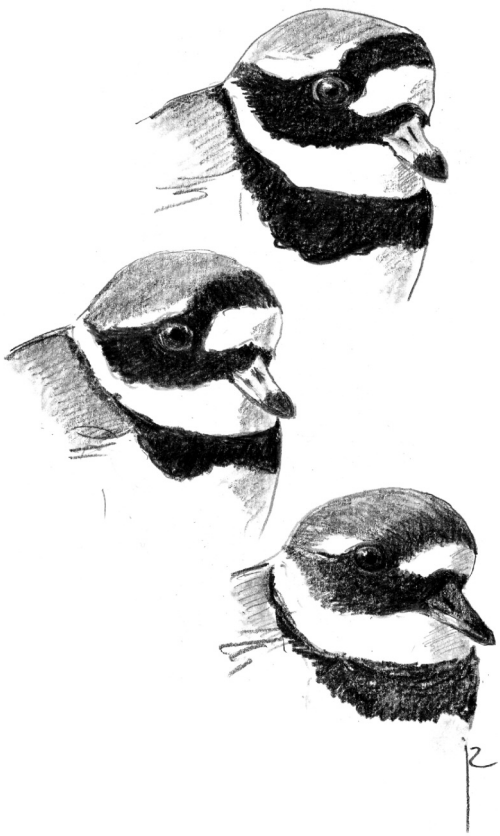
Population and subspecies differentiation in a high latitude breeding wader, the Common Ringed Plover *Charadrius hiaticula*

Leon Thies[1], Pavel Tomkovich[2], Natalie dos Remedios[3], Terje Lislevand[4], Pavel Pinchuk[5], Johan Wallander[6], Juliana Dänhardt[7], Böðvar Þórisson[8], Donald Blomqvist[9,*] & Clemens Küpper[1,10,*]



Thies L., Tomkovich P., dos Remedios N., Lislevand T., Pinchuk P., Wallander J., Dänhardt J., Þórisson B., Blomqvist D. & Küpper C. 2018. Population and subspecies differentiation in a high latitude breeding wader, the Common Ringed Plover *Charadrius hiaticula*. Ardea 106: 163–176. doi:10.5253/arde.v106i2.a8

Exploring the patterns of genetic structure in the context of geographical and phenotypic variation is important to understand the evolutionary processes involved in speciation. We investigated population and subspecies differentiation in the Common Ringed Plover *Charadrius hiaticula*, a high latitude wader that breeds in arctic and temperate zones from northeast Canada across Eurasia to the Russian Far East. Three subspecies, *hiaticula*, *tundrae* and *psammodromus*, are currently widely recognised, whereas a fourth subspecies, *kolymensis*, has been proposed based on geographic isolation and phenotypic differences. We genotyped 173 samples from eleven Common Ringed Plover breeding sites, representing all four putative subspecies, at eight polymorphic microsatellite loci to examine the patterns of population and subspecies differentiation. Bayesian clustering identified three genetic clusters among samples, corresponding to the breeding sites of the three currently recognised subspecies. The existence of the subspecies *kolymensis* was not supported. We also detected the presence of a previously unknown hybridisation zone extending from Northern Scandinavia to Belarus. Differentiation of the subspecies *tundrae* and *hiaticula* most likely occurred in allopatry on the Eurasian continent during past glaciation events, followed by population expansion leading to colonisation of Iceland and Greenland. The lack of genetic differentiation within the *tundrae* subspecies is consistent with ongoing range expansion and high gene flow maintained through migratory behaviour. We discuss the importance of historic climate changes, migratory behaviour and mating system on shaping the observed pattern of genetic differentiation.

Key words: subspecies delineation, population differentiation, microsatellites, *Charadrius*

[1]Institute of Zoology, University of Graz, Universitätsplatz 2, 8010 Graz, Austria; [2]Zoological Museum, M.V. Lomonosov Moscow State University, Bolshaya Nikitskaya Str. 6, 125009 Moscow, Russia; [3]Department of Animal and Plant Sciences, University of Sheffield, Western Bank, Sheffield S10 2TN, UK; [4]University Museum of Bergen, Department of Natural History, University of Bergen, P.O. Box 7800, N-5020 Bergen, Norway; [5]Belarus Bird Ringing Centre, National Academy of Sciences, Academichnaya Str. 27, 220072 Minsk, Belarus; [6]Swedish Board of Agriculture, 551 82 Jönköping, Sweden; [7]Centre for Environmental and Climate Research, Lund University, Sölvegatan 37, 223 62 Lund, Sweden; [8]South Iceland Research Centre, University of Iceland, Lindarbraut 4, 840 Laugarvatn, Iceland; [9]Department of Biological and Environmental Sciences, University of Gothenburg, Box 463, 405 30 Gothenburg, Sweden; [10]Max Planck Institute for Ornithology, Eberhard Gwinner Str., 82319 Seewiesen, Germany;
*corresponding authors (ckuepper@orn.mpg.de, donald.blomqvist@bioenv.gu.se)

Reproductive isolation and genetic differentiation are important precursors of speciation (Mayr 1942, Coyne & Orr 2004). Most terrestrial species with large geographic distributions show moderate to strong genetic differentiation (Avise 2000). Genetic differentiation measured by neutral genetic markers usually arises either because of limited dispersal resulting in isolation-by-distance, or due to spatial barriers interrupting the geographic distribution of a species (vicariance). However, a number of widespread terrestrial species with large continental distributions appear to completely lack genetic differentiation (Estoup *et al.* 1996, Reudink *et al.* 2011, Küpper *et al.* 2012).

Across animal taxa, dispersal ability is positively correlated with gene flow and hence negatively correlated with genetic differentiation (Bohonak 1999). Two dispersal processes, natal and breeding dispersal (Greenwood & Harvey 1982), have the largest influence on gene flow. Of these, natal dispersal is considered more important since natal dispersal distances are usually greater than breeding dispersal distances. Interestingly, in birds both natal and breeding dispersal are negatively correlated with geographic range but positively related to migration (Paradis *et al.* 1998). Dispersal behaviour is shaped by a suite of life history characteristics, including social traits such as mating behaviour (Greenwood 1980, D'Urban Jackson *et al.* 2017, Kempenaers & Valcu 2017).

Phenotypic differentiation does not necessarily imply genetic differentiation (Marthinsen *et al.* 2007, Rheindt *et al.* 2011, Woltmann *et al.* 2014, Hossein *et al.* 2017). Examining the coherence of genetic and phenotypic variation across populations is important to understand the evolutionary processes involved with speciation. Generally, incongruence between genotypes and phenotypes can have two explanations. First, phenotypically monomorphic taxa may exhibit deep genetic divergences, for example when a recognised species harbours several cryptic species (Tolley *et al.* 2008). Second, morphologically distinct taxa can appear to lack genetic differences (Woltmann *et al.* 2014), for example, when the diagnostic differences are encoded by only a small number of genes.

Genetic data are routinely used to assess the validity of species and subspecies delineation. Traditionally, subspecies have been used to distinguish populations with morphological differences (Phillimore & Owens 2006, Winker 2010, Haig *et al.* 2011). In a number of species, population genetic data and subspecies delineation are in agreement, although the majority of avian subspecies have not been subject to genetic evaluation. Correct taxonomy and delineation of species and populations is important for conservation policies and management. Species, subspecies or distinct populations are often used as the basis of practical management and legislation (Höglund 2009, Haig & D'Elia 2010). Examination of genetic diversity can help to assess the viability of small populations (Blomqvist *et al.* 2010) and if necessary inform translocations of animals to supplement endangered populations.

Waders, or shorebirds, are characterised by high dispersal abilities as well as large variation between species and populations in mating systems, migration and breeding behaviour (Piersma 1987, Piersma & Lindström 2004, Székely *et al.* 2007, Thomas *et al.* 2007). Waders are a challenging group of birds for taxonomists, with poor resolution especially at the tips of the wader phylogeny (dos Remedios *et al.* 2015). In a number of wader species, genetic variation does not match phenotypic variation across geographic clines (Marthinsen *et al.* 2007, Küpper *et al.* 2009, Rheindt *et al.* 2011). Variation in mating and migratory behaviour has also been invoked to explain genetic differentiation and subspecies number in waders (Kraaijeveld 2008, D'Urban Jackson *et al.* 2017) although genetic evaluation of subspecies delineation is yet to be conducted for most wader species.

We studied genetic differentiation and subspecies delineation in the Common Ringed Plover *Charadrius hiaticula*, (Ringed Plover from here on) a high latitudinal monogamous wader (Wallander *et al.* 2001, Wiersma *et al.* 2018). As the ancestral *Charadrius* plover (dos Remedios *et al.* 2015), Ringed Plovers have an Arctic breeding distribution, stretching from eastern Canada across the Palearctic to the Russian Far East. The breeding distribution also extends into temperate climate zones in Europe (Wiersma *et al.* 2018) and Siberia (Lappo *et al.* 2012). The global population is estimated at 360,000–1.3 million individuals (Delany *et al.* 2006), and Iceland holds the largest European population (up to 50,000 breeding pairs; Wiersma *et al.* 2018). Return rates of juveniles to their hatching site is relatively low, implying high natal dispersal, whereas adult return rates to breeding sites seem to be variable (Laven 1940, Wallander & Andersson 2003, Lislevand *et al.* 2017, Tomkovich *et al.* 2017). Western Palearctic Ringed Plovers show leap-frog migration, with the northern breeding populations generally wintering further south than southern populations (Taylor 1980). It is likely, however, that individuals from different breeding populations mix during winter, at least in Iberia and north-western Africa (Thorisson *et al.* 2012).

Despite its large breeding distribution, the Ringed Plover shows relatively little morphological variation

Female Common Ringed Plover of the *tundrae* subspecies (Chukotka, 6 June 2015).

(Salomonsen 1930, Engelmoer & Roselaar 1998, Meissner 2007, Meissner *et al.* 2010, Wiersma *et al.* 2018). Consequently, the number of distinct subspecies has been debated, ranging from two to seven (Engelmoer & Roselaar 1998, Meissner 2007, Lappo *et al.* 2012, Wiersma *et al.* 2018) with most authors suggesting three subspecies: *C. h. hiaticula* breeding from southern Scandinavia and the Baltic south to the British Isles and north-western France, *C. h. tundrae* from northern Scandinavia across northern Russia, and *C. h. psammodromus* in northeast Canada, Greenland, Iceland and Faeroe Islands. The subspecies differ in morphometrics (particularly wing, secondary and tail length), moult cycle and plumage colouration of the upper parts (Engelmoer & Roselaar 1998). The exact subspecies delineation is contentious, for example Scandinavian populations have been proposed to be transition forms between the nominate and *tundrae* subspecies (Haftorn 1971). Moreover, the Siberian distribution of Ringed Plovers is interrupted between 145°E and 160°E, with only a few scattered breeding sites found in between (Figure 1). Interestingly, Ringed Plovers from Chukotka, in the Far East, differ in morphometrics and chick plumage from *C. h. tundrae* (Engelmoer & Roselaar 1998, Lappo *et al.* 2012). Chick plumage is often a valid indicator of species differences (Jehl 1968, Küpper *et al.* 2009) and the geographically isolated populations in Chukotka are tentatively referred to as subspecies *kolymensis* (Lappo *et al.* 2012). A thorough genetic analysis is required to verify this taxonomic hypothesis.

Here, we examine population genetic structure in Ringed Plovers, sampling a substantially larger part of the breeding distribution than any previous study (cf. D'Urban Jackson *et al.* 2017). We analyse genetic variation within and among eleven breeding populations, from Iceland in the west to Chukotka in the Russian Far East. Using data from eight polymorphic microsatellite markers, we evaluate genetic subspecies delineation and examine patterns of genetic differentiation in the Ringed Plover. We discuss factors influencing population subdivision and gene flow.

METHODS

We collected blood, tissue or feather samples from Ringed Plover adults or chicks in eleven sampling areas across Eurasia (Table 1, Figure 1). Our sampling included areas from the three currently recognised subspecies, *hiaticula* (breeding areas HAL, TUR; area abbreviations in Table 1), *psammodromus* (WES) and *tundrae* (LAP, VAR, VOR, TAY), and the suggested fourth subspecies *kolymensis* (NWC, SEC, ECC, NEC). The smallest distance between sampling areas was around 230 km (ECC and SEC) and the maximum distance between samples within an area was 132 km.

Blood sampling was the main source of DNA, with the exception of WES, where we obtained feather samples instead. Adult plovers were caught on their

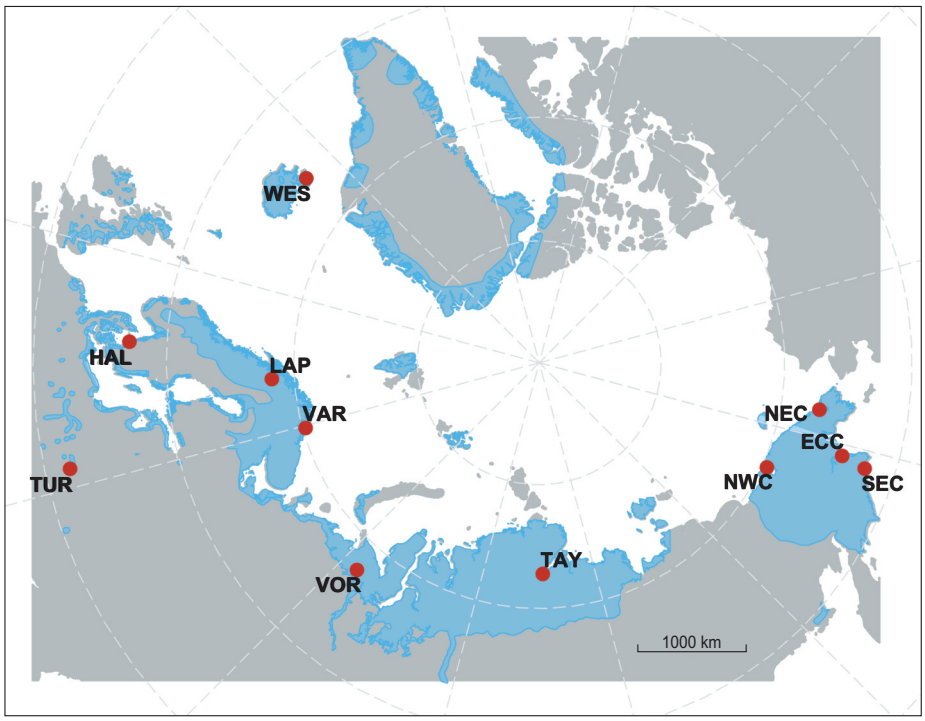


Figure 1. Polar projection of the breeding range and the sampled Common Ringed Plover breeding sites. Breeding range of the species is shown based on distributions given in Delany *et al.* (2009), Valcu *et al.* (2012) and Lappo *et al.* (2012).

Table 1. Characteristics of sampled Common Ringed Plover breeding areas. Latitude and longitude are means of sampling area; *n*: number of individuals successfully genotyped; H_E: expected heterozygosity across final six microsatellite markers; H_O: observed heterozygosity across final six microsatellite markers.

Site	Country	Abbreviation	Latitude (°N)	Longitude (°E)	*n*	H_O	H_E
Westfjords	Iceland	WES	66.06	–23.35	42	0.70	0.68
Halland	Sweden	HAL	57.33	12.06	25	0.64	0.68
Lapland	Sweden	LAP	68.35	18.49	10	0.47	0.70
Turov	Belarus	TUR	52.07	27.73	12	0.73	0.71
Varanger	Norway	VAR	70.36	30.59	12	0.60	0.68
Vorkuta	Russia	VOR	67.72	63.64	10	0.58	0.70
Taymyr	Russia	TAY	72.87	105.96	15	0.70	0.66
NW Chukotka	Russia	NWC	69.72	170.33	2	NA	NA
SE Chukotka	Russia	SEC	62.54	177.00	24	0.69	0.71
E Central Chukotka	Russia	ECC	64.50	177.92	11	0.65	0.66
NE Chukotka	Russia	NEC	67.07	–174.50	10	0.73	0.72

nests using funnel traps and automatic clap-net traps. Chicks were caught at hatching or during opportunistic encounters in the field. We obtained a small blood sample (25–50 μl for adults from the brachial vein, 25 μl for chicks from the tarsal vein). At NWC and SEC, we obtained tissue samples from one embryo from one nest at each site. Blood and tissue were stored in absolute ethanol or Queen's Lysis Buffer (Seutin *et al.* 1991) whilst feather samples were kept refrigerated in sealed plastic bags until DNA extraction.

DNA extraction and microsatellite genotyping followed established protocols (Küpper *et al.* 2009, 2012). In brief, we extracted DNA from blood or feather using an ammonium acetate precipitation protocol following proteinase K digest (Nicholls *et al.* 2000). After two ethanol washes, the DNA was dried and dissolved in TE buffer. We used a 0.7% agarose gel stained with SYBR safe (Invitrogen) to check the quality and establish quantity of the DNA. We amplified fragments of two to four microsatellite loci in multiplex Polymerase Chain Reactions (PCRs) with 1 μl (0.1–100 ng) of DNA solution, 1 μl of primer mix including one fluorescently labelled primer per pair and 4 μl (samples extracted from blood) or 8 μl (samples extracted from feathers) solution of the Qiagen Multiplex PCR Kit (Qiagen). We analysed the fragment length of PCR products using an ABI3730 capillary DNA Analyzer, visualising the fragments with GENEMAPPER software v. 4.1. We avoided the inclusion of first order relatives by genotyping only presumably unrelated parents, or a single chick from broods where the parent had not been caught.

To obtain polymorphic markers we tested available microsatellite primers at the NERC Biomolecular Analysis Facility at University of Sheffield that had shown amplification in other *Charadrius* plovers previously (Funk *et al.* 2007, Küpper *et al.* 2007, 2008, Dawson *et al.* 2010). The final microsatellite data consisted of eight autosomal polymorphic microsatellite markers (Table 2). We only included samples from which we obtained at least 75% of genotypes from all markers. All excluded samples were feather samples from Iceland (n = 18), likely reflecting the higher degradation or lower DNA yield from these samples (Harvey *et al.* 2006). All subsequent calculations were made using the remaining 173 individuals from eleven sites (Table 1).

Linkage disequilibrium, null alleles and deviation from neutrality may have an impact on estimates of population differentiation (Luikart *et al.* 2003, Chapuis & Estoup 2007). We therefore tested the microsatellite loci for these three characteristics for each sampling area where at least ten individuals were successfully genotyped, before conducting the population genetic analyses. We used GENEPOP v. 4.3 (Raymond & Rousset 1995) to test for linkage disequilibrium, adjusting the significance levels with the Bonferroni correction to account for multiple testing in pairwise comparisons between loci. We assessed the proportion of null alleles in MICROCHECKER v. 2.2.3 (Van Oosterhout *et al.* 2004) with 0.95 confidence interval and 1000 bootstrap iterations. We then assessed the selective neutrality of unlinked markers that showed no significant null alleles using the software LOSITAN (Antao *et al.* 2008), which employs an F_{ST}-outlier approach to test for loci under selection. The confidence interval was set to 0.99 and we tested for conformance to neutrality under the stepwise mutation model with the options 'neutral mean F_{ST}' and 'force mean F_{ST}'.

Table 2. Characteristics of the eight microsatellite markers used to genotype Common Ringed Plovers. Genotyping success refers to samples included in the final data set. Loci marked with an asterisk were excluded from population genetic analyses due to the presence of null alleles. *A* refers to the number of alleles, and Length to allele length in base pairs.

Locus name	*A*	Length (bp)	Genotyping success	Reference
Calex7	15	137–167	99%	Küpper *et al.* (2007)
Calex10	3	210–214	100%	Küpper *et al.* (2007)
Calex17	4	190–196	100%	Küpper *et al.* (2007)
Calex20*	8	176–183	86%	Küpper *et al.* (2007)
Calex23	7	227–239	88%	Küpper *et al.* (2007)
Calex40*	21	124–237	90%	Küpper *et al.* (2007)
C201	18	154–192	98%	Funk *et al.* (2007)
C204	9	185–201	99%	Funk *et al.* (2007)

We used ARLEQUIN v. 3.5.2.2 (Excoffier *et al.* 2005) to compute indices of genetic variation within and among populations, including observed heterozygosity (H_O) and expected heterozygosity (H_E). We then evaluated population differentiation among breeding areas in several steps. To account for (1) expected variance in gene flow in pairwise comparisons that feature sites of the same or different subspecies, (2) variation in genetic diversity and (3) type of genetic markers employed, we calculated three pairwise indices of genetic variation: F_{ST}, R_{ST} and Jost's D_{ST} (henceforth D) between breeding areas. F_{ST}, a summary statistic introduced by Wright (1943), is the method most frequently used for assessing

population differentiation (Meirmans & Hedrick 2011) and we provide values for the widely used F_{ST}-estimator that is appropriate for multiallelic loci (Weir & Cockerham 1984). R_{ST} is an F_{ST}-analogue reflecting the mutational processes of microsatellite markers. For its calculation the assumption is made that marker variation is generated through a stepwise mutation model and hence R_{ST} values may provide more accurate differentiation estimates when markers adhere to the stepwise mutation process (Balloux & Lugon-Moulin 2002). However, F_{ST}-based estimates may perform better than R_{ST} when there are few loci and small sample sizes (Gaggiotti *et al.* 1999). *D* measures the actual differentiation in allele frequencies among populations (Jost 2008). Unlike F_{ST}, it does not decline with increasing marker polymorphism, as the maximum possible F_{ST}-value is determined by the amount of within-population diversity (Meirmans & Hedrick 2011). Pairwise F_{ST}- and R_{ST}-values were calculated using ARLEQUIN with 1000 random permutations. *D*-values were calculated using the DEMEtics package v. 0.8-7 (Gerlach *et al.* 2010) for R v. 3.2.2. (R Core Team 2015) with 1000 bootstrap resamplings to estimate *P*-values. All F_{ST}-, R_{ST}- and *D*-values were tested at a Bonferroni adjusted α threshold of 0.05 to account for multiple testing.

Differentiation among all breeding areas was also assessed through an analysis of molecular variance (AMOVA) implemented in ARLEQUIN. We assessed to what extent molecular variance would be explained by variance among individuals, breeding areas or subspecies in two models: (1) across all breeding areas and (2) while grouping the areas into the three traditional subspecies based on their geographic location.

We tested for isolation-by-distance by performing Mantel-tests. Pairwise geographic distances between breeding areas were calculated based on geographic coordinates. As a measure of genetic distance, we calculated pairwise F_{ST}, R_{ST} and *D*. Mantel-tests were calculated using the ecodist package v. 1.2.9 (Goslee & Urban 2007) in R and statistical significance was assessed using 10,000 random permutations.

We used the Bayesian clustering software STRUCTURE v. 2.3.4 (Pritchard *et al.* 2000) to determine population structure and the number of genetic clusters *K* in our sample set. We ran two sets of models: (1) without location prior and (2) with location prior grouping samples within a distance of 500 km, merging VAR and LAP as well as SEC, ECC and NEC, resulting in eight regions with specific location priors. Using the location prior has been shown to identify meaningful structure when the amount of available genetic data (samples or markers) is low (Hubisz *et al.* 2009). We used the admixture model with correlated allele frequencies (Falush *et al.* 2003). We tested models from $K = 1$ to $K = 11$ (the number of breeding areas). For each model, we ran ten replicates with a burn-in period of 100,000 followed by 1 million generations. We combined the ten replicates to assess summary statistics for each model, including assignment probabilities, log-likelihoods and delta *K* (Evanno *et al.* 2005), using STRUCTURE HARVESTER (Earl & vonHoldt 2012). Results of the ten runs for each *K* were summarised using CLUMPP (Jakobsson & Rosenberg 2007) and visualised with DISTRUCT (Rosenberg 2004).We then identified the most plausible K from inspection of the plots and summary statistics of each model.

Table 3. Pairwise F_{ST}- (below diagonal) and R_{ST}-values (above diagonal) among Common Ringed Plover sampling areas. Abbreviations are given in Table 1. Bold values indicate significant ($P < 0.05$) differentiation after Bonferroni correction.

	WES	**HAL**	**LAP**	**TUR**	**VAR**	**VOR**	**TAY**	**NWC**	**SEC**	**ECC**	**NEC**
WES		**0.41**	–0.01	–0.03	0.10	0.13	–0.01	0.04	–0.02	0.01	–0.03
HAL	0.02		**0.28**	**0.40**	0.15	**0.29**	**0.39**	0.24	**0.35**	**0.40**	**0.34**
LAP	–0.02	0.01		–0.02	–0.01	0.03	–0.03	–0.08	0.00	–0.02	–0.04
TUR	–0.01	0.03	0.00		0.07	0.09	–0.03	–0.02	–0.02	–0.03	–0.04
VAR	0.01	0.03	–0.02	0.01		0.06	0.07	–0.04	0.08	0.08	0.03
VOR	0.02	0.05	0.02	0.01	0.05		0.10	–0.07	0.06	0.07	0.04
TAY	–0.01	0.04	–0.01	0.00	0.01	0.03		–0.01	–0.01	–0.03	–0.04
NWC	0.06	0.08	0.04	0.08	0.09	0.13	0.05		–0.07	–0.06	–0.09
SEC	0.00	0.02	–0.01	0.01	0.02	0.02	0.01	0.06		–0.02	–0.02
ECC	0.02	0.02	0.00	0.00	0.01	0.04	0.00	0.07	0.00		–0.04
NEC	0.00	0.03	0.01	0.01	0.04	0.00	0.01	0.06	–0.01	0.03	

RESULTS

All pairwise tests for linkage disequilibrium were non-significant after Bonferroni correction. Loci Calex20 and Calex40 showed a significant proportion of null alleles in at least three breeding areas and consequently both loci were excluded from further analyses. The remaining six loci appeared to be selectively neutral as selection tests were not significant when accounting for multiple testing.

The final six loci had a mean of 9.3 alleles per locus ranging from three at Calex10 to 18 alleles at locus C201 (Table 2). H_E showed little variation between sites, ranging from 0.66 in ECC to 0.72 in NEC, whereas we observed more variation at H_O, ranging from 0.47 in LAP to 0.73 in NEC, TAY and TUR (Table 1).

Pairwise comparisons of F_{ST}- and D-values among breeding areas revealed no significant genetic differentiation after Bonferroni correction (F_{ST}: mean = 0.02, range: –0.02–0.13; D: mean = 0.06, range: –0.12–0.16; Table 3, Table S1). R_{ST}-values were generally higher (mean = 0.06, range: –0.09–0.40) and we detected eight significant comparisons, all between HAL in southwestern Sweden and other breeding areas (Table 3). In fact, for R_{ST} the ten highest pairwise comparisons all involved HAL whereas there was no clear pattern in F_{ST} or D.

The AMOVAs showed differences in attributing genetic variation to the different hierarchical levels. For the first comparison, with breeding areas as the highest hierarchical level, the R_{ST} ($P < 0.001$) but not F_{ST} model ($P = 0.42$) attributed significant genetic variation to differences among breeding areas. The second set of models, with a priori grouping of the sites according to the three putative subspecies and geographic information, explained no significant amount of variation in F_{ST} ($P = 0.124$) or R_{ST} ($P = 0.238$; Table 4). We found no isolation by distance effect using F_{ST} ($r = 0.03$, $P = 0.40$), R_{ST} ($r = 0.13$, $P = 0.13$) or D ($r = 0.18$, $P = 0.07$).

The results of the Bayesian clustering differed according to whether a location prior was specified or not. Without location prior the best model was $K = 1$, suggesting no population differentiation across sites. With the help of the location prior we detected further plausible genetic structure. Comparison of log-likelihoods, the Evanno method and the graphical output suggested that the best model was $K = 3$ (Figure 2). Ringed Plovers breeding in Iceland (WES), that represented the subspecies *psammodromus*, formed one cluster, plovers from HAL, representing subspecies *hiaticula*, formed the second cluster and plovers from Russia (VOR, TAY, NWC, SEC, ECC, NEC), that belong to the subspecies *tundrae*, formed the third. There was, however, no support for the *kolymensis* subspecies. Interestingly, *tundrae* individuals from LAP, VAR and *hiaticula* plovers from TUR were not clearly assigned to any cluster. Instead, consistently across all ten individual runs, proportions of their genotypes were assigned to all three clusters, but the sites did not form an additional fourth cluster (see bottom panel of Figure 2). Interestingly, the genetic profiles of putative *tundrae* plovers from Lapland and Varanger appeared more similar to the *hiaticula* cluster, whereas the putative *hiaticula* individuals from TUR were more related to the *tundrae* cluster (Figure 2).

DISCUSSION

Our genetic evaluation of subspecies delineation in the Ringed Plover based on microsatellite markers provided some support for the presence of subspecies *hiaticula*, *tundrae* and *psammodromus*, but no support for repro-

Table 4. Results of AMOVA-models for F_{ST} and R_{ST} in the Common Ringed Plover. The top hierarchical grouping for each model is given in the Model column.

		F_{ST}		R_{ST}	
Model	**Source of variation**	% var	*P*	% var	*P*
(1) breeding areas only	Among breeding areas	1.84	0.420	13.00	<0.001
	Among individuals	3.81	0.016	6.99	0.060
	Within individuals	94.35	<0.001	80.01	<0.001
(2) subspecies	Among subspecies	0.62	0.124	5.83	0.238
	Among breeding areas	1.41	0.009	8.78	<0.001
	Among individuals	3.81	0.014	6.86	0.056
	Within individuals	94.16	<0.001	78.52	<0.001

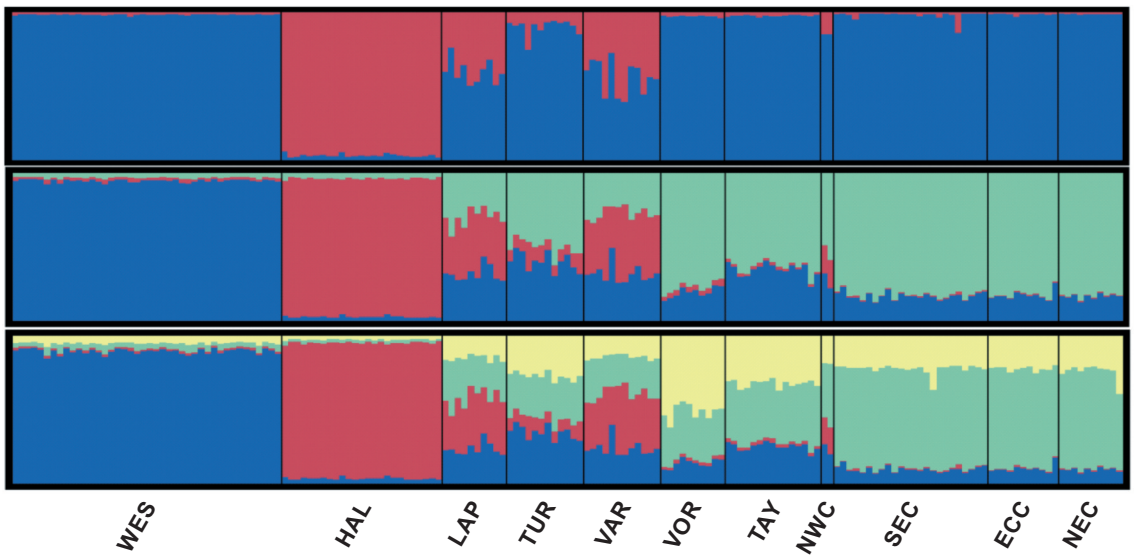


Figure 2. Results of the Bayesian clustering analysis conducted in STRUCTURE for Common Ringed Plovers grouped by breeding areas. Results are displayed as averages of ten runs for models $K = 2$ (top), $K = 3$ (middle) and $K = 4$ (bottom). Model $K = 3$ has highest biological plausibility, clustering the samples according to subspecies and indicating an admixture zone spanning from Northern Scandinavia (LAP and VAR) to Belarus (TUR). There was no support for a divergence of the subspecies kolymensis (NWC, SEC, EEC and NEC).

ductive isolation of *kolymensis* Ringed Plovers. The Bayesian clustering analyses showed a reasonable match between geographic and genetic data, corroborating the three established subspecies, although the AMOVA results for subspecies delineation were not significant. As *kolymensis* Ringed Plovers are geographically separated and show distinct morphological features from *C. h. tundrae*, the observed lack of genetic differentiation among breeding sites in Siberia implies a mismatch between genetic and phenotypic data across the distribution range of this species. Such mismatches are commonly reported in other waders that breed at high latitudes. In Dunlins *Calidris alpina* and Purple Sandpipers *Calidris maritima*, two sandpipers that breed at similar latitudes as Ringed Plovers, subspecies delineation based on phenotypic characters is poorly supported by genetic markers (Marthinsen *et al.* 2007, Barisas *et al.* 2015, LeBlanc *et al.* 2017). In contrast, in several temperate or tropic waders, subspecies delineation is in agreement with patterns of genetic differentiation (Ottvall *et al.* 2005, Funk *et al.* 2007, Höglund *et al.* 2009, Küpper *et al.* 2009, Miller *et al.* 2010, but see Rheindt *et al.* 2011, dos Remedios *et al.* 2017).

Why do mismatches between phenotypic and genetic characters occur more frequently in high latitude species than in low latitude species? Historic climate oscillations had particularly strong impacts on habitats at higher latitudes, including those the Ringed Plover occupies today. These changes are thought to have resulted in repeated expansions and contractions of species ranges. Current within-species patterns of genetic differentiation in boreal and arctic species are thought to have been shaped by Pleistocene climate cycles in particular (Avise & Walker 1998, Lovette 2005). Due to contrasting habitat demands, historic population changes are likely to exhibit opposing patterns for boreal/temperate and arctic species (Kraaijeveld & Nieboer 2000). Advancing ice sheets during glaciation periods forced populations of boreal/temperate species into reproductively isolated refugia where they started to diverge in isolation. In contrast, glaciation periods were likely a time of population range shifts and often expansions for subarctic/ arctic breeders such as the Ringed Plover. Although northern breeding areas were lost to the expanding ice, suitable habitat became available in southern areas leading to population expansions southwards. In warm periods, vegetation growth created environmental barriers and shifted available habitat further north meaning that populations became restricted to higher latitudes or isolated mountain areas where they diverged through isolation by environment (Kraaijeveld & Nieboer 2000).

Based on our results, we tentatively suggest that in Ringed Plovers, the isolation period may have first led to differentiation between *tundrae* and *hiaticula* subspecies in allopatry during the last glaciation (the

Weichselian glacial). During this period, ice sheet coverage was very heterogeneous and varied regionally (Kraaijeveld & Nieboer 2000, Svendsen *et al.* 2004). Coverage was greatest in central Siberia 80,000 to 90,000 years ago (Svendsen *et al.* 2004) whereas northern Europe was fully ice-covered only during the last glacial maximum (18,000 to 20,000 years ago). Remarkably, during this late glacial period nearly all of Siberia was ice free (Tarasov *et al.* 2000, Svendsen *et al.* 2004), presumably providing ample breeding habitat for Ringed Plovers. While *hiaticula* may have found refuge in western Europe on ice free parts of the British Isles or in the North Sea area, *tundrae* was likely restricted to the ice free parts of Siberia. In eastern Europe, boreal forests almost reached the ice sheets (Kraaijeveld & Nieboer 2000). Ice sheets and forests hence would have provided a geographic barrier that separated *hiaticula* and *tundrae* plovers.

The current breeding range of *psammodromus* was fully covered by a large ice sheet during the last glacial maximum (Patton *et al.* 2016), meaning that the Iceland colonisation by Ringed Plovers must have occurred after the ice retreated. Pairwise R_{ST}-values and hierarchical Bayesian analysis indicate that the Icelandic Ringed Plovers (ssp. *psammodromus*) are more closely related to *tundrae* than to *hiaticula*, suggesting that *tundrae* ancestors colonised Iceland despite the larger distance (c. 800 km vs. c. 1100 km to the closest breeding areas of *hiaticula* and *tundrae*, respectively). Alternatively, the subspecies *psammodromus* could have first diverged during an interglacial period from *hiaticula* and then colonised Siberia through leap-frog dispersal. Given that Greenland and Eastern Canada were covered by ice sheets, both refugia would have been located nearby in Northwest Europe. We consider this scenario unlikely since populations would likely have had secondary contact before differentiation took place.

After the retreat of the ice sheet, population expansions led to secondary contact between the subspecies. The hierarchical Bayesian analysis conducted in STRUCTURE suggests that the secondary contact zone runs through Northern Scandinavia and Eastern Europe; Ringed Plovers at three locations (LAP, VAR and TUR) show an admixture profile, indicating that these sites are part of an intergradation zone between the three subspecies (Figure 2). Currently, the three populations are either classified as *tundrae* (LAP and VAR) or *hiaticula* (TUR) based on phenotypic characters (Pinchuk *et al.* 2016). For the Scandinavian sites LAP and VAR this supports observations of intermediate phenotypic characters in these populations (Haftorn 1971, Engelmoer & Roselaar 1998). We note that the Belarusian breeders (TUR) show a higher genetic similarity to the Siberian Ringed Plovers sampled at VOR and TAY than to the other *hiaticula* population sampled at HAL. Indeed, the pairwise comparisons HAL vs. TUR were moderate (F_{ST}, D) to high (R_{ST}; Table 4, Table S1) suggesting substantial differentiation. Based on the clustering results we hypothesize that the admixture zone runs further to the west, e.g. through Poland or the Eastern Baltic countries Lithuania, Estonia or Latvia.

Given the lack of genetic differentiation among the Far East Siberian plovers, we suggest that the disjunct distribution of Ringed plovers in Siberia is the result of recent colonisation and expansion (Lappo *et al.* 2012). The gap in their distribution between 145°E and 160°E could be either the result of leap migration over unsuitable breeding habitat, or due to the past extinction of small population segments in this area. Interestingly, plovers recently started to close this gap in the breeding distribution using artificial gravel or sand mounds created by humans near villages, suggesting that the colonisation process is still ongoing (Lappo *et al.* 2012). The current migration routes of Ringed Plovers breeding in Chukotka support the idea of their post-glacial eastward expansion: Chukotka Ringed Plovers have been found to winter in Africa and the Middle East, but during both spring and autumn migration, they migrate through Siberia following the West Asian – East African Flyway (Tomkovich *et al.* 2017).

The pattern of genetic differentiation is similar to the one observed in other Palearctic temperate- and arctic-breeding wader species (e.g. Ottvall *et al.* 2005, Marthinsen *et al.* 2007, Höglund *et al.* 2009, Rönkä *et al.* 2012, Verkuil *et al.* 2012, Conklin *et al.* 2016). Interestingly, the geographic ranges of genetic lineages or subspecies of waders tend to be highly variable, suggesting that different species used different refugia or displayed different recolonisation dynamics. As in other plover species (D'Urban Jackson *et al.* 2017), we observed low genetic differentiation over large geographic distances in the Ringed Plover. Previous work demonstrated that breeding dispersal, which is closely linked to mating system in many waders, is related to the degree of genetic differentiation. Polygamous wader species often show remarkable long-distance breeding dispersal within a season (Kempenaers & Valcu 2017) and have lower genetic differentiation than monogamous species (D'Urban Jackson *et al.* 2017). Based on its monogamous mating system (Wallander *et al.* 2001), we expected the Ringed Plover to exhibit moderate to high genetic structure and a pattern of isolation by distance. In contrast to these

predictions, we observed a complete lack of genetic differentiation within the *tundrae* subspecies, for which many samples from a large geographic distribution were analysed. Ringed Plovers have low natal philopatry, associated with high juvenile dispersal (Laven 1940, Pienkowski 1984, Foppen *et al.* 2006). Such high juvenile dispersal will result in high gene flow and prevent population divergence since as little as one migrant per generation is commonly assumed to prevent populations from diverging (Mills & Allendorf 1996). Recent studies have also pointed to individual variation in migratory behaviour within subspecies with variation in routes, periods and selection of stop over sites, leading to shorter 'hops' and longer 'jumps' even for individuals from the same populations (Hedh & Hedenström 2016, Lislevand *et al.* 2017, Tomkovich *et al.* 2017). It is not known how individual migration schedules develop but it is plausible that juveniles from different natal sites mix in the wintering areas and then simply follow adults from their winter group to their breeding grounds.

The genetic differentiation of Icelandic Ringed Plovers from continental populations is in line with observed patterns among island populations of many other bird species (Gill 2014). The ocean seems particularly effective in preventing or inhibiting gene flow even for proficient dispersers such as waders that migrate annually over large water bodies. In plovers, populations across island archipelagos are often genetically differentiated and this may eventually lead to allopatric speciation through isolation by environment (Küpper & dos Remedios in press). Isolation in allopatry, on continents or islands separated by water, has led to the emergence of closely related sister species such as the Kentish Plover *Charadrius alexandrinus* superspecies complex (Rheindt *et al.* 2011, Almalki *et al.* 2017).

In this study, we used eight polymorphic microsatellites originally developed for other plover species (Funk *et al.* 2007, Küpper *et al.* 2007). Two of these markers showed a high frequency of potential null alleles. There is always a trade-off between minimizing the errors that arise from non-conforming markers by keeping loci, and reducing statistical power by reducing the number of loci (Selkoe & Toonen 2006). However, a clustering analysis including the presumed null allele markers lead to the same qualitative results in regards of number of genetic clusters and admixture zone (Figure S1). We did not find any support for isolation-by-distance patterns either across the species range or within subspecies (own unpubl. data). This seems at first remarkable, given that we sampled across large geographic distances. We note that estimates of genetic differentiation based on R_{ST} were generally higher than those based on F_{ST}. This may be explained by a better fit of the stepwise mutation model for the employed microsatellite markers (Slatkin 1995, Balloux & Lugon-Moulin 2002). Alternatively, the low pairwise F_{ST}-values may reflect the high amount of within-population diversity found in Ringed Plover populations (Meirmans & Hedrick 2011). However, this may also be a consequence of the generally low to moderate genetic differentiation observed, and could change if more markers were employed and more sites added in future studies. Additional markers would also be needed to robustly test our outlined phylogeographic hypothesis on the impact of glaciation on subspecies differentiation.

In conclusion, we found genetic support for the three currently recognised subspecies of the Ringed Plover based on microsatellite markers. An admixture zone of all three subspecies runs through Northern Scandinavia and Eastern Europe. The existence of a fourth suggested subspecies, with different phenotypic traits, breeding in Chukotka was not supported by the genetic data. We suggest that the current three subspecies arose only during and after the latest glaciation event. The apparent mismatch between genetic and phenotypic characters in the Ringed Plover may be explained by its evolutionary history, which most likely included repeated population expansions and contractions. Future studies with DNA sequence markers and further sampling sites are needed to shed light on the timing of population differentiation, the exact location and dynamics of the admixture zone and may also clarify the colonisation history of this species.

ACKNOWLEDGEMENTS

Alexei Dondua, Egor Loktionov, Yulia Karagicheva, Vladimir Morozov, Viktor Golovnyuk, Angela Pauliny, Mikael Larsson, Uno Unger, Natalia Karlionova, Eugenia Luchik and Ivan Bogdanovich helped with collection of samples. Mihai Valcu kindly provided an updated map of the breeding distribution and the R-code to draw Figure 2. We thank Roos Kentie and two anonymous reviewers for constructive comments on an earlier version of this manuscript. Fieldwork was funded through Elis Wides foundation (to JD) and BirdsRussia (to PT). PT was supported by the Russian Science Foundation project no. 14-50-00029. DB was supported by the Swedish research council Formas (grants no. 215-2009-463 and 215-2013-752). Microsatellite genotyping was funded and conducted at the NERC Biomolecular Analysis Facility, University of Sheffield. We thank Terry Burke and Tamás Székely for initial discussion of the study. CK was supported by the Max Planck Society.

REFERENCES

Almalki M., Kupán K., Carmona-Isunza M.C., López P., Veiga A., Kosztolányi A., Székely T. & Küpper C. 2017. Morphological and genetic differentiation among Kentish Plover *Charadrius alexandrinus* populations in Macaronesia. Ardeola 64: 3–16.

Antao T., Lopes A., Lopes R.J., Beja-Pereira A. & Luikart G. 2008. LOSITAN: A workbench to detect molecular adaptation based on a F_{ST}-outlier method. BMC Bioinformatics 9: 323.

Avise J.C. (ed.) 2000. Phylogeography: The history and formation of species. Harvard University Press, Cambridge.

Avise J.C. & Walker D.E. 1998. Pleistocene phylogeographic effects on avian populations and the speciation process. Proc. R. Soc. Lond. B. 265: 457–463.

Balloux F. & Lugon-Moulin N. 2002. The estimation of population differentiation with microsatellite markers. Mol. Ecol. 11: 155–165.

Barisas D.A., Amouret J., Hallgrímsson G.T., Summers R.W. & Pálsson S. 2015. A review of the subspecies status of the Icelandic Purple Sandpiper *Calidris maritima littoralis*. Zool. J. Linnean Soc. 175: 211–221.

Blomqvist D., Pauliny A., Larsson M. & Flodin L.-Å. 2010. Trapped in the extinction vortex? Strong genetic effects in a declining vertebrate population. BMC Evol. Biol. 10: 33.

Bohonak A.J. 1999. Dispersal, gene flow, and population structure. Q. Rev. Biol. 74: 21–45.

Chapuis M.-P. & Estoup A. 2007. Microsatellite null alleles and estimation of population differentiation. Mol. Biol. Evol. 24: 621–631.

Conklin J.R., Reneerkens J., Verkuil Y.I., Tomkovich P.S., Palsbøll P.J. & Piersma T. 2016. Low genetic differentiation between Greenlandic and Siberian Sanderling populations implies a different phylogeographic history than found in Red Knots. J. Ornithol. 157: 325–332.

Coyne J.A. & Orr H.A. (eds) 2004. Speciation. Sinauer, Sunderland, Massachusetts.

D'Urban Jackson J., dos Remedios N., Maher K.H., Zefania S., Haig S., Oyler-McCance S., Blomqvist D., Burke T., Bruford M.W., Székely T. & Küpper C. 2017. Polygamy slows down population divergence in shorebirds. Evolution 71: 1313–1326.

Dawson D.A., Horsburgh G.J., Küpper C., Stewart I.R.K., Ball A.D., Durrant K.L., Hansson B., Bacon I., Bird S., Klein A., Krupa A.P., Lee J.W., Martín-Gálvez D., Simeoni M., Smith G., Spurgin L.G. & Burke T. 2010. New methods to identify conserved microsatellite loci and develop primer sets of high cross-species utility – as demonstrated for birds. Mol. Ecol. Res. 10: 475–494.

Delany S., Scott D.A. & Helmink A.T.F. (eds) 2006. Waterbird population estimates - fourth edition. Wetlands International, Wageningen, The Netherlands.

Delany S., Scott D., Dodman T. & Stroud D.A. (eds) 2009. An atlas of wader populations in Africa and Western Eurasia. Wetlands International and International Wader Study Group, Wageningen, The Netherlands.

dos Remedios N., Lee P.L., Burke T., Székely T. & Küpper C. 2015. North or south? Phylogenetic and biogeographic origins of a globally distributed avian clade. Mol. Phylogenet. Evol. 89: 151–159.

dos Remedios N., Küpper C., Székely T., Baker N., Versfeld W. & Lee P.L. 2017. Genetic isolation in an endemic African habitat specialist. Ibis 159: 792–802.

Earl D.A. & vonHoldt B.M. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Res. 4: 359–361.

Engelmoer M. & Roselaar C.S. (eds) 1998. Geographical variation in waders. Kluwer, Dordrecht, The Netherlands.

Estoup A., Solignac M., Cornuet J.M., Goudet J. & Scholls A. 1996. Genetic differentiation of continental and island populations of *Bombus terrestris* (Hymenoptera: Apidae) in Europe. Mol. Ecol. 5: 19–31.

Evanno G., Regnaut C. & Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14: 2611–2620.

Excoffier L., Laval G. & Schneider S. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evol. Bioinform. Online 1: 47–50.

Falush D., Stephens M. & Pritchard J.K. 2003. Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. Genetics 164: 1567–1587.

Foppen R.P.B., Majoor F.A., Willems F.J., Meininger P.L., Houwelingen G.C.V. & Wolf P.A. 2006. Survival and emigration rates in Kentish *Charadrius alexandrinus* and Ringed Plovers *Ch. hiaticula* in the Delta area, SW-Netherlands. Ardea 94: 159–173.

Funk W.C., Mullins T.D. & Haig S.M. 2007. Conservation genetics of snowy plovers (*Charadrius alexandrinus*) in the Western Hemisphere: population genetic structure and delineation of subspecies. Conserv. Genet. 8: 1287–1309.

Gaggiotti O., Lange O., Rassmann K. & Gliddon C. 1999. A comparison of two indirect methods for estimating average levels of gene flow using microsatellite data. Mol. Ecol. 8: 1513–1520.

Gerlach G., Jueterbock A., Kraemer P., Deppermann J. & Harmand P. 2010. Calculations of population differentiation based on G_{ST} and D: forget G_{ST} but not all of statistics! Mol. Ecol. 19: 3845–3852.

Gill F.B. 2014. Species taxonomy of birds: Which null hypothesis? Auk 131: 150–161.

Goslee S.C. & Urban D.L. 2007. The ecodist package for dissimilarity-based analysis of ecological data. J. Stat. Softw. 22: 1–19.

Greenwood P.J. 1980. Mating systems, philopatry and dispersal in birds and mammals. Anim. Behav. 28: 1140–1162.

Greenwood P.J. & Harvey P.H. 1982. The natal and breeding dispersal of birds. Ann. Rev. Ecol. Syst. 13: 1–21.

Haftorn S. (ed.) 1971. Norges fugler. Oslo.

Haig S.M. & D'Elia J. 2010. Avian subspecies and the US endangered species act. Ornithol. Monogr. 67: 24.

Haig S.M., Bronaugh W.M., Crowhurst R.S., D'Elia J., Eagles-Smith C.A., Epps C.W., Knaus B., Miller M.P., Moses M.L. & Oyler-McCance S. 2011. Genetic applications in avian conservation. Auk 128: 205–229.

Harvey M.G., Bonter D.N., Stenzler L.M. & Lovette I.J. 2006. A comparison of plucked feathers versus blood samples as DNA sources for molecular sexing. J. Field Ornithol. 77: 136–140.

Hedh L. & Hedenström A. 2016. Autumn migration strategies and trapping numbers in the Common Ringed Plover *Charadrius hiaticula* in southern Sweden. Ardea 104: 227–237.

Höglund J. (ed.) 2009. Evolutionary conservation genetics. Oxford University Press.

Höglund J., Johansson T., Beintema A. & Schekkerman H. 2009. Phylogeography of the Black-tailed Godwit *Limosa limosa*: substructuring revealed by mtDNA control region sequences. J. Ornithol. 150: 45–53.

Hossein B.-B., Mansour A., Martin I., Yanhua Q., Jamshid D., Tamás S., van Dijk R. & Ericson P. 2017. Phylogeny of penduline tits inferred from mitochondrial and microsatellite genotyping. J. Avian Biol. 48: 932–940.

Hubisz M.J., Falush D., Stephens M. & Pritchard J.K. 2009. Inferring weak population structure with the assistance of sample group information. Mol. Ecol. Res. 9: 1322–1332.

Jakobsson M. & 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.

Jehl J.R. 1968. Relationships in the Charadrii (shorebirds): A taxonomic study based on color patterns of the downy young. San Diego Society of Natural History Memoirs, no. 3.

Jost L. 2008. GST and its relatives do not measure differentiation. Mol. Ecol. 17: 4015–4026.

Kempenaers B. & Valcu M. 2017. Breeding site sampling across the Arctic by individual males of a polygynous shorebird. Nature 541: 528–531.

Kraaijeveld K. 2008. Non-breeding habitat preference affects ecological speciation in migratory waders. Naturwissenschaften 95: 347–354.

Kraaijeveld K. & Nieboer E.N. 2000. Late Quaternary paleogeography and evolution of arctic breeding waders. Ardea 88: 193–205.

Küpper C. & dos Remedios N. In press. Defining species and populations: molecular genetic studies in plovers. Stud. Avian Biol.

Küpper C., Horsburgh G.J., Dawson D.A., Ffrench-Constant R., Székely T. & Burke T. 2007. Characterization of 36 polymorphic microsatellite loci in the Kentish Plover (*Charadrius alexandrinus*) including two sex-linked loci and their amplification in four other *Charadrius* species. Mol. Ecol. Notes 7: 35–39.

Küpper C., Burke T., Székely T. & Dawson D.A. 2008. Enhanced cross-species utility of conserved microsatellite markers in shorebirds. BMC Genomics 9: 502.

Küpper C., Augustin J., Kosztolányi A., Figuerola J., Burke T. & Székely T. 2009. Kentish versus Snowy Plover: Phenotypic and genetic analyses of *Charadrius alexandrinus* reveal divergence of Eurasian and American subspecies. Auk 126: 839−852.

Küpper C., Edwards S.V., Kosztolányi A., AlRashidi M., Burke T., Herrmann P., Argüelles-Tico A., Amat J.A., Amezian M., Rocha A., Hötker H., Ivanov A., Chernicko J. & Székely T. 2012. High gene flow on a continental scale in the polyandrous Kentish Plover *Charadrius alexandrinus*. Mol. Ecol. 21: 5864–5879.

Lappo E.G., Tomkovich P.S. & Syroeckovskiy E.E. (eds) 2012. Atlas of breeding waders in the Russian Arctic. UF Ofsetnaya Pechat, Moscow, Russia.

Laven H. 1940. Beiträge zur Biologie des Sandregenpfeifers (*Charadrius hiaticula* L.). J. Ornithol. 88: 183–287.

LeBlanc N.M., Stewart D.T., Pálsson S., Elderkin M.F., Mittelhauser G., Mockford S., Paquet J., Robertson G.J., Summers R.W. & Tudor L. 2017. Population structure of Purple Sandpipers (*Calidris maritima*) as revealed by mitochondrial DNA and microsatellites. Ecol. Evol. 7: 3225–3242.

Lislevand T., Briedis M., Heggøy O. & Hahn S. 2017. Seasonal migration strategies of Common Ringed Plovers *Charadrius hiaticula*. Ibis 159: 225–229.

Lovette I.J. 2005. Glacial cycles and the tempo of avian speciation. Trends Ecol. Evol. 20: 57–59.

Luikart G., England P.R., Tallmon D., Jordan S. & Taberlet P. 2003. The power and promise of population genomics: from genotyping to genome typing. Nature Rev. Genet. 4: 981–994.

Marthinsen G., Wennerberg L. & Lifjeld J.T. 2007. Phylogeography and subspecies taxonomy of Dunlins (*Calidris alpina*) in western Palearctic analysed by DNA microsatellites and amplified fragment length polymorphism markers. Biol. J. Linnean Soc. 92: 713–726.

Mayr E. (ed.) 1942. Systematics and the origin of species from the viewpoint of a zoologist. Columbia University Press, New York.

Meirmans P.G. & Hedrick P.W. 2011. Assessing population structure: F_{ST} and related measures. Mol. Ecol. Res. 11: 5–18.

Meissner W. 2007. Different timing of autumn migration of two Ringed Plover *Charadrius hiaticula* subspecies through the southern Baltic revealed by biometric analysis. Ringing Migr. 23: 129–133.

Meissner W., Chylarecki P. & Skakuj M. 2010. Ageing and sexing the Ringed Plover *Charadrius hiaticula*. Wader Study Gr. Bull. 117: 99–102.

Miller M.P., Haig S.M., Gratto-Trevor C.L. & Mullins T.D. 2010. Subspecies status and population genetic structure in Piping Plover (*Charadrius melodus*). Auk 127: 57–71.

Mills L.S. & Allendorf F.W. 1996. The one-migrant-per-generation rule in conservation and management. Conserv. Biol. 10: 1509–1518.

Nicholls J.A., Double M.C., Rowell D.M. & Magrath R.D. 2000. The evolution of cooperative and pair breeding in thornbills *Acanthiza* (*Pardalotidae*). J. Avian Biol. 31: 165–176.

Ottvall R., Höglund J., Bensch S. & Larsson K. 2005. Population differentiation in the Redshank (*Tringa totanus*) as revealed by mitochondrial DNA and amplified fragment length polymorphism markers. Conserv. Genet. 6: 321–331.

Paradis E., Baillie S.R., Sutherland W.J. & Gregory R.D. 1998. Patterns of natal and breeding dispersal in birds. J. Anim. Ecol. 67: 518–536.

Patton H., Hubbard A., Andreassen K., Winsborrow M. & Stroeven A.P. 2016. The build-up, configuration, and dynamical sensitivity of the Eurasian ice-sheet complex to Late Weichselian climatic and oceanic forcing. Q. Sci. Rev. 153: 97–121.

Phillimore A.B. & Owens I.P. 2006. Are subspecies useful in evolutionary and conservation biology? Proc. R. Soc. Lond. B. 273: 1049–1053.

Pienkowski M.W. 1984. Behaviour of young Ringed Plovers *Charadrius hiaticula* and its relationship to growth and survival to reproductive age. Ibis 126: 133–155.

Piersma T. 1987. Hop, skip, or jump? Constraints on migration of arctic waders by feeding, fattening and flight speed. Limosa 60: 185–194. (In Dutch with English summary)

Piersma T. & Lindström Å. 2004. Migrating shorebirds as integrative sentinels of global environmental change. Ibis 146: 61–69.

Pinchuk P., Karlionova N. & Meissner W. 2016. Biometry indicates sexual differences in spring migration strategy in Ringed Plovers *Charadrius hiaticula tundrae* captured in the southern Belarus. N.W.J. Zool. 12: 319–324.

Pritchard J.K., Stephens M. & Donnelly P. 2000. Inference of population structure using multilocus genotype data. Genetics 155: 945–959.

R Core Team 2015. R: A Language and Environment for Statistical Computing. Foundation for Statistical Computing, Vienna, Austria (ISBN 3-900051-07-0). www.Rproject.org

Raymond M. & Rousset F. 1995. Genepop (version 1.2): Population genetics software for exact tests and ecumenism. J. Hered. 86: 248–249.

Reudink M.W., Kyle C.J., Nocera J.J., Oomen R.A., Green M.C. & Somers C.M. 2011. Panmixia on a continental scale in a widely distributed colonial waterbird. Biol. J. Linnean Soc. 102: 583–592.

Rheindt F.E., Székely T., Edwards S.V., Lee P.L.M., Burke T., Kennerley P.R., Bakewell D.N., AlRashidi M., Kosztolányi A., Weston M.A., Liu W., Lei W., Shigeta Y., Javed S., Zefania S. & Küpper C. 2011. Conflict between genetic and phenotypic differentiation: the evolutionary history of a 'lost and rediscovered' shorebird. PLoS One 6: e26995.

Rönkä N., Kvist A., Pakanen V.M., Rönkä A., Degtyaryev V., Tomkovich P.S., Tracy D. & Koivula K. 2012. Phylogeography of the Temminck's Stint (*Calidris temminckii*): historical vicariance but little present genetic structure in a regionally endangered Palearctic wader. Divers. Distrib. 18: 704–716.

Rosenberg N.A. 2004. Distruct: a program for the graphical display of population structure. Mol. Ecol. Notes 4: 137–138.

Salomonsen F. 1930. Bemerkungen über die geographische Variation von *Charadrius hiaticula* L. J. Ornithol. 78: 65–72.

Selkoe K.A. & Toonen R.J. 2006. Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. Ecol. Lett. 9: 615–629.

Seutin G., White B.N. & Boag P.T. 1991. Preservation of avian blood and tissue samples for DNA analyses. Can. J. Zool. 69: 82–90.

Slatkin M. 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics 139: 457–462.

Svendsen J.I., Alexanderson H., Astakhov V.I., Demidov I., Dowdeswell J.A., Funder S., Gataullin V., Henriksen M., Hjort C., Houmark-Nielsen M., Hubberten H.W., Ingólfsson Ó., Jakobsson M., Kjær K.H., Larsen E., Lokrantz H., Lunkka J.P., Lyså A., Mangerud J., Matiouchkov A., Murray A., Möller P., Niessen F., Nikolskaya O., Polyak L., Saarnisto M., Siegert C., Siegert M.J., Spielhagen R.F. & Stein R. 2004. Late Quaternary ice sheet history of northern Eurasia. Q. Sci. Rev. 23: 1229–1271.

Székely T., Kosztolányi A., Küpper C. & Thomas G.H. 2007. Sexual conflict over parental care: a case study of shorebirds. J. Ornith. 148: S211–S219.

Tarasov P.E., Volkova V.S., Webb T., Guiot J., Andreev A.A., Bezusko L.G., Bezusko T.V., Bykova G.V., Dorofeyuk N.I., Kvavadze E.V., Osipova I.M., Panova N.K. & Sevastyanov D.V. 2000. Last glacial maximum biomes reconstructed from pollen and plant macrofossil data from northern Eurasia. J. Biogeogr. 27: 609–620.

Taylor R.C. 1980. Migration of the Ringed Plover *Charadrius hiaticula*. Ornis Scand. 11: 30–42.

Thomas G.H., Székely T. & Reynolds J.D. 2007. Sexual conflict and the evolution of breeding systems in shorebirds. Adv. Study Behav. 37: 279–342.

Thorisson B., Eyjólfsson V., Gardarsson A., Albertsdóttir H.B. & Gunnarsson T.G. 2012. The non-breeding distribution of Icelandic Common Ringed Plovers. Wader Study Gr. Bull. 119: 97–101.

Tolley K.A., Chase B.M. & Forest F. 2008. Speciation and radiations track climate transitions since the Miocene Climatic Optimum: a case study of southern African chameleons. J. Biogeogr. 35: 1402–1414.

Tomkovich P.S., Porter R., Loktionov E.Y. & Syroechkovskiy E.E. 2017. Transcontinental pathways and seasonal movements of an Asian migrant, the Common Ringed Plover *Charadrius hiaticula tundrae*. Wader Study 124: 175–184.

Valcu M., Dale J. & Kempenaers B. 2012. rangeMapper: a platform for the study of macroecology of life-history traits. Global Ecol. Biogeogr. 21: 945–951.

Van Oosterhout C., Hutchinson W.F., Wills D.P. & Shipley P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecol. Resour. 4: 535–538.

Verkuil Y.I., Piersma T., Jukema J., Hooijmeijer J.C.E.W., Zwarts L. & Baker A.J. 2012. The interplay between habitat availability and population differentiation: a case study on genetic and morphological structure in an inland wader (Charadriiformes). Biol. J. Linnean Soc. 106: 641–656.

Wallander J. & Andersson M. 2003. Reproductive tactics of the Ringed Plover *Charadrius hiaticula*. J. Avian Biol. 34: 259–266.

Wallander J., Blomqvist D. & Lifjeld J.T. 2001. Genetic and social monogamy – Does it occur without mate guarding in the Ringed Plover? Ethology 107: 561–572.

Weir B.S. & Cockerham C.C. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38: 1358–1370.

Wiersma P., Kirwan G.M. & Boesman P. 2018. Common Ringed Plover (*Charadrius hiaticula*). In: del Hoyo J., Elliott A., Sargatal J., Christie D.A. & de Juana E. (eds) Handbook of the birds of the world alive. Lynx Edicions, Barcelona.

Winker K. 2010. Subspecies represent geographically partitioned variation, a gold mine of evolutionary biology, and a challenge for conservation. Ornithol. Monogr. 67: 6–23.

Woltmann S., Stouffer P.C., Bergeon Burns C.M., Woodrey M.S., Cashner M.F. & Taylor S.S. 2014. Population genetics of Seaside Sparrow (*Ammodramus maritimus*) subspecies along the Gulf of Mexico. PloS One 9: e112739.

Wright S. 1943. Isolation by distance. Genetics 28: 114–138.

SAMENVATTING

Het onderzoeken van patronen in genetische structuren in de context van geografische en fenotypische variatie is belangrijk om de evolutionaire processen te begrijpen die bij soortvorming zijn betrokken. We hebben de differentiatie van populaties en ondersoorten onderzocht in Bontbekplevieren *Charadrius hiaticula*, een steltloper die broedt in de arctische en gematigde streken van het noordoosten van Canada en van Eurazië (tot het Russische Verre Oosten toe). Er worden momenteel drie ondersoorten algemeen erkend (*hiaticula*, *tundrae* en *psammodromus*), terwijl een vierde ondersoort (*kolymensis*) is voorgesteld op basis van geografische isolatie en fenotypische verschillen. We hebben 173 monsters van elf broedlocaties van Bontbekplevieren (die alle de vier vermeende ondersoorten vertegenwoordigen) gegenotypeerd op acht polymorfe microsatelliet-loci om patronen in populaties en ondersoorten te analyseren. Bayesiaanse clustering onderscheidde drie genetische clusters, overeenkomend met de broedplaatsen van de drie momenteel erkende ondersoorten. Het bestaan van de ondersoort *kolymensis* werd niet ondersteund. We ontdekten ook de aanwezigheid van een voorheen onbekende hybridisatiezone die zich uitstrekt van Noord-Scandinavië tot Wit-Rusland. Differentiatie van de ondersoorten *tundrae* en *hiaticula* deden zich hoogstwaarschijnlijk voor in allopatrische populaties op het Euraziatische continent, gevolgd door populatiegroei die leidde tot kolonisatie van IJsland en Groenland. Het ontbreken van genetische differentiatie binnen de ondersoort *tundrae* komt overeen met de voortdurende uitbreiding van het leefgebied en de sterke uitwisseling van genen die door het trekgedrag in stand wordt gehouden. We bespreken het belang van historische klimaatveranderingen, trekgedrag en paringssysteem bij het vormgeven van het waargenomen patroon van genetische differentiatie.

Corresponding editor: Roos Kentie
Received 20 April 2018; accepted 24 July 2018

Supplimentary Material is available online
www.ardeajournal.nl/supplement/s106–163–176