016). Hair samples were collected and later stored at -80 °C for genetic identification of the species (see Bartolommei et al., 2014) and assessment of genetic relatedness between individuals.

Seasonal home ranges (spring-summer: 21 March – 20 September; autumn-winter: 21 September – 20 March) were calculated using the 95% and 50% fixed kernel (FK) density estimator with least-squares cross-validation (Seaman and Powell, 1996). We decided to use the kernel estimator to obtain reliable home range estimates, as we were able to collect a relatively large number of fixes per season per individual, i.e. $n \geq 45$ (Seaman and Powell, 1996). For each pair of individuals we assessed the proportion of overlap between home ranges and calculated the distance between home ranges as the linear distance between their

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arithmetic centres. We used these measures as indices of geographic dispersion. Overlap between home ranges of individual pairs (A and B) was calculated as: $\frac{2 \times (\text{area of overlap of A and B})}{(\text{home range area of A} + \text{home range area of B})}$. We assessed allelic variation at 12 out of 13 species-specific microsatellite loci as described in Natali et al. (2010). Average number of alleles (N_a) per locus was 5.5 ± 0.5 SE (range: from 3 to 8 alleles). Locus Mar15 was not included in the analysis due to the low level of polymorphism recorded in our sample set ($N_a=2$). Average polymorphic information content (Botstein et al., 1980) was 0.59 ± 0.03 SE. Alleles were scored using a multiple-tubes approach (Taberlet et al., 1996). That is a highly reliable method routinely used when non-invasive biological samples such as hair or scats are likely to result in small amount of degraded DNA (e.g. Boggiano et al., 2013). According to this technique, heterozygotes were defined if alleles appeared at least twice after three polymerase chain reaction (PCR) replicates. Samples were instead scored as homozygotes after eight positive and consistent PCR amplifications of the same allele. Genotyping errors due to false alleles, allelic dropout or locus-specific errors were assessed using the bimodal test and the difference in capture history (DCH) test implemented in Dropout (McKelvey and Schwartz, 2005). We used GenAEx 6.501 (Peakall and Smouse, 2012) to derive consensus genotypes from different PCRs of the same sample, estimate probability of identity, identify identical genotypes, calculate observed and expected heterozygosity and estimate the fixation index F_{IS} . The probability that two unrelated individuals, drawn at random from a population, had the same multilocus genotype was $PI=1.3 \times 10^{-10}$, while the same probability for full siblings was $PI_{sibs}=7.6 \times 10^{-5}$. When the number of individual genotypes was plotted against the number of loci distinguishing each pair of individual genotypes, the bimodality test showed a minimal separation distribution with a unimodal mode for samples differing at four loci. There were no multilocus genotypes differing by less than three loci, suggesting that our sample set contained no genotypic errors and all samples belonged to different individuals. The DCH test identifies the number of loci such that PI and PI_{sibs} are very small and all individuals should therefore be uniquely distinguished. A list of unique individuals is then produced and compared to the number of unique individuals generated through adding additional loci or changing the composition of loci. No new individuals were generated through this process indicating that no locus had significantly more errors than any other locus. Average observed and expected heterozygosities were 0.64 ± 0.04 SE and 0.67 ± 0.3 SE, respectively. Fixation index was $F_{IS}=0.042$. Deviation from Hardy-Weinberg (HW) equilibrium was assessed using Genepop 4.2.1 (Rousset, 2008). No departure from HW proportions was recorded for either the studied population ($p=0.108$) or any of the 12 loci after Bonferroni correction for multiple comparisons ($p>0.004$). We used Monte Carlo simulations implemented in Coancestry (Wang, 2011) to generate, from known allele frequencies, a set of multilocus genotypes consisting of 100 pairs each with seven relatedness values equal to 1) parent-offspring, 2) fullsib, 3) halfsib, avuncular (any of the four combinations of aunt-nephew, aunt-niece, uncle-nephew, uncle-niece), grandparent-grandchild, 4) double first cousin, 5) first cousin, 6) second cousin and 7) unrelated individuals. These genotypes were comparatively analysed by two maximum likelihood estimators (Milligan, 2003; Wang, 2007) and five moment relatedness estimators (Lynch, 1988; Lynche and Ritland, 1999; Queller and Goodnight, 1989; Ritland, 1996; Wang, 2002). The Queller and Goodnight (1989) index (R_{xy} =twice the probability that an allele from individual x is identical by descent to an allele from individual y) yielded the highest correlation to the true relatedness value of the 100 simulated dyads and was selected for subsequent pairwise relatedness analyses. We also assessed whether pine martens were more related than expected in a random sample from a panmictic group of individuals by comparing the distribution of pairwise relatedness coefficients with a null distribution obtained after 10^4 permutations of genotypes using a Monte Carlo resampling procedure implemented in Identix 1.1 (Belkhir et al., 2002).

The socio-spatial organization of pine martens (distance and overlap between home ranges) was analysed as function of the degree of kinship (R_{xy}) of each individual pair tracked during the same season ($n=91$).

Table 1 – Parameter estimates (β) for GLMs of overlap and distance between home ranges and distance between core areas of pine marten pairs. For the categorical factors (i.e. season and sex of pairs) spring-summer season and male-male pair were chosen as the reference category. Estimate and standard error (SE) are reported for intercept and covariates. Statistically significant p values are indicated in bold.

Home range distance	β	SE	p
(Intercept)	7.205	0.168	<0.001
Kinship	-1.352	0.447	0.003
Season: Aut-Win ^a	-0.235	0.118	0.049
Pair sex: female-female	0.259	0.193	0.184
Pair sex: male-female	0.186	0.180	0.304
Core area distance			
(Intercept)	7.076	0.178	<0.001
Kinship	-1.184	0.472	0.01
Season: Aut-Win ^a	-0.399	0.124	0.01
Pair sex: female-female	0.354	0.204	0.295
Pair sex: male-female	0.187	0.190	0.327
Home range overlap			
(Intercept)	0.106	0.071	0.140
Kinship	0.633	0.189	<0.001
Season: Aut-Win ^a	0.026	0.050	0.601
Pair sex: female-female	0.048	0.082	0.552
Pair sex: male-female	0.016	0.076	0.834

^a Aut-Win: autumn-winter season.

Data analysis included 36 pairs (9 individuals) tracked in spring and summer and 55 pairs (11 individuals) tracked in autumn and winter. The distance between home range centres (95% and 50% FK) was modelled using generalized linear models (GLM) with Gamma distribution (log link). The mean overlap between home ranges (95% FK) was modelled using GLM with normal distribution (identity link). The mean overlap between core areas (50% FK) was excluded from the analysis because of the low number of overlaps recorded. Predictor variables of the GLMs included: pairwise kinship, season (spring-summer and autumn-winter) and sex of individual pairs (male-male, female-female, male-female). Kinship, distance between home ranges and proportion of home range overlap were compared between intrasexual pairs (male-male vs female-female) and between inter- and intra-sexual pairs (male-male vs male-female; female-female vs male-female) using Student's t -test for independent samples. Statistical analyses were performed using R (R Core Team, 2015, release 3.2.2), while spatial data were processed using ArcView 9.2 (ESRI) with the Animal Movement, Home Range and XTool extensions.

Spatial analysis was based on a total of 2235 locations. Average home range distance of marten pairs was 1257 ± 110 SE m (range: 49–3002 m) and average distance of core areas was 1062 ± 112 SE m (range: 37–3232 m). Average overlap between home ranges was 21% (from 0% to 74% overlap) and average overlap between core areas was 8% (from 0% to 62% overlap). Mean kinship between pine martens ($R_{xy}=0.098 \pm 0.013$ SE) was lower than values expected for first cousins ($R_{xy}=0.125$) and higher than kinship values for second cousins ($R_{xy}=0.031$). Moreover, average relatedness did not differ significantly from the value expected in a panmictic population ($p>0.05$).

Proximity between home ranges and core areas of pine martens increased with the degree of kinship (Tab.1). Distance between core areas was also influenced by season, meaning that martens increased the proximity between their centres of activity during autumn-winter season (Tab.1). The degree of kinship had also a significant effect on home range overlap, which increased with genetic relatedness between individuals (Tab.1). Season and sex had no effect on either distance or overlap between home ranges (Tab.1), suggesting that the spatial distribution of martens was primarily affected by genetic relatedness.

Female pairs ($n=31$) overlapped their home and core ranges more than male pairs ($n=12$) ($df=41$, $t=-1.24$, $p=0.009$ for 95% FK; $df=30$, $t=-3.33$, $p=0.002$ for 50% FK). No differences were recorded in the degree of overlap between intra and inter-sexual pairs ($n=48$). Sixty one percent and 32% of female pairs overlapped their home ranges and core areas, respectively. Among these, 63% and 50%, respectively were genetically related ($R_{xy}>0$). As for male pairs, no overlap of core areas was recorded, and only 33% of pairs overlapped their home

ranges. Of these, 20% were related. Forty six percent and 17% of male-female pairs overlapped their home ranges and core areas, respectively. Among these, 68% and 75%, respectively were genetically related.

Relatedness between males was lower than that between females ($df=35$, $t=-3.71$, $p=0.001$) and of inter-sexual pairs ($df=57$, $t=-4.04$, $p<0.001$). The degree of kinship did not differ between female pairs and pairs of opposite sex. Overall, 65% of female pairs and 50% of inter-sexual pairs had mean kinship (0.199 ± 0.028 SE and 0.196 ± 0.014 SE respectively) between values expected for first cousins ($R_{xy}=0.125$) and for half-siblings, avuncular (any of the four combinations of aunt-nephew, aunt-niece, uncle-nephew, uncle-niece) and grandparents-grandchildren ($R_{xy}=0.25$). Maximum kinship (recorded in 35% of related females pairs and in 20% of related inter-sexual pairs) matched expected values for parents-offspring and full-siblings pairs ($R_{xy}=0.5$). The proportion of related individuals in male pairs was 33%, with average relatedness (0.041 ± 0.017 SE) comparable to that expected for second cousins. Maximum kinship in male pairs was $R_{xy}=0.25$ and it was recorded in 20% of related pairs.

Genetic and radio-tracking data from this study suggested that spatial distribution of European pine martens was affected by genetic relatedness, so that individuals that lived in greater proximity or occupied the same area were also more likely to be closely related. The effect of kinship on spatial organization of mammals has already been described in solitary species, including carnivores (Ratnayeke et al., 2002; Støen et al., 2005; Moyer et al., 2006; Janečka et al., 2007; Schmidt et al., 2016). In several species of mammals, females are more likely to be philopatric than males, so that sex-biased dispersal would result in higher relatedness between pairs of females and a shorter distance between their home ranges. In pine martens, although sex had no effect on distance between home ranges, female pairs had a higher proportion of overlap of their home and core ranges than male pairs. Male pairs were also less related than female and inter-sexual pairs, showing a pattern of space use consistent with male-biased dispersal and female philopatry as for other medium size solitary carnivores (e.g. raccoon *Procyon lotor*, Ratnayeke et al., 2002). However, kinship of intersexual pairs was comparable to that of females, suggesting that a number of males were highly related to some of the adult females. Male dispersal may therefore not always be the rule, as some adult males may remain in or close to their natal area (Ratnayeke et al., 2002).

Most of the females that overlapped their home ranges and the half with overlapped core areas were genetically related, while only 20% of the few male pairs occupying the same territories were kin. However, female pine martens with either high or low relatedness values had >50% home range overlap. A similar pattern was found in raccoon, for which a positive association between home range overlap and relatedness was considered a consequence of common female philopatry rather than kin-biased tolerance (Ratnayeke et al., 2002). It is largely unknown whether solitary mammals choose conspecifics on the basis of relatedness, however it is commonly thought that opportunities to recognise kin are limited. Kin recognition, as well as philopatry and dispersal patterns are considered to be part of inbreeding avoidance mechanism (Schmidt et al., 2016). However, the social structure of solitary mammals is based on individuals having a low chance of encountering conspecifics. Therefore, even if female philopatry occurs, it may have different consequences in terms of inbreeding avoidance than in gregarious species. In this study, male-female pairs had the highest percentage of related individuals overlapping their home and core ranges. Thus, although we have not monitored all individuals simultaneously, our results seem to indicate that spatial segregation is unlikely to play a role in preventing encounters between kin. Moreover, the acquisition of a territory may be a priority over the need of avoiding inbreeding.

There may be several factors involved in the relationship between relatedness and spatial organization, such as population density, home range size and fidelity, population turnover, mating strategies, risk of dispersal as well as quality and distribution of available habitat (Waser, 1988; Gompper and Wayne, 1996). Population density and spatial behaviour of pine martens as well as abundance of main prey (Bartolommei et al., 2016) suggested that the sub-Mediterranean deciduous

oak forest of our study area is a suitable habitat for *M. martes*. However, we do not know the actual role played by these factors at affecting kin-related spatial organization observed in our pine marten population. Nevertheless, our results highlight that individuals with a higher kinship degree tend to distribute closer to each other. Long-term and large-scale studies combining field data with molecular techniques are necessary to obtain further insight into kin-based spatial organization of solitary carnivores. ☞

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