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Increasing populations of wild boar and feral domestic pigs *Sus scrofa* have evoked growing concern due to their potential as disease reservoir and as an origin of agricultural damages. Reliable population estimates are needed for effective management measures of this species. As an alternative to traditional methods, non-invasive genetic population estimation approaches based on hair or faeces sampling have yielded promising results for several species in terms of feasibility and precision. We developed and applied a non-invasive population estimation approach based on wild boar faeces in a study area situated in the Palatinate Forest, southwestern Germany. We collected 515 faeces samples along transects in January 2008. We carried out genotyping using six microsatellite markers to discriminate between individuals. During the trial, we identified 149 individual wild boar. Using multimodel inference and model averaging, we obtained relatively consistent estimates. Population densities calculated using the estimated population sizes ranged from 4.5 (2.9-7.8) to 5.0 (4.0-7.0) wild boar/km^2. In the future, to further improve the precision of population estimates based on wild boar faeces, the detection probability should be increased. However, even when comparing a conservative population estimate to the hunting bag, our results show that the present hunting regime in our study area is not effective in regulating the wild boar population. The method which we present here offers a tool to calibrate hunting or other management measures for wild boar.

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Wild boar and feral domestic pigs *Sus scrofa* have moved into the focus of wildlife management in many countries all over the world (Bieber & Ruf 2005). Population numbers are rapidly increasing, resulting in agricultural damages and also in increased spread of diseases such as e.g. the Classical Swine Fever and Aujeszki's disease (Acevedo et al. 2007, Saez-Royuela & Telleria 1986, Schley et al. 2008, Toigo et al. 2008). Several factors have been discussed as causes of the population increase, i.e. favourable climatical conditions, increased cultivation of crops like e.g. maize, which can serve as food resource for wild boar, and artificial feeding (Sàez-Royuela & Telleria 1986, Schley et al. 2008). At

present, the main regulatory mechanism for the growing wild boar populations is hunting, especially in regions where natural predators are lacking e.g. in most parts of Central Europe (Boitani et al. 1995, Toigo et al. 2008). However, so far, there is no efficient method to assess the effectiveness of a given hunting regime in regulating wild boar populations. Hunting bags do not necessarily reflect actual population sizes. Furthermore, it is crucial to obtain reliable population estimates to enable efficient management measures and for epidemiologic reasons (Baber & Coblentz 1986, Sweitzer et al. 2000, Truvé 2004, Acevedo et al. 2007). Based on reliable population estimates, modified hunting regimes or other regulatory mechanisms, such as fertility control via immuno-contraceptives and a ban of artificial feeding, could be enforced (Massei et al. 2008). Hunting bag statistics and other traditional approaches such as counts of tracks, faeces or farrowing nests yield only relative numbers or population trends (Acevedo et al. 2007). These can be sufficient for an effective management under some circumstances, but absolute numbers are preferable for population control and for epidemiological reasons. Capture-mark-recapture (CMR) approaches can yield absolute population numbers (Otis et al. 1978, Seber 1982, Pollock et al. 1990). However, relatively few CMR studies have been carried out on wild boar (but see Andrzejewski & Jezierski 1978, Baber & Coblentz 1986). The reasons for this might be that CMR is barely feasible for a large, difficult-to-capture and elusive species such as the wild boar (Petit & Valière 2006) and bears a high risk of yielding severely biased results, because capture probabilities are influenced by age, sex and social status (Pollock et al. 1990, Briedermann 2008, Ebert et al. 2010a,b). In this context, non-invasive DNA-based methods, which have been widely applied for estimation of population size (often using a CMR framework) in the last 10 years, have yielded promising results (Taberlet et al. 1999, Beja-Pereira et al. 2009). These methods are said to be particularly advantageous in case of rare or endangered animal species, because for these species, obtaining reliable population estimates with conventional methods is especially difficult, and in some cases the risk of damage through invasive approaches like e.g. removal methods or CMR may prohibit their use (Puechmaille & Petit 2007, Jacob et al. 2009). However, non-invasive genetic methods may also be beneficial for population estimation of abundant species such as the wild boar, because they may yield less biased and more representative estimates compared to most traditional approaches (McKelvey & Schwartz 2004, Fickel & Hohmann 2006, Petit & Valière 2006, Zhan et al. 2006). Nevertheless, several issues are crucial for the successful application of non-invasive methods. One of them is to ensure a reliable laboratory protocol with careful error-checking for DNA sample analysis, because genotyping errors like allelic drop-out and false alleles can severely compromise population estimation (Waits & Leberg 2000, Creel et al. 2003, Lukacs & Burnham 2005). Another one is that it may be difficult to obtain a sufficiently large sample size and a sufficiently high detection probability with a feasible effort (Ebert et al. 2009, Harris et al. 2010).

For mammals, the main sources for non-invasively obtainable DNA samples are hairs and faeces. After having conducted pilot studies of both hair and faeces sampling for wild boar (Ebert et al. 2009, 2010a), we decided to focus on faeces, because hair sampling using baited hair traps seemed to be strongly influenced by individual age and group status of the animals. In our pilot study on wild boar faeces sampling (Ebert et al. 2009), a small sample size and rather high genotyping error rates resulted in inconsistent and partly imprecise estimates. We therefore aimed at increasing the sample size and improving our genotyping protocol. In this paper, the results of a faeces sampling trial are shown, in which we used a simplified form of adaptive cluster sampling (Thompson 1991) to increase the number of faeces samples. Furthermore, we used an improved laboratory protocol for DNA analysis and included a step of quality prescreening by determining the amount of target DNA in each sample after extraction by quantitative real-time PCR (qRTPCR). This approach allows an accurate determination of usable DNA per sample regardless of the total amount of DNA (Beja-Pereira et al. 2009, Morin et al. 2001).

We compare the resulting population estimates to the bag record in the study area in order to assess the potential use of the method as a calibration of management measures. In this case, the hunting regime in the study area is evaluated with respect to its success in regulating wild boar numbers.

Material and methods

Study area
We carried out faeces sampling in a site covering 2,500 ha and situated in the Palatinate Forest in the

federal state of Rhineland-Palatinate, southwestern Germany (49°12'N, 7°45'E). Elevation ranged between 210 m and 609 m a.s.l. The predominant native plant community was beech *Fagus sylvatica* forest (Luzulo-Fagetum). The area was covered with forest by approximately 90% (i.e. 44% beech, 26% pine *Pinus* sp., 10% Norwegian spruce *Picea abies*, 12% Sessile oak *Quercus petraea* and 8% common oak *Q. robur*; Reis 2006). Several small settlements with surrounding open areas occurred in the periphery of the study area. The annual average temperature is 8-9°C (Weiß 1993) and the annual precipitation approximates 600-1,000 mm. Artificial feeding of wild boar was forbidden in the federal state, but baiting was legal for hunting at registered sites when ≤ 1 litre of maize was given/site and day.

The annual harvest of wild boar in the state-managed hunting areas between 1999 and 2009 averaged 2.4 individuals/km^2 (range: 1.14-5.23 individuals/km^2 and year; Reis 2006, G. Scheffler, Forestry Office of Hinterweidenthal, pers. comm.). The hunting bag in the year before our faeces sampling was comparably low (1.8 wild boar/km^2), whereas it was rather high in the year of our study (3.5 wild boar/km^2). Hunting was carried out both via single hunts all year round and via drive hunts. Drive hunts were carried out in segments of 3-5 km^2 between mid-October and the end of January every two to three weeks, covering the whole study area during one season.

Field sampling

We carried out sampling during 14-31 January 2008. No hunts were carried out within our sampling period. We collected wild boar faeces along 16 transects of approximately 6-8 km length each (Fig. 1). Transects were installed parallel to each other in a north-south orientation (overall length: 104 km). Trails, small roads or streams were crossed, if

necessary, but we avoided conducting transects along trails or roads to prevent potential bias of sampling results. We chose the parallel north-south transect design with the aim to cover the study area as representatively as possible by including all occurring habitat types and altitudinal levels. We aimed at maximising the collection of fresh faeces by walking the same transect routes in every repetition. We marked transect routes using spray paint on trees. The transect width, which could be effectively searched for wild boar faeces by a walking person, was approximately 3 m. We searched each transect for faeces every 48 hours and thus a total of six times during 12 days of one trial. The transects were walked by eight persons altogether, each covering 1 transect/day.

To increase our faeces finding rate, we applied a simplified form of adaptive cluster sampling (Thompson 1991). Every time we found a faeces sample, the field worker paused walking the transect and searched the area surrounding the sample in a radius of approximately 5-6 m. If further wild boar faeces were found within this radius, the search was extended in the respective direction. After having completed a cluster, the field worker continued walking the regular transect route. Thereby, we aimed at accounting for the fact that wild boar, at least females and their offspring, regularly occur in groups, and thus often faeces of more than one individual can be found in close proximity (Briedermann 2008). The maximum distance from the main transect route for cluster sampling was approximately 30 m.

We collected whole faeces using inverted freezer bags, which were then reversed and closed. Samples were stored frozen (at -19°C) in the sealed freezer bags for 6-8 months until analysis.

DNA extraction and genotyping

We extracted genomic DNA from all faecal samples using the NucleoSpin soil kit (Macherey-Nagel,

Figure 1. Transect design for collection of wild boar faeces for use in non-invasive genetic population estimation. The transects (represented by parallel black lines) are orientated in north-south direction. The area covered by transects together with the buffer (visible as a thin grey line around the transect grid) represents the effectively sampled area. Streets are indicated in bold grey and rivers as thin lines. The study area is situated in the federal state of Rhineland-Palatinate in southwestern Germany.
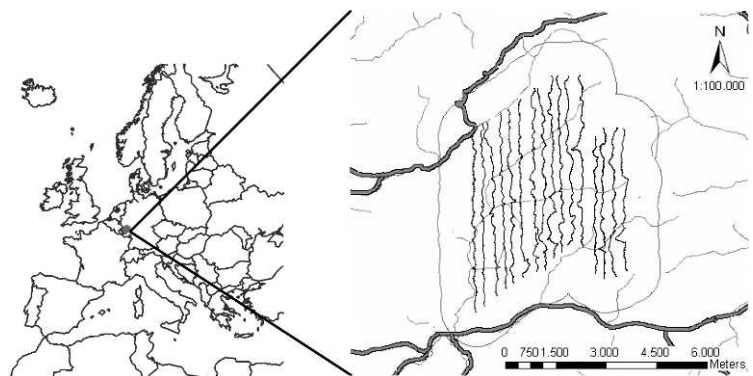
Düren, Germany) following the manufacturer's instructions. For extraction, we scraped off material from the surface of the faecal pellets with a fresh scalpel for every sample. We established a qRTPCR on transgelin as a single copy gene (Acc. No. XM_003357308, *Sus scrofa* smooth muscle protein 22-alpha, mRNA) and used PRIMER3 software (Rozen & Skaletsky 2000) to design a suitable primer pair. The oligonucleotide sequences of this primer pair were as follows: 5'-TAT CCT GAC GGC TCC AAA CCC-3' (forward, TAGLN1sus) and 5'-CAG TCT TGG TGA CGC CAT AGT CC-3' (reverse, TAGLN2sus). We performed qRTPCR reactions in a total reaction volume of 15 µl containing 9 µl LC480 Sybrgreen Mix (Roche, Mannheim, Germany), 0.03 µl of each primer (100 µM), 2.94 µl H_2O and 3 µl DNA (diluted 1:5). We used a Light-Cycler®480 II (Roche) under the following conditions for qRTPCR: initial denaturation for 10 minutes at 95°C, followed by 45 amplification cycles with 15 seconds of 95°, 25 seconds of 59° and 35 seconds at 72°. We carried out the final melting curve assessment during a temperature increase from 60 to 95°C. We calibrated the absolute DNA quantification using a dilution series of a tissue derived DNA standard as described in Hausknecht et al. (2009) and defined a cycle threshold of 0.01 ng ($C_t = 32$) target DNA to exclude low quality samples from further analysis.

We used all samples containing a sufficient amount of target DNA according to the qRTPCR for further analyses. For microsatellite analysis, we used Gene-Amp®PCR System 9700 Cycler (Applied Biosystems, Darmstadt, Germany) at the following PCR conditions: initial denaturation at 95°C for 15 minutes followed by 45 cycles of 30 seconds at 94°C, 30 seconds of 57°C and 60 seconds at 72°C, and a terminal elongation step at 60°C for 30 minutes. We performed amplification reactions in triplicates, each in a total volume of 12 µl using the Qiagen Multiplex PCR kit (Qiagen, Hilden, Germany). We used the primers at concentrations of 0.075 µM to 0.3 µM. We ran amplification products on an ABI3730 Sequencer using the ABI GS500LIZ size ladder, and we analysed them using the software GeneMapper v 3.7 to determine allele lengths (Applied Biosystems, Darmstadt, Germany). We carried out individual identification based on six microsatellite loci: TNFB, CGA, S0005 (Lowden et al. 2002), SW2496, SW2021 and SW742 (Rohrer et al. 1994, Kolodziej et al. 2011; see Table 2). For sex determination, we amplified the PigSRY gene (Kawarasaki et al. 1995). We combined the selected markers and co-amplified markers in one multiplex PCR. The markers S005 and SW2496 were amplified together in one additional run for every sample.

We calculated the probability of identity (P_{ID}) and the P_{ID} between siblings (P_{IDsibs}) using GIMLET (Valière 2002) to test if the combination of loci was sufficient to discriminate between individuals for the purpose of population estimation in our studied population (Waits et al. 2001). All samples were typed three times, and we determined a consensus genotype from the three results by applying the following rules: a sample was assigned a heterozygote when both alleles were observed at least twice, and to be classified as homozygous, the allele had to be observed three times. All samples showing ambiguous results after the first three repeats were typed another three times. We discarded samples which failed to amplify for two or more loci or did not show an unambiguous genotype after six (for markers S0005 and SW2496 eight) repeats from further analysis. We analysed genotyping results using GENECAP (Wilberg & Dreher 2004) and calculated genotyping error rates (allelic dropout ADO and false allele FA) according to Broquet & Petit (2006).

Using these error rates, we calculated global error rates (E_T and E_{1or2}) and final error rates (E_T-E_{1or2}) as developed in Puechmaille & Petit (2007) using an R script provided by S.J. Puechmaille (unpubl. report).

Population size estimation

We used maximum likelihood mixture models for closed captures with heterogeneity (Pledger 2000) implemented in program MARK (White & Burnham 1999) to estimate population size. We defined a set of plausible candidate models with varying assumptions concerning capture probability (p), choosing nomenclature according to Otis et al. (1978) for simplicity: M_{Null} as the most parsimonious model with capture probablity being constant over time and among individuals, M_h (heterogeneity; a mixture model incorporating two groups of animals with differing p), M_t (p varying over time) and M_{th} (heterogeneity and p varying over time). For each of these four basic models, we considered two different cases: 'basic model only' and 'basic model including sex' (two attribute groups).

Furthermore, we constructed capture histories for population estimation in two ways: For the first, we included only detections of the same individuals on different sampling days as 'recaptures', i.e. multiple captures during one sampling day were pooled to a

single capture for each of the six sampling events. In the following, we will refer to this approach as 'detections on different days only', or DDO. For the second, we included all possible recaptures, i.e. plus multiple detections of individuals on the same day (see also Miller et al. 2005, Ruell et al. 2009), and shifted one of these detections back in time one day to fit in the capture history (the maximum number of detections recorded for the same individual per day was two). We will refer to the second approach as 'maximum number of detections' or MND.

For each of the two approaches, we calculated population size using all eight models. Additionally, we calculated model averages (i.e. weighted average over all models according to their model weight and thus according to their GOF; Burnham & Anderson 2002). As in program MARK, confidence intervals (CI) for model averages do not account for the minimum number of wild boar observed in the sampling area, we calculated CI using the unconditional SE and the equations reported in Rexstadt & Burnham (1991:19). For management reasons, we additionally aimed at obtaining CI for the total population (male + female). The population sizes and CI are estimated separately for both sexes, when sex is included as a grouping variable as it is the case in our analysis. Therefore, we calculated the sum of a random number of the female and male probability distribution, iterated this 10.000 times and calculated mean (total population size) and standard error from the resulting distribution. We used mean and standard error to calculate 95% CI based on the corresponding (Rexstadt & Burnham 1991) equations.

In addition to the closed capture mixture model estimates, we calculated population estimates using a single sampling session Bayesian model (Gazey & Staley 1986, Petit & Valière 2006), which is especially suitable for non-invasive data because it can make full use of all sampling information in the data set. To examine the assumption of capture homogeneity, we carried out a test in which we simulated the sampling process under the assumption of homogeneity and that the expected number of captures is compared with the observed number of captures/individual (Puechmaille & Petit 2007). We performed the Bayesian estimate and the test using the R package (Ihaka & Gentleman 1996) and a script provided by E. Petit (pers. comm.).

Population density

For the purpose of comparison with other wild boar populations and other studies as well as for comparison with the hunting bag per km^2 in the study area, we calculated population densities. Due to the short time span of sampling and because there were no drive hunts and most probably no births during each sampling trial, we consider the assumption of demographic closure as met in our study (Otis et al. 1978). However, there was no possibility to obtain topographic boundaries for the study area; thus, it can not be considered as geographically closed. The small sample size prevented us from applying models for open populations for population estimation (Luikart et al. 2010). Therefore, we added a buffer zone around the transect grid to calculate the effectively sampled area (ESA). We then used the ESA for calculating population density. We determined the width of the buffer using VHF- and GPS-telemetry data collected from wild boar tracked in our study area (C. Ebert, unpubl. data). We chose the radius of a mean monthly home range (95% Minimum Convex Polygon) as a buffer for calculating the ESA (see e.g. Tioli et al. 2009). This resulted, with a mean monthly home-range radius of 1,000 m, in an ESA of 52 km^2.

Hunting efficiency

To evaluate the efficiency of the hunting regime in our study area, we calculated an estimate of the reproductive output for comparison to the hunting bags for the given year. To calculate reproductive output, we assumed a population growth rate of 250% per year which was derived from combined data on wild boar reproduction in our study area and in a similar forested habitat also situated in southwestern Germany (Gethöffer et al. 2007). As a basis for the calculation of reproductive output, we used the Bayesian population estimate for each study year, because it seemed to be the most robust and conservative approach with our data.

Results

Field sampling and genotyping

During the 2008 sampling period, we collected 515 wild boar faeces (i.e. 0.23 samples/km of transect searched). Based on the qRTPCR-results, 270 samples (i.e. 52%) contained a sufficient amount of target DNA and were used for further analysis. Of these, 244 (90%) yielded a complete 7-locus consensus genotype. From these, we identified 149 individuals, 66 males and 83 females, equivalent to a sex ratio of 1:1.26 (Table 1). P_{ID} and P_{IDsibs} were 4.73×10^{-8} and

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Table 1. Results of the faeces sampling in a wild boar population in the Palatinate Forest, southwestern Germany.

	Male	Female	Both sexes
Number of samples genotyped successfully	98	146	244
Number of individuals	66	83	149
Number of recaptures on different days only	20	33	53
Number of all possible recaptures	32	63	95

Table 2. Characteristics of the microsatellite markers used for individual identification of wild boar (Hexp = expected heterozygosity; Hobs = observed heterozygosity). Mean error rates (ADO and FA; in %) were calculated after Broquet & Petit (2006).

Marker	Alleles	Hexp	Hobs	ADO	FA
SW2021	6	0.69	0.76	3.75	0.001
TNFB	7	0.74	0.81	6.46	0.004
SW2496	10	0.81	0.88	6.15	0.004
SW742	10	0.84	0.89	5.16	0.001
S0005	16	0.85	0.79	5.83	0.000
CGA	11	0.80	0.85	7.10	0.003
Mean	10	0.79	0.83	4.72	0.002

0.00134, respectively. Of the 149 individuals, 53 (36%) were detected more than once, and the number of detections/individual ranged from one to six.

Mean ADO rate per locus was between 0.037 and 0.071 (Table 2). The observed FA rates ranged from 0 to 0.004. The multilocus error rate (E_T) estimated using equation 6 in Puechmaille & Petit (2007) was 0.0384733. This represents the probability of a genotype containing at least one error. The probability of a genotype containing exactly one or two errors (E_{1or2}) was 0.0384676. Consequently, the probability of a genotype containing more than two errors is 0.000005747. Because genotypes potentially containing one or two errors can be found in most cases by carefully rechecking all genotypes differing only at one (1-MM) or 2 (2-MM) loci, the latter represents an approximation of the residual error probability. According to these estimates and the number of analysed samples, the expected number of genotypes containing one or more errors (under the assumption that consensus genotypes are correct) is 9.4 with a 99.9% chance of representing exactly one or two errors.

Population size estimation

When comparing the maximum likelihood estimates for the DDO approach, the most supported model is M_h, which received 35% of the total model weight, followed by models M_{Null} and M_b with model weights of 20 and 15%, respectively (Table 3). The other models were considerably less supported and had AIC_c of > 2. None of the models with separated parameters for each sex received considerable support. The model averaged population estimate in the case of DDO indicates a total population size of 261 (207-366) wild boar. For the MND approach, only the M_h and M_{hsex} models received considerable support (sharing 99.5% of the model weight of which 77.3% are attibuted to M_h) with all other models showing AIC_c of > 10. The model-averaged population estimate indicates a total population size of 235 (199-297) wild boar. For both the DDO and MND approach, the male population estimates are less accurate than those for the females (see Table 3).

Table 3. Top five candidate models for population estimates of wild boar in the Palatinate Forest, derived from genotyped faeces samples and calculated using program MARK. The model selection was based on Akaike's Information Criterion corrected for small sample sizes (AIC_c). Further parameters given are w_i (model weights), K (number of parameters) and N (estimated population size) for both sexes including 95% confidence intervals. For a detailed description of the estimation models see text. DDO = capture history created using detections on different days only; MND = capture history created using maximum number of detections.

| Data set	Model	K	ΔAIC_c	w_i	N̂ male	N̂ female	Both sexes, N̂ total	SE	Density
DDO	M_h	5	0.000	0.350	124 (101-163)	156 (128-203)	280 (240-337)	24.22	5.38 (4.6-6.5)
	M_{Null}	4	1.072	0.204	119 (98-155)	150 (124-192)	269 (233-320)	21.89	5.17 (4.5-6.2)
	M_b	5	1.754	0.146	90 (76-124)	113 (95-155)	203 (178-251)	18.01	3.90 (3.4-4.8)
	$M_{b\,sex}$	8	3.031	0.083	141 (81-431)	97 (11-124)	238 (171-508)	72.29	4.58 (3.3-9.8)
	$M_{Null\,sex}$	6	3.59	0.063	136 (103-202)	138 (114-181)	274 (228-346)	29.28	5.27 (4.4-6.6)
	Model average				120 (85-213)	141 (108-217)	261 (207-366)	39.03	5.02 (4.0-7.0)
MND	M_h	5	0.000	0.733	104 (86-139)	130 (109-173)	234 (202-285)	20.61	4.50 (3.9-5.5)
	$M_{h\,sex}$	8	2.059	0.262	119 (88-196)	118 (101-149)	237 (197-311)	28.19	4.56 (3.8-6.0)
	M_{th}	15	10.105	0.005	104 (86-135)	130 (109-168)	234 (204-279)	18.75	4.50 (3.9-5.4)
	M_{Null}	4	19.740	0.000	90 (79-108)	113 (101-134)	203 (185-229)	10.97	3.90 (3.6-4.4)
	M_b	5	20.242	0.000	81 (72-103)	102 (91-129)	183 (167-215)	11.70	3.52 (3.2-4.1)
	Model average				108 (84-162)	127 (105-171)	235 (199-297)	24.23	4.52 (3.8-5.7)
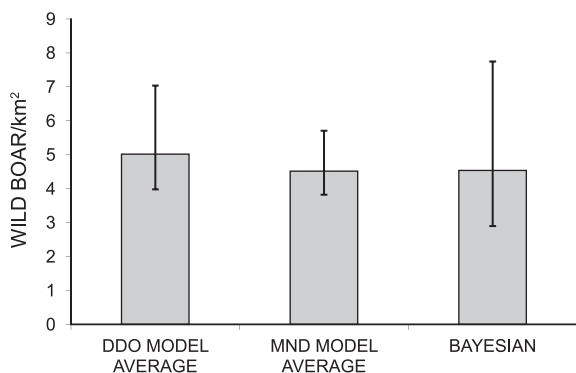
Figure 2. Estimated wild boar population densities in the Palatinate Forest, southwestern Germany, for three approaches: 'DDO model average' = capture mark recapture model average using closed capture maximum likelihood models including recaptures on different days only; 'MND model average' = capture mark recapture model average using closed capture maximum likelihood models with the maximum number of recaptures, i.e. including samples found the same day; 'Bayesian' = Bayesian population estimate after Puechmaille & Petit (2007). Error bars represent 95% CI.

The Bayesian approach yielded an estimated population size of 236 (151-403) wild boar. In the simulation test, at least one of the observed values was outside the 95% CI of the expected values, thus indicating capture heterogeneity (Appendix I, Fig. 1).

Population density and hunting efficiency

Population density calculated using the model averaged population estimates is 5.0 wild boar/km^2 (95% CI: 4.0-7.0) for the DDO approach and 4.5 wild boar/km^2 (95% CI: 3.8-5.7) for the MND approach (see Table 3). Using the Bayesian population estimate yielded a population density of 4.5 (95% CI: 2.9-7.8) wild boar.

The reproductive output estimated from the Bayesian population density estimates is 6.8 (95% CI: 4.4-11.6) wild boar/km^2, leading to a total spring population size of 11.3 (95% CI: 7.2-19.3) wild boar/km^2 (Fig. 2). The 2008/2009 harvest density of 3.5 wild boar/km^2 thus corresponds to 31% (95% CI: 48-18%) of the estimated summer population (i.e. population including output) and to 51% (95% CI: 80-30%) of the reproductive output (Fig. 3).

Discussion

Regarding the P_{ID} and P_{IDsibs} determined for our study, the set of markers should allow for discrimination between individuals with sufficient certainty
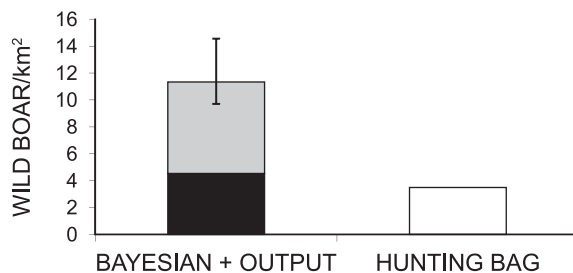

Figure 3. Comparison between estimated wild boar population density (Bayesian estimate) and estimated population growth (output; error bars represent 95% CI) and hunting bag in the corresponding study area between April 2008 and January 2009 (thus in the year after sampling). The reproductive output (light grey) was calculated using a reproduction rate of 250%.

for the purpose of population estimation (Lukacs & Burnham 2005, Woods et al. 1999). We believe the overall misidentification rate to be very low due to the careful genotyping approach with up to eight PCR replicates/locus. This seems to be confirmed by the low estimated residual error rate. Additionally, the use of qRTPCR proved to be very efficient for quality pre-screening, which not only saved cost and effort, but may also have contributed to the quality of the resulting genotypes.

For both maximum likelihood approaches, the capture probabilities (p) were rather low (mean p was 0.12 for DDO and 0.19 for MND), but ranged above the minimum values recommended in literature order to yield reliable and precise estimates (minimum p > 0.1, better p > 0.2; see e.g. Otis et al. 1978). One reason for the low number of samples may be that wild boar have a low defaecation rate compared to other ungulates (Briedermann 2008). In a study carried out in the same area, 3-4 times as many red deer *Cervus elaphus* faeces were collected with a similar effort compared to the wild boar sampling trials, even though red deer density is considerably lower (C. Ebert, unpubl. data). Furthermore, the climate in our study area is rather mild and humid, which limits sample persistance in the field and DNA quality and thus limits sample size (Lucchini et al. 2002, Murphy et al. 2007). Compared to our first attempts of wild boar faeces sampling (Ebert et al. 2009), we achieved a considerably higher sample size due to cluster sampling and probably due to a gain in experience. However, future studies should aim at further increasing the sampling probability, e.g. by increasing sampling intensity and success or by using other approaches in combination with faeces sampling. The latter may not only be valuable to increase

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the sample size, but also to yield data with low overall sampling bias (Dreher et al. 2007, Boulanger et al. 2008). For wild boar, this could be achieved e.g. by collecting tissue samples from hunted individuals. It may also be promising to stratify the faeces sampling by searching more intensively along wild boar passes, at wallows or baiting sites (in case baiting is carried out for hunting) in addition to walking transects. Such incidental or opportunistic sampling can provide a valuable additional sample (Gervasi et al. 2008). Since in our study we did not increase the sample size using additional strategies, we applied the MND approach in order to exploit all the available capture information. We consider the MND approach in the case of sparse data as useful and assume that in our case, it has improved the estimates because the MND estimates have smaller standard errors and narrower confidence intervals compared to those of the same models generated using the DDO approach (see Table 3 and Fig. 2).

Like the MND approach and in contrast to the traditional CMR (DDO) approach, the Bayesian model developed by Gazey & Staley (1986) allows using every single observation of each individual for population estimation. Another single session approach which has been developed for non-invasive population estimation is program CAPWIRE (Miller et al. 2005), which, however, is best suited for populations of < 100 individuals and tends to produce overestimates for large populations and when sample size is low. We therefore decided to use the Bayesian single session model for comparison to the mixture results. The Bayesian estimate is very similar to result of the MND model average, but shows considerably larger 95% CI and thus less precision. In simulation tests, the Bayesian estimator behaved better than other methods (including maximum-likelihood models like those applied in MARK; Petit & Valière 2006). This may be due to the fact that Bayesian models use more information from the data and that they are parsimonious compared to the more complicated of the maximum-likelihood models. However, in presence of capture heterogeneity, the Bayesian model tends to produce slight underestimates of population size (Puechmaille & Petit 2007). As in our case, the Bayesian estimator matches with the MND model average, which includes models that accomodate for capture heterogeneity, we do not believe it to be severely underestimating the population. Furthermore, the population estimates are relatively consistent for all three approaches, even though we used

two totally different methods (DDO and MND, which are both maximum likelihood methods, and the Bayesian method). This can be a hint that the population is adequately represented by the estimates.

Management implications
We selected a conservative estimate of population density as well as a moderate reproduction rate (Bieber & Ruf 2005) to calculate reproductive output for comparing to the hunting bag in the study area. However, the number of harvested wild boar corresponded to only 31% of the estimated summer population size, and even when taking into account the lower confidence interval of the population estimate, the harvest rate is approximately 48% of the estimated summer population size. Thus, the current hunting regime in our study area does not seem to be sufficient for regulating the wild boar population, even though the state agencies in charge aim to reduce the population (G. Scheffler, Forestry Office of Hinterweidenthal, pers. comm.). Since 1999, the mean hunting bag in the study area has increased almost threefold. To anticipate a further increase in population size, and even more to reduce the wild boar numbers, either the hunting regime will have to be changed (e.g. hunt more females of all age classes, especially piglets; Gethöffer et al. 2007, Toigo et al. 2008) or other regulatory mechanisms will have to be established (e.g. contraceptives; Massei et al. 2008). Artificial feeding is most probably no important factor in our study area, as feeding is forbidden and baiting only allowed in very restricted amounts, but may be so in other areas. In this case, the first thing to counteract population increase is to stop feeding wild boar. The data which we present here certainly are only a 'snapshot' of the wild boar population size in the study area and thus the management implications are of rather exemplary character. In order to effectively draw conclusions for management, a population should be monitored over several consecutive years.

Our study area is only one example; it is a known problem in many regions that hunting is not efficient in regulating wild boar populations. Nevertheless, until now there has been no measure for the extent to which hunting can achieve a reduction of a population or how far it is away from achieving a sufficient reduction. The method presented in our paper represents a tool to quantify the success of hunting or other management measures and thus serve as a calibration for wild boar management.

However, in order to allow more reliable and precise population estimates and thus more fine-grained conclusions for management, the sampling probability will have to be increased. Further studies should focus on the development of sampling strategies that allow a better representation of the sampled population in terms of number of unambiguously identified genotypes. Thus, faeces sampling efficiency and the combination with other strategies (e.g. genetic sampling of the hunting bag) are relevant parameters for research, but also the improvement of genotyping success.

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Appendix I

Results of the simulation test for capture heterogeneity (*cf.* Puechmaille & Petit 2007) for non-invasive genetic population estimation of wild boar in south western Germany.
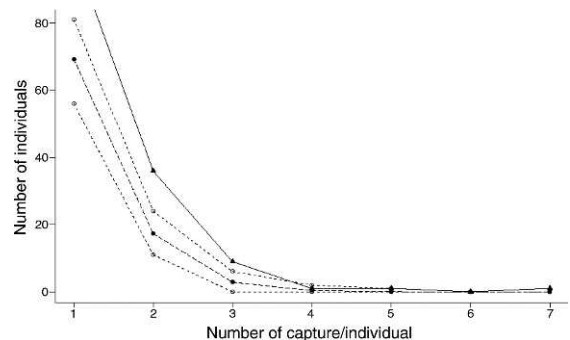

Figure 1. The test for heterogeneity of capture probabilities is carried out by simulating the sampling process under the assumption of homogeneity and comparing the expected number of captures with the observed number of captures per individual. In the figure, the expected number of captures per individual is symbolised by black circles and a dashed line. The 95% confidence interval of the expected number of captures is represented by open circles. The observed number of individuals captured once, twice and three times (as represented by triangles and a solid line) differs significantly from the expected values, indicating the occurrence of heterogeneity.