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NONKIN ASSOCIATIONS IN WILD BOAR SOCIAL UNITS

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We investigated the social organization of wild boars (*Sus scrofa*) using genetic and spatial data from a study population in Tuscany, Italy. In total, 120 wild boars of different sexes and age classes were captured and monitored from 2002 to 2006. All of them were genetically analyzed by using 10 polymorphic microsatellites ($H_E = 0.693$, $k = 6.6$) and a matrix of pairwise relatedness was calculated. In addition, a reference sample of fully related individuals was created by genotyping 11 adult females and their fetuses ($n = 56$). Spatial data were gathered for 65 animals that had been fitted with either radiocollars or ear transmitters. Sixteen social units were identified by capture data and confirmed by observations and telemetry. A correlation between interindividual spatial distance and relatedness was observed only in summer–early autumn and seemed to be associated to the presence of piglets. The prediction of matrilinearity in wild boar social units was not confirmed, because a low degree of relatedness among boars was observed within groups. Aggregations of unrelated adult females (with their litters) were detected in the study population. The high turnover in the population due to human-caused mortality seems to be the main factor responsible for this altered social structure. Accordingly, we suggest that the observed social organization would result from grouping of unrelated survivors that is promoted by the presence of wolves in the area.

Key words: genetic relatedness, microsatellites, social structure, *Sus scrofa*, wild boar

Gene dynamics within a population are strongly influenced by breeding system, social structure, and dispersal patterns (Apollonio and Hartl 1993; Chepko-Sade and Tang Halpin 1987; Storz 1999). Polygynous breeding and female philopatry are the rule in mammals, and a huge variation can be observed in their social structures, ranging from primarily solitary to highly social (Eisenberg and Kleiman 1983). Social systems also may differ from population to population, as a response to different ecological constraints and management practices (Lott 1991; Pope 1998). In any case, this variation has a profound impact on the genetic features of populations (Dobson 1998; Storz 1999). Conversely, the study of the genetic structure of a population at a fine scale can prove helpful in describing its social organization (Sugg et al. 1996). In particular, knowledge of genetic relationships among individuals in a population can disclose hidden social interactions that are important to fully understand the behavioral ecology of the species (e.g., nonkin-based cooperation—Blundell et al. 2004).

The application of molecular techniques provides a tool to establish kin relationships within a population, thus enabling evaluation of multiple hypotheses in relation to the spatial and social organization of the species under study. Examination of molecular data has revealed close spatial associations among kin in several mammal species including the Florida black bear (*Ursus americanus floridanus*—Moyer et al. 2006), raccoons (*Procyon lotor*—Ratnayeke et al. 2002), gray mouse lemurs (*Microcebus murinus*—Wimmer et al. 2002), and African lions (*Panthera leo*—Spong and Creel 2004). However, the hypothesis that relatedness influences spatial organization in mammals has not always been confirmed, such as in studies on snowshoe hares (*Lepus americanus*—Burton and Krebs 2003), white-tailed deer (*Odocoileus virginianus*—Comer et al. 2005), and bobcats (*Lynx rufus*—Janečka et al. 2006).

The wild boar (*Sus scrofa*) is an important wildlife species, in both economical and ecological terms. Its widespread recovery across Europe during the last 50 years has raised concerns about management of this species, which is considered a pest by some and a resource by others. Effective management strategies should take into account several aspects of this species' biology, its social behavior being one of the most important.

The social organization of wild boars is centered around philopatric adult females, which are facultative cooperative breeders. According to Briedermann (1986), the basic social unit is a matrilineal group, with 1 or more related adult females, and 1 or more cohorts of offspring. After weaning,

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most females stay with their mothers, and only about 20% of yearling females leave the natal group and disperse (Kaminski et al. 2005). Once yearling females have achieved the appropriate growth condition, they are likely to reproduce while still in the social group with their mother (Kaminski et al. 2005). However, genealogical relationships in female groups have been poorly investigated thus far and deviations from this commonly accepted scheme have been rarely documented (see Gabor et al. 1999).

Female wild boars typically maintain long-term fidelity to relatively small home ranges (Spitz and Janeau 1990), and a high percentage of adjacent females exhibit overlapping home ranges (Boitani et al. 1994). Accordingly, one would expect overlapping home ranges to reflect a common female lineage, and genetic relatedness should be inversely correlated with the spatial distance between individuals.

In comparison to other ungulates, wild boars are characterized by several peculiarities such as very high reproductive output (3–6 piglets per litter), early reproduction in females, and a weak mother–offspring bond (Carranza 1996; Cousse et al. 1994; Kaminski et al. 2005). These features obviously affect their social structure, influencing both the size and the composition of social groups, and the duration of interindividual associations. In addition, the organization of wild boar groups can vary temporally, with splitting into subgroups and merging of subgroups occurring frequently in a population (Gabor et al. 1999; Kaminski et al. 2005). Importantly, however, the role of demographic and extrinsic factors (e.g., hunting) possibly affecting both the composition and the stability of social groups has not been systematically investigated.

The primary objective of this study was to evaluate the nature of wild boar associations in relation to the genetic relatedness among individuals. We 1st verified the correlation between geographic and genetic distance among individuals in a population, and then considered the spatial behavior of social units in relation to their composition and the intragroup degree of relatedness. Specifically, we addressed the following questions: Is the geographic distance between individuals inversely correlated to their genetic relatedness? Is genetic relatedness higher for individuals belonging to the same social unit than for individuals belonging to different social units? Are all adult females in a social group close relatives (mother–offspring or full sisters)?

Materials and Methods

Study area.—The study was carried out in the Alpe di Catenaia, a 12,000-ha mountainous area along the Apennines in Tuscany, Italy (43°48′N, 11°49′E). The area includes a natural reserve (2,730 ha) and nearby zones that are open to hunting (Fig. 1). Elevation ranges from 490 to 1,414 m above sea level; the climate is temperate, with hot, dry summers, and cold, rainy winters. Most of the study area (85%) is covered by forests, whereas the remaining 15% consists of scrubland, cultivated areas, orchards, vineyards, olive groves, and human settlements. The only other wild ungulate species in the area is roe deer (*Capreolus capreolus*), and wolves (*Canis lupus*) are the only predator. Wild boars represented the staple prey item for wolves in this area (Mattioli et al. 1995, 2004), and a wolf pack of 5 or 6 individuals established its territory and maintained a presence in the area throughout the study period.

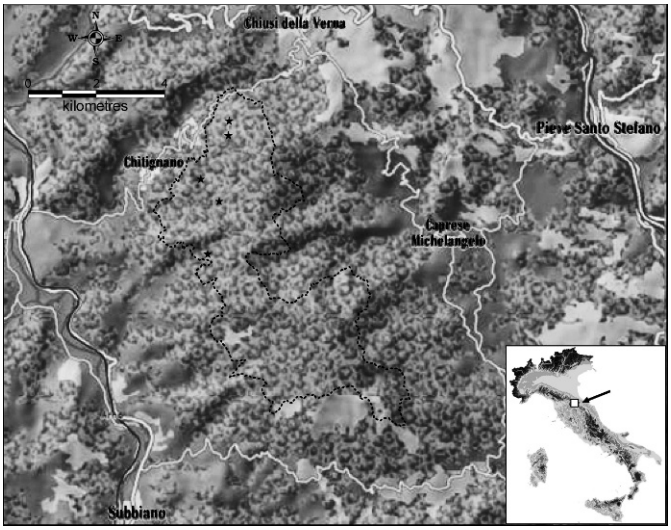

Fig. 1.—Study area in the Alpe di Catenaia, Arezzo, Italy. Borders of the natural reserve (hatched line) and location of cage traps used to capture wild boars (*Sus scrofa*; asterisks) are shown.

The wild boar is a game species that is intensively hunted in Tuscany. Outside the protected area of the natural reserve, wild boars are managed by local hunters. Drive hunts with dogs are conducted from September to January, when 300–900 animals are legally killed in the area each year.

Animal captures, radiotracking, and group definition.—This study was carried out from spring 2002 to winter 2005–2006. Wild boars were captured by cage traps baited with maize, except in February–March when they were captured using a vertical drop net. Cage traps allowed for the simultaneous capture of up to 9 individuals per capture event. We determined sex of captured animals and classified them into 1 of 3 age classes: piglets (from birth to about 12 months; hereinafter referred to as PGL), yearlings (12–24 months old; YRL), or adults (>24 months). Upon capture, individuals were blindfolded, fitted with ear tags (Allflex, Northfield, Minnesota), weighed, and measured, and age was determined by teeth eruption and wear patterns (Briedermann 1986). Zoletil (Virbac SAS, Carros Cedex, France; 10 ml/10 kg) was used to immobilize relatively large animals (≥35 kg). Hair samples for genetic analyses were collected and stored in plastic envelopes at −20°C. Sixty-five wild boars were radiocollared. Thirty-one animals (≥30 kg) were fitted with TXV-10 radiocollars (Televilt, Lindesberg, Sweden), whereas 24 animals (<30 kg) were fitted with TXP-R ear transmitters (Televilt). The procedures we used in this work conform to all relevant Italian wildlife and animal welfare legislation, and meet guidelines approved by the American Society of Mammalogists (Gannon et al. 2007).

We determined locations of radiocollared animals by triangulation from 3 different reference points (White and Garrott 1990). A minimum of 8 locations per animal per month (range 8–14 locations) was collected. Locations were distributed uniformly over the day (discontinuous telemetry—Swihart and Slade 1985), with consecutive positions separated by $\geq$12 h. We plotted all locations onto a 1:10,000 digital map of the study area. We estimated the accuracy of locations by locating test transmitters that had been placed in different habitats within the study area (Harris et al. 1990). Error for positions was in the range of $\pm$100 m for fair signals within the study area.

Genetic analysis.—One hundred twenty captured wild boars were analyzed from hair (n = 99) or tissue (n = 21) samples. The 21 tissue samples were obtained from marked animals killed by hunters, or found dead in the study area. We also analyzed tissue reference samples from 11 pregnant females killed during the hunting season and their fetuses (from 4 to 6 fetuses per female). Total genomic DNA was extracted using GenElute Mammalian Genomic DNA Miniprep kit (Sigma-Aldrich, St. Louis, Missouri) for tissue samples and Instagene Matrix (Bio-Rad, Hercules, California) for hair samples, and kept at -20°C.

All individual animals were typed by a panel of 10 polymorphic microsatellites selected for the analysis: s090, s155, sw24, sw122, sw461, sw2021, sw2492, sw2496, sw2532, and IGF1 (details at http://www.thearkdb.org). Each polymerase chain reaction was performed in a 10-µl reaction volume, containing 3 µl of DNA solution, 0.5 U of *Taq* DNA polymerase (Euroclone, Siziano, Italy), 1$\times$ polymerase chain reaction buffer (Euroclone), 2.5 mM $MgCl_2$, 100 µM of each deoxynucleosite triphosphate, and 2 pmol of each primer. The forward primer of each pair was labeled with an ABI fluorescent dye (6-FAM, HEX, or TET; Applied Biosystems, Foster City, California). The amplification profile was set up with an initial step of denaturation at 95°C for 3 min, followed by 35 cycles of 92°C for 45 s, annealing temperature (52–65°C) for 45 s, and 72°C for 30 s. A further extension step of 72°C for 10 min concluded the reaction. Polymerase chain reaction–amplified microsatellite alleles were sized using capillary electrophoresis in an ABI PRISM 3100-Avant automatic sequencer (Applied Biosystems). GeneMapper software (Applied Biosystems) was used to analyze electrophoretic data.

Data analysis.—The program Ranges 6 (Kenward et al. 2003) was used to estimate monthly home ranges based on the 95% minimum convex polygon method (Southwood 1966). Minimum convex polygon home range was preferred over the kernel method because of the limited number of fixes available for animals each month (Kernohan et al. 2001). However, in calculating home-range centroids from fix locations, the kernel method was preferred over alternative methods (harmonic or arithmetic mean), because this method incorporates information on the density of locations.

Individual wild boars were partitioned into social units according to capture data, which were subsequently confirmed by observations and telemetry. We assumed that individuals captured together in the same trap or moving together when caught in the nets were part of a social unit. We took into account only those associations that were confirmed by visual observations or telemetry data during the 1st month after the animals' capture. To confirm subsequent groupings, we evaluated the concurrence and the distribution of locations of each pair of individuals over 1 month. Accordingly, we assumed that 2 individual wild boars were associated in a social unit during a specific month when >50% of their locations during a 2-h time period were closer than 500 m. The occurrence and composition of each social unit was checked each month from July 2002 to February 2006. Individual locations and home-range overlaps were visualized in ArcView GIS 3.2 (ESRI, Redlands, California).

We evaluated the level of genetic variability of the population based on observed heterozygosity (H_O) and expected heterozygosity (H_E), which were estimated by GenAlEx 6 (Peakall and Smouse 2005). GENEPOP 3.2 (Raymond and Rousset 1995) was used to estimate the inbreeding coefficient (F_{IS}; ranging between -1 and 1) and to test loci for Hardy–Weinberg equilibrium and linkage equilibrium. The sequential Bonferroni correction was applied to correct significance thresholds in case of multiple tests (Rice 1989).

We used GenAlEx to calculate a matrix of pairwise relatedness for all the sampled individuals in the population. As coefficient of relatedness, we chose the unbiased r_{xy} statistics introduced by Queller and Goodnight (1989). Pairwise r_{xy} values range from -1 to +1, with 0 indicating the relatedness in a random draw of alleles from the population. Theoretically, in a randomly mating population a relatedness value of 0.5 is expected for parent–offspring and full siblings. Actually, deviations from such expectation are common and this value may vary considerably (Queller and Goodnight 1989). The relatedness matrix was calculated for the data set including all available genotypes (n = 120). In addition, we obtained an empirical data set of fully related individuals (parent–offspring and full siblings) by genotyping 11 adult females killed during the hunting season, together with their fetuses (4–6 fetuses per female, n = 56). The distribution of r_{xy} values of these ''true'' family groups (n = 163 comparisons) was used as reference.

Our a priori prediction was that the geographic distance between individuals in the population would be inversely proportional to their genetic distance, based on the idea that closely related animals would either belong to the same social unit or occupy home ranges in relatively closer proximity than more distantly related animals. We tested this hypothesis by estimating the correlation between pairwise relatedness values and spatial distances among monthly home-range centroids in our sample of radiotagged animals (n = 65). This analysis was restricted to the period April 2003–March 2005, because this was the time span during which we had a fairly large sample size. To account for the lack of independence among pairwise values, we performed a Mantel test for matrix correspondence in GenAlEx, testing significance of the correlation coefficient by 9,999 random permutations (Smouse et al. 1986), and applied Bonferroni correction. Because piglets typically move

in close association with their mothers during their 1st year of life (Briedermann 1986), the inclusion of piglets in this analysis may obscure the effect of relatedness in the postweaning establishment of individual home ranges. Therefore, the Mantel test was repeated after removing from the matrix all comparisons that included piglets.

Moreover, we assessed the degree of relatedness between all the members of each social unit identified by field data. Accordingly, we classified each pairwise interaction over a monitored period into either ''group'' when the 2 individuals joined the same social unit for at least 1 month, or ''nongroup'' when the 2 individuals were never detected in the same social unit. We compared the 2 corresponding relatedness distributions between each other and to the reference sample, to evaluate the deviation from a state of full relatedness (i.e., from a theoretic r_{xy} value of 0.5). Finally, we explored levels of relatedness within groups including adult females (FAD), by comparing PGL-FAD, YRL-FAD, and FAD-FAD associations with those within reference families (mothers + fetuses). Likewise, we evaluated the possible composition of groups of subadults by comparing the relatedness in YRL-YRL associations with those obtained for PGL-PGL, and for the reference true siblings (fetuses from the same female). Two-sample randomization tests (10,000 iterations) were used in PopTools 2.7.5 (Hood 2006) to test for differences between means. Descriptive statistics and graphs were performed using SPSS version 13.0 (SPSS Inc., Chicago, Illinois). Inferring kinship from relatedness coefficients can be misleading, because results can deviate from actual pedigree relationships (Blouin 2003; Csillery et al. 2006; Van Horn et al. 2008). Nonetheless, Van Horn et al. (2008) showed that the identification of unrelated dyads is poorly affected by errors, being the chance of misclassification higher at higher degrees of kinship. Accordingly, we used the program Kingroup 2 (Konovalov et al. 2004) to test the hypothesis of unrelatedness between pairs of adult females. The program uses a simulation routine to calculate a ratio between the likelihoods associated to 2 specific alternative hypotheses (e.g., unrelated versus full siblings). Using allele frequencies in the real population, Kingroup generates simulated distributions of r_{xy} for each of the kinship categories corresponding to the null hypothesis (e.g., unrelated) and the primary hypothesis (e.g., full siblings). From these distributions, it calculates the confidence threshold of the likelihood ratio (i.e., the values needed to reject the null hypothesis). We considered that 2 females were unrelated when, for a given pair, the null hypothesis of unrelatedness could not be rejected, whereas the alternative hypothesis (i.e., mother–offspring, full siblings, and half siblings) could confidently be discarded.

Results

Sample composition and spatial data.—We captured a total of 120 wild boars, 65 of which were fitted with radiotransmitters and radiotracked between 2002 and 2006. Sixteen social units were identified in our sample on the basis of capture and spatial associations. Mortality from hunting and poaching was high and caused 86% of the deaths of the study animals. Annual mortality amounted to 47% for adults, 75% for yearlings, and 48% for piglets (mortality in the first 2 months of life was not considered because very young piglets could not be radiotagged). Consequently, each single wild boar was monitored for an average of 8.6 months. In the 2-year period (April 2003–March 2005), the average number of monthly locations of each radiocollared individual was 8.3 ± 2.3 *SD* and a total of 4,546 radiolocations were obtained.

The composition of our sample in the 1st year (2003) differed markedly from that in the 2nd year (2004), especially in relation to the proportion of juveniles, which dropped from an average of 50% of the sample in 2003 to only 8% in 2004 (Fig. 2b). Because of this difference we treated the 2 years separately in the statistical analysis. Monthly home ranges differed between years as well, averaging 187.1 ha ± 209.6 *SD* in 2003 and 50.7 ± 65.1 ha in 2004 (Fig. 2c). Similarly, the mean overlap between home ranges was twice as high in 2003 compared to 2004 (30.0% versus 15.6%; Fig. 2d).

Genetic variation.—A total of 66 different alleles were found at the analyzed loci (minimum = 3 and maximum = 12 per locus, $k = 6.6$). Average H_O and H_E were similar, amounting to 0.688 and 0.693, respectively. The overall F_{IS} in the population was very close to 0 (0.006). The population did not show any significant deviation from Hardy–Weinberg equilibrium, both at single loci and overall (Fisher's method, $P = 0.377$), whereas linkage disequilibrium resulted only for 3 (out of 45) loci combinations (sw2532–sw2496, s090–sw2496, and sw122–sw2532). However, each of these markers was mapped in a different chromosome, so that physical linkage could be excluded. Accordingly, in the statistical analyses, we assumed that alleles at different loci were independent.

The coefficient of relatedness in our sample of 120 wild boars averaged −0.010 ± 0.209 *SD*. The reference sample represented by 11 adult females and their litters (fetuses) provided a mean relatedness of 0.599 ± 0.130 *SD*, slightly higher than the value of 0.5, which is theoretically expected for comparisons between 1st-degree relatives (parent–offspring and full siblings).

Relatedness, spatial patterns, and social units.—The correlation between spatial distance and genetic relatedness, as resulting from the Mantel test, fluctuated during the study period (Table 1), proving significantly negative only in summer and autumn (July–October) 2003. This could be related to the presence of piglets, given that no correlation was observed in 2004 when the sample composition was biased toward subadults and adults (Fig. 2). Moreover, the repetition of this analysis without piglets resulted in a complete lack of significance during the 2-year study period.

The hypothesis that individuals belonging to the same social unit were more related than nonassociated individuals was confirmed. The randomization test showed a significantly higher relatedness among individuals of the same social unit ($P < 0.001$), although the width of the range suggests that unrelated individuals can group together (Fig. 3a). In fact,

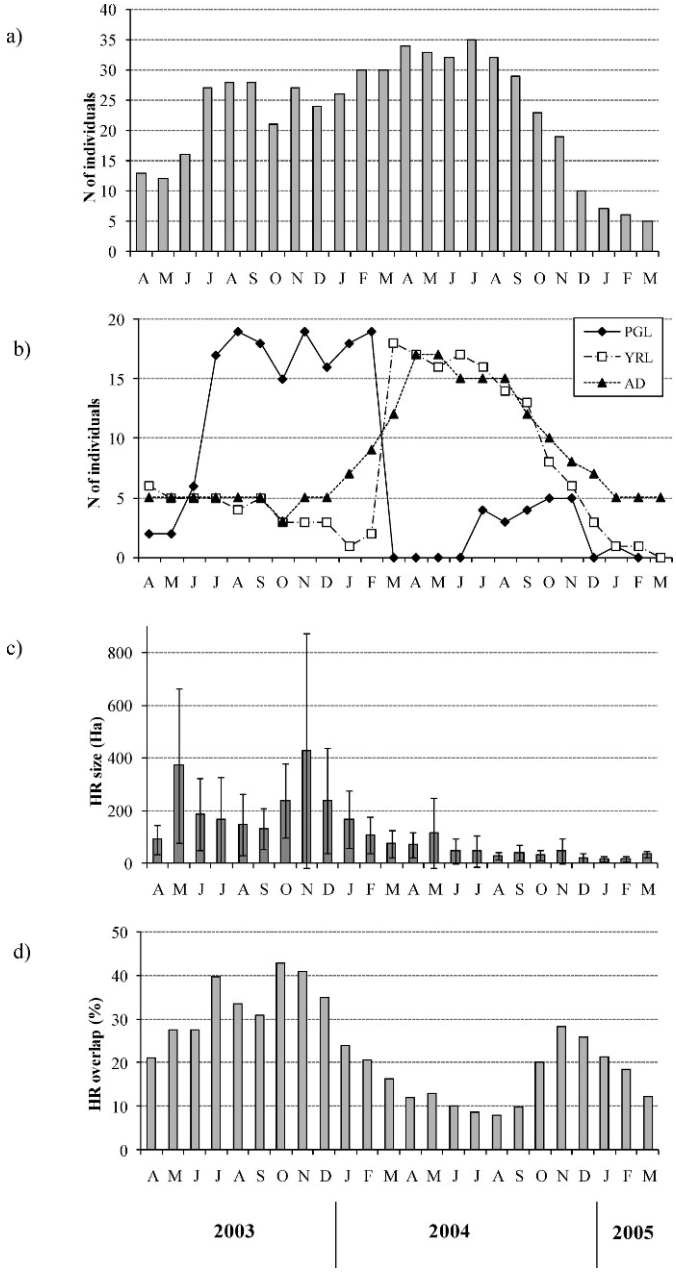

Fig. 2.—Sample variation and its spatial behavior during the study period (April 2003–March 2005) in the Alpe di Catenaia wild boar (*Sus scrofa*) population: a) sample size; b) sample composition (AD = adults, YRL = yearlings, PGL = piglets); c) home-range size; and d) home-range overlap. Home-range size is shown as the mean (± *SD*) of all individual home ranges calculated by the minimum convex polygon method using 95% of fix locations. Home-range overlap refers to the average percentage of all pairwise overlaps among monthly individual home ranges.

intragroup comparisons differed from the reference sample ($P < 0.001$), thus suggesting a deviation from the full-relatedness hypothesis.

As regards the type of association within putative matrilineal social units, YRL-FAD associations showed the highest levels of relatedness, followed by PGL-FAD and FAD-FAD (Fig. 3b). The relatedness between adult females in a group was significantly lower than the relatedness observed in YRL-FAD ($P = 0.017$) and PGL-FAD ($P = 0.026$) associations, but each of them significantly differed from the distribution observed in the reference families (all $P < 0.001$). Similarly, pairs of yearlings (YRL-YRL) showed a low average level of relatedness when compared to PGL-PGL associations and to control sibling pairs (Fig. 3c), thus deviating from the expectation of sibship. The average relatedness of FAD-FAD dyads was 0.082 ± 0.155 (mean ± *SD*). The likelihood analysis with Kingroup allowed us to confidently exclude 5 of 9 pairs from being represented by close relatives (r_{xy} ranging between −0.212 and 0.140). Indeed, for all of them, the null hypotheses of full siblings, half siblings, and parent–offspring could be rejected at a 95% confidence. Three unrelated pairs were found in association for <3 months (usually because of the death or signal loss of 1 female in the pair), whereas the other 2 dyads persisted for as long as 6 consecutive months.

Table 1.—Temporal variation (April 2003–March 2005) in the correlation between geographic distance and relatedness (Mantel test) in wild boars (*Sus scrofa*) of the Alpe di Catenaia, Italy. Correlations are computed both including and excluding piglets (PGL). Significant correlations, evaluated over 9,999 random permutations, are in boldface type ($P_{0.05} < 0.00213$, $P_{0.01} < 0.0004$; Bonferroni correction for 24 tests).

	With PGL		Without PGL	
Month	*R*	*P*	*R*	*P*
April 2003	−0.070	0.252	−0.224	0.104
May 2003	0.009	0.513	−0.024	0.393
June 2003	−0.155	0.058	−0.058	0.306
July 2003	−0.235	**0.001**	−0.034	0.373
August 2003	−0.219	**0.001**	−0.034	0.370
September 2003	−0.260	**0.000**	−0.083	0.229
October 2003	−0.329	**0.000**	−0.083	0.288
November 2003	−0.167	0.014	0.082	0.314
December 2003	−0.114	0.110	0.192	0.148
January 2004	−0.075	0.208	0.147	0.261
February 2004	−0.052	0.261	0.085	0.256
March 2004	−0.011	0.436	−0.002	0.505
April 2004	−0.050	0.222	−0.034	0.292
May 2004	−0.011	0.420	−0.035	0.271
June 2004	−0.166	0.009	−0.167	0.011
July 2004	−0.134	0.028	−0.080	0.122
August 2004	−0.076	0.141	−0.012	0.400
September 2004	−0.043	0.274	−0.010	0.421
October 2004	−0.005	0.409	0.097	0.169
November 2004	−0.177	0.080	−0.056	0.298
December 2004	0.019	0.518	0.112	0.270
January 2005	−0.026	0.376	−0.111	0.210
February 2005	−0.154	0.299	−0.154	0.297
March 2005	0.403	0.128	−0.154	0.303

Discussion

Social organization can vary under different ecological conditions (Lott 1991), and the goal of this study was to combine genetic and radiotelemetry data for evaluating the

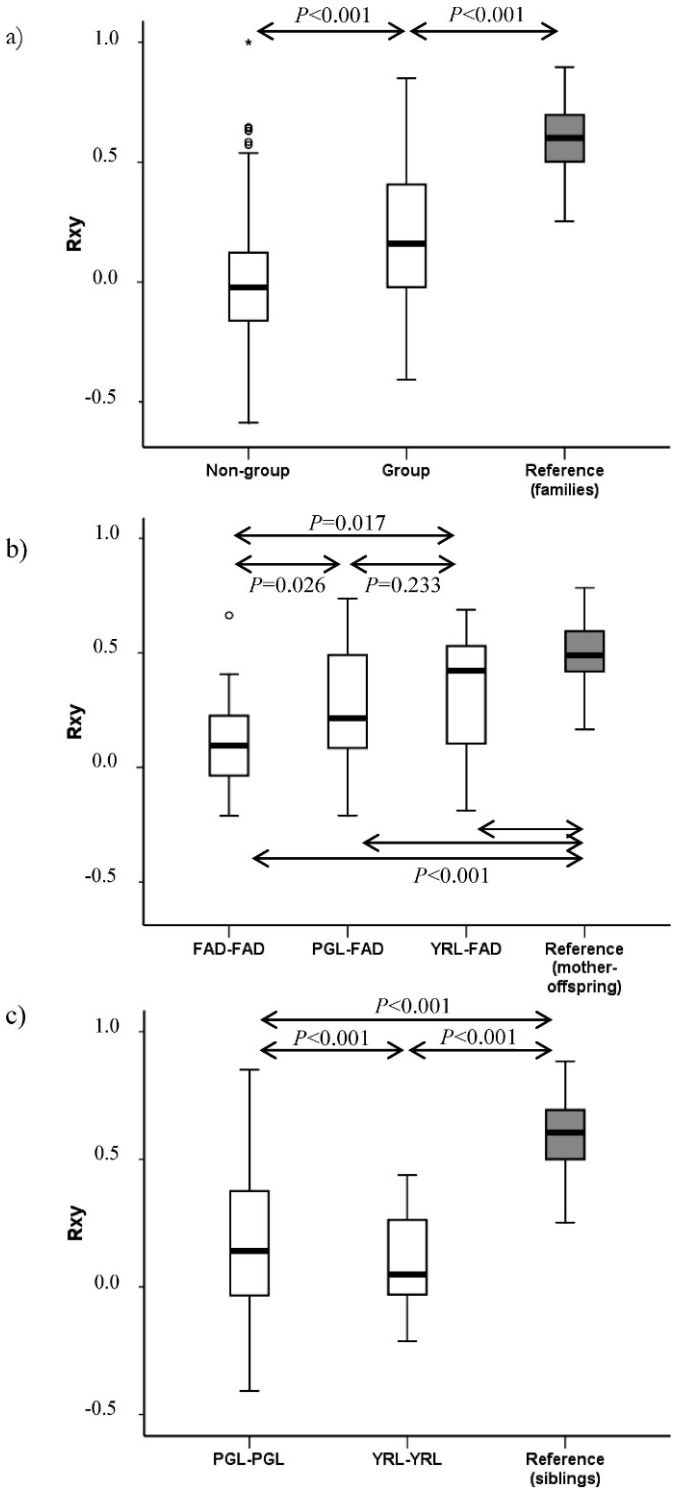

Fig. 3.—Distributions of relatedness values (r_{xy} [Queller and Goodnight 1989]). a) Comparisons between wild boars (*Sus scrofa*) joining the same social unit (''Group''; $n = 215$ dyads) and between wild boars moving separately in the study area (''Nongroup''; $n = 1{,}598$). As reference, relatedness observed in 11 mother–fetuses families ($n = 163$) is reported. b and c) Different age class associations within wild boar social units: PGL = piglet, YRL = yearling, FAD = adult female. Reference relatedness distributions are reported for mother–offspring (mother–fetuses, $n = 61$) and sibling dyads (fetuses in a litter, $n = 118$). *P*-values refer to 2-sample randomization tests for differences between means ($n = 10{,}000$ iterations).

social organization of wild boars based on estimates of genetic relatedness among individuals of the same and different social groups. Based on what was previously known for the behavior of wild boars, we expected social units in our study population to be composed of relatives, and that individual social units would have a higher chance of being surrounded by related than unrelated individuals. Because wild boars are social and only adult males are solitary, the overall spatial segregation among individuals can be predicted to correlate with genetic relatedness.

We observed a negative correlation between geographic distance (i.e., distance between home-range centroids) and genetic relatedness only in summer and early autumn, that is, during the period between parturition and weaning of juvenile wild boars. This also was the period when the social affinity in groups appears stronger (Kaminski et al. 2005), as observed in other suids (Byers 1983; Somers et al. 1995). A remarkable difference was observed between years. The correlation between distance and relatedness was statistically significant in the period July–October 2003 but not during year 2004. The most obvious explanation for the observed correlation in 2003 was that this pattern resulted from the higher number of piglets in social units during July–October, when they were strongly associated with their mothers. A very small number of piglets were included in our sample during 2004 (Fig. 2) because of low capture success. However, we cannot exclude other ecological factors (e.g., food availability, climate, etc.) from causing the observed difference between years.

The overall weak and temporally limited correlation between genetic and spatial distance does not fit a model of social structure where relatives tend to stay close even though they occasionally belong to different groups. A similar pattern was found in white-tailed deer (Comer et al. 2005), where the observed weak correlation between genetic relatedness and spatial association in females contradicted the ''rose-petal'' hypothesis of social organization in this species (Porter et al. 1991). Comer at al. (2005) considered this apparent contradiction as a possible effect of heavy harvesting, suggested by the altered age structure in the female population, that could have limited the occurrence of persistent and cohesive social groups. Hunting also could help explain the pattern observed in our study population, as suggested by the relatedness analyses within social units (see below). The wild boar population we studied was characterized by an overall high mortality rate, mostly due to hunting and poaching (86% of deaths in our sample). The resulting high turnover could have affected the distribution of genes in the population, accounting for the observed deviation from the expected pattern.

Individuals in a group were more related than individuals that were never found in association. This result agrees with the expectation of matrilineal social units, although the divergence from the reference families suggests that low-related or unrelated individuals also could be found in association. When we evaluated intragroup relatedness with respect to the age class of individuals, we obtained unexpected results. In particular, in 5 of 9 cases, adult females joining the

same social unit were neither sisters, half sisters, nor mother–daughter. Furthermore, for each age class combination that we took into consideration, the range of relatedness values suggested the simultaneous presence of unrelated and fully related individuals. Thus, contrary to common expectation, associations of both unrelated adult females and unrelated yearlings appeared to be frequent in our study population. The higher level of relatedness shown by the YRL-FAD with respect to FAD-FAD dyads (Fig. 3b) suggested that the individuals remaining in association with adult females after the 1st year of age usually were their offspring. The high number of piglets in a group implied that multiple litters of different adult females often were associated. This could easily explain the low relatedness of PGL-FAD dyads when compared to the control mother–offspring groups.

The occurrence of nonkin associations within groups was reported for feral pigs in Texas (Emlen 1997; Gabor et al. 1999) but has never been demonstrated for European free-living wild boars. Temporal associations of unrelated individuals can be accounted for by the possible benefits deriving from group living, beyond the fitness consequences of kin-based cooperative behavior (Griffin and West 2002). For instance, herding represents an effective antipredator strategy, commonly adopted against cooperative-hunting predators (Hamilton 1971). Wolves are the most important natural predators of wild boars in Europe, where they basically select for young boars (Jędrzejewski et al. 2000; Mattioli et al. 1995). Grouping as an antipredator response to wolves is common in ungulates (Creel and Winnie 2005; Lingle 2001; Mech and Peterson 2003). Adult wild boars, because of their size and aggressiveness, are less vulnerable to wolf predation and are expected to have lower benefits from grouping than young individuals (Mech and Peterson 2003). This could be a key factor explaining the solitary life of adult males. On the contrary, adult females also have to warrant protection for their litter. When piglets are present, mothers will often react aggressively against predators (Heck and Raschke 1980), like other large-sized ungulate mothers (e.g., female moose [*Alces alces*—Stephenson and Van Ballenberghe 1995]). Reaction by adult females to a wolf attack can be more effective if they are in a group. Therefore, they might be urged by the presence of predators to join other females, regardless of kinship. This could well be the case in our study area, where a wolf pack was consistently present during the study and mainly relied on wild boars as a prey species (Apollonio and Mattioli 2006).

Human activities also are likely to influence the social structure of wild boars. Hunting was shown to affect both the social and spatial behaviour of wild boars, increasing social affinity (Kaminski et al. 2005) and inducing variation in home-range size, as well as temporary departures from traditional resting sites (Baubet et al. 1998; Maillard and Fournier 1995; Sodeikat and Pohlmeyer 2002). These effects are more evident when drive hunting is practiced, as revealed by a parallel study on spatial patterns of roe deer in the same area (Bongi et al. 2007). Furthermore, losses due to the high mortality rate modified the size and the composition of social units and, as a consequence, nonkin associations might have been formed so as to replace dead individuals and maintain the advantages of group living. This explanation would entail a high turnover and a dynamic composition of social units, where the loss of 1 or more relatives is compensated by the acceptance of unrelated individuals to the group or by merging of groups. These processes may be enhanced by the high proportion of females killed during the hunting season in the study area (in a bag of 2,648 kills, 31% were represented by subadult or adult females). In fact, unlike in other European countries (e.g., Germany), Italian legislation allows hunters to kill adult females leading groups.

In addition to the above-mentioned explanations, average similarity within a social unit also can be lower than expected because of multiple paternity. Indeed, when piglets in a litter have different fathers (i.e., they are half siblings), the overall intralitter relatedness will be lower than when they are full siblings (i.e., a single father). The effect can be more pronounced when it involves different cohorts within the same social unit. Although observations of adult females breeding with several males are poorly documented (Barrett 1978), in a recent study Delgado et al. (2008) confirmed that this phenomenon may occur at low frequencies, detecting limited signs of multiple paternity in a wild boar population in Portugal.

To summarize, our results suggest that interactions between kin do not play an exclusive role in wild boar sociality. The matrilineal structure of social units in this species may thus exhibit exceptions under certain conditions. In our study area, the weak correlation between genetic relatedness and spatial distance, and the occurrence of unrelated adult females within a group, suggest a frequent deviation from matrilinearity. We believe that rearrangements of wild boar social groups were likely due to the combination of high human-caused mortality, and constant exposure to predation risk.

Further studies are warranted to investigate the temporal and spatial dynamics of nonkin associations, their occurrence under different conditions (e.g., hunting versus nonhunting areas), and the role of predators as a driving force in promoting group formation in wild boars. Finally, the presence of unrelated individual wild boars in a social unit opens a series of questions regarding the possible fitness benefits associated with cooperative breeding in this species.

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Literature Cited

Apollonio, M., and G. B. Hartl. 1993. Are biochemical–genetic variation and mating system related in large mammals? Acta Theriologica Supplement 2:175–185.

Apollonio, M., and L. Mattioli. 2006. The wolf in the Arezzo Province, Italy. Editrice Le Balze, Montepulciano, Italy.

Barrett, R. H. 1978. The feral hog at Dye Creek Ranch, California. Hilgardia 46:283–355.

Baubet, E., S. Brandt, and C. Touzeau. 1998. Effet de la chasse sur les stratégies d'occupation de l'espace des sangliers (*Sus scrofa*). Analyses préliminaires. Gibier Faune Sauvage 15:655–658.

Blouin, M. S. 2003. DNA-based methods for pedigree reconstruction and kinship analysis in natural populations. Trends in Ecology & Evolution 18:503–511.

Blundell, G. M., M. Ben-David, P. Groves, R. T. Bowyer, and E. Geffen. 2004. Kinship and sociality in coastal river otters: are they related? Behavioral Ecology 15:705–714.

Boitani, L., L. Mattei, D. Nonis, and F. Corsi. 1994. Spatial and activity patterns of wild boars in Tuscany, Italy. Journal of Mammalogy 75:600–612.

Bongi, P., S. Grignolio, S. Ciuti, and M. Apollonio. 2007. Influence of hunting with dogs on roe deer spatial behaviour: differential responses according to sex and age classes. Hystrix—Italian Journal of Mammalogy (New Series) 1, Supplement 2007:238.

Briedermann, L. 1986. Schwartzwild. Neumann-Neudamm Verlag, Berlin, Germany.

Burton, C., and C. J. Krebs. 2003. Influence of relatedness on snowshoe hare spacing behavior. Journal of Mammalogy 84:1100–1111.

Byers, J. 1983. Social interactions of juvenile collared peccaries, *Tayassu tajacu* (Mammalia: Artiodactyla). Journal of Zoology (London) 201:83–96.

Carranza, J. 1996. Sexual selection for male body mass and the evolution of litter size in mammals. American Naturalist 148:81–100.

Chepko-Sade, B. D., and Z. Tang Halpin (eds.). 1987. Mammalian dispersal patterns: the effects of social structure on population genetics. University of Chicago Press, Chicago, Illinois.

Comer, C. E., J. C. Kilgo, G. J. D'Angelo, T. C. Glenn, and K. V. Miller. 2005. Fine-scale genetic structure and social organization in female white-tailed deer. Journal of Wildlife Management 69:332–344.

Cousse, S., F. Spitz, M. Hewison, and G. Janeau. 1994. Use of space by juveniles in relation to their postnatal range, mother, and siblings: an example in the wild boar, *Sus scrofa* L. Canadian Journal of Zoology 72:1691–1694.

Creel, S., and J. A. Winnie. 2005. Responses of elk herd size to fine-scale spatial and temporal variation in the risk of predation by wolves. Animal Behaviour 69:1181–1189.

Csillery, K., et al. 2006. Performance of marker-based relatedness estimators in natural populations of outbred vertebrates. Genetics 173:2091–2101.

Delgado, R., P. Fernández-Llario, M. Azevedo, A. Beja-Pereira, and P. Santos. 2008. Paternity assessment in free-ranging wild boar (*Sus scrofa*)—are littermates full-sibs? Mammalian Biology 73:169–176.

Dobson, F. S. 1998. Social structure and gene dynamics in mammals. Journal of Mammalogy 79:667–670.

Eisenberg, J. F., and D. G. Kleiman. 1983. Advances in the study of mammalian behavior. Special Publication 7, The American Society of Mammalogists.

Emlen, S. T. 1997. Predicting family dynamics in social vertebrates. Pp. 228–253 in Behavioural ecology (J. R. Krebs and N. B. Davies, eds.). Blackwell Scientific, London, United Kingdom.

Gabor, T. M., E. C. Hellgren, R. A. Van Den Bussche, and N. J. Silvy. 1999. Demography, sociospatial behaviour and genetics of feral pigs (*Sus scrofa*) in a semi-arid environment. Journal of Zoology (London) 247:311–322.

Gannon, W. L., R. S. Sikes, and the Animal Care and Use Committee of the American Society of Mammalogists. 2007. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy 88:809–823.

Griffin, A. S., and S. A. West. 2002. Kin selection: fact and fiction. Trends in Ecology & Evolution 17:15–21.

Hamilton, W. D. 1971. Geometry of the selfish herd. Journal of Theoretical Biology 31:295–311.

Harris, S., W. J. Cresswell, P. G. Forde, W. J. Trewhella, T. Woollard, and S. Wray. 1990. Home-range analysis using radio-tracking data—a review of problems and techniques particularly as applied to the study of mammals. Mammal Review 20:97–123.

Heck, L., and G. Raschke. 1980. Die Wildsauen: Naturgeschichte, Ökologie, Hege und Jagd. Verlag Paul Parey, Hamburg, Germany.

Hood, G. M. 2006. PopTools version 2.7.5. http://www.cse.csiro.au/poptools. Accessed 24 February 2009.

Janečka, J. E., T. L. Blankenship, D. H. Hirth, M. E. Tewes, C. W. Kilpatrick, and L. I. Grassman, Jr. 2006. Kinship and social structure of bobcats (*Lynx rufus*) inferred from microsatellite and radio-telemetry data. Journal of Zoology (London) 269:494–501.

Jędrzejewski, W., B. Jędrzejewska, H. Okarma, K. Schmidt, K. Zub, and M. Musiani. 2000. Prey selection and predation by wolves in Białowieża Primeval Forest, Poland. Journal of Mammalogy 81:197–212.

Kaminski, G., S. Brandt, E. Baubet, and C. Baudoin. 2005. Life-history patterns in female wild boars (*Sus scrofa*): mother–daughter postweaning associations. Canadian Journal of Zoology 83:474–480.

Kenward, S. E., A. B. South, and S. S. Walls. 2003. Ranges6 v. 1.2: for the analysis of tracking and location data. Anatrack Ltd., Wareham, United Kingdom.

Kernohan, B. J., R. A. Gitzen, and J. J. Millspaugh. 2001. Analysis of animal space use and movements. Pp. 126–168 in Radio tracking and animal populations (J. J. Millspaugh and J. M. Marzluff, eds.). Academic Press, San Diego, California.

Konovalov, D. A., C. Manning, and M. T. Henshaw. 2004. KINGROUP: a program for kinship reconstruction and kin group assignments using genetic markers. Molecular Ecology Notes 4:779–782.

Lingle, S. 2001. Anti-predator strategies and grouping patterns in white-tailed deer and mule deer. Ethology 107:295–314.

Lott, D. F. 1991. Intraspecific variation in the social systems of vertebrates. Cambridge University Press, Cambridge, United Kingdom.

Maillard, D., and P. Fournier. 1995. Effects of shooting with hounds on size of resting range of wild boar (*Sus scrofa* L.) groups in Mediterranean habitat. Ibex, Journal of Mountain Ecology 3:102–107.

Mattioli, L., M. Apollonio, V. Mazzarone, and E. Centofanti. 1995. Wolf food habits and wild ungulate availability in the Foreste Casentinesi National Park; Italy. Acta Theriologica 40:387–402.

Mattioli, L., C. Capitani, E. Avanzinelli, I. Bertelli, A. Gazzola, and M. Apollonio. 2004. Predation by wolves (*Canis lupus*) on roe deer (*Capreolus capreolus*) in north-eastern Apennine, Italy. Journal of Zoology (London) 264:249–258.

MECH, L. D., AND R. O. PETERSON 2003. Wolf–prey relations. Pp. 131–160 in Wolves: behavior, ecology, and conservation (L. D. Mech and L. Boitani, eds.). University of Chicago Press, Chicago, Illinois.

MOYER, M. A., J. W. MCCOWN, T. H. EASON, AND M. K. OLI. 2006. Does genetic relatedness influence space use pattern? A test on Florida black bears. Journal of Mammalogy 87:255–261.

PEAKALL, R., AND P. E. SMOUSE. 2005. GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. Australian National University, Canberra, Australia.

POPE, T. R. 1998. Effects of demographic change on group kin structure and gene dynamics of red howling monkey populations. Journal of Mammalogy 79:692–712.

PORTER, W. F., N. E. MATHEWS, H. B. UNDERWOOD, R. W. SAGE, AND D. F. BEHREND. 1991. Social organization in deer: implications for localized management. Environmental Management 15:809–814.

QUELLER, D. C., AND K. F. GOODNIGHT. 1989. Estimating relatedness using genetic markers. Evolution 43:258–275.

RATNAYEKE, S., G. A. TUSKAN, AND M. R. PELTON. 2002. Genetic relatedness and female spatial organization in a solitary carnivore, the raccoon, *Procyon lotor*. Molecular Ecology 11:1115–1124.

RAYMOND, M., AND F. ROUSSET. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248–249.

RICE, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223–225.

SMOUSE, P. E., J. C. LONG, AND R. R. SOKAL. 1986. Multiple regression and correlation extensions of the Mantel test of matrix correspondence. Systematic Zoology 35:627–632.

SODEIKAT, G., AND K. POHLMEYER. 2002. Temporary home range modifications of wild boar family groups (*Sus scrofa* L.) caused by drive hunts in Lower Saxony (Germany). Zeitschrift für Jagdwissenschaft 48:161–166.

SOMERS, M. J., A. E. RASA, AND B. L. PENZHORN. 1995. Group structure and social behaviour of warthogs, *Phacochoerus aethiopicus*. Acta Theriologica 40:257–281.

SOUTHWOOD, T. R. E. 1966. Ecological methods. Methuen and Co., London, United Kingdom.

SPITZ, F., AND G. JANEAU. 1990. Spatial strategies: an attempt to classify daily movements of wild boar. Acta Theriologica 35:129.

SPONG, G., AND S. CREEL. 2004. Effects of kinship on territorial conflicts among groups of lions, *Panthera leo*. Behavioral Ecology and Sociobiology 55:325–331.

STEPHENSON, R. O., AND V. VAN BALLENBERGHE. 1995. Defense of one twin calf against wolves, *Canis lupus*, by a female moose, *Alces alces*. Canadian Field Naturalist 109:251–253.

STORZ, J. F. 1999. Genetic consequences of mammalian social structure. Journal of Mammalogy 80:553–569.

SUGG, D. W., R. K. CHESSER, F. S. DOBSON, AND J. L. HOOGLAND. 1996. Population genetics meets behavioral ecology. Trends in Ecology & Evolution 11:338–342.

SWIHART, R. K., AND N. A. SLADE. 1985. Testing for independence of observation in animal movements. Ecology 66:1176–1184.

VAN HORN, R. C., J. ALTMANN, AND S. C. ALBERTS. 2008. Can't get there from here: inferring kinship from pairwise genetic relatedness. Animal Behaviour 75:1173–1180.

WHITE, G. C., AND R. A. GARROTT. 1990. Analysis of wildlife radiotracking data. Academic Press, San Diego, California.

WIMMER, B., D. TAUZT, AND P. M. KAPPELER. 2002. The genetic population structure of the gray mouse lemur (*Microcebus murinus*), a basal primate from Madagascar. Behavioral Ecology and Sociobiology 52:166–175.

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