

Supplementary Information to:

J van Schaik, S Schuler, K Stienstra, R Janssen, D Dekeukeleire, JPC Boshamer, B Noort, J Steenbergen, S Lagerveld. **Diverse but declining? Population genetic structure and genetic diversity of *Pipistrellus nathusii* along the Dutch coastline during the autumn migration period.** *Mammalian Biology*

Figure S1: Estimated null allele frequency per locus

Figure S2: Structure and DAPC plots

Table S1: Summary information for loci removed from the analysis

Table S2: Hardy-Weinberg test per locus

Table S3: Index of association between loci

Table S4: Parent-offspring assignments with positive LOD scores

Table S5: Raw data file (provided as a separate csv)

Figure S1 Null allele frequency estimate (Brookfield 1996) for all loci, as implemented in PopGenReport (Adamack & Gruber 2014).

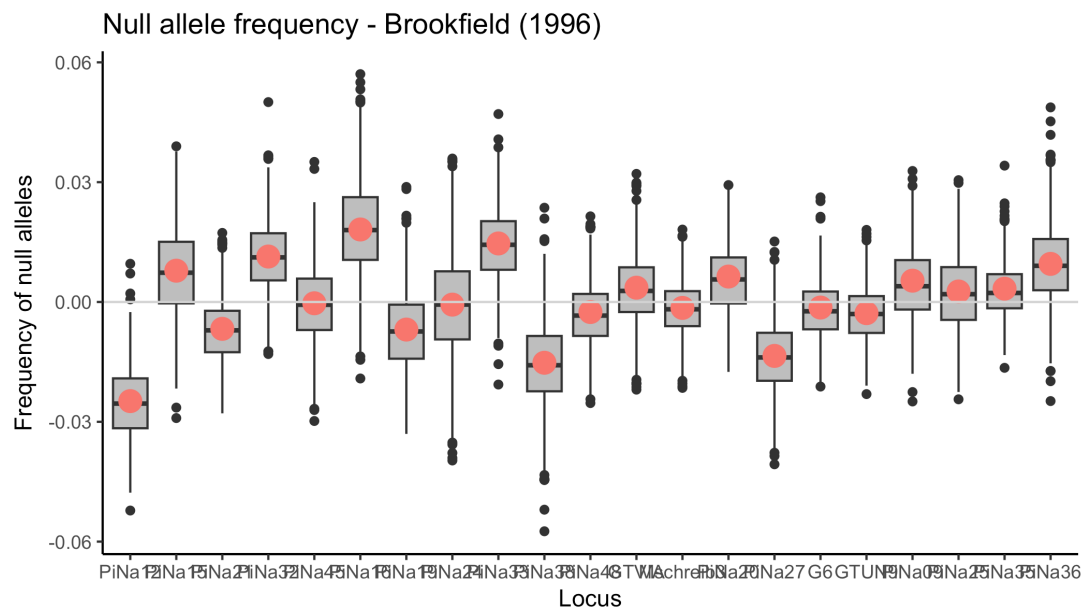


Figure S2 Population sub-structuring of analyzed *Pipistrellus nathusii* samples as assessed by Structure (A+B) and K-means clustering (C-E). In the Structure analysis, K=1 yielded the highest log-likelihood value (A), a plot of individual cluster assignment for K=2 is provided in B, and illustrates that additional clusters do not partition out biologically meaningful groups or individuals. In the k-means clustering approach, the lowest BIC-score was obtained for K=3 (C). To visualize this assignment, the number of PCs to be retained in the DAPC was determined using the optim-a-score approach (D), yielding a DAPC with no discernible separation between the inferred clusters (E).

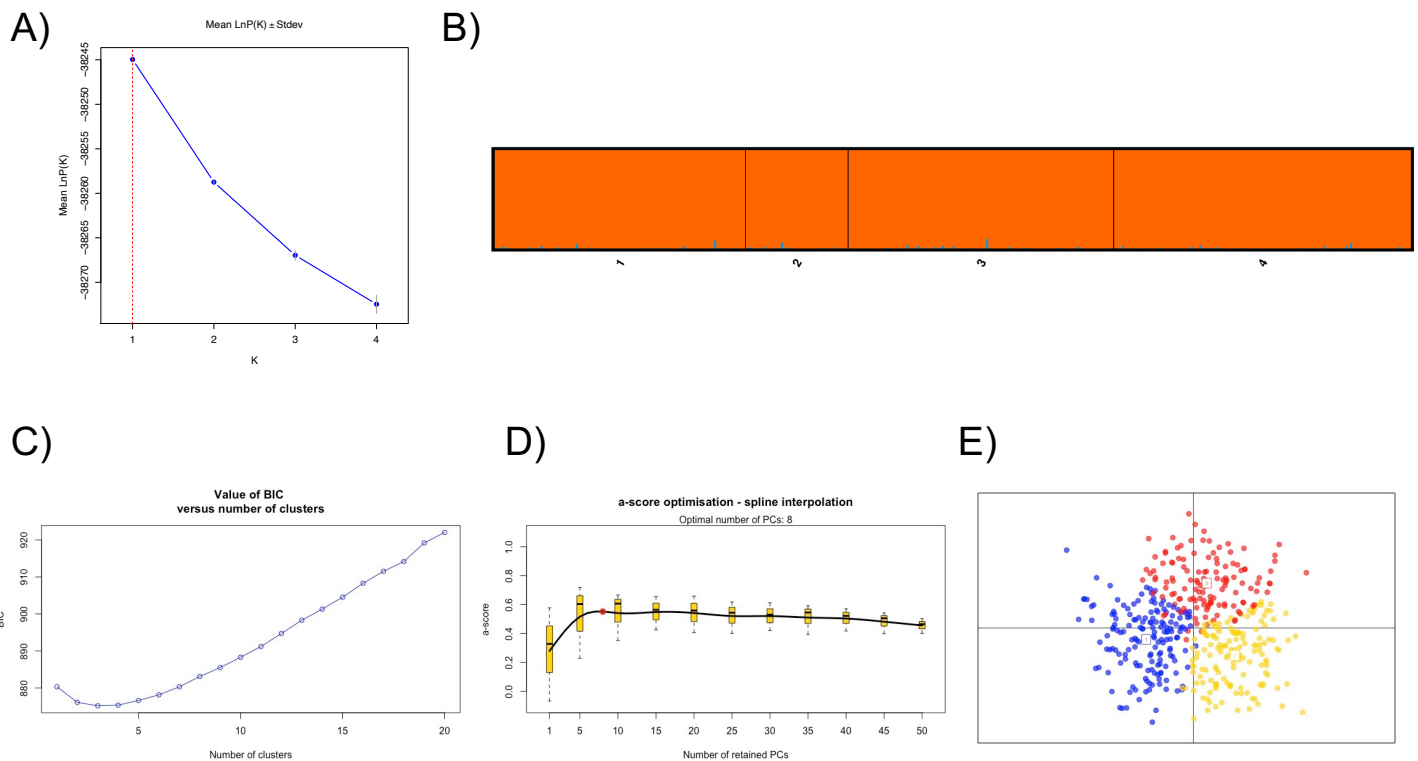


Table S1: Microsatellite loci removed from the analysis due to inconsistent scoring or deviations from Hardy-Weinberg Equilibrium. Markers PiNa03, PiNa14, PiNa41 showed strong deviations from HWE, PiNa44 could not be scored reliably due to single base-pair insertions/deletions and stuttering.

Locus	Forward / Reverse (5' - 3')	Source / Accession No.	Fl. label / Size range	Multiplex / PCR Conc.	Repeat Motif	No. Alleles	H _o	H _e	F _{is}
PiNa03	F: GCCATACCTAATCTAGTGCAGC	this study	FAM	1	(AT) ₂₀	6	0.3924	0.6464	0.394
	R: TGCCTGGCTAAGTACATGGT		80-140	0.12 µM					
PiNa14	F: AGCAGAATGTTGCAGCATAACA	this study	PET	1	(AC) ₁₁	12	0.6532	0.8611	0.2425
	R: AGTCAAATGCTGAGAATAGTGCC		130-200	0.1 µM					
PiNa41	F: ACGGGCTCAAAGATTCTGGT	this study	PET	1	(AC) ₄ AA(AC) ₉	14	0.5314	0.6051	0.1229
	R: ACTTCCAGTCTTAACCTGCCA		230-290	0.2 µM					
PiNa44	F: CCCTCAAGGCCCATTCATGT	this study	FAM	4	(AG) ₈	58	0.9328	0.946	0.0154
	R: GGCAGCTCGGTTGATTAGAGT		230-300	0.5 µM					

Table S2: Chi-squared test for deviation from Hardy-Weinberg equilibrium per locus.

P-values were adjusted using the False Discovery Rate approach of Benjamini and Hochberg.

Locus	X²	df	Pr(X²)	Adjusted p-value
PiNa09	111.267	190	0.999999	0.84567
PiNa12	26.923	45	0.985059	0.84567
PiNa15	71.046	105	0.995474	0.84567
PiNa16	19.144	21	0.575903	0.84567
PiNa19	83.492	78	0.31457	0.84567
PiNa20	167.815	231	0.999379	0.84567
PiNa21	168.892	231	0.99923	0.84567
PiNa24	12.064	21	0.937855	0.84567
PiNa25	60.383	78	0.930402	0.84567
PiNa27	69.123	105	0.99732	0.8964
PiNa32	92.425	105	0.804682	0.84567
PiNa33	143.123	105	0.00795	0.5082
PiNa35	991.127	780	3.83E-07	0.84567
PiNa36	109.394	153	0.996926	0.84567
PiNa38	8.778	10	0.553255	0.84567
PiNa45	87.477	105	0.891981	0.84567
PiNa48	108.870	136	0.958007	0.84567
G6	231.427	210	0.148185	0.0546
GTUN9	139.523	136	0.400443	0.8078
GTVIA	70.438	105	0.996149	0.84567
Mschreib3	610.670	561	0.07201	0.84567

Table S3: Pairwise index of association (I_a : index of association; $\bar{r}_d$: standardized index of association) for the 20 loci-pairs with the highest associations. P-values were adjusted using the False Discovery Rate approach of Benjamini and Hochberg.

Marker Pair	I_a	$\bar{r}_d$	Adjusted p-value
PiNa24:PiNa27	0.0349	0.0350	0.6587
PiNa32:PiNa25	0.0272	0.0275	0.6287
PiNa12:PiNa25	0.0244	0.0244	0.9440
PiNa21:PiNa16	0.0235	0.0236	0.6287
PiNa24:G6	0.0221	0.0227	0.6287
PiNa32:PiNa19	0.0215	0.0217	0.6287
PiNa19:PiNa24	0.0216	0.0216	0.9440
PiNa27:PiNa25	0.0203	0.0204	0.9440
PiNa38:PiNa36	0.0198	0.0198	0.9440
PiNa38:PiNa35	0.0168	0.0175	0.9440
PiNa32:PiNa20	0.0167	0.0167	0.6587
PiNa16:PiNa27	0.0162	0.0162	0.9440
PiNa24:Mschreib3	0.0153	0.0160	0.9440
PiNa15:PiNa21	0.0158	0.0160	0.9440
PiNa15:Mschreib3	0.0144	0.0150	0.9440
PiNa33:PiNa35	0.0148	0.0150	0.6587
PiNa45:Mschreib3	0.0141	0.0143	0.9440
PiNa48:PiNa20	0.0143	0.0143	0.8383
PiNa21:PiNa25	0.0134	0.0135	0.9440
PiNa45:PiNa25	0.0133	0.0134	0.9440

Table S4 Potential parent-offspring assignments with positive LOD scores based on analysis in Cervus (v3.0.7). As all potential pairs contained at least 2 mismatching loci, none were considered to be true parent-offspring pairs.

Pair										
Offspring	Candidate	Pair loci	Pair loci	LOD	Pairwise	Offspring	Mother	Offspring	Mother	Offspring
ID	mother ID	compared	mismatching	score	Relatedness	Location	Location	Date	Date	sex
Pnat_0376	Pnat_0005	21	3	0.973	0.3319	Wil	Wil	18.9.2021	8.9.2021	Female
Pnat_0189	Pnat_0180	21	3	2.322	0.4120	Kor	Kor	21.9.2022	7.9.2022	Female
Pnat_0481	Pnat_0205	21	3	3.955	0.3232	Noh	Wil	16.9.2023	9.9.2021	Female
Pnat_0150	Pnat_0292	21	3	1.047	0.3483	Wil	Kor	7.9.2022	7.9.2021	Female
Pnat_0016	Pnat_0327	21	4	0.508	0.4180	HvH	Wil	13.9.2020	16.9.2020	Female
Pnat_0513	Pnat_0539	21	3	0.919	0.3065	Kor	Goe	4.9.2023	20.9.2023	Female
Pair										
Offspring	Candidate	Pair loci	Pair loci	LOD	Pairwise	Offspring	Father	Offspring	Father	Offspring
ID	father ID	compared	mismatching	score	Relatedness	Location	Location	Date	Date	sex
Pnat_0068	Pnat_0004	21	2	4.953	0.3394	Wil	Cal	10.10.2021	20.8.2021	Male
Pnat_0537	Pnat_0008	21	3	1.245	0.3542	Goe	Nor	20.9.2023	20.8.2021	Male
Pnat_0535	Pnat_0527	21	3	2.889	0.3734	Goe	Goe	20.9.2023	20.9.2023	Female