



ORIGINAL INVESTIGATION

**Paternity assessment in free-ranging wild boar
(*Sus scrofa*) – Are littermates full-sibs?**

Rodrigo Delgado^a, Pedro Fernández-Llario^b, Marisa Azevedo^c,
Albano Beja-Pereira^c, Pedro Santos^{a,*}

^aDepartamento de Ecologia, Colégio Luís António Verney, Universidade de Évora, R. Romão Ramalho, 59, 7000-671, Évora, Portugal

^bDepartamento de Biología y Geología, Instituto de Enseñanza Secundaria “Santa Lucía del Trampal”, 10160 Alcuéscar, Cáceres, Spain

^cCentro de Investigação em Biodiversidade de Recursos Genéticos, Campus Agrário de Vairão, R. Monte-Crasto, 4485-6661 Vairão, Portugal

Received 11 December 2006; accepted 19 July 2007

Abstract

Multiple paternity within litters occurs in various groups of mammals exhibiting different mating systems. Using seven genetic markers (i.e., microsatellites), we investigated the paternity of littermates in free-ranging wild boar (*Sus scrofa*) in a Mediterranean habitat. Using the software CERVUS 2.0 we estimated the probability of detecting multiple paternity across all loci (D), the probability of paternity (W) and a statistic Δ that allows the assignment of paternity to the most likely male with strict and relaxed levels of confidence. Multiple paternity was inferred for one of the nine analysed litters at the 80% confidence level. This suggests that a single male may control the access to receptive adult females and it shows that multiple paternity is not very common in the studied free-ranging wild boar population. Despite the possible occurrence of sperm competition and/or female cryptic choice, mate guarding seems to play a significant role in sexual selection. To better understand the wild boar's mating strategies further studies analysing the reproductive success of both sexes and under different environmental conditions should be conducted.

© 2007 Deutsche Gesellschaft für Säugetierkunde. Published by Elsevier GmbH. All rights reserved.

Keywords: *Sus scrofa*; Mating system; Sexual selection; Mate guarding; Sperm competition

Introduction

Female sexual promiscuity seems to be rather common and several evolutionary benefits have been proposed for multi-male mating (Jennions and Petrie 2000; Gomendio 2002; Wolff and Macdonald 2004). The increasing use of molecular tools revealed that multiple paternities within single broods or litters are

frequent in a large range of different taxa (Birkhead and Møller 1998). Recently, microsatellite analysis revealed multiple paternity in natural ungulate populations, such as in wild pronghorn antelopes (*Antilocapra americana*) (Carling et al. 2003) and in white-tailed deer (*Odocoileus virginianus*) (Sorin 2004).

The social organization of wild boar (*Sus scrofa*) is very much related to its reproductive cycle, and accordingly adult males only join females groups in the rutting period (Dardaillon 1988). When an anoestrous period ends the females belonging to the same

*Corresponding author.

E-mail address: aps@uevora.pt (P. Santos).

social unit resume cycling at the same time (Delcroix et al. 1990). According to the same authors, there is an extreme accurate synchronization that appears to result from interactions between females of the same group and is likely to have a functional significance. Bearing in mind that the oestrous period in the domestic sow is about 44 h (Sorensen Jr 1982), most probably in each group, formed by a few females (Fernández-Llario 1996), a single dominant boar is able to monopolize all the receptive females not allowing any other male to copulate. In fact, there is empirical evidence that dominant males exhibit agonistic behaviours during the rutting period in order to safeguard the sexual access to the receptive females, and that if necessary they will fight sneakers (males trying to usurp a copulation) using their large canine teeth (Barret 1986; Rushen and Pajor 1987). The walling behaviour in adult males during the rutting period may have a sexual function (Fernández-Llario 2005); the dry mud, mainly clay acts as a protection shield during the fights over the females. Male–male competition for accessing reproduction seems to begin early in life since pregnant females tend to invest more in males than in females foetuses, and also since across litters the heaviest piglet is a male in the large majority of cases (Fernández-Llario et al. 1999; Santos 2002). These results support the Trivers and Willard (1973) model's first prediction, showing that pregnant females in polygynous species tend to invest more in the sex with the higher biotic potential.

Besides male–male competition over copulation partners, post-copulatory competition may also be involved in sexual selection on wild boar. In fact, when compared to other livestock, adult domestic boars have relatively larger testes and produce higher semen volume (Sorensen Jr 1982), and it was shown that wild boar semen is not different from that of domestic boars (Kozdrowski and Dubiel 2004). These facts suggest sperm competition assuming that fertilization success of a given male depends on the relative amount of semen it can ejaculate. The testes size and the volume of sperm produced by wild boar could be male morphological adaptations to sperm competition and the consequence of a polyandrous mating behaviour. According to this hypothesis, multiple paternity could be a common event since heterospermic artificial insemination already showed that it may occur in domestic pig (Berger et al. 1996; Stahlberg et al. 2000). Another male adaptation to sperm competition might be the gel-like fraction of the boar ejaculate that forms a plug in the vagina of the mated sows (Hafez 1993). According to common knowledge, these copulatory plugs do not act like “chastity belts” neither seem to reduce female attractiveness to rival. However, it remains uncertain whether they can avoid further male insemination and egg fertilization or not. Nevertheless, multiple sired litters have been reported when gilts mate in rapid succession with different boars (Martin and Dzuk 1977). It is

frequently difficult to predict the present role of sperm competition-related male traits since they may compromise female fitness and so a fast evolutionary arms race between sexes in continuously ongoing (Chapman et al. 2003), and this is why so many different functions have been attributed to them in distinct species.

Though wild boar is generally considered a polygynous mammal, some morphological and physiological sperm competition-related male traits seem to point towards the possible occurrence of multiple paternity. This features inconsistency makes either single or multiple paternity plausible hypotheses. The aim of the present study was to investigate if multiple paternity within single broods occurred in foetuses litters on a Mediterranean environment (Alentejo, Portugal), using seven microsatellite markers.

Material and methods

Sample collection

Samples were collected in Alentejo (38°22'–38°35'N; 7°35'–7°43'W), a Portuguese county belonging to the Mediterranean Ibero-Atlantic Province where the Thermo-mediterranean bioclimatic type prevails (Rivas-Martínez and Loidi 1999). The elevation ranges from sea level to about 1000 m, the annual rain fall ranges from 500 to 700 mm, decreasing from the coastal to the inner zones, and the annual mean temperature ranges from 15 to 17 °C. The summer drought from June to September favours sclerophyllic evergreen Mediterranean vegetation and a secondary type of forest called *montado*, characterized by species like cork oak (*Quercus suber*) and holm oak (*Quercus rotundifolia*), dominates the landscape. In arable lands, oats (*Avena sativa*) and wheat (*Triticum* sp.) in autumn/winter, and sunflower (*Helianthus annuus*) and maize (*Zea mays*) in spring/summer, are the main crops. In rugged hilly areas mature stages of vegetation can still be seen. The hunting pressure is high and so is the number of wild boars taken per 100 ha of shooting area (Fernández-Llario et al. 2003). The hunting method is the so-called “*montaria*” and it consists in dog teams chasing wild boar towards hunters standing at fixed points spread along the shooting area.

Samples were obtained during the hunting season of 2001/2002 and were collected in different “*montarias*”. The uteri of pregnant females were removed during field necropsy and stored at –20 °C until genomic DNA extraction. These females were weighted (total life weight) and their age assessed according to the chronology of teeth eruption (Santos et al. 2006). For parentage analysis, samples of tissue were taken from foetuses (belonging to different litters) and from the respective mothers. The most prolific females were selected – litter size equal or bigger than five – in order to increase the chance of detecting multiple paternity.

Genetic analysis

(a) DNA extraction

DNA was extracted from tissue using Proteinase K digestion followed by extraction with standard

Phenol-chloroform or high-salt procedures, from tissue samples from 59 individuals (nine females and 50 foetuses).

(b) Microsatellite loci genotyping

We amplified by PCR seven dinucleotide microsatellites – SW240, SW951, S0101, S0005, S0215, S0218, and S0228. These genetic markers are independently segregated (Nechtelberger et al. 2001), and thus linkage disequilibrium – non-random associations of alleles at two or more loci – is not expected. PCR products were separated by electrophoresis in 8% denaturing polyacrylamide gels, followed by silver staining (Bassam et al. 1991). Allele identification and counting were made directly from the gels and, to avoid misidentification of alleles, two reference samples were always used.

Paternity analysis

DNA of adult males is difficult to collect since these animals are frequently absent from montarias’s hunting bags. Thus, comparing each foetus in a litter with the mother’s genotype allowed the identification of paternal alleles and the alleged fathers’ genotypes were established accordingly. When paternal alleles could not be unambiguously determined, either because of missing maternal data or because mother and all offspring in a litter shared the same heterozygous genotype, they were considered missing data in the software used for paternity inference (Marshall et al. 1998).

If more than two paternal alleles are necessary to explain the genetic variation in a litter, multiple paternity can be ascertain for that locus. Bearing in mind that the presence of only one or two paternal alleles in a litter is not enough to exclude multiple paternity, we have also calculated a detection index (*d*) (Burton 2002). This index gives the probability of detecting alleles from more than one presumed father and was calculated as

$$d = 1 - 2a_2 + a_3 + 3(a_2a_3 - a_5) - 2(a_2^2 - a_4),$$

where $a_x = \sum_{i=1}^n pi^x$ and *pi* is the frequency in the population of the *i*th allele for *n* alleles (Westneat et al. 1987; Burton 2002). Following the same authors, the probability of detecting multiple paternity across all loci (*D*) was calculated as

$$D = 1 - \prod_{i=1}^m (1 - di)$$

for *m* loci.

Besides direct counts of paternal alleles, we also used two likelihood-based paternity inference methods (Krützen et al. 2004). In both methods Paternity Indexes (PIs) were generated using the software CERVUS 2.0 (Marshall et al. 1998), laying the difference in the way in which significance is applied to the indexes. The first method uses the Bayesian’ theorem to calculate the probability of paternity of an alleged father:

$$W = p_{prior} \prod PI / [p_{prior} \prod PI + (1 - p_{prior})],$$

where *p*_{prior} is the probability prior to considering the genetic evidence. We assumed a prior probability of 0.5, which is the practice in most paternity testing.

The second method (the so-called CERVUS method) uses a simulation to define a statistic *Δ* based on the observed loci allele frequencies and determines the most likely father between the two males with the highest PIs with strict (95%) and relaxed (80%) levels of confidence (Marshall et al. 1998). The simulation parameters were the following: 10,000 cycles, five candidate parents, 0.6 proportion of candidate parents sampled, 0.93 proportion of loci typed, 0.1 proportion of loci mistyped. The number of candidate parents was estimated considering previous reports on wild boar space use and home ranges (Santos et al. 2004), and the proportion of candidate parents sampled derived accordingly considering the number of alleged fathers genotyped in the area.

Results

Nine pregnant females and the respective litters (a total of 50 foetuses) were analysed (Table 1). Eight females were adults (more than 2 years old) and one was yearling (aged between 1 and 2 years). The sample mean life body weight was 76.11 (±16.64 SD) and the mean number of foetuses per litter was 5.56 (±0.73 SD). These females were hunted from November to February in four different “montarias” that took place in an area of about 30,000 ha.

All seven microsatellite loci were useful for paternity inference, since likelihood ratios are calculated on the basis that the Hardy–Weinberg equilibrium holds (Table 2). SW240, S0101 and S0005 were the most informative loci, combining the biggest overall number of alleles with the highest overall heterozygosity (Table 2). Across all seven

Table 1. Characteristics of the analysed wild boar pregnant females and the month in which they were hunted

Female	Age	Weight (kg)	Litter size	Hunting month
Female A	More than 2 years	80	5	November
Female B	More than 2 years	70	5	November
Female C	More than 2 years	75	5	November
Female D	More than 2 years	55	7	January
Female E	More than 2 years	85	6	January
Female F	Between 1 and 2 years	60	6	February
Female G	More than 2 years	100	6	February
Female H	More than 2 years	75	5	February
Female I	More than 2 years	85	5	February

loci, the probability of detecting an allele from more than one putative father was high ($D = 0.95$).

Paternity inference

Considering the minimum number of paternal alleles, none of the nine analysed litters showed evidence of multiple paternity ($D = 0.95$). In all litters any offspring's (foetuses) genotype at each of the studied loci could be explained by only one or two different paternal alleles (Table 3). The paternal alleles identified at the different loci for one given litter were considered the alleles of that litters' alleged father. The alleged fathers of the studied litters were nine different individuals.

LOD scores for 49 offspring–mother–alleged father trios were positive and paternities reliably determined for most foetuses according to Bayesian inference: 20 cases at the 80% confidence level and 27 cases at the 95% confidence level (Table 4). In seven of nine litters

the probability of paternity (W) was larger than 80% for all offspring–mother–alleged father trios, namely in LitA, LitB, LitC, LitE, LitF, LitG and LitH. However, in LitE, W was higher for the trio LitE3–FemE–MalG than for the trio LitE3–FemE–MaleE. Also in litters LitD and LitI, trios composed by other males than the alleged father obtained higher W for some foetuses than the trios in which the male was the alleged father (Table 4). These paternities disputes were resolved using the CERVUS method (Marshall et al. 1998).

The CERVUS method showed high power to assign paternity: 17 offspring were assigned fathers with 80% confidence level and 28 offspring were assigned fathers with 95% level of confidence. In six of nine litters, offspring from the same litter were assigned to the same male – the respective alleged father. In litters LitD, LitE and LitI offspring from the same litter were assigned to different parents, however only in LitD paternities were assigned to other male than the alleged father (two out of seven) at 80% confidence level though these paternities assignments were not secure at 95% confidence level. Multiple paternity was not established from the CERVUS method at a strict confidence level while at a relaxed (80%) confidence level it was inferred for one of the nine litters (11%). In this litter, although LitD1 paternity was assigned by CERVUS to MaleI, the trio LitD1–FemD–MaleI probability of paternity was smaller than 80% and so the true father of LitD1 can be another male from the same population. In the same litter, LitD6 paternity was assigned by CERVUS to MaleH and this male is likely the true father of that offspring since the LitD6–FemD–MaleH probability of paternity is nearly 90%.

Paternity inference based on direct exclusion seems congruent with the two likelihood-based paternity inference methods used. The small differences found can be explain by the fact that the first approach determines minimum paternal numbers while the second type of approach estimates real numbers of paternities.

Table 2. Summary of the genetic variability at the seven microsatellites studied

Locus	NA	H_o	H_e	d	Hardy–Weinberg	Null freq.
SW240	6	0.697	0.707	0.468	NS	+0.0188
SW951	3	0.582	0.511	0.260	NS	–0.0934
S0101	4	0.731	0.689	0.438	NS	–0.0363
S0005	5	0.682	0.626	0.398	NS	–0.0533
S0215	4	0.712	0.595	0.302	NS	–0.0991
S0218	3	0.339	0.457	0.209	NS	+0.1468
S0228	4	0.475	0.443	0.246	NS	–0.0704
Overall	4.14	0.603	0.576	$D = 0.945$		

NA: number of alleles; H_o : observed heterozygosity; H_e : expected heterozygosity; d : probability of detection for each of the seven loci; D : probability to detect multiple paternity across all loci. It is also given the Hardy–Weinberg chi-square statistic and the estimation of null allele frequency.

Table 3. Results of paternity assessment by comparison of littermates' genotypes with their mother's genotype, referring the minimum number of paternal alleles at each locus, the minimum number of fathers and the alleged father code of each litter

Minimum number of paternal alleles at each locus										
Litter code	Mother code	SW240	SW951	S0101	S0004	S0215	S0218	S0228	Minimum number of fathers	Alleged father code
LitA	FemA	2	1	2	2	1	2	2	1	MaleA
LitB	FemB	2	1	2	2	2	2	1	1	MaleB
LitC	FemC	2	2	2	2	2	2	1	1	MaleC
LitD	FemD	2	1	2	2	2	2	1	1	MaleD
LitE	FemE	2	1	2	2	2	1	2	1	MaleE
LitF	FemF	1	2	2	2	2	1	1	1	MaleF
LitG	FemG	2	1	2	2	2	1	1	1	MaleG
LitH	FemH	1	2	2	1	1	2	1	1	MaleH
LitI	FemI	2	1	2	2	1	2	1	1	MaleI

Table 4. Results of paternity assessment using Bayesian inference and CERVUS method

Offs	Moth.	Cand. Fath.	LOD	<i>L</i>	<i>W</i> pp 0.5	<i>Δ</i>	Confid.
LitA1	FemA	MaleA	1.77	5.87	0.8545	0.2130	–
LitA2	FemA	MaleA	3.08	21.69	0.9559	3.0767	*
LitA3	FemA	MaleA	1.93	6.89	0.8733	0.2618	–
LitA4	FemA	MaleA	3.078	21.71	0.9560	3.0706	*
LitA5	FemA	MaleA	2.61	13.58	0.9314	2.6085	*
LitB1	FemB	MaleB	3.20	24.57	0.9609	1.6188	+
LitB2	FemB	MaleB	3.07	21.51	0.9556	3.0685	*
LitB3	FemB	MaleB	3.90	49.44	0.9802	3.9008	*
LitB4	FemB	MaleB	2.70	14.87	0.9370	0.2876	–
LitB5	FemB	MaleB	3.17	23.80	0.9597	1.5974	+
LitC1	FemC	MaleC	2.9693	19.4778	0.9512	2.9693	*
LitC2	FemC	MaleC	2.9693	19.4778	0.9512	2.9693	*
LitC3	FemC	MaleC	2.9693	19.4778	0.9512	2.9693	*
LitC4	FemC	MaleC	2.9693	19.4778	0.9512	2.9693	*
LitC5	FemC	MaleC	2.0923	8.1039	0.8902	2.0923	+
LitD1	FemD	MaleI	0.8059	2.2386	0.6912	0.8059	+
LitD1	FemD	MaleD	–0.7001	0.4966	0.3318	0.0000	
LitD2	FemD	MaleD	3.2298	25.2737	0.9619	3.0231	*
LitD3	FemD	MaleD	3.7513	42.5747	0.9771	1.8773	+
LitD4	FemD	MaleD	1.9193	6.8164	0.8721	1.3926	+
LitD5	FemD	MaleD	3.5432	34.5766	0.9719	2.0640	+
LitD6	FemD	MaleH	2.0922	8.1029	0.8901	0.8482	+
LitD6	FemD	MaleD	1.2440	3.4695	0.7763	0.0000	
LitD7	FemD	MaleD	1.7656	5.8453	0.8539	1.3926	+
LitE1	FemE	MaleE	3.2182	24.9822	0.9615	3.2182	*
LitE2	FemE	MaleE	3.0030	20.1464	0.9527	3.0030	*
LitE3	FemE	MaleG	1.8624	6.4390	0.8656	0.2905	–
LitE3	FemE	MaleE	1.5719	4.8157	0.8281	0.0000	
LitE4	FemE	MaleE	1.9618	7.1118	0.8767	1.7895	+
LitE5	FemE	MaleE	1.6703	5.3138	0.8416	1.6703	+
LitE6	FemE	MaleE	2.5427	12.7144	0.9271	2.5427	*
LitF1	FemF	MaleF	5.3401	208.5300	0.9952	5.3401	*
LitF2	FemF	MaleF	4.3527	77.6894	0.9873	3.1826	*
LitF3	FemF	MaleF	3.3279	27.8804	0.9654	0.9919	+
LitF4	FemF	MaleF	5.3480	210.1919	0.9953	1.0758	+
LitF5	FemF	MaleF	2.5004	12.1878	0.9242	2.5004	*
LitF6	FemF	MaleF	5.2240	185.6793	0.9946	5.2240	*
LitG1	FemG	MaleG	2.3084	10.0582	0.9096	2.3084	*
LitG2	FemG	MaleG	2.0913	8.0951	0.8901	2.0913	+
LitG3	FemG	MaleG	4.1782	65.2480	0.9849	3.7107	*
LitG4	FemG	MaleG	4.1782	65.2480	0.9849	3.7136	*
LitG5	FemG	MaleG	2.1265	8.3853	0.8935	2.1265	+
LitG6	FemG	MaleG	2.3084	10.0582	0.9096	2.3084	*
LitH1	FemH	MaleH	1.5970	4.9383	0.8316	1.5970	+
LitH2	FemH	MaleH	4.1244	61.8278	0.9841	4.1244	*
LitH3	FemH	MaleH	3.0183	20.4561	0.9534	3.0183	*
LitH4	FemH	MaleH	3.8474	46.8705	0.9791	3.8474	*
LitH5	FemH	MaleH	3.0786	21.7285	0.9560	3.0786	*
LitI1	FemI	MaleH	1.0577	2.8798	0.7423	0.0748	–
LitI1	FemI	MaleI	0.9829	2.6722	0.7277	0.0000	
LitI2	FemI	MaleI	2.3469	10.4528	0.9127	2.3469	*
LitI3	FemI	MaleI	2.4638	11.7499	0.9216	2.4638	*
LitI4	FemI	MaleI	2.3614	10.6055	0.9138	1.7038	+
LitI5	FemI	MaleI	2.4638	11.7499	0.9216	2.4638	*

Offs: offspring code; Moth.: mother code; Cand. Fath.: candidate father code (alleged father and, if applicable, males with higher LOD score than the alleged father); LOD: scores of the logarithm of the likelihood ratio; (*L*): paternity index; (*W*): probability of paternity; (*Δ*): difference in LOD scores between most likely and second most likely candidate and referring confidence level of CERVUS paternity assignments – strict (*) and relaxed (+).

The Bayesian and the CERVUS approaches were concordant and complementary. The second method was useful to resolve paternity when different males presented positive LOD scores for a certain offspring, while the first method sanctioned the paternity of a male fulfilling the Δ statistic criterion only if that male attained a minimum paternity probability.

Discussion

This study reveals that multiple sired litters are less frequent than full-sibs litters in free-ranging wild boar populations that are under intensive game management in southern Portugal. This conclusion cannot be explained by lack of informative loci since the used seven microsatellites markers have a high combined level of detecting multiple paternity. Noteworthy, that the same number or fewer polymorphic markers were enough to detect multiple paternity in several other species (Say et al. 1999; Burton 2002; Kraaijeveld-Smit et al. 2002; Dean et al. 2006). Further, the size of all studied litters was larger than the suggested minimum of tree littermates necessary to detect multiple paternity (Burton 2002). False paternity exclusions due to null alleles are not likely, given that no mismatches were found between mother and offspring. Thus, failure to detect multiple paternity cannot result from deficient power to assigned paternities reliably, either. In fact, a substantial success of accurate paternity inference was observed, resulting from the small number of candidate fathers combined with the relative high proportion of males “sampled”. However, stronger conclusions can only be drawn if a large proportion of candidate fathers are sampled in fact (not derived from the alleged fathers as in this study).

A high mean foetal litter size was expected given that we have selected the most prolific females in order to increase the chance to detect multiple paternity. An average of 5.5 foetuses per litter contrasts with the observed in several different regions of the Iberian Peninsula where it ranges from 3.6 to 4.3 (see for example Abaigar 1992; Fernández-Llario and Mateus-Quesada 1998; Santos 2002; Fonseca et al. 2004). According to the same authors, fertility is strongly related to body weight, and litter size increases from young to adult age class. Thus, the obtained litter size reveals that the proportion of adult females in our sample exceeds their frequency in the population. In fact, eight out of nine females were adult animals in good body condition that came from a population where this age class represents just about 60% of the breeding females (Santos 2002). Therefore, our results should be interpreted cautiously and not generalized to all population because the mating system might be different in younger and smaller breeding females.

Paternity analysis and mating system

Our results suggest that once a boar succeeds in copulating it may try to prevent the access to receptive females. Otherwise, if females mated in sequence with different boars, stronger evidence of multiple paternity would be found since heterospermic inseminations produce littermates sired by different males (Berger 1995; Stahlberg et al. 2000), and multiple paternity occurs when gilts mate in succession with different boars (Martinl and Dzuk 1977). Huck et al. (1985) proposed that female precopulatory guarding should evolve if there is first male sperm precedence, and conversely that post-copulatory mate guarding should evolve if there is last male sperm precedence. In *S. scrofa* as both first and last-copulatory males are able to sire offspring it seems likely that a boar would gain fertility advantage over competitors by expending long time in copulatory mate guarding, either in pre and post-copulatory guarding. Such a strategy is consistent with the male-biased sexual dimorphism observed in adult wild boar (Fonseca 1999) and with the large size of adult male canine teeth (Barret 1986; Rushen and Pajor 1987). Nevertheless, multiple paternity is a frequent occurrence in other male-biased sexual dimorphic natural populations, such as pronghorn antelope (Carling et al. 2003) and white-tailed deer (Sorin 2004). Thus, there is no clear pattern relating sexual size dimorphism to littermate's paternity in ungulates.

Sperm competition seems to play a minor role in sexual selection on the studied population, considering that the semen gel-fraction does not act as an effective copulatory plug. Nevertheless, male–male competition for fertilization a given set of ova may have occurred in one of the examined litters. It is likely that at least two boars fathered the offspring belonging to that singular litter, and differences in sperm fertilizing capacity were noticed. One of the males sired approximately 70% (five out of seven) of the foetuses in the litter. This result is similar to other obtained after heterospermic insemination with pooled semen of boars exhibiting comparable semen parameters (Stahlberg et al. 2000). Besides sperm competition, cryptic female semen choice can also explain these results, and so is possible that there is an ongoing co-evolutionary sexual conflict (Ball and Parker 2003).

Genetic evidence, either of mate guarding or of sperm competition, is consistent with a polyandrous mating system. However, a review of the non-*suidae* mammalian literature suggests that our results should not be generalized to other environments. Say et al. (2002) found that the mating system of feral cats (*Felis catus*) in a sub-Artic environment could be confined to monogamy, contrary to what was observed in environments under strong human influence where the mating system is polygynous or promiscuous (Yamane 1998; Say et al.

1999). The mating system can change according to environmental conditions and with population density, and thus can change the number (percentage) of offspring in a litter sired for different males. The study of both male and female reproductive success variance in *S. scrofa*, considering different age classes, in free-ranging wild boar populations and in enclosed domestic breeds, may shed more light on the species mating strategies and help to understand the role of mate guarding, sperm competition and female cryptic semen choice on sexual selection.

Acknowledgements

We thank the estates' owners, game managers and field staff who helped in tissue collection and Patrick Fitze for the translation of the abstract into German. A. Beja-Pereira was supported by research Grant SFRH/BPD/17822/2004 from Fundação para a Ciência e Tecnologia, Portugal.

Zusammenfassung

Vaterschaftsanalysen bei freilebenden Wildschweinen (*Sus scrofa*) – Sind Jungtiere Vollgeschwister?

Mehrfachvaterschaft innerhalb eines Wurfs kommt in verschiedenen Gruppen von Säugetieren und in verschiedenen Paarungssystemen vor. Mittels sieben genetischer Marker (Mikrosatelliten) untersuchten wir die Vaterschaft von Foeten in freilebenden Wildschweinen (*Sus scrofa*) des mediterranen Raumes. Wir benutzten das Programm CERVUS 2.0. zur Berechnung der Entdeckungswahrscheinlichkeit von mehrfacher Vaterschaft unter Einbeziehung aller Loci (D), zur Berechnung der Wahrscheinlichkeit der Vaterschaft (W), sowie zur Berechnung des statistischen Δ , welches die Zuteilung der Vaterschaft zum wahrscheinlichsten Männchen mit strikten und weniger strikten Konfidenzintervallen erlaubt. Mehrfache Vaterschaft konnte nur in einem von neun Würfen nachgewiesen werden (CERVUS Konfidenzintervall = 80%). Unsere Resultate lassen deshalb vermuten, dass ein Keiler den Zugang zu empfängnisbereiten adulten Bachen unter bestimmten Bedingungen kontrollieren kann, und sie zeigen, dass mehrfache Vaterschaften in wildlebenden Wildschweinpopulationen relativ ungewöhnlich sind. Trotz des möglichen Vorkommens von Spermienkonkurrenz und/oder kryptischer Weibchenwahl scheint das "mate guarding" eine bedeutende Rolle in der sexuellen Selektion zu spielen. Um die Paarungsstrategien der Wildschweine besser zu verstehen, ist es nötig, weitere Studien durchzuführen, welche den reproduktiven Erfolg beider Geschlechter unter verschiedenen Umweltbedingungen messen.

References

- Abaigar, T., 1992. Paramètres de la reproduction chez le sanglier (*Sus scrofa*) dans le sud-est de la Péninsule Ibérique. *Mammalia* 56, 245–250.
- Ball, M.A., Parker, G.A., 2003. Sperm competition games: sperm selection by females. *J. Theor. Biol.* 224, 27–42.
- Barret, C., 1986. Fighting behaviour of wild boar *Sus scrofa*. *J. Mammal.* 67, 512–525.
- Bassam, B.J., Caetano-Anollés, G., Gresshoff, P.M., 1991. Fast and sensitive silver staining of DNA in polyacrilamide gels. *Anal. Biochem.* 80, 81–84.
- Berger, T., 1995. Proportion of males with lower fertility spermatozoa estimated from heterospermic insemination. *Theriogenology* 43, 769–775.
- Berger, T., Anderson, D.L., Penedo, M.C.T., 1996. Porcine sperm fertilizing potential in relationship to sperm functional capacities. *Anim. Reprod. Sci.* 44, 231–239.
- Birkhead, T.R., Møller, A.P., 1998. *Sperm Competition and Sexual Selection*. Academic Press, New York.
- Burton, C., 2002. Microsatellite analysis of multiple paternity and male reproductive success in the promiscuous snowshoe hare. *Can. J. Zool.* 80, 1948–1956.
- Carling, M.D., Wiseman, P.A., Byers, J.A., 2003. Microsatellite analysis reveals multiple paternity in a population of wild prohorn antelopes (*Antilocapra americana*). *J. Mammal.* 84, 237–243.
- Chapman, T., Arnqvist, G., Bangham, J., Rowe, L., 2003. Sexual conflict. *Trends Ecol. Evol.* 18, 41–47.
- Dardaillon, M., 1988. Wild boar groupings and their seasonal changes in the Camargue, Southern France. *Z. Jagdwiss.* 53, 22–30.
- Dean, M.D., Ardlie, K.G., Nachman, M.W., 2006. The frequency of multiple paternity suggests that sperm competition is common in house mice (*Mus domesticus*). *Mol. Ecol.* 15, 4141–4151.
- Delcroix, I., Mauget, R., Signoret, J.P., 1990. Existence of synchronization of reproduction at the level of the social group of the european wild boar (*Sus scrofa*). *J. Reprod. Fertil.* 89, 613–617.
- Fenández-Llario, P., 1996. Ecology of wild boar in Donña: reproductive parameters and environmental impact. Dissertation Thesis, University of Extremadura (in Spanish).
- Fernández-Llario, P., 2005. The sexual function of wallowing in male wild boar (*Sus scrofa*). *J. Ethol.* 23, 9–14.
- Fernández-Llario, P., Mateus-Quesada, P., 1998. Body size and reproductive parameters in the wild boar *Sus scrofa*. *Acta Theriol.* 43, 439–444.
- Fernández-Llario, P., Carranza, J., Mateus-Quesada, P., 1999. Sex allocation in a polygynous mammal with large litters: the wild boar. *Anim. Behav.* 58, 1079–1084.
- Fernández-Llario, P., Mateos-Quesada, P., Silvério, A., Santos, P., 2003. Habitat effects and shooting techniques on two Wild Boar (*Sus scrofa*) populations in Spain and Portugal. *Z. Jagdwiss.* 49, 120–129.
- Fonseca, C., 1999. Ecology of wild boar (*Sus scrofa* L, 1758) in the Centre of Portugal. Dissertation Thesis, University of Coimbra (in Portuguese).
- Fonseca, C., Santos, P., Monzón, A., Bento, P., Alves da Silva, A., Alves, J., Silvério, A., Soares, A.M.V.M., Petrucci-Fonseca, F., 2004. Reproduction in the wild boar

- (*Sus scrofa* Linnaeus, 1758) populations of Portugal. *Galemys* 16 (Special Issue), 53–65.
- Gomendio, M., 2002. Competición espermática. In: Soler, M. (Ed.), *Evolución: la Base de la Biología*. Proyecto Sur de Ediciones SL, Granada, pp. 261–270.
- Hafez, E.S.E., 1993. Semen evaluation. In: Hafez (Ed.), *Reproduction in Farm Animals*. Lea & Febiger, Philadelphia, pp. 405–423.
- Huck, U.W., Quin, R.P., Lisk, R.D., 1985. Determinants of mating success in the golden hamster (*Mesocricetus auratus*). *Behav. Ecol. Sociobiol.* 17, 239–252.
- Jennions, M.D., Petrie, M., 2000. Why do females mate multiply? A review of genetic benefits. *Biol. Rev.* 75, 21–64.
- Kozdrowski, R., Dubiel, A., 2004. The effect of season on the properties of wild boar (*Sus scrofa* L.) semen. *Anim. Reprod. Sci.* 80, 281–289.
- Kraaijeveld-Smit, F.J.L., Ward, S.J., Temple-Smith, P.D., 2002. Multiple paternity in a field population of a small carnivorous marsupial, the agile antechinus, *Antechinus agilis*. *Behav. Ecol. Sociobiol.* 52, 84–91.
- Krützen, M., Barré, L.M., Connor, R.C., Mann, J., Sherwin, W.B., 2004. ‘O father: where art thou?’ – paternity assessment in an open fission–fusion society of wild bottlenose dolphins (*Tursiops* sp.) in Shark Bay, Western Australia. *Mol. Ecol.* 13, 1975–1990.
- Marshall, T.C., Slate, J., Kruuk, L., Pemberton, J.M., 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Mol. Ecol.* 7, 638–655.
- Martin, P.A., Dzuk, P.J., 1977. Assessment of relative fertility of males (cockereles and boars) by competitive mating. *J. Reprod. Fertil.* 49, 323–329.
- Nechtelberger, D., Kaltwasser, C., Stur, I., Meyer, J.-N., Brem, G., Mueller, M., Mueller, S., 2001. DNA microsatellite analysis for parentage control in Austrian pigs. *Anim. Biotechnol.* 12, 141–144.
- Rivas-Martínez, S., Loidi, J., 1999. Biogeography of the Iberian Peninsula. *Itinera Geobot.* 13, 49–67.
- Rushen, J., Pajor, E., 1987. Offence and defence in fights between young pigs (*Sus scrofa*). *Agress. Behav.* 13, 329–346.
- Santos, P., 2002. Criteria for the wise use of wild boar, *Sus scrofa* L. 1758, in Mediterranean ecosystems. Dissertation Thesis, University of Évora (in Portuguese).
- Santos, P., Mexía-de-Almeida, L., Petrucci-Fonseca, F., 2004. Habitat selection by wild boar *Sus scrofa* L. In Alentejo, Portugal. *Galemys* 16 (Special Issue), 167–184.
- Santos, P., Fernández-Llario, P., Fonseca, C., Monzón, A., Bento, P., Soares, A.M.V.M., Mateus-Quesada, P., Petrucci-Fonseca, F., 2006. Habitat and reproductive phenology of wild boar (*Sus scrofa*) in the western Iberian Peninsula. *Eur. J. Wildl. Res.* 52, 207–212.
- Say, L., Pontier, D., Natoli, E., 1999. High variation in multiple paternity of domestic cats (*Felis catus* L.) in relation to environmental conditions. *Proc. R. Soc. London B. Biol. Sci.* 266, 2071–2074.
- Say, L., Devillard, S., Natoli, E., Pontier, D., 2002. The mating system of feral cats (*Felis catus* L.) in a sub-Antarctic environment. *Polar Biol.* 25, 838–842.
- Sorensen Jr., M., 1982. *Reproducción Animal – Principios y Práctica*. McGraw-Hill, City of Mexico.
- Sorin, A.B., 2004. Paternity assignment for white-tailed deer (*Odocoileus virginianus*): mating across age classes and multiple paternity. *J. Mammal.* 85, 356–362.
- Stahlberg, R., Harlizius, B., Weitze, K.F., Waberski, D., 2000. Identification of embryo paternity using polymorphic DNA markers to assess fertilizing capacity of spermatozoa after heterospermic insemination in boars. *Theriogenology* 53, 1365–1373.
- Trivers, R.L., Willard, D.E., 1973. Natural selection of parental ability to vary the sex ratio of offspring. *Science* 179, 90–92.
- Westneat, D.F., Fredrick, P.C., Havnen, W.R., 1987. The use of genetic markers to estimate the frequency of successful alternative reproductive tactics. *Behav. Ecol. Sociobiol.* 21, 35–45.
- Wolff, J.O., Macdonald, D.W., 2004. Promiscuous females protect their offspring. *Trends Ecol. Evol.* 19, 127–134.
- Yamane, A., 1998. Male reproductive tactics and reproductive success of the group living cat (*Felis catus*). *Behav. Proc.* 43, 239–249.