Supplementary Information of the paper entitled "Estimating abundance of a recovering transboundary brown bear population with capture-recapture models" by Vanpé et al.

Materials and Methods

Genetic analyses from 2017 to 2020

From 2017 to 2020, genetic analyses were conducted in our laboratory at ANTAGENE (https://www.antagene.com/en). DNA extraction was conducted according to a sterile process in a designated extraction room free of DNA. For each sample, disposable sterile tools were used and the bench was cleaned with bleach to avoid cross-contamination. Each sample was transferred to a sterile labelled microtube to proceed to DNA extraction. Sample tubes were surrounded by positive and negative extraction controls and lysed overnight at 56°C according to the manufacturer's instructions (Nucleospin 96 Tissue Kit, Macherey-Nagel, Düren, Germany). DNA was isolated and purified using purification columns and vacuum filtration (Nucleospin 96 Tissue Kit, Macherey-Nagel, Düren, Germany). DNA was eluted with 100 µL of elution buffer to obtain final concentrations between 20-100 ng/µl. Extracts were stored in labelled 96-tube strip plates in a -20°C freezer.

For each DNA sample, 13 microsatellites and 3 sex identification markers (ZFX, 318.2 and SMCY) were amplified by two multiplex PCRs (polymerase chain reaction) four times and analyzed in two runs (one for each multiplex) with an automated sequencer (Table S6). Because the genetic sex marker described in the scientific publication De Barba et al. (2017) proved to be not very reproducible, the ANTAGENE laboratory uses a system of three pairs of primers allowing the amplification by PCR of two specific regions of the Y chromosome and one specific region of the X chromosome, according to a method developed and validated in all bear species (Bidon 2013). This system provides an internal positive control for all individuals, with the amplification of a region of the X chromosome present in males (XY) and in females (XX) and to amplify in duplicate a specific region of the Y chromosome present only in males (XY). This triple amplification guarantees an excellent recognition of the Y chromosome and therefore of males, and increases the reliability of characterization of the genetic sex, especially on DNA from degraded samples (hair, scats, etc.).PCR reactions were prepared step-by-step according to a unidirectional workflow starting in a clean room

with positive air pressure to prepare sensitive reagents (enzymes and DNA primers) and continued in a pre-PCR room for combining DNA and reagents using filtered tips. Three negative and positive controls were included per PCR reaction. PCR amplifications were then performed in a dedicated post-PCR area in 96-well microplates at 10 µl final volumes containing 5 µl of mastermix Taq Polymerase (Type-It Microsatellite PCR Kit, Qiagen, Hilden, Germany), and either 0.80 µL of a first pool of 8 pairs of primers or 0.36 µl of a second pool of 8 pairs of primers at a concentration from 0.08 to 0.60 µM each, and a mean of 30 ng of genomic DNA (Table S6). Each pair of primers was coupled with a fluorescent dye (Table S6). Our PCR thermal protocol consisted of 95°C for 15 min, followed by 8 touchdown cycles of 95°C for 30 s, 62°C to 55°C for 90 s (decreasing 1°C per cycle), and 72°C for 30 s, then followed by 35 cycles of 95°C for 30 s, 55°C for 90 s, and 72°C for 30 s, ending with an extension of 60°C for 30 min. PCR products were resolved on an ABI PRISM 3130 XL capillary sequencer (ThermoFisher Scientific, Waltham, Massachusetts) under denaturing conditions (Hi-DiTM Formamide, ThermoFisher Scientific, Waltham, Massachusetts) with an internal size marker prepared once and dispatched equally in all sample wells of each multiplex run. The four electropherograms for each sample were analyzed using GENEMAPPER 4.1 (ThermoFisher Scientific, Waltham, Massachusetts) and analyzed independently by two analysts to determine the allele sizes for each marker of each individual. When the genotypes determined by each analyst did not agree, the electropherograms were read again, reading errors were resolved, and in case of persistent disagreement, ambiguous results were considered as missing data.

Dating of bear signs

For photos and videos, we used the metadata from the automatically triggered camera traps or cameras to define accurately the date of bear presence. For hair collected on baited hair traps, we used photo data collected on camera traps set up in front of baited hair traps when available to identify date when hair were left. From those specific bear signs, month of bear presence could be determined accurately based on the date when signs were left.

For other types of bear signs, we could not know precisely the date when signs were left and we relied on an evaluation of the time period when sign could have been left by the bear. More specifically, when hair collected on baited hair trap were not associated with any photo or video, we considered that the bear had left the hair during the time period included between the date of the last visit of the hair trap when barbed wire was cleaned and the date of the visit when hair were collected. If this time period was larger than 2 months, we discarded the hair sample from our analyses. We also discarded hair samples collected spontaneously outside systematic monitoring design, because the time interval during which they might have been left by the bear could not be evaluated precisely (bear hair deteriorates very slowly in the field), except in the case hair were associated with damage to livestock or beehives, in which case the estimated date of the damage provided the estimated date of hair deposition. Finally, we estimated the time interval when scats were dropped (≤2 weeks) by evaluating the freshness of the scat when collected in the field, using expert judgement in relation to the color and appearance of the scat, recent weather conditions (rain, sunshine, snow, temperature, etc.) and type of habitat (directly exposed to sun, under vegetation cover, etc.) (e.g., Sergiel et al. 2020 for a similar approach). When the time period during which hair or scat could have been left overlapped two different months, we considered as a proxy the month of the median date between maximum and minimum date of the time period as the month of bear presence, since this should not affect much our estimation of population size with capture-recapture analyses. Note that we selected preferentially fresher scats (with less DNA degradation) to send to the molecular laboratory, allowing a better genotyping success and identifying more individuals genetically (Sentilles, Vanpé & Quenette 2021). In France, we collected in total 4,022 hair or scat samples from 2008 to 2020, among which about 5.5% were excluded from our analyses due to inaccurate dating.

Compilation of monthly detection history of bears

Matching genotypes were considered to arise from the same individual and classified as recaptures as the combined non-exclusion probability of the 13 microsatellites for independent individuals and

for sibships were negligeable (Lukacs & Burnham 2005). Importantly, we did not consider location data from GPS collar or VHF transmitters to compile detection history to avoid large inter-individual differences in monitoring pressure between bears, since it concerns respectively 5 bears and 1 bear for a period ranging from several months to a few years. Orphan cubs that were captured in the field and kept in captivity for a while for care before being released in the wild were considered as still present and detected in the population during the months of captivity (this concerns only 1 orphan cub during two months of captivity). For individuals for which we knew the date of death (N = 9), we used this information and right censored them in the corresponding detection histories. For translocated bears originating from Slovenia (N = 3), the first month of potential detection was the month of release in the Pyrenees.

References

Bidon, T., Frosch, C., Eiken, H.G., Kutschera, V.E., Hagen, S.B., Aarnes, S.G., ... & Hailer, F. (2013). A sensitive and specific multiplex PCR approach for sex identification of ursine and tremarctine bears suitable for non-invasive samples. *Mol. Ecol. Res.* 13(3), 362-368.

De Barba, M., Miquel, C., Lobréaux, S., Quenette, P. Y., Swenson, J. E., & Taberlet, P. (2017). High-throughput microsatellite genotyping in ecology: Improved accuracy, efficiency, standardization and success with low-quantity and degraded DNA. *Mol. Ecol. Res.* 17(3), 492-507.

Table S1. Systematic monitoring effort in the French Pyrenees in terms of number of transects (including 6 hair traps per transect in average), total length of transects (km), number of camera traps, number of baited hair traps per year between 2008 and 2020.

Year	# transects	total length of transects (km)	# camera traps	# baited hair traps
2008	30	300*	7	59
2009	36	360*	10	73
2010	60	600*	12	86
2011	68	615	26	90
2012	68	615	18	0
2013	48	426	49	0
2014	50	411	39	0
2015	44	358	40	0
2016	47	376	48	0
2017	53	414	45	0
2018	57	441	45	0
2019	56	424	59	0
2020	58	428	60	0

Note: * Estimated based on an average transect length of 10 km.

Table S2. Total number of validated non-invasive brown bear signs (e.g., scats, hair, tracks, visual observations, damages, photos / videos) collected in the Pyrenees, total number of validated brown bear samples (i.e. scats and hair) collected in the Pyrenees, number of samples (among collected sampled) genetically analysed by the French molecular laboratory LECA or ANTAGENE, number of brown bear samples (among analysed samples) successfully genotyped and number of different brown bear genotypes identified (among successfully genotyped samples) per year between 2008 and 2020.

Year	# validated bear signs collected	# bear samples	# genetically analysed samples	# successfully genotyped samples	# different genotypes identified
2008	743	210	125	73	11
2009	712	229	84	42	12
2010	939	323	167	106	15
2011	1152	518	209	122	15
2012	1239	521	224	153	15
2013	1318	521	137	77	14
2014	1243	571	193	96	21
2015	1567	870	152	110	24
2016	1854	874	179	137	32
2017	1394	569	134	105	34
2018	1625	601	158	109	35
2019	2450	830	314	209	41
2020	2783	1116	448	309	45
TOTAL	19019	7753	2524	1648	314

111 **Table S3.** Combination of microsatellite markers used in each PCR mix and type of fluorescent dye
112 used for each microsatellite marker from 2017 to 2020.

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Mix	Locus name	Dye	Publication
A	UA03	6FAM	De Barba *et al*. 2017
A	UA06	6FAM	De Barba *et al*. 2017
A	UA25	NED™	De Barba *et al*. 2017
A	UA67	NED™	De Barba *et al*. 2017
A	UA64	PET™	De Barba *et al*. 2017
A	UA63	PET™	De Barba *et al*. 2017
A	UA16	VIC™	De Barba *et al*. 2017
A	UA14	VIC™	De Barba *et al*. 2017
B	UA17	6FAM	De Barba *et al*. 2017
B	UA57	6FAM	De Barba *et al*. 2017
B	UA51	NED™	De Barba *et al*. 2017
B	UA65	PET™	De Barba *et al*. 2017
B	UA68	VIC™	De Barba *et al*. 2017
B	Our-ZFX	6FAM	Bidon *et al*. 2013
B	Our-318	6FAM	Bidon *et al*. 2013
B	Our-SMCY	6FAM	Bidon *et al*. 2013

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115 References:

116 Bidon, T., Frosch, C., Eiken, H. G., Kutschera, V. E., Hagen, S. B., Aarnes, S. G., Fain, S.R., Janke,
117 A. & Hailer, F. (2013). A sensitive and specific multiplex PCR approach for sex identification of
118 ursine and tremarctine bears suitable for non-invasive samples. *Mol.Ecol. Res.* 13(3), 362-368.

119 De Barba, M., Miquel, C., Lobréaux, S., Quenette, P. Y., Swenson, J. E., & Taberlet, P. (2017). High-
120 throughput microsatellite genotyping in ecology: Improved accuracy, efficiency, standardization and
121 success with low-quantity and degraded DNA. *Mol.Ecol. Res.* 17(3), 492-507.

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Table S4. Summary statistics of the 58 different genotypes found in the Pyrenean brown bear population in 2020 for each of the 13 microsatellite loci provided by the allele frequency analysis of CERVUS software (Marshall et al. 1998).

Locus	N	k	HObs	HExp	NE-I	NE-SI	PIC	F(Null)
UA03	58	4	0.655	0.622	0.215	0.495	0.549	-0.0218
UA06	58	4	0.724	0.657	0.173	0.467	0.6	-0.0653
UA14	58	4	0.759	0.705	0.144	0.437	0.645	-0.0458
UA16	58	6	0.414	0.461	0.328	0.604	0.424	0.0908
UA17	58	3	0.517	0.497	0.308	0.581	0.442	-0.0189
UA25	58	5	0.483	0.427	0.364	0.629	0.392	-0.0989
UA51	58	4	0.603	0.537	0.269	0.551	0.483	-0.0608
UA57	58	3	0.552	0.45	0.399	0.627	0.354	-0.1089
UA63	57	6	0.719	0.694	0.146	0.442	0.639	-0.0178
UA64	58	2	0.534	0.492	0.381	0.601	0.369	-0.0455
UA65	58	4	0.621	0.595	0.246	0.516	0.513	-0.0358
UA67	58	3	0.517	0.571	0.266	0.533	0.488	0.0589
UA68	58	5	0.724	0.734	0.121	0.417	0.68	-0.0054
MEAN		4.08	0.602	0.572	0.258	0.531	0.506	-0.0289

Note: N: number of individuals typed, k: the number of alleles, Hobs: observed heterozygosity, Hexp: expected heterozygosity, NE-I: average exclusion probabilities for each locus for identity, NE-SI: average exclusion probabilities for each locus for sib identity, PIC: polymorphic information content, F(Null): the frequency of null alleles. The combined non-exclusion probabilities for identity and sib identity were 9.10^{-9} and 0.000235, respectively.

Reference:

Marshall, T.C., Slate, J.B.K.E., Kruuk, L.E.B. & Pemberton, J.M. (1998). Statistical confidence for likelihood-based paternity inference in natural populations. *Mol. Ecol.* 7(5), 639-655.

Table S5. Parameters of the model in which temporary emigration is random, survival is age-dependent and there is heterogeneity in the detection process, estimated using a Bayesian robust-design capture-recapture (CR) approach.

	Mean	SD	Naive SE	Time-series SE
beta[1]	8.52E-01	0.0402	0.8991	0.0014
beta[2]	9.33E-01	0.0333	0.0007	0.0009
beta[3]	9.46E-01	0.0175	0.0004	0.0004
deviance	2.31E+03	17.9349	0.4010	0.5163
gamma	6.62E-02	0.0218	0.0005	0.0006
mean.p	4.29E-01	0.0272	0.0006	0.0019
pstar[1]	8.50E-01	0.0188	0.0004	0.0009
pstar[2]	8.50E-01	0.0188	0.0004	0.0009
pstar[3]	8.50E-01	0.0188	0.0004	0.0009
pstar[4]	8.50E-01	0.0188	0.0004	0.0009
pstar[5]	8.50E-01	0.0188	0.0004	0.0009
pstar[6]	8.50E-01	0.0188	0.0004	0.0009
pstar[7]	8.50E-01	0.0188	0.0004	0.0009
pstar[8]	8.50E-01	0.0188	0.0004	0.0009
pstar[9]	8.50E-01	0.0188	0.0004	0.0009
pstar[10]	8.50E-01	0.0188	0.0004	0.0009
pstar[11]	8.50E-01	0.0188	0.0004	0.0009
pstar[12]	8.50E-01	0.0188	0.0004	0.0009
sdeps	2.10E-01	0.0214	0.0005	0.0007

Note: beta[i]: age-specific survival for age i (with 1: cubs, 2: subadults, 3: adults), gamma: probability of emigration; mean.p: mean detection probability, sdeps: SD of the random effect, pstar[j]: averaged detection over individuals for year j, with j ranging from 2008 to 2019.

Table S6. Comparison of the annual abundance of the Pyrenean brown bear population, estimated from Bayesian Pollock's robust design (PCRD) capture-recapture (CR) approach (with associated 95% Credible Interval), with Minimum Detected Size (MDS, total number of different individuals detected in the population during the year) and Minimum Retained Size (MRS, reassessment of the MDS in the light of the information collected in subsequent years) values from 2008 to 2020.

Year	PCRD Estimate	95% CI	MDS value	MRS value
2008	13.0	12.8 - 13.3	16	15
2009	17.4	17.0 - 17.8	17	16
2010	16.3	15.9 - 16.7	18	20
2011	19.5	19.1 - 20.0	22	23
2012	23.9	23.4 - 24.4	22	24
2013	21.7	21.3 - 22.2	25	25
2014	26.0	25.5 - 26.7	31	31
2015	29.3	28.7 - 30.0	29	32
2016	41.2	40.4 - 42.2	39	41
2017	41.2	40.4 - 42.2	43	48
2018	39.1	38.3 - 40.0	40	52
2019	49.9	48.9 - 51.1	52	59
2020	66.2	64.8 - 67.8	64	68

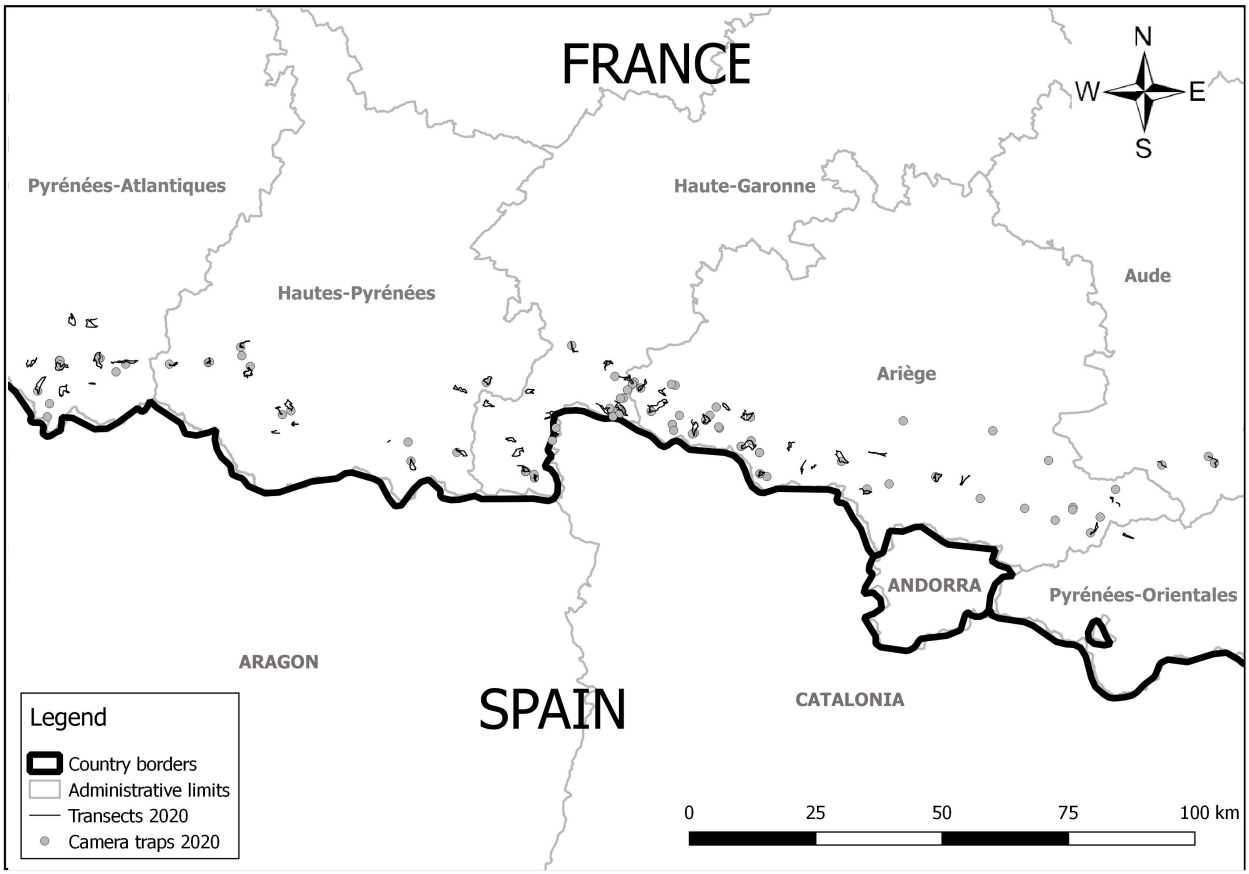
Note: MRS count for 2020 is provisional and probably slightly underestimated.

Table S7. Evolution of the sex ratio of the Pyrenean brown bear population from 2008 to 2020 among all individuals and among adult only.

	# adult males	# adult females	Adult sex ratio	# males	# females	Sex ratio
2008	5	4	1.25	8	6	1.33
2009	6	5	1.20	8	8	1.00
2010	5	6	0.83	8	12	0.67
2011	6	6	1.00	8	14	0.57
2012	6	7	0.86	9	14	0.64
2013	6	9	0.67	9	14	0.64
2014	8	11	0.73	11	17	0.65
2015	7	12	0.58	13	19	0.68
2016	7	13	0.54	19	21	0.90
2017	8	14	0.57	21	26	0.81
2018	8	19	0.42	22	28	0.79
2019	11	21	0.52	23	28	0.82
2020	12	23	0.52	29	29	1.00

155 **Figure. S1.** Map of the camera traps and transects used in 2020 in France within the framework of

156 the systematic monitoring of the Pyrenean brown bear population.



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