Near panmixia at the distribution-wide scale but evidence of genetic differentiation in a geographically isolated population of the Terek Sandpiper *Xenus cinereus*

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Abstract

Populations at different parts of the species range may vary in their genetic structure, variation and dynamics. Geographically isolated populations or those located at the edge of the range may differ from the populations located at the core, and even hold genetic variation important for the adaptive potential of the species. We studied the distribution-wide population genetic structure of the Terek Sandpiper (*Xenus cinereus*) using 13 microsatellite loci and the mitochondrial DNA (mtDNA) control region. In addition, we estimated whether genetic variation changes from the core towards the edge of the main breeding range. We used the results to evaluate the management need of the sampled populations. Distribution-wide structure was negligible, and the only population that showed significant genetic differentiation was the geographically isolated Dnieper River basin population in Eastern Europe. The genetic variation of microsatellites decreased towards the edge of the distribution supporting the abundant-center hypotheses in which the core area of the distribution preserves the most variation, but no such trend could be seen with mtDNA. Overall genetic variation was low, and there were signs of past population contractions followed by expansion; a pattern found in most northern waders. The current effective population size (N_e) is large, and therefore global conservation measures are not necessary. However, the marginal Dnieper River population needs to be considered as its own management unit. In addition, the Finnish population warrants conservation actions due to its extremely small size and degree of isolation from the main range, which makes it vulnerable to genetic depletion.

Keywords: core-edge, management unit, microsatellites, mtDNA, population structure, phylogeography, wader

Introduction

Many species with wide ranges show substantial morphological, demographic and genetic variation. This kind of variation may result from adaptations to different environments the species face and be important for the survival of the species (e.g. Kirkpatrick & Barton 1997). Some species, such as many northern waders, are nevertheless monotypic or have very low spatial variation, even when their ranges span across continents (e.g. Buehler and Baker 2005, Wennerberg & Burke 2001, Rönkä *et al.* 2012, Verkuil *et al.* 2012a, Conklin *et al.* 2016). As noted already by Mayr (1954), the difference between monotypic and polytypic species can be attributed to the effects of local selection pressures, genetic drift or dispersal, i.e. gene flow that can lead to either mixing or isolation of local populations. These mechanisms can often be connected with historical processes such as past climate changes, causing population size changes, range shifts and/or diversification events, especially at the northern hemisphere (Hewitt 2004). In both monotypic and polytypic species, edge populations or those isolated from the main range often show different patterns of genetic divergence and variability compared to populations at the core areas (Sagarin & Gaines 2002, Vuetich & Waite 2003, Eckert *et al.* 2008, Kark *et al.* 2008).

Genetic, morphological and ecological connectivity to other populations define the conservation value of a population (Lesica & Allendorf 1995), and can further be used as criteria for defining units for conservation by assessing both current and historical, ecological and genetic information (e.g. Moritz 1994, Crandall *et al.* 2000, Funk *et al.* 2012). Differentiated populations are commonly considered valuable in conservation, because they may contain evolutionary potential not present elsewhere, and possess the ability to adapt to the ongoing fast environmental changes, such as the climate change (Hampe & Petit 2005). The management of differentiated populations may therefore be beneficial for the evolutionary potential of the species (Lesica & Allendorf 1995).

Isolated and edge populations usually have lower levels of genetic diversity and they are more differentiated from each other than core populations (Eckert *et al.* 2008) due to the leading-edge

effect occurring during colonization (e.g. Hewitt 2000, 2004), long distances to the main range, possible source-sink or metapopulation structures, genetic drift and differential selection pressures. Low connectivity may also reinforce inbreeding within the isolated populations, further reducing their genetic diversity (Segelbacher *et al.* 2010), and ultimately even leading to extinction (e.g. Rogers & Slatkin 2017). The spatial differences in genetic variation may result from historical distribution and demography, but current dispersal behavior also clearly influences how easily species extend their ranges and how much gene flow there is between populations (e.g. Frankham *et al.* 2002). Among birds, these kind of patterns are most often observed on islands (e.g. Frankham 1997, 1998 and references therein, Küpper *et al.* 2012) or on resident species (Lira-Noriega & Manthey 2014, Pironon *et al.* 2017), and few studies so far have found distribution-wide patterns on migratory birds (Lira-Noriega & Manthey 2014).

We used 13 nuclear microsatellite loci and the mitochondrial DNA (mtDNA) control region to 1) examine the patterns in genetic diversity, population differentiation and demographic and phylogeographic history across the large breeding range of the Terek Sandpiper (*Xenus cinereus*; Fig. 1). The species is currently considered monotypic, despite biometric data that indicate some geographic differentiation of populations on wintering grounds in Australia (Higgins & Davies 1996), differences in breeding habitat use (Lappo *et al.* 2013, Meissner *et al.* 2013), and old suggestions of at least two subspecies: the western *X. c. cinereus* (Güldenstädt 1775) and eastern *X. c. javanicus* (Horsfield 1821, Clancey 1984). The core of the breeding range starts from Volga River basin in the west and continues east along the large rivers dissecting through Siberia, but there are at least two populations that are geographically separated from the main distribution (Tomkovich *et al.* 2016): in Finland and in Dnieper River basin (hereafter Dnieper River) in Belarus, Ukraine and Russia (Cramp & Simmons 1983). We 2) studied if these populations have lowered genetic diversity and increased population differentiation as might be expected, and evaluated their need for conservation. Finally, we 3) examined whether genetic variation shows any spatial patterns in the core-edge continuum in

the main distribution. We expected the variation to decrease towards the edges, according to the abundant-center hypothesis (e.g. Brussard 1984): edge and/or isolated population should be more differentiated from each other, have lower genetic variation and effective population size (N_e), and even show signs of inbreeding (e.g. Vuetich & Waite 2003) compared to core populations.

Methods

Study species and sampling

The Terek Sandpiper is one of the least studied waders in the Palearctic and its status is poorly known, which is reflected by the uncertainty of the global population size estimate (100 000–1 200 000 individuals; Delany *et al.* 2009, Wetlands International 2016, BirdLife International 2017). The first records of the Finnish population date back to the 1880s (Krank 1898). When detailed research begun in the 1970s and 1980s, the Finnish population consisted of ca. 30 pairs, but it has since declined to about five, maximum 10 pairs (Valkama *et al.* 2011, Veli-Matti Pakanen pers. obs.). The Terek Sandpiper is classified as critically endangered in the EU, including Finland, and is subjected to special conservation measures (Directive 2009/147/EC, Annex I; Tiainen *et al.* 2016, HELCOM 2013, Birdlife International 2015). The Dnieper River population is restricted to southern Belarus, northern Ukraine and a small portion of Russia (the Bryansk Region; Mongin *et al.* 1998, Tomkovich *et al.* 2016). In Belarus, the population consisted of only 20–40 pairs in the 1990s but it has since grown to 150–200 pairs in the 2010s (Thorup 2006, Birdlife International 2017). The population trend in Ukraine has also been positive, and the population size was estimated at 300–500 pairs in the beginning of the 21st century (BirdLife International 2017). In the Bryansk region, the estimated number of breeding birds is very low, perhaps up to 10 pairs at the most (Pavel Tomkovich pers. comm.). Prior to this study, nothing was known about the genetic connectivity between these isolated populations in Europe and the main distribution. Wintering sites are distributed along the coasts of Africa, southern Asia and Australia (Delany *et al.* 2009, Pakanen 2016).

We sampled eight locations spanning the whole breeding range of the Terek Sandpiper (Fig. 1, Table 1) between 2003 and 2013 ($n = 98$; either adults or one chick per nest). Sampling of blood, feather and muscle tissues followed national laws, and were collected with permission from respective authorities (Table 1). Two sites (Vorkuta and Lower Ob) are relatively close to each other (ca. 150 km), but are located on the opposite sides of the Ural Mountains. Distances to the edge of the range for each population within the main breeding area were measured according to the sampling locations and the edge of the range as shown in the distribution maps by Golovatin *et al.* (2010) and Lappo *et al.* (2013). Unless otherwise mentioned, the sample from Yenisei was excluded from the analyses of pairwise differentiation and demography due to its small sample size.

DNA extraction

DNA was extracted from blood or muscle tissue using the UltraClean Blood Spin Kit or Tissue & Cells DNA Isolation Kit (MoBio), respectively. DNA was extracted from feathers with the QuickExtract solution (Epicentre).

Microsatellites

Microsatellite amplification and preliminary analyses

Thirteen microsatellite loci, originally designed for the Dunlin (*Calidris alpina*: Calp2; Wennerberg & Bensch 2001, CA009877; Blomqvist and Pauliny, unpublished), Ruff (*Calidris pugnax*: Ruff1; Thuman *et al.* 2002), Barn Swallow (*Hirundo rustica*: Hru2; Primmer *et al.* 1995), Red Knot (*Calidris canutus*: Pgt83; Blomqvist *et al.* 2010), Pectoral Sandpiper (*Calidris melanotos*: Cme1, Cme2, Cme5, Cme6, Cme9 and Cme10; Carter & Kempenaers 2007), Great Snipe (*Gallinago media*: Sn13; Verkuil *et al.* 2012a) and Black Oystercatcher (*Haematopus bachmani*: Hbau6; Williams *et al.* 2012), were amplified in 10 µl reaction volumes containing 10 to 250 ng of DNA, 1.0 µM of each primer, 0.1 mM of each dNTP, 1 µl of 10 × PCR buffer, 2–2.5 mM of MgCl₂, and 0.1 U of DNA polymerase (Biotools

DNA-polymerase). The amplification profile was 94 °C for 1 min followed by 35 cycles of 94 °C for 30 s, locus-specific annealing temperatures (Supporting Online Material Table S1) for 30 s and 72 °C for 45 s for synthesis, ending with a final extension at 72 °C for 5 min. The amplifications were analyzed on an ABI 3730 automatic sequencer and alleles were scored with GENEMAPPER v. 4.0 (Applied Biosystems).

Error rates (repeatability) were calculated from 24 to 48 % of repeated PCRs per locus. The data were checked for scoring errors, null-alleles and large allelic dropouts with MICROCHECKER v. 2.2.3 (van Oosterhout *et al.* 2004). Linkage disequilibrium and deviations from the Hardy-Weinberg equilibrium for each locus and population were assessed using GENEPOP v. 4.2 (Raymond & Rousset 1995, Rousset 2008).

Genetic variation and structure

Expected (H_e) and observed (H_o) heterozygosities as well as the numbers of alleles were calculated with ARLEQUIN v. 3.5 (Excoffier *et al.* 2005), and the allelic richness (AR) with FSTAT v. 2.9.3.2 (Goudet 2002). Inbreeding coefficient (F_{IS} , i.e. excess homozygosity relative to a random mating population of the same size) was estimated with GENEPOP.

Population structure was first studied with ARLEQUIN by calculating population pairwise F_{ST} -values. Significance was tested with 1 000 permutations and Bonferroni corrected. When using fixation indices such as F_{ST} , differentiation can be underestimated in highly variable markers such as microsatellites (Hedrick 1999, Jost 2008, Bird *et al.* 2011; but see Kronholm *et al.* 2010, Meirmans & Hedrick 2011, Whitlock 2011). A differentiation index, D_{est} , should be a better estimator of population differentiation than F_{ST} (Jost 2008, Bird *et al.* 2011, Whitlock 2011). Therefore, also D_{est} was calculated using the *DEMEtics* package (Gerlach *et al.* 2010) in R v. 3.0.3 (R Development Core Team 2014). The number of boot-strap replicates was set to 1 000.

Genetic structure was also examined with the Bayesian clustering analysis in STRUCTURE v. 2.3.4 (Pritchard *et al.* 2000). In STRUCTURE, uneven sampling can lead to wrong inferences of population structure, as populations with reduced sampling can be merged together despite their genetic distinctiveness, and individuals from extensively sampled populations can be split despite belonging to the same panmictic population (Puechmaillie 2016). The data set was therefore pruned to contain the same number of individuals per population, i.e. eight (according to the smallest sample after Yenisei, Chukotka). The analysis was performed with the admixture model, correlated allele frequencies and prior information on the sampling locations, i.e. the LOCPRIOR model that has been shown to assist genetic clustering when the number of samples is low, by assuming that the data points dispersed closely together are likely from one population (Hubisz *et al.* 2009). The number of genetic clusters (K) to test was set from one to ten and the program was run for 10 iterations for each K with 1 000 000 Markov Chain Monte Carlo (MCMC) repeats and a burn-in of 500 000. STRUCTURE HARVESTER (Earl & von Holdt 2012) was then used to estimate ΔK between the consecutive numbers of Ks using a method of Evanno *et al.* (2005): the highest ΔK should be the best estimator of the actual K.

Population structure was further studied with the Discriminant Analysis of Principal Components (DAPC), a multivariate method for identifying genetically related individuals. DAPC was run with the package *adeigenet* (Jombart 2008, Jombart *et al.* 2010) in R. While STRUCTURE usually identifies the highest level of genetic structure, DAPC can also reveal finer-level structure (Jombart *et al.* 2010). The *find.cluster* command was used to find out the most supported genetic clustering based on the Bayesian information criterion (BIC). The optimal number of principal components (PCs) retained was determined after the preliminary run by using the *optim.a.score* command and re-running the DAPC using the suggested number of PCs. Yenisei was included in the DAPC analysis.

Isolation-by-distance (IBD) was tested with SpaGeDi (Hardy & Vekemans 2002). The mean pairwise kinship coefficients of Loiselle *et al.* (1995) between eight distance classes were estimated and regressed against the mean geographic distances of the classes. In addition, an intra-group kinship coefficient was calculated. Significance was tested with 10 000 permutations, and standard errors for each class were derived with a jackknife procedure over loci.

Demographic changes and the effective population size

The existence of genetic bottlenecks was examined using BOTTLENECK v. 1.2.02 (Cornuet & Luikart 1996) for populations with a sample size of over 10 individuals. The program compares the observed heterozygosity to the expected under the assumption of a constant population size. In a bottlenecked population, the observed heterozygosity should be higher than expected, since rare alleles, which do not contribute much to the overall heterozygosity are lost by chance more frequently than the common ones (Cornuet & Luikart 1996). The comparisons were performed under the two-phase mutation model (TPM, with 50% of single-step mutation model) and significance tested with the Wilcoxon test. The current effective population sizes were estimated with program NeEstimator (Do *et al.* 2014) using the linkage disequilibrium method and assuming random mating.

Mitochondrial DNA

Amplification of partial sequences from domains I and II of the mtDNA control region was performed with primers XCL140 (5'-ATCCATGTTCCAATTCCATTAA-3') and XCH680 (5'-GTCTTAGATTGTATTCACCCGTTG-3'), which we designed for this study based on the control region sequence in GenBank (accession number AY524806). Control region was selected because it is more variable than the rest of the mitochondrial genome (e.g. Parker *et al.* 1998), and due to its rapid coalescent time, it can be used to study within-species phylogeographic events on a relatively recent time scale (e.g. during the past 250 000 years; Kraaijeveld & Nieboer 2000, Emerson

& Hewitt 2005). In addition, it has been shown to be a powerful tool in revealing population structures in northern waders (e.g. Wenink *et al.* 1994, Buehler & Baker 2005). Sequences were amplified in 10 µl volumes containing 10–250 ng of the template DNA, 1 µl of 10 x PCR-buffer, 0.2 mM of each dNTP, 2 mM of MgCl₂, 0.1 µM of each primer and 0.1 units of DNA polymerase (Biotools). The amplification profile was 94 °C for 1 min, followed by 35 cycles at 94 °C for 30 sec, 50 °C for 45 sec and 72 °C for 1 min and ending with a final extension of 72 °C for 5 min. The control region was amplified and sequenced twice for all individuals. Sequencing was performed using the Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems) and reactions were analyzed on an ABI 3730 automatic sequencer. Sequences were aligned manually with BioEdit v. 7.2.5 (Hall 1999).

Genetic variation and structure

Haplotype diversity (Hd ; Nei 1987) and nucleotide diversity (π ; Nei 1987) were calculated with DnaSP v. 5.10.1 (Librado & Rozas 2009). Double peaks were observed in several nucleotide sites in 10 individuals, indicating amplification of a nuclear or mitochondrial copy. These individuals were excluded from the analyses.

Haplotype frequencies and pairwise Φ_{ST} values were calculated with ARLEQUIN. Significance was tested with 1 000 permutations and Bonferroni corrected. Kimura 2-parameter substitution model with a γ value of 0.05 was used, based on the model selection results obtained in MEGA v. 6.06 (Tamura *et al.* 2013). Program TCS v. 1.21 (Clement *et al.* 2000) was used to construct a parsimony network of the haplotypes.

Demographic changes and the effective population size

Past population size changes were examined with DnaSP by calculating Fu's F_s values (Fu 1997), Ramos-Onsins and Rozas's R_2 statistics (Ramos-Onsins & Rozas 2002) and mismatch distribution (Harpending 1994). F_s and R_2 were used because they are shown to be very powerful statistical tests

for detecting population growth, and the R_2 is shown to perform better than F_S when the sample size is small (Ramos-Onsins & Rozas 2002). Long-term changes in female effective population sizes (N_{ef}) were studied by constructing a Bayesian skyline plot in BEAST v. 1.8 (Drummond *et al.* 2012). The mutation rate was set to 7.32×10^{-8} /nucleotide/year, the same one used by Buehler and Baker (2005) for the Red Knot and Dunlin. The underlying population size function was fitted using the piecewise constant function. Out of the three available substitution models in BEAST, we used the Hasegawa-Kishino-Yano (HKY) model, since it was the most suitable one according to the model test in MEGA. Markov chains were run for 10 000 000 generations and sampled every 10 000 generations, with a burn-in of 1 000 000 generations. Posterior distribution, convergence and effective sample sizes were examined with TRACER v. 1.6 (Drummond *et al.* 2012).

Genetic variation in relation to the distance to the edge

Relationships between the indices of genetic diversity (AR , H_e , H_o and F_{IS} for microsatellites, and π and Hd for the mtDNA, arcsin transformed except for F_{IS}) and the distance to the edge of the distribution (km, log transformed) were examined using linear regression models in R. Yenisei was excluded from all comparisons except allelic richness due to its small sample size. Yenisei could be included in the allelic richness comparison, because when calculating AR , the rarefaction option was used to standardize AR to the smallest n in the comparison (Leberg 2002). In addition, Finland and Dnieper River were excluded because they are not part of the continuous range, but geographically separated, and their population genetics and dynamics therefore differ from the populations at the main range, which might give misleading results. Furthermore, it is impossible to estimate their distances from the edge in a logical way, whereas those estimated for the populations in the main range are comparable with each other.

Results

Microsatellites

We found no evidence of scoring errors, null-alleles or allelic dropouts except for three loci, each in a different population. The mean error rate across markers was low (0.036, SD = 0.039; Supporting Online Material Table S1). Twenty-five percent of these errors were due to allelic dropouts, which were then corrected in the data. All other types of non-concordant alleles between two or more runs were discarded from the data ($n = 29$). Linkage disequilibrium was not found.

Genetic variation and structure

Allelic richness was the highest in Chuvash (3.329, Table 2) and lowest in Dnieper River (2.961). Dnieper River also had the lowest observed heterozygosity (0.608), and F_{IS} was significantly positive at this site ($p = 0.032$). F_{IS} was also significant in Chuvash ($p = 0.035$). Observed heterozygosity was the highest in Finland and Lower Ob (both 0.723). Yenisei possessed nearly as much variation as the most variable populations ($AR = 3.231$, $H_o = 0.718$). Yakutia and Lower Ob both possessed four private alleles, Finland and Chuvash both three, and Chukotka one. Dnieper River, Vorkuta and Yenisei had no private alleles.

The overall F_{ST} was low (0.0030, $p = 0.107$), and no significant pairwise differences were found (Table 3). D_{est} values were concordant with F_{ST} values, but up to an order of magnitude higher, and many of them were significant: Finland differed from Vorkuta ($p = 0.018$); Dnieper River from Vorkuta ($p = 0.022$), Lower Ob ($p = 0.020$) and Yakutia ($p = 0.002$); and Chuvash from Yakutia ($p = 0.007$, Table 3). The only population that did not differ significantly from any of the others was Chukotka.

The Bayesian clustering analysis indicated no genetic structure: the best support was for $K = 1$ ($\ln P[K] = -2038.17$). The highest ΔK was for $K = 2$, but as ΔK cannot be used to estimate $K = 1$

(Evanno *et al.* 2005), and as no pattern could be distinguished from the bar plots at $K = 2$ (nor any other given K), the most likely number of clusters obtained from STRUCTURE remained at $K = 1$.

Based on the DAPC analysis, the lowest BIC value was obtained for $K = 3$. However, this clustering was not strong, as $K = 1$, $K = 2$ and $K = 4$ were also supported ($\Delta BIC < 2$ for $K = 1-4$; BIC values for clusters $K = 1-10$ are shown in Supporting Online Material Fig. S1). Assignment of individuals into the clusters at $K = 2-4$ revealed no pattern either, except perhaps a very weak west-east trend at $K = 3$ (assignment of individuals per populations into genetic clusters at $K = 2-4$ are shown in Supporting Online Material Fig. S2). The differences in the proportions of assigned individuals were nevertheless very small. The scatterplots indicating the genetic differentiation of individuals showed more and more overlap after $K = 3$ (scatterplots for $K = 3$ and $K = 4$ are shown in Supporting Online Material Fig. S3).

The intra-group kinship coefficient and the kinship coefficients of the first two distance classes were positive, but only the intragroup value was significant ($p = 0.019$, Fig. 2). The kinship coefficients of the larger distance classes were all negative but close to zero ($p = 0.3703$; $R^2 < 0.001$).

Demographic changes and effective population size

The Dnieper River ($p = 0.003$) and Yakutia ($p = 0.029$) had significant heterozygote excess in relation to the number of alleles (Table 2). The N_e estimates were low in Finland (52.3 individuals, Table 4) and Yakutia (12.5) and moderate in Dnieper River (130.6). In all other populations, N_e was infinite.

Mitochondrial DNA

A 499 bp sequence of the control region was obtained from 85 individuals (GenBank accession numbers will be provided upon the acceptance of the manuscript). There were 11 variable sites forming 19 different haplotypes of which 10 were unique (Fig. 3). Six of these singletons were found in the Lower Ob. Finland and Dnieper River shared one haplotype not present anywhere else.

Genetic variation and structure

Nucleotide diversity was on average 0.0030 (Table 5). It was the highest in Lower Ob and lowest in Yakutia. Yenisei had the highest haplotype diversity, with all three individuals having a different haplotype. Pairwise Φ_{ST} values varied from -0.2127 to 0.2371 (average 0.0327, $p = 0.131$, Table 3). Dnieper River differed significantly from every population except Finland and Chuvash. The differentiation values between Finland and the rest were of the same order of magnitude as those between Dnieper River and the rest, albeit not significant. All the other comparisons were non-significant, and most of them close to zero.

Demographic changes and effective population size

Demographic analyses indicated population growth (Table 5): the mismatch distributions followed the curves expected for past population expansion, and the haplotype tree was somewhat star-shaped (Fig. 3), as expected for a population that has grown in size. In addition, Fu's F_s values were negative in every population, and significantly so in most. R_2 statistics were significant only in Chukotka.

The effective population size (N_{ef}) of the Terek Sandpiper across its distribution was estimated to be currently at nearly 790 000 individuals. It grew from ca. 60 000 to 160 000 between 10 000 and 5 000 years ago, after which the population growth rate increased (Fig. 4). The posterior distribution of the analysis was unimodal and the effective sample size (ESS) 278.

Genetic variation in relation to the distance to the edge

Distance to the edge of the distribution showed a statistically significant positive relationship with allelic richness ($df = 4$, $t = 3.339$, $p = 0.030$, Adj. $R^2 = 0.67$) and nearly significant with expected heterozygosity ($df = 3$, $t = 3.165$, $p = 0.051$, Adj. $R^2 = 0.69$, Fig. 5a), but not with observed heterozygosity or F_{IS} . Haplotype diversity ($df = 3$, $t = -1.837$, $p = 0.164$, Adj. $R^2 = 0.37$) and nucleotide

diversity ($df = 3$, $t = -1.241$, $p = 0.303$, Adj. $R^2 = 0.12$, Fig. 5b) seemed to increase towards the edge, but not significantly.

Discussion

Genetic variation and structure

Our results, for the first time describing the population genetics of the Terek Sandpiper in its entire breeding range showed low overall structure and variation. No genetic indication of possible subspecies was detected, supporting the prevailing concept that the Terek Sandpiper is a monotypic species. Most of the mtDNA haplotypes and microsatellite alleles were shared even between the furthestmost populations, and the distribution-wide differentiation was therefore negligible. The only population showing any consistent differentiation was the geographically separated Dnieper River.

More positive pairwise differences were found with microsatellites than mtDNA. With the latter, only Dnieper River showed any differentiation, although the Φ_{ST} values between Finland and most of the others were at similar level or even higher, just not significant. The high differentiation of Finland and Dnieper River with the others is likely affected by the one haplotype that was shared between these two isolated populations but not present anywhere else. However, this haplotype differed from the most common one by only two nucleotide substitutions. Thus, even though the pairwise differentiation values were rather high, no geographic structure could be detected from the haplotype network. With microsatellites, none of the F_{ST} values were significant, but many such emerged when estimated with D_{est} . This was expected, because D_{est} has proven to be a better estimator of actual population differentiation and give a more reliable picture of the genetic uniqueness of populations than F_{ST} (Jost *et al.* 2008, Bird *et al.* 2011, Whitlock 2011).

While the significantly positive intra-group kinship coefficient suggests that philopatry might have an effect on genetic structure at least to some extent, the kinship coefficients for the eight distance classes were all close to zero, and no indications of an IBD pattern were therefore detected.

Moreover, no support for distribution-wide genetic structuring was obtained with either the DAPC or Bayesian clustering analyses. The Terek Sandpiper breeds in ephemeral habitats such as river flood plains (Golovatin *et al.* 2010, Meissner *et al.* 2012, 2013), in which deterioration of breeding sites may lead to desertions of breeding sites and recolonization of new areas. This would increase both female and male gene flow between the breeding sites and decrease genetic differentiation between populations.

In addition, Terek Sandpipers originating from different breeding locations may share wintering sites, i.e. have weak migratory connectivity (Delany *et al.* 2009, Pakanen 2016), possibly facilitating movements between populations. For example Ruffs (*Calidris pugnax*) use various flyways between their wintering and breeding grounds, and individuals originating from the same wintering site can end up in a vast range of breeding areas (Verkuil *et al.* 2012b). If Terek Sandpipers from a certain breeding area mix during wintering and end up in different breeding grounds in the consecutive breeding season, it could lead to the low overall genetic structure observed here. Continent-scale mixing may nevertheless be less likely because the eastern populations migrate along the eastern Pacific coast to Australia, whereas the western populations fly over the Caspian region and the Middle East, or between the Ural and Volga rivers to the coasts of the Indian Ocean (Snow & Perrins 1998).

Demographic changes and phylogeography

The Terek Sandpiper had low mtDNA variation, signs of population expansion and low level of genetic structure. These kind of genetic patterns can be caused by selective sweeps (e.g. Simonsen *et al.* 1995), but as similar features are often observed in northern species (Avice 2000, Hewitt 2004), including many waders (see below), the most likely scenario for the Terek Sandpiper might be that it has experienced repeated population contractions and consequent expansions during the climate changes during the past glacial periods. Similar patterns have been found in the Temminck's Stint

(*Calidris temminckii*, Rönkä *et al.* 2008, 2012), Red Knot (*Calidris canutus*, Buehler & Baker 2005), White-rumped Sandpiper (*Calidris fuscicollis*, Wennerberg *et al.* 2002), Curlew Sandpiper (*Calidris ferruginea*, Wennerberg & Burke 2001), Ruddy Turnstone (*Arenaria interpres*, Wenink *et al.* 1994), Buff-breasted Sandpiper (*Calidris subruficollis*, Lounsberry *et al.* 2013) and Sanderling (*Calidris alba*, Conklin *et al.* 2016). Therefore, even though the Terek Sandpiper breeds mainly in the boreal region (Snow & Perrins 1998, Golovatin *et al.* 2010, Lappo *et al.* 2013), it shares more similarities with the northern species than more southern ones such as the Kentish Plover (*Charadrius alexandrinus*), which has higher genetic variation indicating that it has not been subjected to severe population contractions in the past (Küpper *et al.* 2012).

In contrast to many Arctic waders that are dependent on tundra, the Terek Sandpiper can breed in diverse riverine habitats from low Arctic to temperate regions. This suggests that more suitable habitats for the Terek Sandpiper may have been available during the warm interstadial or interglacial periods than during the cold phases of the glacial cycles. Therefore, it is unlikely that the Terek Sandpiper has undergone population size changes comparable to those in strictly Arctic waders. For example, the Red Knot and Ruddy Turnstone have undergone a bottleneck during the warm period of the early Holocene 8 000–6 000 years ago (Kraaijeveld & Nieboer 2000, and references therein). The effective population size of the Terek Sandpiper was the lowest at the end and after the latest glaciation ca. 10 000 years ago, after which the population started to grow. However, as the confidence intervals of the skyline plot were large, this should be considered only as a rough estimate. It nevertheless seems that the Terek Sandpiper population has decreased in size and subsequently expanded, spreading ancestral polymorphism throughout the range. The seemingly homogenous overall genetic structure can therefore reflect recent shared ancestral polymorphism and incomplete lineage sorting rather than current global panmixia (e.g. Bulgin *et al.* 2003): genetic structure may be evolving, but we just cannot see it yet.

Genetic variation in relation to the distance to the edge

We found that allelic richness and expected heterozygosity of microsatellites decreased towards the edge of the main distribution. These results, together with the low N_e of the Finnish and Dnieper River populations, are in accordance with the abundant-center hypothesis, which builds on the assumption that conditions affecting fitness are optimal at the core, but suboptimal at the edge (Brussard 1984). Populations closer to the edge of the distribution should have lower densities, be more fragmented and isolated, and consequently their genetic diversity and N_e should be lower compared to the core populations (Vuetich & Waite 2003). Surprisingly, the Yakutian population, located in a presumably high-density region in the core area (Golovatin *et al.* 2010), had extremely low N_e , and the population showed signs of a bottleneck.

The slightly different patterns revealed by mitochondrial and microsatellite data is perhaps not very surprising given the differences between the markers: mitochondrial markers have lower effective population size and mutation rates than microsatellites, and mtDNA is maternally inherited whereas microsatellites are bi-parentally inherited. Hence, some kind of differences in the diversity indices might also have been expected. The pattern observed in microsatellites is nevertheless interesting given the small sample size used in this study, and warrants further studies on the core-edge-continuum of genetic diversity especially on long-distance migratory bird species, because most studies reporting such differences have so far been conducted on resident birds (meta-analyses of Lira-Noriega & Manthey 2014 and Pironon *et al.* 2017 and references therein), less mobile animal species (Munwes *et al.* 2010) or plants (Vakkari *et al.* 2009).

Conservation implications

On a global scale, the conservation status of the Terek Sandpiper is Least Concern (Birdlife International 2017), and due to its vast distribution and large population size, both census and effective, conservation measures are not needed at a distribution-wide scale, even though the global

population trend is decreasing (BirdLife International 2017). However, the Terek Sandpiper is critically endangered in the EU, based on the presence of only one small and geographically separated population in Finland (with occasional breeding in Latvia; Birdlife International 2015). The Red List assessment calls for research on migration, locating the breeding sites and estimating population parameters of the Finnish population (HELCOM 2013, Birdlife International 2015). Based on our results indicating a lack of differentiation from the main range, fairly high polymorphism in nuclear and mitochondrial markers and a higher N_e than the census population size, the Finnish population may receive immigrants. Indeed, colour-ringing data indicate that previously unringed displaying males are occasionally observed, but in some years mates can be so scarce that close inbreeding is witnessed (Veli-Matti Pakanen pers. obs.). If immigration exists, it is possibly very stochastic in nature, and might be dependent on straying individuals during migration. Some Terek Sandpipers breeding in northwestern Russia migrate southwest through continental Europe and possibly also through Finland (Mauer & Ijzendoorn 1987).

The isolated Dnieper River population was significantly differentiated from the main distribution (with the exception being Chuvash, although the fixation and differentiation indices were all positive and at the same level as between the other populations). Furthermore, it had homozygosity excess, the lowest allelic richness, low N_e , and there was evidence of a population bottleneck. These results fit the previous suggestion that this population is a recently founded discrete breeding population (Snow & Perrins 1998). The south of Bryansk Region, where this population is closest to the main range, is located approximately 400 km from the main range in the Voronezh and northeastern Smolensk Region, Russia (Tomkovich *et al.* 2016).

Even though Dnieper River did not possess private alleles and there was no structure based on the mitochondrial haplotypes (except for the one haplotype shared only with Finland), it is geographically isolated and differs from most of the main range populations significantly with two different markers. Therefore, it fulfills the criteria of a management unit (Moritz 1994). In addition,

even though Dnieper River seems to have large enough N_e to avoid immediate risk of extinction due to e.g. inbreeding (suggested threshold N_e of 50), it is not enough for the persistence of the long-term evolutionary potential (suggested N_e of 500; Franklin 1980). Moreover, even though in Belarus the population has grown from few tens to 150–200 pairs in a couple of decades (Thorup 2006, Birdlife International 2017), and the Ukrainian population has also been growing (Birdlife International 2017), local decreases have also been observed. In the Middle Pripyat River in Belarus, the number of breeding pairs has declined from 20–25 in the beginning of the 21st century to only one breeding pair in years 2010–2017 (Szurlej-Kielńska *et al.* 2017). The small population sizes and large distances to the main breeding range of both Finnish and Dnieper River populations make them vulnerable to genetic depletion, especially if gene flow is restricted, warranting conservation actions for preserving them (see also AlmalkI *et al.* 2017).

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Table 1. Details of the sampled Terek Sandpiper (*Xenus cinereus*) populations and sampling permit information. Year = years during which the sampling was conducted; DistE = approximate distance to the edge of the distribution (km), or indication if the population is separated from the main range.

Population	<i>n</i>	Year	Latitude	Longitude	DistE	Permits
Finland	13	2006–2013	64°59'–66°43'	25°25'–27°24'	separated	Finland , Regional Centres for Economic Development, Transport and the Environment in North-Ostrobothnian regional Centre for Economic Development, Transport and the Environment (PPO-2006-L-206-254, POPELY/172/07.01/2010) and Lapland (LAP-2006-L-717-254, LAPELY/112/07.01/2011) Belarus , Institute of Zoology, National Academy of Sciences of Belarus Russia , Permit No. 21 issued on 10 May 2009 for the Zoological Museum of Moscow State University by the Russian "Federal Service for Control in the Scope of Nature Management"
Dnieper River	19	2007–2009	52°04'	27°44'	separated	
Chuvash	9	2009–2012	54°45'–55°24'	46°12'–46°43'	640	
Vorkuta	9	2007–2011	67°21'–67°30'	62°02'–64°40'	80	
Lower Ob	22	2010	66°25'–66°43'	66°17'–66°49'	130	
Yenisei	3	2010–2011	62°18'–62°20'	89°01'–89°00'	760	
Yakutia	15	2009–2010	57°41'–62°00'	129°48'–132°17'	400	
Chukotka	8	2003–2012	62°32'–64°18'	174°06'–178°03'	50	

Table 2. Genetic diversity and demographic history of the Terek Sandpiper (*Xenus cinereus*) populations, measured with 13 microsatellite markers. NA = mean number of alleles; AR = allelic richness; H_e/H_o = expected/observed heterozygosity; F_{IS} = excess level of homozygosity relative to a random mating population of the same size; TPM = p -values for the Wilcoxon test for heterozygosity excess under the two-phase model. Significance $p < 0.05$ is shown in bold. – = not estimated.

Population	n	NA	AR	H_e/H_o	F_{IS}	TPM
Finland	13	5.385	3.172	0.682/0.723	-0.064	0.153
Dnieper River	19	4.923	2.961	0.646/0.608	0.062	0.003
Chuvash	9	5.077	3.329	0.722/0.676	0.069	–
Vorkuta	9	4.154	3.010	0.675/0.624	0.074	–
Lower Ob	22	6.231	3.155	0.681/0.723	-0.062	0.137
Yenisei	3	3.231	3.231	0.697/0.718	-0.037	–
Yakutia	15	5.154	3.131	0.687/0.648	0.059	0.029
Chukotka	8	4.308	3.039	0.669/0.663	0.009	–

Table 3. Population pairwise F_{ST}/D_{est} values, respectively, estimated from the microsatellite data above the diagonal and Φ_{ST} values based on mitochondrial DNA control region sequences below the diagonal for the Terek Sandpiper (*Xenus cinereus*). Significant values ($p < 0.05$) are shown in bold.

	Finland	Dnieper River basin	Chuvash	Vorkuta	Lower Ob	Yakutia	Chukotka
Finland		-0.0027/0.0200	0.0040/0.0150	0.0130/ 0.0652	-0.0120/-0.0135	-0.0121/-0.0009	-0.0183/-0.0355
Dnieper River	-0.0763		0.0094/0.0302	0.0141/ 0.0489	0.0062/ 0.0319	0.0172/ 0.0681	0.0132/0.0226
Chuvash	0.2227	0.1668		-0.0081/-0.0057	0.0023/0.0358	0.0189/ 0.0707	0.0148/0.0436
Vorkuta	0.2371	0.1887	-0.1153		-0.0093/0.0088	0.0058/0.0285	-0.0098/-0.0209
Lower Ob	0.0241	0.0246	-0.0324	-0.0166		-0.0037/0.0035	-0.0094/-0.0319
Yakutia	0.1441	0.1117	-0.0170	0.0443	-0.2127		-0.0062/-0.0107
Chukotka	0.1408	0.1505	-0.0354	-0.0189	-0.1429	-0.0080	

Table 4. Effective population size (N_e) estimated from microsatellite data and census size estimates for the Terek Sandpiper (*Xenus cinereus*) populations.

Population	N_e (individuals)	Census size ¹ (pairs)
Finland	52.3 ($\pm$ 15.2)	5–10
Dnieper River	130.6 ($\pm$ 35.4)	450–700
Chuvash	∞	Western Russia: 15 000–50 000
Vorkuta	∞	
Lower Ob	∞	
Yakutia	12.5 ($\pm$ 7.7)	
Chukotka	∞	

¹Birdlife International (2017). Census size estimates are lacking for populations within the main distribution because population size is difficult to define without specific borders.

Table 5. Mitochondrial DNA control region diversity estimates for each Terek Sandpiper (*Xenus cinereus*) population. NH, number of haplotypes; π , nucleotide diversity; Hd = haplotype diversity; F_S = Fu's F_S statistics. R_2 = Ramos-Onsins and Roza's R_2 statistics. Significance at $p < 0.05$ is shown in bold. SD is given in parentheses for π and Hd . – = not determined.

Population	n	NH	π	Hd	F_S	R_2
Finland	9	5	0.0026 (0.0004)	0.861 (0.087)	-1.886	0.206
Dnieper River	11	5	0.0029 (0.0004)	0.855 (0.066)	-1.143	0.140
Chuvash	8	4	0.0021 (0.0006)	0.750 (0.139)	-1.164	0.210
Vorkuta	9	5	0.0028 (0.0005)	0.806 (0.120)	-1.686	0.157
Lower Ob	22	14	0.0043 (0.0005)	0.935 (0.036)	-10.308	0.110
Yenisei	3	3	0.0027 (0.0009)	1.000 (0.272)	–	–
Yakutia	15	5	0.0018 (0.0004)	0.695 (0.109)	-1.808	0.152
Chukotka	8	6	0.0030 (0.0007)	0.893 (0.111)	-3.443	0.110
Overall	85	19	0.0030 (0.0002)	0.841 (0.027)	-13.170	0.074

Figure legends

Figure 1. The main breeding distribution (light grey) and sampling sites (black circles) of the Terek Sandpiper (*Xenus cinereus*).

Figure 2. Kinship coefficients of the eight distance classes of the Terek Sandpiper (*Xenus cinereus*) from the 13 microsatellite loci studied. Whiskers depict SE. Distance class zero indicates the intra-group value. * = $p < 0.05$.

Figure 3. Maximum parsimony network based on 499 bp of mitochondrial DNA control region sequences of 85 individuals of the Terek Sandpiper (*Xenus cinereus*) collected from eight breeding populations. Lines linking haplotypes and/or small open circles indicate nucleotide substitutions. Number of individuals belonging to each haplotype is indicated next to those haplotypes that are represented by more than one individual.

Figure 4. Estimates of the female effective population size for the global Terek Sandpiper (*Xenus cinereus*) population during the past 10 000 years based on the mitochondrial DNA control region sequences. Dashed lines indicate 95 % confidence intervals.

Figure 5. Relationship between the distance to the edge of the distribution (km, log transformed) and a) allelic richness and expected heterozygosity of 13 microsatellite loci (arcsin transformed), and b) nucleotide and haplotype diversities of the mitochondrial DNA control region (arcsin transformed) estimated for different Terek Sandpiper (*Xenus cinereus*) populations in the main distribution.