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Multiple paternity in domestic pigs under equally probable natural matings – a case study in the endangered Gochu Asturcelta pig breed

J. Menéndez[1], I. Álvarez[2], I. Fernández[2], B. de la Roza[3], and F. Goyache[2]

[1]Asociación de Criadores de Gochu Asturcelta (ACGA), 33403 Avilés, Spain
[2]Área de Genética y Reproducción Animal, SERIDA-Deva, 33203 Gijón, Spain
[3]Área de Nutrición, Pastos y Forrajes, SERIDA-Villaviciosa, 33300 Villaviciosa, Spain

Correspondence to: F. Goyache (fgoyache@serida.org)

Abstract. Here we provide evidence of multiple paternities in naturally mated sows under conditions ensuring that (a) sows had the same probability of being mated by any of the available boars and (b) no differences in hybrid vigour existed. Total DNA was obtained from 19 Gochu Asturcelta piglets from three different sows, each with the same chance of natural mating with two different boars. A set of 20 microsatellites were typed on all the individuals. The program CERVUS was used to asses the informative ability of the microsatellite set and to perform paternity assignment. Allelic frequencies at population level were obtained using a total of 141 Gochu Asturcelta individuals. Offspring were always assigned to a candidate boar with high statistical confidence. All litters had different parents. Our results show that multiple paternities are possible in domestic pigs under natural mating. Furthermore, the current study can be useful to further understand the mating system of the wild boar.

1 Introduction

Whatever the cause (male copulatory competition, post-copulatory competition or differences in semen quantity and quality), multiple paternity in wild boar has been widely reported (see Delgado et al., 2008, for a review). However, this issue has not been fully addressed with regard to domestic pigs. Heterospermic artificial inseminations give litters with multiple paternities in domestic pigs (Berger et al., 1996; Stahlberg et al., 2000). In addition, Aguilera-Reyes et al. (2006) have reported multiple paternities in naturally mated sows under a planned mating system with intervals of 12 h between each mating. The experiment by Aguilera-Reyes et al. (2006) included a few variables, namely the hybrid vigour of the most successful boar and the interval among subsequent matings that could affect the reported results. Here we give evidence of multiple paternities in naturally mated sows under the following conditions: (a) assuming the same vigour, sows had the same probability of being mated for all the available boars, and (b) no differences in hybrid vigour existed. Results were obtained from the endangered Asturcelta pig breed (Royo et al., 2008; Santos e Silva et al., 2008), reared in very traditional conditions in Asturias (Spain).

2 Materials and methods

Two groups (A and B) of Asturcelta individuals were assessed. Group A consisted of four full sibs (two sows – A_{72} and A_{73} – and two boars – A_{74} and A_{75}), born on 5 August 2005 and kept together, under loose housing, from weaning to 6 months old, in cubicles of about 450 m^2. Group B consisted of two full sibs, born on 30 December 2005 (one sow – B_{69} – and one boar – B_{76}); after they were 6 months old, they were kept in a paddock together with an adult boar (B66, born on 1 October 2003). There was no contact or mating restrictions within groups. Litter size was six for A_{73} and B_{69} and seven for A_{72}. The offspring of group A were born on 19 April (A_{72}) and 22 April (A_{73}) 2006, and those belonging to group B were born on 10 December 2005.

Skin samples were obtained from the 26 individuals. Total DNA was isolated from samples following standard procedures (Sambrook et al., 1989). Individuals were genotyped with a set of 20 microsatellites (IGF1, S0002,

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Table 1. Description of the informative ability of the 19 polymorphic microsatellites genotyped.

Locus	k	H_{Obs}	H_{Exp}	PIC	NE-1P	NE-2P	NE-PP	NE-I	NE-SI	HW	F(Null)
IGF1	8	0.490	0.601	0.565	0.793	0.616	0.420	0.195	0.500	NS	+0.105
S0002	9	0.600	0.801	0.772	0.562	0.386	0.200	0.067	0.368	–	+0.142
S0026	5	0.562	0.740	0.691	0.683	0.507	0.332	0.116	0.410	–	+0.139
S0071	6	0.688	0.747	0.702	0.664	0.488	0.306	0.108	0.405	NS	+0.028
S0101	11	0.452	0.511	0.492	0.847	0.669	0.467	0.258	0.560	NS	+0.039
S0155	6	0.712	0.708	0.656	0.708	0.538	0.356	0.137	0.431	NS	−0.007
S0225	5	0.431	0.521	0.475	0.858	0.705	0.540	0.276	0.560	NS	+0.077
S0226	10	0.609	0.714	0.679	0.682	0.498	0.298	0.115	0.423	NS	+0.063
S0227	4	0.119	0.535	0.454	0.855	0.736	0.598	0.298	0.558	–	+0.646
S0228	6	0.250	0.304	0.290	0.952	0.831	0.703	0.499	0.723	NS	+0.145
SW240	9	0.636	0.787	0.756	0.585	0.406	0.216	0.075	0.377	NS	+0.101
SW632	10	0.688	0.787	0.754	0.590	0.413	0.227	0.077	0.377	NS	+0.065
SW911	7	0.421	0.618	0.565	0.791	0.628	0.451	0.198	0.492	–	+0.205
SW936	9	0.669	0.793	0.760	0.583	0.405	0.220	0.074	0.373	NS	+0.084
SW951	4	0.324	0.323	0.298	0.947	0.832	0.713	0.484	0.710	NS	+0.001
SW857	9	0.581	0.821	0.794	0.532	0.358	0.179	0.057	0.355	–	+0.175
S0005	5	0.485	0.532	0.480	0.856	0.703	0.539	0.271	0.556	NS	+0.024
S0090	3	0.576	0.533	0.415	0.862	0.777	0.664	0.335	0.571	NS	−0.061
SW24	3	0.938	0.520	0.396	0.869	0.794	0.689	0.354	0.582	–	−0.298

k: number of alleles per locus; H_{Obs}: observed heterozygosity; H_{Exp}: expected heterozygosity; PIC: polymorphic information content; NE-1P: combined non-exclusion probability (first parent); NE-2P: combined non-exclusion probability (second parent); NE-PP: combined non-exclusion probability (parent pair); NE-I: combined non-exclusion probability (identity); NE-SI: combined non-exclusion probability (sib identity); HW: deviation from Hardy–Weinberg equilibrium; F(Null): frequency of null alleles.

S0026, S0071, S0101, S0155, S0225, S0226, S0227, S0228, SW240, SW632, SW911, SW936, SW951, SW857, S0005, S0090, S0218 and SW24) in an automatic sequencer (ABI 310, Applied Biosystems). Most microsatellites used were included in the ISAG-FAO panel (http://www-lgc.toulouse.inra.fr/pig/panel/panel2004.htm). Primer sequences and PCR (polymerase chain reaction) conditions can be found on the aforementioned website and in Laval et al. (2000). Microsatellite S0218 was monomorphic in our sample and was not used in further analyses. Microsatellite information and paternity assignment analyses were carried out using the program CERVUS 3.0 (Kalinowski et al., 2007). Allelic frequencies at population level were obtained using a total of 141 Gochu Asturcelta individuals.

3 Results and discussion

Parameters describing the variability and informative ability of the microsatellite set used are given in Table 1. Probabilities of non-exclusion were 0.00384536 when the two parents were unknown, 0.00005149 when one parent was unknown and 0.00000005 when the two parents were known. Simulations showed that the use of the microsatellite set assayed gave 98 % successful paternity assignments with a confidence of 95 % if the genotype of the mother was not known and 100 % when this was known. Six of the microsatellites tested showed significant deviations from the Hardy–Weinberg proportions, probably due to inbred matings. Since the analysed samples came from inbred individuals, this scenario can be considered acceptable. In a recent report, Costa et al. (2012) tested a microsatellite set for parentage analyses in three different populations of European wild boar. Even though such a microsatellite set showed a comparable informative ability to that used in the current analysis (see Table 1 of that paper), Costa et al. (2012) found some failures in detecting any putative father within their samples due to insufficient male sampling. This concern is not relevant to our study.

Table 2 gives the results obtained in paternity assignments. No pair–loci mismatching was identified for any sow–offspring couple. Offspring were always assigned to a candidate boar with high statistical confidence. All litters had two different parents; the boar A_{74} had six offspring, A_{75} had seven offspring, B_{66} had four offspring and B_{76} had two offspring. A chi-square test showed that no statistical differences in paternity success were assessed in group A. The higher paternity success observed for the adult boar B_{66} can be explained by its higher probabilities of success in male–male competition for accessing reproduction. Mate guarding has been reported to play a significant role in sexual selection in wild boar (Delgado et al., 2008). By contrast with previous reports (Aguilera-Reyes et al., 2006), our data were obtained without copulatory restrictions and, particularly in the case of the full sibs forming the analysed group A, no differences in hybrid vigour.

Table 2. Results of paternity assessment obtained using the program CERVUS. The assigned fathers are those with higher LOD score.

Offspring code	Mother code	Pair LOD score[a]	Assigned father	Pair LOD score[b]	Pair Delta[b,c]	Pair confidence[d]
A_{93}	A_{73}	1.35×10^{15}	A_{74}	9.72×10^{14}	9.72×10^{14}	*
A_{94}	A_{73}	1.02×10^{15}	A_{75}	3.82×10^{14}	3.82×10^{14}	*
A_{95}	A_{73}	1.03×10^{15}	A_{75}	5.58×10^{14}	2.92×10^{14}	*
A_{96}	A_{73}	1.12×10^{15}	A_{74}	1.04×10^{15}	6.31×10^{14}	*
A_{97}	A_{73}	1.04×10^{15}	A_{74}	9.88×10^{14}	8.67×10^{14}	*
A_{98}	A_{73}	8.60×10^{14}	A_{75}	4.30×10^{14}	2.25×10^{14}	*
A_{99}	A_{72}	1.23×10^{15}	A_{75}	5.69×10^{14}	2.44×10^{14}	*
A_{100}	A_{72}	1.15×10^{15}	A_{75}	1.95×10^{14}	1.95×10^{14}	*
A_{101}	A_{72}	1.11×10^{15}	A_{74}	8.16×10^{14}	7.88×10^{14}	*
A_{102}	A_{72}	1.13×10^{15}	A_{75}	2.42×10^{14}	2.42×10^{14}	*
A_{103}	A_{72}	9.17×10^{14}	A_{75}	5.26×10^{14}	5.26×10^{14}	*
A_{104}	A_{72}	9.25×10^{14}	A_{74}	7.15×10^{14}	3.08×10^{14}	*
A_{105}	A_{72}	1.08×10^{15}	A_{74}	8.93×10^{14}	3.25×10^{14}	*
B_{50}	B_{69}	1.42×10^{15}	B_{76}	1.19×10^{15}	1.19×10^{15}	*
B_{51}	B_{69}	9.23×10^{14}	B_{66}	4.74×10^{14}	9.59×10^{12}	*
B_{52}	B_{69}	6.91×10^{14}	B_{66}	7.09×10^{14}	4.77×10^{14}	*
B_{53}	B_{69}	1.41×10^{15}	B_{76}	1.15×10^{15}	9.86×10^{14}	*
B_{54}	B_{69}	7.16×10^{14}	B_{66}	5.41×10^{14}	1.03×10^{14}	*
B_{55}	B_{69}	9.46×10^{14}	B_{66}	6.16×10^{14}	2.79×10^{14}	*

[a] LOD: scores of the logarithm of the likelihood ratio; [b] figures must be multiplied by 10^{14}; [c] Delta is defined as the difference in LOD scores between most likely and the second most likely candidate; [d] confidence level of CERVUS paternity assignments. An asterisk indicates a statistical confidence on paternity assignment higher than 95 % using the "strict" criterion implemented in CERVUS. Note that the alternative relaxed criterion would mean a statistical confidence higher than 80 %.

Our results clearly show that (a) multiple paternities are possible in domestic pigs under natural mating and (b) when no differences in animal size, development, hybrid vigour or probabilities of mating exist, no differences in paternity success are to be expected. Our results can also be useful to further understand the mating system of the wild boar (Costa et al., 2012). There is extensive evidence of multiply sired litters in wild board that are likely to be due to ethological rather than to biological factors (Aguilera-Reyes et al., 2006; Delgado et al., 2008; Poteaux et al., 2009).

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