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On the evolutionary consequences of increasing litter size with multiple paternity in wild boar (*Sus scrofa scrofa*)

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Understanding how some species may be able to evolve quickly enough to deal with anthropogenic pressure is of prime interest in evolutionary biology, conservation, and management. Wild boar (*Sus scrofa scrofa*) populations keep growing all over Europe despite increasing hunting pressure. In wild boar populations subject to male-selective harvesting, the initially described polygynous mating system may switch to a promiscuous/polyandrous one. Such a change in the mating system, where potentially more males sire a litter at one reproductive event, may be associated with the retention of high genetic diversity and an increase of litter size. We tested these hypotheses by estimating the number of sires per litter based on a six-year long monitoring of a wild boar population subject to particularly high harvesting pressure. Our results show a high and stable genetic diversity and high rates of multiple paternity compared to other populations, thus depicting a promiscuous/polyandrous mating system in this population. We also show that litter size is positively linked to the number of sires, suggesting that multiple paternity increases fecundity. We finally discuss that multiple paternity may be one of the factors allowing rapid evolution of this population by maintaining both genetic and phenotypic diversity.

KEY WORDS: **Fecundity, harvesting, mating system, polyandry, selective hunting.**

Human exploitation, through hunting or fishing, affects the size of free-ranging populations. High harvesting pressures lead to the removal of a large proportion of individuals, inducing a strong yearly decline of population size. Because genetic diversity is linked to population size (Frankham 1996), intensively harvested populations undergo great genetic loss every year (Harris et al. 2002). This may affect their adaptive potential (Amos and Balmford 2001; Barrett and Schluter 2008), leading to a demographic decline and, in the worst case scenario, to extinction (Gilpin and Soulé 1986; Rosser and Mainka 2002; see Spielman et al. 2004 for a meta-analysis on 170 taxa) if the populations are unable to respond to the new selective pressures. For example, overexploitation is the main factor that induced the collapse of several fisheries in the past century (Hutchings and Myers 1995; Jackson et al. 2001). In addition, for populations facing anthropogenic pressures through exploitation, some modifications have also been observed in phenotypic traits (Coltman et al. 2003; Douhard et al. 2016), demography (Milner et al. 2007; Servanty et al. 2011), or genetic characteristics (Harris et al. 2002; Allendorf et al. 2008).

Selective harvesting, the intensification of harvest efforts geared toward individuals showing phenotypic traits favored by hunters (Milner et al. 2007), may affect the structure of populations. For instance, in populations subject to size-selective harvesting, where the largest adults are preferentially removed,

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the age and sex structure is biased toward the young and females (Milner et al. 2007). Such a change in age and sex distribution may have strong consequences on the mating system (Kokko and Rankin 2006; Milner et al. 2007). In ungulate populations, the mating system is known to be influenced by density of males and females (Isvaran 2005). For species showing a polygynous mating system with female monopolization by males, intrasexual competition is diminished when larger males are removed and younger males are more likely to obtain paternities by harassment of females (Isvaran 2005). The mating system switches to a promiscuous/polyandrous one and the multiple paternity rate (*MPR*; defined as the proportion of litters showing more than one father) increases. As the number of males accessing reproduction grows, the variance of male reproductive success decreases, allowing greater genetic diversity to pass on from one year to the other compared to the polygynous mating system (Nunney 1993; Sugg and Chesser 1994; Pearse and Anderson 2009). We hypothesize that these processes (promiscuous mating system and multiple paternity) may have the potential to buffer the loss of genetic diversity due to intensive harvesting. However, multiple paternity effects have been the topic of a debate. On the one hand, in their review, Jennions and Petrie (2000) described most of the genetic benefits for females, including the increase of genetic diversity within a litter, which tends to increase the genetic diversity at the population scale (Pearse and Anderson 2009). On the other hand, Lotterhos (2011) tempered this result by showing that the positive link between multiple paternity and effective population size, which reflects population genetic diversity, is not always true but depends on the litter size, the number of reproductive events, and the female's number of mates over her lifetime.

Surprisingly, wild boars (*Sus scrofa scrofa*) do not fit into the classical frame of reduced population size due to intensive harvesting. Their populations are growing all over Europe despite the continuous increase of hunting pressure (Massei et al. 2014). The mating system of this species has been originally described as polygynous (Mauget 1980; Dardaillon 1984), which is consistent with the sexual dimorphism displayed by the species (Ralls 1977). Interestingly, with the rise of molecular genetic techniques, a growing literature has shown that multiple paternity occurs (Delgado et al. 2008; Poteaux et al. 2009) and may be common in some populations of wild pigs (Delgado-Acevedo et al. 2010; Costa et al. 2012). A positive effect of multiple paternity on litter size has even been highlighted in the domestic pig (*Sus scrofa domesticus*), which is the domestic counterpart of the wild boar. Due to its economic importance (Orr and Shen 2006), the pig is the subject of many studies that aim at understanding the mechanisms underlying reproduction to improve production. The number of artificial insemination events is known to have a positive effect on litter size (Kemp and Soede 1996; Corrêa et al. 2002). Moreover, several sires are commonly used in pig husbandry to increase litter size, hence productivity (Badinel 2010). However, until now, only a few studies have focused on the link between litter size and number of fathers in the wild (DiBattista et al. 2008; Thonhauser et al. 2014, but see Waller and Bilkei 2002 for evidence of larger litter sizes with increasing number of sires per litter in free-ranging pigs).

Recent studies conducted on the wild boar population of Châteauvillain-Arc-en-Barrois, which suffers from a particularly high and male-selective harvesting pressure (Toïgo et al. 2008; Servanty et al. 2011), have shown that selection for both earlier birth date and earlier sexual maturity in females could occur over just a few generations to adapt to the harvesting regime (Servanty et al. 2009; Gamelon et al. 2011). But whether multiple paternity may (1) have a positive effect on wild boar fecundity through larger litter size as shown in pigs, and (2) maintain a high genetic diversity through time, which is then transmitted to each generation, thereby buffering yearly genetic loss and allowing a high ability to respond to new selective pressure, remain understudied questions. Hence, using six years of data sampling, we addressed these questions. We expected a high level of genetic diversity together with a high rate of multiple paternity in this population, compared to other populations of the same species, triggered by a likely disruption of the polygynous mating system. Moreover, we investigated how the litter size is related to the number of sires within litters and predicted a larger litter size when the number of sires increases.

Material and Methods

STUDY SITE AND SAMPLE COLLECTION

The wild boar population is located in the 11,000 ha Châteauvillain-Arc-en-Barrois open forest (48°02′N; 4°55′E, France) surrounded by agricultural fields, thus immigration rate is low (Baubet unpubl. data). The number of individuals was estimated to be between 1200 and 1500 (Gamelon et al. 2011). In this heavily hunted population, wild boars have a 40% probability of being shot every year, rising to 70% for adult males (Toïgo et al. 2008). Moreover, the population exhibits a particularly short generation time for an ungulate that was previously estimated to be 2.27 years (Servanty et al. 2011). During six hunting seasons (2007–2012), the number of wild boar killed annually ranged from 567 to 794 (mean 635). Tissue samples were collected from 165 hunted pregnant females and their full litters (845 fetuses, mean litter size = 5.1 $\pm$ 1.63 SD), 264 nonbreeding females (included only in the genetic diversity analyses), and from 627 putative reproductive males with a dressed body mass (i.e., without the digestive system, heart, lungs, liver, reproductive tract, and blood) higher than 30 kg (Gamelon et al. 2012). Body mass has been shown to be

a structuring factor, more appropriate than age for this species (Gamelon et al. 2012), so weight was recorded for individuals.

MOLECULAR ANALYSIS

All tissue samples were stored in alcohol in an individual hermetic straight container of 25 mL and then genotyped for 12 microsatellite loci (Supporting Information 1). For each sample, 20–80 ng/μL of total genomic DNA was extracted using a buffer lyse. A few milligrams of tissue were pounded and then incubated first at 56°C for 2–3 h and then at 72°C for 20 min in 200 μL volumes containing 4 μL of Tris HCL 1 M, 0.3 μL of $MgCl_2$ 1 M, 5μL of KCl 1 M, 1 μL of Tween 20 and 1 μL of K proteinase (20 mg/mL, EUROBIO). Selective amplification was carried out for 12 microsatellite loci divided into two polymerase chain reaction (PCR) multiplexes. PCR were conducted in 96-well microtiter plates in final volumes of 20 μL containing 10 μL of PCR Multiplex Master Mix (2×, Qiagen), 0.6 μL of each primer (10 mM), and 2 μL of the extraction product. PCR was conducted using a BIOBLOCK PTC 100 thermal cycler with the following program: 95°C/15 min; 30 cycles with 94°C/30 sec; 57°C/1.30 min; and 72°C/1 min denaturing, annealing, and extension temperatures, respectively, and finally 60°C/30 min. The sizes of PCR amplified products were resolved by GENOSCREEN (http://www.genoscreen.fr) using an APPLIED BIOSYSTEMS 3730 × l DNA Sequencing Analyzer (Supporting Information 1). Three mother–litter pairs and two males were removed from the analysis due to the high number of missing genotypes.

GENETIC AND PATERNITY ANALYSIS

CERVUS 3.0.7 (Kalinowski et al. 2007) was used to compare observed (*Ho*) and expected (*He*) heterozygosity (using only adult genotypes to avoid biases from family genetic links, Supporting Information 1), to identify mother–offspring mismatches, to estimate the null allele rate, and to conduct maximum likelihood paternity analyses. CERVUS compares potential sires using mismatches in the fetus–mother–male trio and likelihood ratio scores. Males were considered to be a fetus's genetic sire when there was no father–offspring mismatch, when the fetus–mother–male trio had a positive likelihood of detection (LOD) and when a ΔLOD higher than the 80% critical likelihood ratio (determined by simulations [Supporting Information 2]). Putative sires for fetuses sampled in a given hunting season i were composed of all males sampled during the hunting season i, all yearlings and adult males sampled in the hunting season $i + 1$, and adult males sampled in the hunting season $i + 2$. Overall, the numbers of putative sires were 141, 233, 307, 346, 332, and 122 for the six hunting seasons (2007–2012), respectively. The number of sires per litter N_C, estimated with CERVUS, was recorded as the number of identified fathers.

To circumvent CERVUS failures to identify sires for all fetuses within a litter, GERUD 2.0 (Jones 2005) was also used to provide a second measure of the minimum number of males contributing to each litter N_G. Using known maternal genotypes, GERUD calculates the minimum number of fathers contributing to a given litter by subtracting the known maternal alleles from fetus genotypes, simulating all possible paternal genotypes, and determining the combinations of the remaining alleles that yield the fewest possible sires (Jones 2005). The error rate was estimated by simulations using GERUDsim (Supporting Information 2). Due to computational limitations, we used for each mother–litter array only the five most polymorphic loci showing no missing data, when possible. In 87 of 160 mother–litter arrays, the five loci were the five most variable ones overall. Among the 73 remaining mother–litter arrays, 45 were analyzed with one other locus, 19 using two other loci and nine with all loci showing no missing data. Within these constraints, we performed an exhaustive search for the number of possible combinations of fathers that could explain each progeny array and recorded the minimum number of sires for each litter N_G. Because GERUD uses exclusion to estimate the number of male genotypes contributing to a given progeny array, estimates using this program are considered very conservative and should never overestimate the number of sires N_G for a litter (Jones 2005).

To circumvent CERVUS failures to identify sires of all fetuses within a litter and GERUD conservatism, we complemented our analysis with a less conservative analytical approach based on the maximal number of paternal alleles. Each fetus inherits one allele from its father and one allele from its mother so that the maximum number of alleles in a monopaternal litter is four if both parents are heterozygous and a maximum of two paternal alleles would be identified if single paternity occurs. For each litter and each locus, we calculated the number of alleles, from which we retrieved the known number of maternal alleles to estimate the number of paternal alleles. The maximal number of paternal alleles over the 12 loci was retained to obtain N_{PA}. We acknowledge that this number is also conservative, as only one allele in a litter at a given locus does not preclude multiple paternities.

Three different estimates of the *MPR* could thus be obtained, allowing us to evaluate consistency: the proportion of litters having more than one putative sire (CERVUS, MPR_C), the proportion of litters having a minimum number of sires N_G higher than one (GERUD, MPR_G) and the proportion of litters having more than two paternal alleles for at least one locus (maximal number of paternal alleles approach, MPR_{PA}).

STATISTICAL ANALYSIS

To identify the key variables driving the variability of the litter size *LS* across the litters, we performed a Poisson regression model where the response variable was *LS* and in which the locus-specific

Table 1. List of the eight papers including the present study dealing with multiple paternity in *Sus scrofa* populations (on 25 September 2015).

Sub-species	Sample size	N_{loci}	*A*	*Ar*	*Ho*	*He*	$N_{litters}$	*LS*	*MPR*	Harvesting pressure information in the study	Study (population)
S.s.d	354	14	8.14 [4–17]	9.96 [2–25]	0.575 [0.367–0.756]	**0.68** [0.504–0.833]	11	5.64[1] [3–10]	0% (0)	A population "sampling rate" of 70% was assumed on the basis of published estimates of feral pig capture rates from studies that used very similar trapping methods to those used in this study	Hampton et al. (2004)
S.s.d	55	13	4.62 [2–6]	7.55 [1–20]	**0.663** [0.333–0.889]	**0.641** [0.5–0.822]	21	No data	**48%** (10)	Prior to our study, this region was subjected to at least two years of intensive feral pig control, mainly through aerial baiting with 1080 (sodium monofluoroacetate) baits	Spencer et al. (2005)
S.s.d	409	12[2]	8.3[2] [4–12]	10.12 [2–25]	**0.595**[2] [0.294–0.78]	**0.68**[2] [0.39–0.838]	2	5.4[2] [2–11]	**50%** (1)	Feral pig were trapped, harvested or removed by aerial and ground shooting as part of damage-control management activities and sport hunting.	Delgado-Acevedo et al. (2010) (Brooks)
							5		40% (2)		Delgado-Acevedo et al. (2010) (Cameron)
							2		0% (0)		Delgado-Acevedo et al. (2010) (Coryell)
							8		13% (1)		Delgado-Acevedo et al. (2010) (Dimmit)
							1		0% (0)		Delgado-Acevedo et al. (2010) (Hidalgo)

(*Continued*)

Table 1. Continued.

Sub-species	Sample size	N_{loci}	*A*	*Ar*	*Ho*	*He*	$N_{litters}$	*LS*	*MPR*	Harvesting pressure information in the study	Study (population)
							1		**100%** (1)		Delgado-Acevedo et al. (2010) (Kerr)
							11		27% (3)		Delgado-Acevedo et al. (2010) (Kleberg)
							16		38% (6)		Delgado-Acevedo et al. (2010) (McMullen)
							18		39% (7)		Delgado-Acevedo et al. (2010) (San Patricio)
S.s.s	9	7	4.14 [3–6]	4.63 [1–13]	**0.603** [0.339–0.731]	0.576 [0.443–0.707]	9	5.56[3] [5–7]	11% (1)	The hunting pressure is high and so is the number of wild boars taken per 100 ha of shooting area (Fernandez-Llario et al. 2003) From this study: 9.49 ± 8.65/100 ha ($n = 17$ hunt, mean ± SD)	Delgado et al. (2008)
S.s.s	488	12	6.58 [2–16]	10.32 [2–25]	0.518 [0–0.83]	0.552 [0.21–0.87]	21	4.05 [2–6]	10% (2)	40% probability of being shot up to 70% for males from Toigo et al. (2008) 5.21/100 ha (±2.66, range: 0.64–9.27) from Servanty et al. (2009)	Poteaux et al. (2009) (Population of the study)
S.s.s	49		6.21 [3–14]	7.42 [1–20]	**0.718** [0.444–1]	**0.698** [0.396–0.901]	5	5.8	40% (2)	No information	Costa et al. (2012) (Hungary)
	72	14[2]	5.07 [3–10]	7.95 [2–21]	0.542 [0.268–0.861]	0.552 [0.259–0.776]	5	6.2	20% (1)	No information	Costa et al. (2012) (Portugal)
	46		5.5 [4–12]	7.3 [1–20]	**0.647** [0.364–0.909]	**0.646** [0.411–0.906]	5	4.8	40% (2)	No information	Costa et al. (2012) (Spain)

(*Continued*)

Table 1. Continued.

Sub-species	Sample size	N_{loci}	*A*	*Ar*	*Ho*	*He*	$N_{litters}$	*LS*	*MPR*	Harvesting pressure information in the study	Study (population)
		14[2]	9.64[2]	10.76	0.548	0.553	27	4.3	Different estimates		Pérez-González et al. (2014)
S.s.s	181		[5–24]	[2–25]	[0.451–0.833][2]	[0.412–0.837][2]			not directly comparable with ours (MPR defined as		(Spain Western Iberian Peninsula)
	188				**0.658** [0.451–0.833][2]	**0.632** [0.412–0.837][2]	35	3.9	number of sires		Pérez-González et al. (2014) per sow) (Spain Azagala)
	74				**0.634** [0.451–0.833][2]	**0.632** [0.412–0.837][2]	13	3.5		Past experience with the study areas suggests a sampling intensity between 10 and 20%	Pérez-González et al. (2014) (Spain Santa Amalia)
	260				**0.683** [0.451–0.833][2]	**0.692** [0.412–0.837][2]	35	5.9			Pérez-González et al. (2014) (Hungary)
S.s.s	1054	12	11.25 [2–25]	–	0.590 [0.107–0.845]	0.602 [0.125–0.891]	160	5.1 [1–10]	$MPR_G = 61\%$ $MPR_C = 44\%$ $MPR_{NPA} = 34\%$	40% probability of being shot up to 70% for males from Toigo et al. (2008) 5.21/100 ha (±2.66, range: 0.64–9.27) from Servanty et al. (2009)	Present study

The subspecies (*S.s.d* for *S. s. domesticus* corresponds to feral pig populations, *S.s.s* for *S. s. scrofa*), the sample size, the number of microsatellite loci (N_{loci}), the mean number of alleles (*A*), the mean allelic richness (*Ar*, obtained from 1000 random subsamplings of our dataset corresponding to the sample size of the study cited), the mean observed heterozygosity (*Ho*), the mean expected heterozygosity (*He*), the mean number of litters (N_{litter}), the mean litter size (*LS*), the *MPR* with the number of litters showing multiple paternity in brackets, information about harvesting pressure found in the study, and the reference of the study (with information about population's location when required) are provided. Values in brackets show the range of the values when available. Bold values for *A*, *Ar*, *Ho*, and *He* show values higher than the ones obtained in the present study. Bold values for *MPR* show values higher than the mean value of our three methods (i.e., 46%).

[1]Only litters with three or more piglets/fetuses were analyzed.

[2]No data per population available.

[3]Only litters with five or more fetuses were selected.

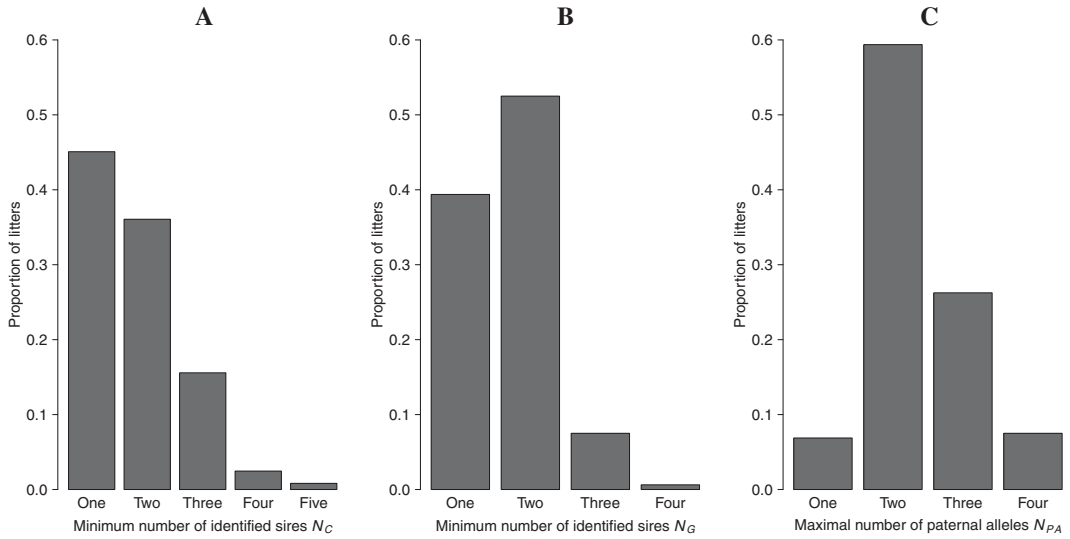

Figure 1. Distribution of the estimation of the minimum number of sires per litter using (A) CERVUS (N_C, $n_{litters} = 122$); and (B) GERUD (N_G, $n_{litters} = 160$) and (C) the maximal number of paternal alleles per litter (N_{PA}, $n_{litters} = 160$).

Table 2. Estimates, SEs, *z* statistics, and *P* values of parameters linked with litter size (*LS*). Significant parameters are in bold ($n_{litters} = 160$). (A) Values for the number of alleles *A*, the mother dressed body mass BM_m, and the maximal number of paternal alleles N_{PA} were obtained from the model strongly supported by the data (Table S3). (B) Values for the mother dressed body mass BM_m and the number of father estimated by GERUD (N_G) were obtained from the model strongly supported by the data (Table S4).

(A)				
Parameter	Estimate	SE	*z*-test statistic	*P*-value
Intercept	0.475	0.222	2.14	–
A	**0.017**	**0.007**	**2.30**	**0.02**
BM_m	**0.010**	**0.002**	**4.34**	**<0.001**
N_{PA}	**0.112**	**0.047**	**2.36**	**0.02**
(B)				
Intercept	0.852	0.153	5.57	–
BM_m	**0.011**	**0.002**	**4.63**	**<0.001**
N_G	**0.132**	**0.054**	**2.44**	**0.015**

Ho and the observed number of alleles *A* for the locus showing the maximal number of paternal alleles in each litter were included as confounding variables. The mother dressed body mass BM_m and, finally, the maximal number of paternal alleles N_{PA}, as a proxy of the number of fathers, were included as main biological effects. We started model selection from the full additive model and then we selected the model with the lowest AICc to get estimates and SE for each predictor variable.

To ensure that the pattern of relationship revealed between *LS* and N_{PA} was not an artifact due to the positive structural relationship between N_{PA} and *LS* (i.e., the impossibility to observe a N_{PA} higher than *LS*), we used a permutation test. We performed 10,000 random permutations of N_{PA}, *Ho*, and *A* values kept together as a triplet against the pair of *LS* and BM_m values in the range of possible values (permutations where N_{PA} was higher than *LS* were not allowed). Each permutated dataset was analyzed using the model selected from the analysis of the observed dataset (model with the lowest AICc) to obtain the averaged coefficient associated to N_{PA}. The effect of N_{PA}, obtained with our observed dataset, was tested by calculating the exact *P*-value, against the distribution of permuted values, using the method described by Phipson and Smyth (2010).

The same analyses were performed using N_G along with BM_m as biological effect in the model to explain *LS* variability. The same permutation approach was also carried out by permuting N_G values against *LS* and BM_m values in the range of possible values. The analysis was not performed with CERVUS due to the few litters with all fathers identified. Moreover, litters with few fathers are more likely to be fully resolved than litters with a higher number of fathers. All analyses were performed in R 3.1.3 software (R Core Team 2015).

Results

PATERNITY ANALYSES AND *MPR*

Overall, mean allelic diversity was $A = 11.25$ alleles per locus, ranging from two to 25 and mean expected heterozygosity was $He = 0.602$, ranging from 0.125 to 0.891 (Table 1, see Supporting Information 1 for details). Ten out of 12 loci showed very small deviations from expected heterozygosity and 11 out of 12 a low frequency of null alleles (<0.05 per locus; Supporting Information

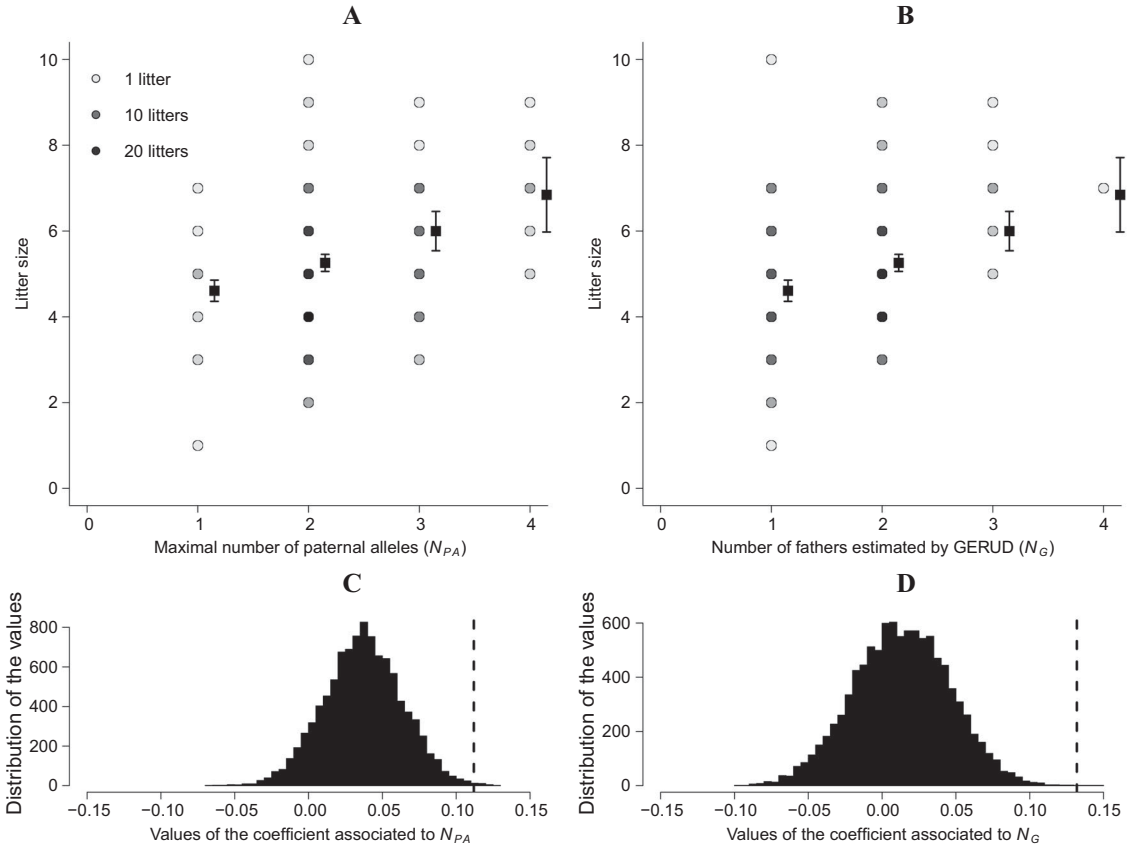

Figure 2. **Effect of the number of sires per litter estimated by (A) the maximal number of paternal alleles (N_{PA}) and (B) the number of fathers (N_G) on the litter size ($n_{\text{litters}} = 160$). Circles, whose color indicates the number of litters, correspond to observations, and squares (± SE) correspond to predicted litter sizes. Note that predicted values were obtained assuming a mother body mass (BM_m), and a number of alleles *A* equal to the mean observed values (i.e., 50.24 kg, and 21.35, respectively). Distribution of the values of the coefficient associated to (C) N_{PA} and (D) N_G, obtained from 10,000 random permutations of the dataset. The dashed lines correspond to the observed averaged values of the coefficients ($N_{PA} = 0.112$ and $N_G = 0.132$). Note that $p(N_{PA\ \text{permuted}} > N_{PA\ \text{observed}}) = 0.002$ with 10,000 values of $N_{PA\ \text{permuted}}$ and $p(N_{G\ \text{permuted}} > N_{G\ \text{observed}}) < 0.001$ with 10,000 values of $N_{G\ \text{permuted}}$.**

1). Two mothers showed loci mismatches with all their presumed offspring, leading us to consider that a sampling mistake occurred at the collecting site. Therefore, they were removed from further analyses. Overall, the sire of 44.77% of the fetuses was identified among the set of candidate fathers by CERVUS and 10% of the litters (i.e., 16 of 160) were fully resolved. Albeit, CERVUS failed to identify any father for 23.75% of the litters (i.e., 38 of 160). The number of sires N_C ranged from one to five for the litters with at least one father identified (mean $N_C = 1.78$ sires per litter ± 0.86 SD, $n_{\text{litter}} = 122$, Fig. 1A). The results obtained with GERUD were very similar. The minimum number of sires N_G ranged from one to four (mean $N_G = 1.69$ sires per litter ± 0.63 SD, $n_{\text{litter}} = 160$, Fig. 1B). Using the maximal number of paternal alleles' approach, N_{PA} ranged from one to four (mean $N_{PA} = 2.34$ sires per litter ± 0.72 SD, $n_{\text{litter}} = 160$, Fig. 1C). The *MPR* obtained with GERUD ($MPR_G = 0.606$, $n = 160$) was higher than with CERVUS ($MPR_C = 0.438$, $n = 16$ fully resolved litters). With the last approach, the *MPR* was the lowest ($MPR_{NPA} = 0.338$, $n = 160$).

FACTORS EXPLAINING THE VARIABILITY OF N_{PA} AND N_G

One model including the mother body mass BM_m, the observed number of alleles *A* for the locus showing the maximal number of paternal alleles and the maximal number of paternal allele N_{PA} was supported by the data (ΔAICc < 2, Supporting Information 3, Table S3). *LS* increased significantly with BM_m ($\beta = 0.010 \pm 0.002$, $P < 0.001$, Table 2A) and A ($\beta = 0.017 \pm 0.007$, $p = 0.021$, Table 2A). Once the effect of BM_m and *A* was removed, *LS* was positively linked to N_{PA} ($\beta = 0.112 \pm 0.047$, $P = 0.018$, Fig. 2A). The probability for the random effect of N_{PA} on *LS* to be greater than the observed effect of N_{PA} was small ($p(N_{PA\ \text{permuted}} > N_{PA\ \text{observed}}) = 0.002$ with 10,000 values of beta from the permutated dataset). The positive influence of increasing value of N_{PA} on *LS* was significantly higher than the basal link (Fig. 2C).

Only the full model was supported by the data with the GERUD approach (Supporting Information 4, Table S4). BM_m

was positively linked with *LS* ($\beta = 0.011 \pm 0.002$, $P < 0.001$, Table 2B). Again, *LS* increased significantly with the number of fathers ($\beta = 0.132 \pm 0.054$, $P = 0.015$, Table 2B, Fig. 2B) and the probability for the random effect of N_G to be greater than the observed effect of N_G on *LS* was small ($p[N_{G\ \text{permuted}} > N_{G\ \text{observed}}] < 0.001$, with 10,000 values of beta, Fig. 2D). Thus, the positive effect of N_G was significantly higher than the basal link between *LS* and the number of sires. Overall, the higher the number of sires in a litter, the larger the litter size.

Discussion

Our results show that the average number of alleles per locus and heterozygosity were high and moderate, respectively, in this wild boar population, despite intensive hunting. The rates of multiple paternity varied between 0.338 and 0.606 depending on the approach used for estimating the number of sires. Regardless of the method used, we found larger litter sizes with increasing number of sires.

The average number of alleles and the allelic richness we reported are the highest among all the studies dealing with multiple paternity in wild boar (Table 1). It is noteworthy that the average number of alleles is sensitive to the sample size (our sample size is twice as big as the largest dataset), and both the average number of alleles and the allelic richness may vary with the loci analyzed (Table 1). Regarding the average heterozygosity, we found a moderate value ($He = 0.602$) compared to other studies (Table 1). Remarkably, this value is closer to the average heterozygosity reported for 14 nonthreatened taxa ($He = 0.699$) than to the one reported for their 14 taxonomically related threatened taxa ($He = 0.407$) provided in Frankham et al. (2002). Therefore, despite the strong hunting pressure, the genetic characteristics of this population are similar to those of other wild boar populations (Table 1) characterized, for some of them, with a weaker hunting pressure. Thus, our studied population definitely does not show any characteristics of endangered taxa. However, we acknowledge that despite the fact that comparing heterozygosities is less sensitive to sample size than comparing allelic richness, the comparison may still be sensitive to the loci used. Interestingly, four microsatellite loci used in our study have also been used by Poteaux et al. (2009) on data collected between 1999 and 2001 in the same population. The expected heterozygosity remains constant through time according to the four common loci ($He_{\ 1999–2001} = 0.518$ vs. $He_{\ 2007–2012} = 0.548$), while the average allelic richness is higher ($A_{\ 1999–2001} = 7.75$ vs. $Ar_{\ 2007–2012} = 9.64$ from 1000 subsamplings of 488 individuals). Thus, both allelic number and expected heterozygosity showed no decrease over time despite the fact they are separated by at least twice the length of the generation time of the population and six hunting seasons. Such findings highlight that this heavily hunted population does not display any evidence of genetic loss over time on the studied loci.

Around 60.6% of the litters showed multiple paternity with GERUD, 43.7% with CERVUS, and this rate was only 33.8% with the maximal number of paternal alleles' approach. This might suggest that the *MPR* is underestimated with this last method for which at least three paternal alleles have to be identified within a litter to classify it as a litter with multiple paternity. However, in our dataset, most of the litters display two paternal alleles, which could be obtained with one or more fathers. To our knowledge, these rates of multiple paternity obtained from the first long-term study at the population level, are among the highest ever reported in the species (Table 1). *MPR*s are high (Table 1), but they likely underestimate the proportion of females that mate with more than one male. Indeed, *MPR*s only measure the number of successful matings that lead to multiple sired litters, and do not correspond to the proportion of females that mate with more than one male. Such a proportion may potentially be higher than the reported *MPR*s, suggesting that the mating system in this population is predominantly promiscuous/polyandrous.

In this population where intensive hunting pressure, especially targeting males, exists for a long time relative to the short generation time (2.27 years; Servanty et al. 2011), we observed both promiscuous/polyandrous mating system and stable genetic characteristics. We raise the hypothesis that such a mating system might have appeared as an evolutionary response to high hunting pressure due to the lack of dominant males, and be preserved because it has the ability to maintain high genetic variability within a litter (Pérez-González et al. 2014). It is also possible that this mating system appeared due to a tendency for females to mate promiscuously in the absence of dominant males, and it is preserved by the continual removal of large dominant males from the population. Equations from Nunney (1993) showed that multiple paternity (likened through random union of gametes) can increase effective population size by 10% and up to 50% when compared to harem polygyny depending on the proportion of males in the population. This was measured considering a constant population size, a generation time of 2.5 years, harem sizes of one (monogamy) and five females, thus consistent with group size of female wild boars (Dardaillon 1988; Podgórski et al. 2014). Thus, we hypothesize that the high rates of multiple paternity measured in our study favor the retention of a high genetic diversity through year. Unfortunately, without genetic monitoring of our population before the beginning of intensive hunting, it is impossible to quantify the change of the mating system and its influence on genetic diversity. Moreover, the population studied here is nonfenced and thus open to emigration and immigration. Even if the immigration rate is known to be low in our population (Baubet unpubl. data), it is difficult to unravel the relative contribution of

mating system and migration to the genetic diversity. We strongly encourage further studies to investigate the link between hunting pressure and mating systems. One exciting perspective could be to analyze *MPR*s among populations with different hunting pressures, as well as in nonhunted ones, to strengthen the link between hunting intensity and mating system.

After some evidence in domestic pigs (e.g., Waller and Bilkei 2002), we provide here the first empirical evidence of a positive link between multiple paternity and litter size in a free-ranging population. This finding was supported by the permutation tests, which showed that the observed relation is stronger than any structural relationship between litter size and the number of sires. The pig illustrates the capacity of this species to cope with strong directional selective pressures (Gepts and Papa 2002). It is now well known that the time lapse for optimal fertilization is very short in domestic sows (Soede et al. 1995; Nissen et al. 1997). Therefore, the probability of presence of healthy sperm at ovulation time in the female genital tract increases with the number of artificial insemination events, thereby maximizing the number of fertilized ovules (Kemp and Soede 1996; Corrêa et al. 2002). However, the sperm quality of the boar strongly decreases after one ejaculation for, at least, the next two days (Frangež et al. 2005). Increasing the number of sires for a female is thus used in pig husbandry to obtain optimal fertilization and maximal litter sizes with natural reproduction (Badinel 2010). The underlying behavioral and physiological mechanisms involved in free-ranging wild boars remain to be studied.

In conclusion, high rates of multiple paternity are measured under intensive harvesting regime where rapid evolutionary changes were previously observed (Servanty et al. 2009; Gamelon et al. 2011). This lead us to hypothesize that multiple paternity might be a key basis for exploited populations of wild boar to display evolution over just a few generations (Gamelon et al. 2011; Servanty et al. 2011). It allows the population to withstand the harvesting pressure (Gamelon et al. 2012) through an increase in the number of reproductive males, an unusual pattern in ungulates (Ginsberg and Milner-Gulland 1994), which, we show here, induces larger litter sizes. It is noteworthy that litter size is a key life-history trait of fecundity, a major component in demography. Therefore, multiple paternity could be one of the factors contributing to the actual increase of wild boar abundance (Massei et al. 2014). However, the access to reproduction for younger and/or weaker males that would normally not garner matings may have long-term negative consequences. Indeed, these males may carry and transmit poor-quality genes that could be deleterious on the long run for the population. This study raises question about other ungulate species' strategies to buffer negative consequences of size and sex selective harvesting (Hard et al. 2006), because they are generally unable to modulate the number of offspring produced per reproductive event. Some changes of mating systems can be expected with a decrease of harem size allowing more males to reproduce each year when hunting is intensive. In contrast, many game species from birds to small mammals produce several offspring per reproductive event; investigating to what extent the pattern observed here applies to these species in intensive harvesting context is an interesting challenge.

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DATA ARCHIVING

The Dryad doi for our data is 10.5061/dryad.ps4m1.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Number of alleles *A*, observed *Ho*, and expected *He* heterozygosity, Hardy–Weinberg equilibrium (NS = equilibrium; * = nonequilibrium) and estimated frequency of null alleles for each locus as provided by CERVUS for adult wild boars ($n = 1054$ genotypes).

Appendix S2. Estimation of confidence for paternity analysis with CERVUS and GERUD.

Table S3. Model selection to test the effect of the locus-specific observed heterozygosity *Ho* and observed number of alleles *A*, the mother dressed body mass $BM_{m,}$ and the maximal number of paternal alleles in a litter N_{PA} on the litter size (*LS*) in the wild boar (*Sus scrofa*) population of Châteauvillain, France.

Table S4. Model selection to test the effects of the mother dressed body mass BM_m and the number of fathers estimated by GERUD N_G on the litter size (*LS*) in the wild boar population of Châteauvillain, France.