

Procházka, P., Stokke, B. G., Jensen, H., Fainová, D., Bellinvia, E. Fossøy, F., Vikan, J. R., Bryja, J. and Soler, M. 2011. Low genetic differentiation among reed warbler *Acrocephalus scirpaceus* populations across Europe. – J. Avian Biol. 42: 103–113.

Online supplementary material

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Details on PCR conditions

In the Trondheim lab, a separate Polymerase Chain Reaction (PCR) was carried out for each locus with buffer and primer concentrations as described in Jensen et al. (2003). Two-step temperature “touch-down” PCR profiles were used for all loci. Accordingly, we used five cycles at the higher temperature (62° C for Aar8, POCC2; 65° C for Aar5, Ase58, FhU2, Pdoµ1, Pca3, and Ppi2; 67° C for Aar4 and Ase34), then decreased the annealing temperature by 1° C per cycle (for 10 cycles), followed by 25 cycles at the lower temperature (50° C for Aar8, POCC2; 53° C for Aar5, Ase58, Fhu2, Pdoµ1, Pca3 and Ppi2; 55° C for Aar4 and Ase34). In the Studenec lab, primers for different loci were combined in a single reaction (i.e. multiplex PCRs) whenever possible. Accordingly, locus Aar4 was amplified in duplex with Ase34, Aar8 with POCC2, and Ase58, FhU2 and Pca3 in a triplex reaction, with the remaining three loci amplified separately. The PCR conditions in the Studenec lab are detailed in Procházka et al. (2008). In the Trondheim lab, each reverse primer was labelled with either 6-FAM (Invitrogen; Aar5, Aar8, Ase34, Pca3, POCC2, Ppi2), VIC (Applied Biosystems; Ase58, FhU2) or NED (Applied Biosystems; Aar4, Pdoµ1). In the Studenec lab, each forward primer was labelled at the 5' end with one of the following fluorescent dyes (Applied Biosystems): VIC (Aar5, Pca3, Ppi2), NED (Aar8, FhU2, Pdoµ1), PET (Ase34, POCC2) or 6-FAM (Aar4, Ase58). PCR products were pooled in two mixes prior to electrophoresis in

the Trondheim lab; one mix contained Aar4, Aar5, Ase34, FhU2 and Pca3, the other mix contained Aar8, Ase58, Pdoµ1, POCC2 and Ppi2. In the Studenec lab, PCR products were also pooled in two mixes prior to electrophoresis: one mix included the products of two multiplex reactions (Aar4 and Ase34; Ase58, FhU2 and Pca3), the other contained the products of one duplex (Aar8 with POCC2) and three single reactions (Aar5, Pdoµ1 and Ppi2).

Details on Bayesian clustering programmes

BAPs and STRUCTURE consider multilocus genotypes and attempt to minimize linkage disequilibrium and Hardy–Weinberg disequilibrium by introducing the population structure. In STRUCTURE, we ran five iterations for each $K = 1–10$ (100 000 burn-in, 500 000 repetitions) using the admixture model, correlated allele frequencies and no prior population information. In BAPs, we performed ten independent runs of spatial clustering of groups of individuals (collected at the same locality). In this program the most likely values of K (in this case the number of clusters of groups) are estimated automatically. TESS performs a maximum likelihood assignment test, which uses geographical information and has been shown to perform very well when F_{ST} values are small (Chen et al. 2007). We followed the suggestions in the software documentation and first ran models without admixture (interaction parameter $\psi = 0.6$) to estimate the number of genetic clusters in the data. For each $K_{max} = 2–10$, we executed 20 runs and used 20 000 iterations as burn-in and 100 000 subsequent sweeps. Afterwards, as suggested by the authors of the programme, we ran models with admixture using 100 runs for K with the highest likelihood. Twenty runs with the highest likelihood were then averaged in CLUMPP 1.1, a cluster matching and permutation programme (Jakobsson and Rosenberg 2007), and displayed the individual membership coefficients in DISTRICT 1.1 (Rosenberg 2004).

Table S1. Information on 26 microsatellite loci tested for variability in the reed warbler *Acrocephalus scirpaceus*. The loci were tested in 6 individuals; one nestling from each of two separate nests in each of three populations (France, Romania and Denmark). PCR reactions were carried out on a GeneAmp PCR system 9700 (Applied Biosystems). Each 10 μ L reaction mix included 0.35 units of AmpliTaq DNA polymerase (Applied Biosystems), 8 μ L buffer with final concentration of 20 mM (NH₄)₂SO₄, 75 mM Tris-HCl pH 8.8, 0.15 mg mL⁻¹ DNase free BSA, 10 mM β -mercaptoethanol, 2.5 mM MgCl₂, 0.14 μ M dNTPs (Applied Biosystems), 0.6 μ M of each primer, and approximately 20 ng of genomic DNA. All loci were tested using a touch-down PCR profile which started with 4 min at 94 °C followed by 12 cycles of 30 s at 94 °C, 90 s initially at a temperature of 62 °C (then decreasing by one degree every cycle), and 60 s at 72 °C. This was followed by 20 cycles of 30 s at 94 °C, 90 s at 50 °C, and 60 s at 72 °C. Finally there was a final elongation step at 72 °C for 5 min. PCR products were separated by electrophoresis on 6% denaturing acrylamide gels and visualized by silver staining (Bassam et al. 1991).

Locus	Source species	Reference	Repeat motif in source species	Approximate allele size	Observed number of alleles	Observed allele size*	Description of quality
Aar2	Great reed warbler <i>Acrocephalus arundinaceus</i>	Hansson et al. 2000	(CA) _x	132	2?	Not variable	Not variable
Aar3	Great reed warbler <i>Acrocephalus arundinaceus</i>	Hansson et al. 2000	(CA) _x AA(CT) _y CA(CT) _z	194	2?	Not variable	Not variable
Aar4	Great reed warbler <i>Acrocephalus arundinaceus</i>	Hansson et al. 2000	(CA) _x	118	8	100–175	Good
Aar5	Great reed warbler <i>Acrocephalus arundinaceus</i>	Hansson et al. 2000	(CA) _x		2	70–80	Good
Aar8	Great reed warbler <i>Acrocephalus arundinaceus</i>	Hansson et al. 2000	(CA) _x (GA) _y	115	6	90–120	Good
Ase18	Seychelles warbler <i>Acrocephalus sechellensis</i>	Richardson et al. 2000	(GT) _x	176	?	Much unspecific amplification	Much unspecific amplification
Ase34	Seychelles warbler <i>Acrocephalus sechellensis</i>	Richardson et al. 2000	(CT) _x	220	7	225–350	Good
Ase48	Seychelles warbler <i>Acrocephalus sechellensis</i>	Richardson et al. 2000	(CCTTCT) _x	270	1?	Much unspecific amplification	Much unspecific amplification
Ase58	Seychelles warbler <i>Acrocephalus sechellensis</i>	Richardson et al. 2000	(CTTTT) _x	311	7	175–300	Good
Ase63	Seychelles warbler <i>Acrocephalus sechellensis</i>	Richardson et al. 2000	(GAGAA) _x (GA) _y	400	1	Long	Not variable
Ase64	Seychelles warbler <i>Acrocephalus sechellensis</i>	Richardson et al. 2000	(AGGG) _x (ATGG) _y	412	1	Long	Not variable
Fhu2S	Pied flycatcher <i>Ficedula hypoleuca</i>	Ellegren 1992	(TC) _x		6	125–175	Good
Hru2	Barn swallow <i>Hirundo rustica</i>	Primmer et al. 1995	(GA) _x (GT) _y (TG) _z	137	1	Not variable	Not variable
Hru5	Barn swallow <i>Hirundo rustica</i>	Primmer et al. 1995	(GT) _x	133	5	100–150	Weak amplification
Hru6	Barn swallow <i>Hirundo rustica</i>	Primmer et al. 1995	(AAAG) _x (AC) _y (AAAG) _z	193	?	Much unspecific amplification	Much unspecific amplification
Mcy4	Superb fairy-wren <i>Malurus cyaneus</i>	Double et al. 1997	(GT) _x AT(GT) _y	170	?	Weak amplification	Weak amplification
Pca3	Blue tit <i>Parus caeruleus</i>	Dawson et al. 2000	(GT) _x CT(GT) _y CT(GT) _z CT(GT) _u CT(GT) _v	191	7	150–200	Good
Pca4	Blue tit <i>Parus caeruleus</i>	Dawson et al. 2000	(AC) _x AT(AC) _y	191	1	Not variable	Not variable
Pca5	Blue tit <i>Parus caeruleus</i>	Dawson et al. 2000	(CA) _x	132	3?	50–75	Much unspecific amplification
Pocc2	Large crowned leaf warbler <i>Phylloscopus occipitalis</i>	Bensch et al. 1997	(CA) _x	180–190	3	175–225	Good
Pdo1	House sparrow <i>Passer domesticus</i>	Neumann and Wetton 1996	(TG) _x	150–200	4	200–250	Good
Pdo3	House sparrow <i>Passer domesticus</i>	Neumann and Wetton 1996	(TCCA) _x	110–180	?	Much unspecific amplification	Much unspecific amplification
Pdo4	House sparrow <i>Passer domesticus</i>	Neumann and Wetton 1996	(AnGn)n(GAGAGAA) _x (GAAA) _y	230–500	?	Much unspecific amplification	Much unspecific amplification
Pdo5	House sparrow <i>Passer domesticus</i>	Griffith et al. 1999	(CA) _x	230	1	200–250	Not variable
Pdo10	House sparrow <i>Passer domesticus</i>	Griffith et al. 2007	(CA) _x	125	2?	Clearer if optimized?	Clearer if optimized?
Ppi2	Magpie <i>Pica pica</i>	Martinez et al. 1999	(CA) _x		6	275–375	Good

*To size products a 25 bp Gibco BRL DNA ladder was used in every 25th lane on the 100 lane gel. Sizes were not always noted if the locus was non-variable or there was much unspecific amplification.
 † This locus was originally named PTC2 in Ellegren 1992.

Table S2. Pairwise F_{ST} values for all 31 reed warbler populations across 10 microsatellite loci (below the diagonal). P -values are given above the diagonal. Values significant after adjusting for multiple comparisons using the false discovery rate approach are given in bold. For population abbreviations see Table 1.

	NOR	DEN	DEB	PLM	CZH	LIV	FRB	DKA	UKW	CZS	FLR	ESE	ESM	ESA	ESR	ESG	ESB	PTE	ROT	ROD	BCW	HUW	HUC	HUE	SKT	HRD	HRN	ITL	CYP	JOA	TKM					
NOR																																				
DEN	0.034																																			
DEB	0.0041	0.0055																																		
PLM	0.0134	0.0055	0.3902																																	
CZH	0.0089	-0.0039	0.0043	0.0055																																
LIV	0.0117	0.004	0.0045	-0.0015	0																															
FRB	0.0088	-0.0033	0.005	0.0032	0.0041	0.0025																														
DKA	0.0088	-0.0033	0.005	0.0032	0.0041	0.0025	0.1144																													
UKW	0.0165	0.013	0.0141	0.0118	0.0139	0.0077	-0.0021	0.0022																												
CZS	0.0241	0.0061	0.0059	0.0044	0.0133	0.0074	0.0156	0.0108	0.0199																											
FLR	0.0171	0.0199	0.0192	0.0181	0.0138	0.0162	0.0204	0.0127	0.0236	0.0223																										
ESE	0.0187	0.0212	0.0175	0.0166	0.0126	0.0144	0.013	0.0209	0.0287	0.0155	0.0252																									
ESM	0.0015	0.0055	0.0026	0.005	0.009	0.0081	0.0052	0.0111	0.0156	0.0091	0.0152	0.038																								
ESA	0.0274	0.0216	0.0087	0.011	0.0081	0.0052	0.0111	0.0156	0.0091	0.0152	0.017	0.0137	0.0096																							
ESR	0.0174	0.0227	0.0159	0.0239	0.0142	0.0151	0.0109	0.0194	0.0211	0.0187	0.036	0.0298	0.0054	0.015	0.0146	0.008	0.02	0.0003	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	
ESG	0.0349	0.0253	0.0243	0.0251	0.0239	0.0277	0.0179	0.0325	0.0366	0.0227	0.0444	0.0301	0.0107	0.0214	0.0142	0.008	0.0143	0.0012	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	
ESB	0.0231	0.02	0.0147	0.0195	0.017	0.0269	0.0171	0.0286	0.0318	0.0275	0.0337	0.016	0.015	0.0196	0.0181	0.014	0.0143	0.0012	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	
PTE	0.0261	0.0092	0.0141	0.0092	0.0134	0.0134	0.0128	0.0086	0.0248	0.0122	0.0292	0.0205	0.0397	0.0123	0.0343	0.0469	0.04	0.0354	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	
ROT	0.0056	-0.0064	0.0054	0.0003	0.0041	0.007	0.0013	0.0021	0.0173	0.0046	0.0178	0.0046	0.0197	0.0002	0.0185	0.0241	0.0244	0.0216	0.002	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	
ROD	0.0169	0.0095	0.0226	0.0058	0.0113	0.0112	0.0105	0.0161	0.0289	0.0102	0.0227	0.012	0.0171	0.0171	0.02	0.0221	0.0244	0.0244	0.0175	0.002	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	
BCW	0.0268	0.0054	0.0146	0.0076	0.0041	0.0029	-0.002	0.0123	0.008	0.0045	0.0118	0.0079	0.012	0.0184	-0.007	0.0149	0.029	0.0238	0.0216	0.0044	-0.0032	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032	
HUW	0.0106	0.0025	0.0067	0.0014	0.0081	0.0045	0.0062	0.0048	0.0165	0.008	0.0149	0.0159	0.0203	0.0083	0.0178	0.0259	0.0389	0.0274	0.0132	0.0016	0.0137	0.0137	0.0137	0.0137	0.0137	0.0137	0.0137	0.0137	0.0137	0.0137	0.0137	0.0137	0.0137	0.0137	0.0137	0.0137
HUC	0.0305	0.0191	0.0316	0.0264	0.0243	0.0274	0.0176	0.014	0.0395	0.0231	0.0213	0.0467	0.0476	0.0248	0.0475	0.0344	0.0539	0.057	0.0163	0.0077	0.0178	0.0178	0.0178	0.0178	0.0178	0.0178	0.0178	0.0178	0.0178	0.0178	0.0178	0.0178	0.0178	0.0178	0.0178	0.0178
HUE	0.0021	0.0032	0.0087	0.0033	-0.0006	0.0059	0.0071	0.0076	0.0222	0.0088	0.0157	0.0157	0.0135	0.0015	0.0121	0.021	0.0198	0.0169	0.0152	-0.0041	-0.0041	-0.0041	-0.0041	-0.0041	-0.0041	-0.0041	-0.0041	-0.0041	-0.0041	-0.0041	-0.0041	-0.0041	-0.0041	-0.0041	-0.0041	-0.0041
SKT	0.0165	0.0001	0.0096	-0.0024	0.0098	0.0046	0.0027	0.0111	0.0145	0.0067	0.0255	0.0065	0.0236	0.011	0.024	0.0254	0.0252	0.0162	0.0152	-0.0041	-0.0041	-0.0041	-0.0041	-0.0041	-0.0041	-0.0041	-0.0041	-0.0041	-0.0041	-0.0041	-0.0041	-0.0041	-0.0041	-0.0041	-0.0041	-0.0041
HRD	0.014	-0.0006	0.0063	0.0029	0.0042	0.008	0.0048	0.012	0.0189	0.0035	0.0155	0.0023	0.0134	0.0101	0.0088	0.0182	0.0153	0.0132	0.0116	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	
HRN	0.0092	-0.0004	0	-0.0034	-0.0036	0.0027	-0.0006	0.0027	0.0039	-0.0011	0.0006	0.0122	0.0063	-0.0034	-0.0019	0.0088	0.0159	0.0116	0.0071	-0.0056	-0.0056	-0.0056	-0.0056	-0.0056	-0.0056	-0.0056	-0.0056	-0.0056	-0.0056	-0.0056	-0.0056	-0.0056	-0.0056	-0.0056	-0.0056	
ITL	0.0431	0.0344	0.0288	0.0369	0.0341	0.0244	0.021	0.0257	0.0272	0.0297	0.0644	0.0469	0.0484	0.048	0.0523	0.0295	0.0519	0.0224	0.0278	0.0278	0.0278	0.0278	0.0278	0.0278	0.0278	0.0278	0.0278	0.0278	0.0278	0.0278	0.0278	0.0278	0.0278	0.0278	0.0278	
CYP	0.017	0.0151	0.0137	0.0135	0.0145	0.0116	0.0118	0.0118	0.0118	0.0118	0.0118	0.0118	0.0118	0.0118	0.0118	0.0118	0.0118	0.0118	0.0118	0.0118	0.0118	0.0118	0.0118	0.0118	0.0118	0.0118	0.0118	0.0118	0.0118	0.0118	0.0118	0.0118	0.0118	0.0118	0.0118	0.0118
JOA	0.0095	0.0013	0.0039	0.0006	0.002	0.0013	0.0049	0.0027	0.0141	0.0082	0.0129	0.0063	0.0135	0.0079	0.0157	0.013	0.0252	0.0191	0.0167	-0.0004	0.003	-0.0006	0.0004	0.0015	0.0047	0	-0.0048	0.0351	0.0164	0.0016	0.0002	0.0002	0.0002	0.0002		
TKM	0.0041	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	

P values above the diagonal, bold significant after adjusting to multiple comparisons
Pairwise F_{ST} values below the diagonal

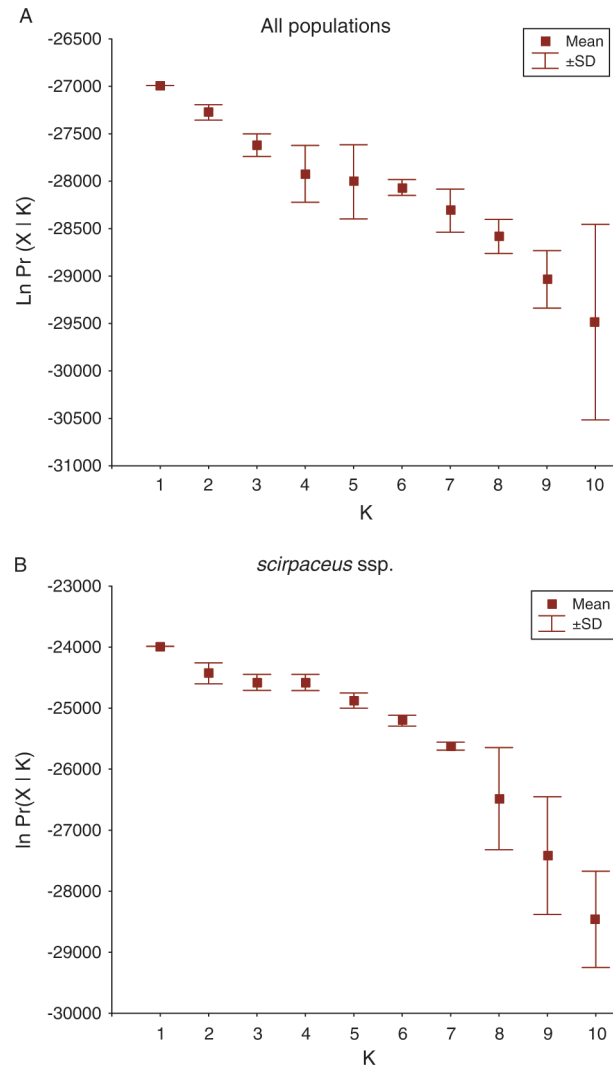


Figure S1. Estimated likelihood for 1–10 genetic population clusters (K) in STRUCTURE (100 000 burn-in, 500 000 repetitions) for (A) all populations and (B) *scirpaceus* populations.

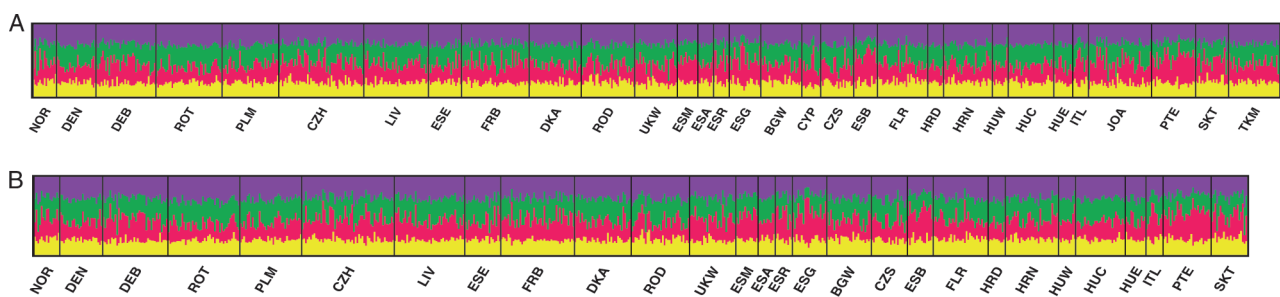


Figure S2. DISTRUCT plot of mean membership coefficients of 20 runs (of 100) with the highest likelihood to four clusters estimated in TESS (admixture, $\psi = 0.6$) and averaged in CLUMPP for 791 reed warbler individuals from 31 breeding populations (a) and 707 individuals from 28 *scirpaceus* populations (b), respectively. Columns represent individuals, colours assignment probabilities of birds to individual clusters, vertical black bars separate sampling sites.

References (if not listed already in the paper)

- Bassam, B. J., Caetanoanollés, G. and Gresshoff, P. M. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. – *Anal. Biochem.* 196: 80–83.
- Double, M. C., Dawson, D., Burke, T. and Cockburn, A. 1997. Finding the fathers in the least faithful bird: a microsatellite-based genotyping system for the superb fairy-wren *Malurus cyaneus*. – *Mol. Ecol.* 6: 691–693.
- Griffith, S. C., Dawson, D. A., Jensen, H., Ockendon, N., Greig, C., Neumann, K. and Burke, T. 2007. Fourteen polymorphic microsatellite loci characterized in the house sparrow *Passer domesticus* (Passeridae, Aves). – *Mol. Ecol. Notes* 7: 333–336.
- Griffith, S. C., Stewart, I. R. K., Dawson, D. A., Owens, I. P. F. and Burke, T. 1999. Contrasting levels of extra-pair paternity in mainland and island populations of the house sparrow (*Passer domesticus*): is there an “island effect”? – *Biol. J. Linn. Soc.* 68: 303–316.
- Jakobsson, M. and Rosenberg, N. A. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. – *Bioinformatics* 23: 1801–1806.
- Jensen, H., Sæther, B.-E., Ringsby, T. H., Tufto, J., Griffith, S. C. and Ellegren, H. 2003. Sexual variation in heritability and genetic correlations of morphological traits in the house sparrow (*Passer domesticus*). – *J. Evol. Biol.* 16: 1296–1307.
- Primmer, C. R., Møller, A. P. and Ellegren, H. 1995. Resolving genetic relationships with microsatellite markers: a parentage testing system for the swallow, *Hirundo rustica*. – *Mol. Ecol.* 4: 493–498.
- Procházka, P., Bellinva, E., Fainová, D., Hájková, P., Elhalah, A. and Alomari, K. 2008. Immigration as a possible rescue of a reduced population of a long-distance migratory bird: reed warblers in the Azraq Oasis, Jordan. – *J. Arid Environ.* 72: 1184–1192.
- Rosenberg, N. A. 2004. DISTRUCT: a program for the graphical display of population structure. – *Mol. Ecol. Notes* 4: 137–138.