Ornis Fennica 93: 146–158. 2016

Genetic assessment of the subspecies status of Eurasian Magpies (*Pica pica*) in Norway

Sang-im Lee, Woohjung Kim, Jae Chun Choe & Magne Husby*

S. Lee, Institute of Advance Machines and Design, Seoul National University, Seoul 151-742, Korea
S. Lee, W. Kim, Laboratory of Behavioral Ecology and Evolution, Seoul National University, Seoul 151-742, Korea
J.C. Choe, Laboratory of Behavior and Ecology, Interdisciplinary Program of EcoCreative, Ewha Woman's University, Seoul 120-750, Korea
*M. Husby, Section of Science, Nord University, 7600 Levanger, Norway. * Corresponding author's e-mail: magne.husby@nord.no*

Received 17 August 2015, accepted 6 April 2016

Based on phenotypes, two subspecies of Eurasian Magpies (*Pica pica*) are recognized in Norway, with nominate *P. p. pica* in southern Norway, and *P. p. fennorum* in northern Norway. In this study, we investigated whether there are genetically distinct groups of Magpies in Norway, which can be considered in the discussion of the subspecies status. We collected DNA from 61 Magpies from seven locations in Norway, and measured genetic diversity using two types of markers: mitochondrial DNA sequences and microsatellites. Genetic differentiation among the Magpies was extremely low. Most of the variance was within populations, and the population identity and the putative subspecies border did not explain the genetic variance among the samples. Although microsatellite markers indicated genetic differentiation, the pattern was not consistent with the geographic locations of the sampling sites. Mismatch analysis suggested that the Magpie populations in Norway were formed by rapid expansion. Our results suggest that all the Magpies in Norway have originated from the same refugia after the last glaciation, their colonization in Norway happened quickly, and that the subspecies status of Magpies in Norway needs to be reconsidered.

1. Introduction

There are three different species of Magpies in the world. The Black-billed Magpie (*Pica hudsonia*) and the Yellow-billed Magpie (*P. nutalli*) live in North America, and the Eurasian Magpie (*P. pica*) lives in the old world (Sibley & Ahlquist 1990). The distribution of Eurasian Magpie is continuous in large parts of Europe and Asia, except for several isolated populations in north-west Africa, Arabian Peninsula and Kamchatka (Birkhead 1991). Although classification at subspecies level can be subjective, currently 11 subspecies of Magpies are recognized in the Eurasian continent (Birkhead 1991, Gill & Donsker 2016).

In many cases, subspecies are defined based on the morphology, probably because many subspecies were described before molecular methods became common in systematics studies (Haig & Winker 2010). The validity of subspecies has been controversial (e.g., Mayr 1982; Frost & Hillis 1990), but currently it is recognized as a discrete

taxonomic category below species that addresses the geographic component of variation and differentiation (Haig & Winker 2010). A recent proposal made by Haig & Winker (2010) finds a general consensus among researchers that a reexamination of subspecies status using modern methods is needed. Thus, although the definition of subspecies may not be necessarily based on the genetic data, the importance of genetic data on defining subspecies is currently well recognized.

Subspecies can be particularly useful when there is genetic differentiation that may lead to speciation. However, dispersal and gene flow among adjacent populations can prevent the establishment of subspecies, and genetic analysis can be used to estimate the amount of gene flow between different populations (Genovart *et al.* 2013). The temperate regions have periodically been covered by extensive ice sheets over the last two million years. Evidence that Magpies survived all these ice ages is found in fossils of a prehistoric Magpie species (*P. mourerae*) that was present on Mallorca, Balearic Islands (Western Mediterranean) 2.5 million years ago (Segui 2001). These expanding and retreating ice sheets can generate isolated populations that may further develop into separate subspecies (Burg *et al.* 2005, McCormack *et al*. 2008, Burg *et al.* 2014). Refugia during the ice ages and their roles in creating genetic variation is evident both in Europe (Hewitt 2004) and Asia (Li *et al.* 2009). Biological processes during and between the ice ages may be the reason why there are so many subspecies of Magpies in Eurasia.

The Magpies in Norway are very sedentary all year round (Collett 1921, Husby 2006), as they are in most of their range (Birkhead 1991). Although it has been reported that some Magpies migrate south in harsh winters (Stegmann 1927, Flint & Stewart 1983), this has not been observed in Norway and Magpies are rare or not observed on islands, even on islands near the mainland (Baines & Anker-Nilssen 1991, Pennington *et al.* 2004, Tveit *et al.* 2004, Williams 2007). Therefore, Magpie populations isolated by fiords or mountains may diverge, and it has been assumed that two subspecies of Magpies exist in Norway, the nominate *P. p. pica* in the south and *P. p. fennorum* in the north (Collett 1907, Lönnberg 1927). However, classification of magpies at the subspecies level is difficult because of their clinal variation (Snow & Perrins 1998). Earlier classification of subspecies was based on morphological differences. *P. p. fennorum* is slightly larger, has more white on the wings and its tail exhibits more bronze and less green on the tail than *P. p. pica* (Coombs 1978). However, clinal variation towards larger size and more white on the wings from south-west to north-east is also recognized (Coombs 1978). von Zedlitz (1925) concluded that the subspecies *P. p. pica* is distributed throughout Sweden, but that there were some mixtures of the subspecies *P. p. pica* and *P. p. germanica* (from central Europe) in southern Sweden based on the variation in grey and white on the rump. Later, Lönnberg examined 101 Magpies from different parts of Sweden, five Magpies from Norway and 55 from Finland, and found that wing length gradually increased northwards (Lönnberg 1927). However, a similar gradient in tail length was not evident. Rump color did not vary geographically in a systematic way either, but was whiter in older birds than in young birds. The characteristic difference in the size of the black tip on the primary feathers between young and old Magpies (which can be used to age the birds; Stegmann 1927, Erpino 1968, Lee *et al*. 2007) did not vary with latitude. Based mainly on the gradual increase in wing length northwards, Lönnberg concluded that there were two subspecies: *P. p. pica* in southern Sweden and *P. p. fennorum* in northern Sweden, with a mixture in between. Although it was argued 45 years ago that further investigations were needed to establish the range of the two subspecies in Norway (Haftorn 1971), no such clarification has been made. Previous studies of the genetic divergence among the subspecies of Eurasian Magpies found an east–west split, but within east and west clades no genetic differentiation was found (Zink *et al.* 1995, Lee *et al.* 2003, Kryukov *et al*. 2004, Haring *et al*. 2007, Zhang *et al.* 2012). These studies included the nominate subspecies *pica* but not *fennorum*.

In this study, we attempt to assess genetic differentiation among the Magpies in Norway; specifically we aim to discern whether there are genetically distinct groups of Magpies, which can be used to determine their subspecies status in Norway. Mitochondrial DNA is an important marker used to detect historical patterns (Pulgarin-R & Burg 2012), and has been successfully used in the classification of species and subspecies of Mag-

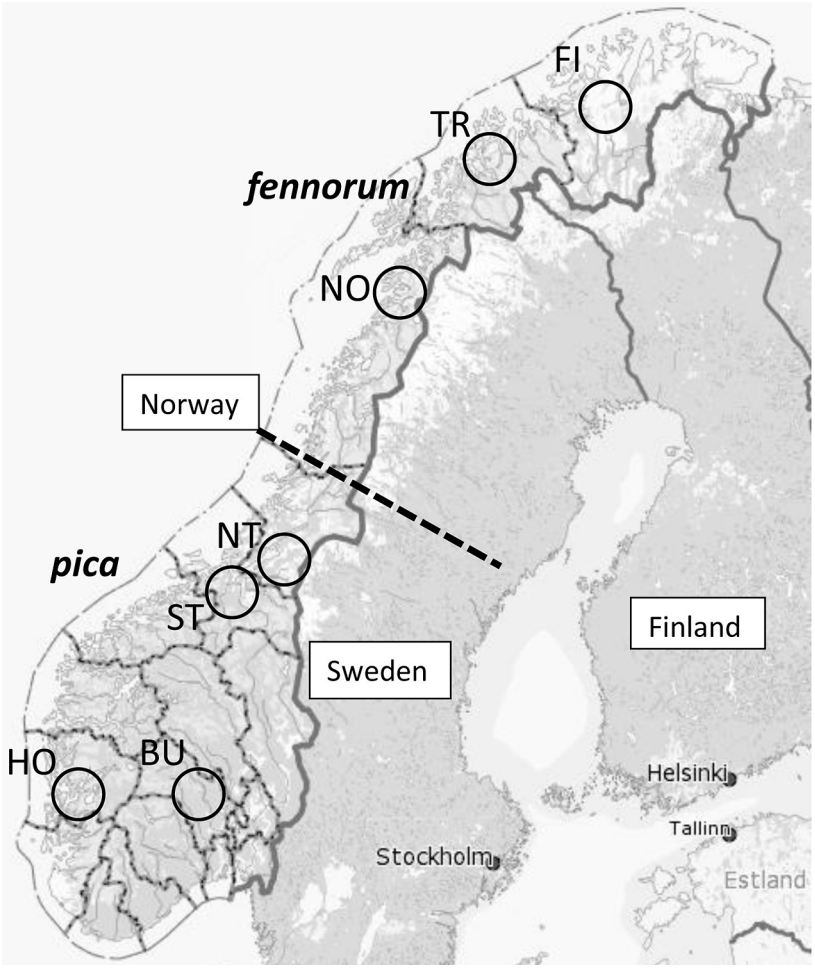

Fig. 1. Location of the sampling sites: Hordaland (HO), Buskerud (BU), Sør-Trøndelag (ST), Nord-Trøndelag (NT), Nordland (NO), Troms (TR) and Finnmark (FI). The subspecies border between *pica* and *fennorum* in Norway suggested by Lönnberg (1927) is marked with a dotted line.

pies globally (Zink *et al*. 1995, Lee *et al.* 2003, Kryukov *et al*. 2004, Haring *et al*. 2007, Zhang *et al.* 2012). However, when the divergence among the populations is small and the range expansion was fast, mitochondrial DNA might not provide sufficient resolution to detect genealogical relationships among the populations. Thus, we analyzed two genetic markers, mitochondrial DNA and microsatellites, to investigate the population divergence in Norway.

2. Material and methods

2.1. Sample collection

We collected Magpies from seven different parts of Norway as described in Fig. 1. 35 birds were collected from four areas south of the border between the subspecies (Lönnberg 1927), and 26 birds were collected from three areas north of the border. Of the 61 birds, 58 were shot in gardens or on rubbish dumps, and three were killed by collisions with cars. Nine hunters delivered the Magpies that were still frozen upon arrival. The distribution of the collected Magpies is not random, but rather adjusted so that both of the possible subspecies are represented in the dataset.

2.2. Genetic analyses

We obtained tissue from liver and pectoral muscle and extracted DNA from the tissue using QIAamp DNA Minikit (QIAGEN) following the protocol provided by the manufacturer.

For mitochondrial DNA, we amplified the D-

Table 1. Information of the microsatellite markers used in this study. No. means number of alleles.

Marker	Primer sequence	Size	No.	Source
Ase18	F: ATCCAGTCTTCGCAAAAGCC R: TGCCCCAGAGGGAAGAAG	223–272	23	Richardson *et al.* 2000
Ppi2	F: CACAGACCATTCGAAGCAGA R: GCTCCGATGGTGAATGAAGT	257–293	18	Martínez *et al.* 1999
Ppi3	F: CCAAACACAAGTACAGCTGCA R: TTTTGCTGGGAGAGGACG	222–272	21	Martínez *et al.* 1999
Ppi016	F: CCAAACACAAGTACAGCTGCA R: TTTTGCTGGGAGAGGACG	229–255	13	Martín-Gálvez *et al.* 2009
Ppi017	F: AAAGCTTTCTGGAGAACAGTGC R: CGTTGCATCTATGAGAGCTGAG	216–234	10	Martín-Gálvez *et al.* 2009
Tgu05	F: GATTGTTCGAGTGCTCTCAATG R: TGGATTTATGCACTTCCAAGC	264–284	9	Martín-Gálvez *et al.* 2009
Tgu06	F: CGAGTAGCGTATTTGTAGCGA R: AGGAGCGGTGATTGTTCAGT	192–204	6	Martín-Gálvez *et al.* 2009
Tgu07	F: CTTCCTGCTATAAGGCACAGG R: AAGTGATCACATTTATTTGAATAT	118–128	6	Martín-Gálvez *et al.* 2009
ApCo46	F: GCTGCCAGCACTCTGAATGTC R: GATTCAGCAAAATAGGGGTCAGAAG	250–252	2	Martín-Gálvez *et al.* 2009

loop region using two primer sets; HJ78 (5'-TCACGAGAACCGAGCTACT-3') and KOR03 (5'-ATGGGGTCAAAGTGCATCAGTG-3') for central domain; and KOR01 (5'-GGGGTCTCTT CAATAAGC-3') and H1248 (5'-CATCTTCA GTGTCATGCT-3'; Tarr 1995) for Domain II. The PCR mixture contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 2.5 mM MgCl2, 200 µM of each dNTP, 1 µM of each primer, 0.5 U Taq DNA polymerase (Biolabs, MA), and 20–250 ng genomic DNA. Total volume of PCR mixtures was adjusted to 50 µl. Thermal conditions for PCRs were as follows; 2 min at 92°C; 30 cycles of 90 sec at 92°C, 50 sec at 52°C (HJ78-KOR03 primers) or 47°C (KOR01-H1248 primers) and 60 sec at 72°C; 10 min at 72°C. We used PTC-100 Programmable Thermal Cycler (MJ Research) for PCR. PCR products were purified with a Gel Extraction Kit (Bioneer, Korea) after electrophoresis on a 2% agarose gel for 60 min at 10 V / cm. PCR amplicons were sequenced on an ABI 3730XL (NICEM, Seoul), aligned using ClustalX (Thompson *et al.* 1997) and edited using Bioedit version 5.0.5 (Hall 1999).

For microsatellites, we amplified nine microsatellite markers (Table 1) using a Multiplex PCR kit (QIAGEN). PCRs were performed using the following conditions: 5 µl of master mix, 1 µl of Q-solution, 0.14 µl each of IRDyes 700 and 800, 4 µl of primer mixture (2 mM for each primer), 5–60 ng of DNA template, and distilled water to adjust total volume to 11 µl. Thermal protocol included an initial Taq polymerase activation step of 15 min at 95°C; 24–30 cycle of 30 sec at 94°C, 90 sec at 48–54°C (depending on the marker), and 60 sec at 72°C, and final extension of 30 min at 60°C. Genotyping was conducted using SAGA-GT Automated Microsatellite Analysis Software (LI-COR, NE) running on LI-COR 4300 DNA analyser.

2.3. Examination of population structure

We aimed to examine whether the individuals from seven localities could be placed into two groups based on the putative subspecies border suggested by Lönnberg (1927). First, we sought phylogenetic trees using mitochondrial D-Loop sequences from 25 Norwegian and 3 Korean samples that were sequenced in this study and 24 sequences that were retrieved from Genbank (further information is given in the Appendix). Trees were constructed in MEGA 4.0 (Tamura *et al*. 2007) using a maximum composite likelihood model. For 25 Norwegian samples, a reduced-median haplotype network was constructed using NETWORK 4.6.1.4 (Bandelt *et al*. 1995).

Genetic diversity indices were calculated with

Table 2. Genetic diversity in the Norwegian Magpie samples (see Fig. 1 for locality). For mitochondrial DNA, number of haplotypes (Hap) and nucleotide diversity (π) are given. For microsatellite markers, average allelic richness (Rs), observed (Ho) and expected (He) heterozygosities are given.

Locality	mtDNA			Microsatellite loci			
	n	Hap	π	n	Rs	Ho	He
FI	3	1	0	10	2.812	0.633	0.738
TR	2	2	0.0048	6	2.819	0.630	0.769
NO	5	2	0.0064	10	2.764	0.695	0.759
NT	3	1	0	10	2.857	0.778	0.743
ST	3	3	0.0064	6	2.922	0.722	0.781
BU	5	1	0	6	2.948	0.759	0.755
HO	4	2	0.0016	5	3.070	0.778	0.806
Total	25	–	–	53	–	–	–

Arlequin 3.0 (Excoffier *et al.* 2005) for mitochondrial DNA and the expected and observed heterozygosity for microsatellite loci were calculated with Fstat 2.9.3.2 (Goudet 2002).

The distribution of genetic variation among the sampled localities, as well as within and among the inferred genetic groups was assessed by an analysis of molecular variance (AMOVA; Excoffier *et al*. 1992) using Arlequin 3.0. In addition, pairwise F_{ST} values were calculated with mtDNA sequences and microsatellite loci using Arlequin 3.0.

In order to identify clusters of genetically similar populations, we implemented a Bayesian model-based estimation using Structure version 2.3.4 (Pritchard *et al*. 2000). We examined the model by assuming admixture with correlated allele frequencies, because this assumption is more appropriate for individuals with admixed ancestries and for populations with similar expected frequencies (Falush *et al*. 2003). Twenty independent analyses were run for each value of K (number of clusters), from $K = 1$ to $K = 10$. Each analysis consisted of 1×10^6 Markov chains with a prior burn-in of 1×10^5. We used the method of Evanno *et al*. (2005) for determining the number of genetically homogeneous groups that best fit the data, by calculating $L(K)$ and ΔK.

We conducted neutrality tests to find any indication of recent population expansion by checking the deviations from selective neutrality using Fu's Fs (Fu 1997) calculated from Arlequin 3.0, and Fu & Li (1993)'s F^* and D^* statistics calculated from DnaSP 5.0 (Rozas *et al*. 2003). We also conducted mismatch analysis using DnaSP 5.0 and Arleiquin 3.0, where recent expansion is indicated by the presence of one common haplotype and others in low frequencies (Rogers & Harpending 1992).

3. Results

3.1. Genetic variation

We sequenced mitochondrial DNA from 25 randomly chosen individuals in Norway using two primer pairs. The two overlapping fragments were assembled resulting in 885 bp sequences (GenBank accession No. DQ473269–473289, KU695565–695568). Aligned mitochondrial DNA sequences contained 11 variable sites (including two deletions; one each in TR1 and ST1) and 3 parsimony-informative sites. Mean base proportions were 32.6% T, 26.8% C, 28.3% A, and 12.3% G. Genetic diversity indices from mitochondrial DNA sequences indicated that the genetic variation among the samples was low (Table 2). From 25 samples, eight haplotypes were detected, and samples from three sites shared one haplotype. Haplotype diversity (H_d) was 0.630 ± 0.103 (mean ± SD). Overall nucleotide diversity (π) calculated from the mtDNA sequences was 0.0015, which was extremely low. On the other hand, observed heterozygosity calculated from microsatellite markers was not particularly low.

With additional 24 sequences obtained from Genbank (see Appendix), we constructed a neighbor joining tree (Fig. 2a). All Norwegian samples

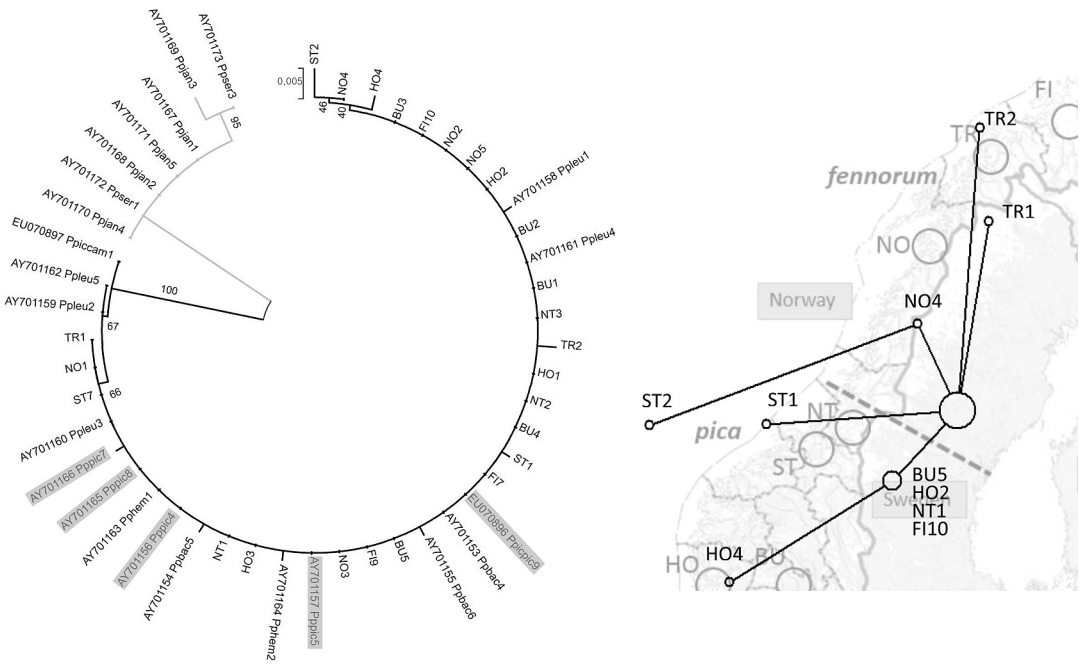

Fig. 2. Genetic relationship among the subspecies of Magpies (a) and within the Magpies in Norway (b). (a) A neighbor-joining tree of the subspecies of the Eurasian Magpies including Norwegian samples (abbreviations are explained in Fig. 1) and 24 sequences retrieved from Genbank (shown with the accession numbers and subspecies names). The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. All positions containing alignment gaps and missing data were eliminated in pairwise sequence comparisons. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (5,000 replicates) are shown next to the branches. Locations of subspecies *pica* are shaded. The optimal tree with the sum of branch length = 0.0818 is shown. The Asian clade is marked with grey branches. (b) A reduced median network drawn with Norwegian samples only. One haplotype was shared by 14 samples (the largest circle) and the identities of other nodes are noted in the figure. The branch lengths were proportional to the number of mutations that included indels.

were grouped together with the subspecies *leucoptera*, *hemileucoptera*, *bactriana*, and *pica*, with *camtshatica* located at the base. Particularly, *pica* (marked with shades) and all Norwegian samples were not closely located in the tree. A reduced-median haplotype network with Norwegian samples only, is given in Fig. 2b. Because one haplotype was predominantly found in all sampling sites, the network was not informative to draw any meaningful pattern of genetic differentiation.

3.2. Testing the presence of putative subspecies border

The results of the AMOVA are shown in Table 3. Both mitochondrial sequences and microsatellite markers suggest that placing the samples into two groups based on the putative subspecies border in the middle of Norway (NO, TR and FI as one, and the rest as the other) does not explain the degree of genetic differentiation in our data. More than 90 percent of the genetic variance is explained within the populations.

Pairwise F_{ST} values based on mtDNA sequences ranged from –0.132 (between NO and FI, NO and NT) to 0.474 (between TR and BU) (Table 4). However, none of the pairwise F_{ST} values from mtDNA sequences were significant. On the other hand, pairwise F_{ST} values based on microsatellite data ranged from –0.015 (between TR and ST) to 0.106 (between NO and TR) (Table 4). NO was the most distinct from all the other populations ($P <$

Table 3. Results of AMOVA based on mitochondrial DNA sequences and microsatellite markers. SSQ denotes 'sum of squares' and Var. comp. are the variance components.

Source of variation	mtDNA				Microsatellite loci			
	df	SSQ	Var. comp.	% variation	df	SSQ	Var. comp.	% variation
Among groups	1	0.427	–0.019	–3.94	1	4.699	–0.018	–0.51
Among populations	5	3.133	0.056	11.89	5	27.003	0.137	3.90
Within populations	18	7.800	0.433	92.04	99	336.100	3.395	96.61
Total	24	11.360	0.471	–	105	367.802	3.514	–

Table 4. Population pairwise F_{ST} values (above diagonal: estimated from mtDNA sequences; below diagonal: estimated from microsatellite markers). Significance levels for F_{ST} values were indicated as * for $0.01 < P < 0.05$, ** for $0.001 < P < 0.01$, and *** for $P < 0.001$.

	FI	TR	NO	NT	ST	BU	HO
FI	–	0.250	–0.132	0.000	0.000	0.000	–0.091
TR	0.086***	–	0.337	0.250	–0.031	0.474	0.172
NO	0.052**	0.106***	–	–0.132	0.084	–0.000	0.025
NT	0.010	0.035*	0.040***	–	0.000	0.000	–0.091
ST	0.044***	–0.015	0.073***	–0.002	–	0.189	–0.008
BU	0.027	0.022	0.054***	0.006	0.019	–	0.063
HO	0.035	0.017	0.048*	0.010	–0.010	–0.000	–

0.05), and FI was significantly differentiated from TR, NO and ST.

The results from Bayesian inference of population structure are shown in Fig. 3a. The results suggest that the pattern of genetic differentiation in the Norwegian Magpie populations, if any, does not conform the geographic locations. Four individuals from Troms (TR), two individuals from Sør-Trøndelag (ST), and one each from Nord-Trøndelag (NT), Buskerud (BU), and Hordaland (HO) could be assigned to different groups than the rest, but this possibility was indicated only when we assumed the presence of three or more subpopulations ($K = 3$ and 4) and this pattern appeared only in several cases out of 20 runs. The average probability of K (L(K)) was the highest at $K = 1$, and it decreased slightly and gradually with $K \geq 2$ (Fig. 3b). ΔK (Fig. 3c) was not informative in deriving the best K value. Based on these results, it seems reasonable to assume that there is no genetic structure among the Magpies in Norway (i.e., $K = 1$).

3.3. Demographic history

Because our data indicate a lack of genetic differentiation among the Norwegian Magpie populations, we examined the possibility of rapid expansion of populations. D^* and F^* test results were not significant ($D^* = –2.2889$, $F^* = –2.5621$, for both $0.05 < P < 0.10$), and Fu's Fs was significantly negative ($Fs = –3.2797$, $P < 0.009$ from 1,000 simulations). These results suggest that there is no background selection and the populations had gone through demographic expansions.

Sum of squared deviations (SSD) and the raggedness index calculated from the mismatch distribution analysis were small (SSD = 0.0089, $P = 0.65$; $r = 0.2381$, $P = 0.60$), which indicates that the mismatch distribution curves fit the sudden expansion model tested (Fig. 4).

Norwegian Magpie populations showed unimodal patterns of mismatch distribution curves, which corroborates the presence of recent population expansion (Fig. 4).

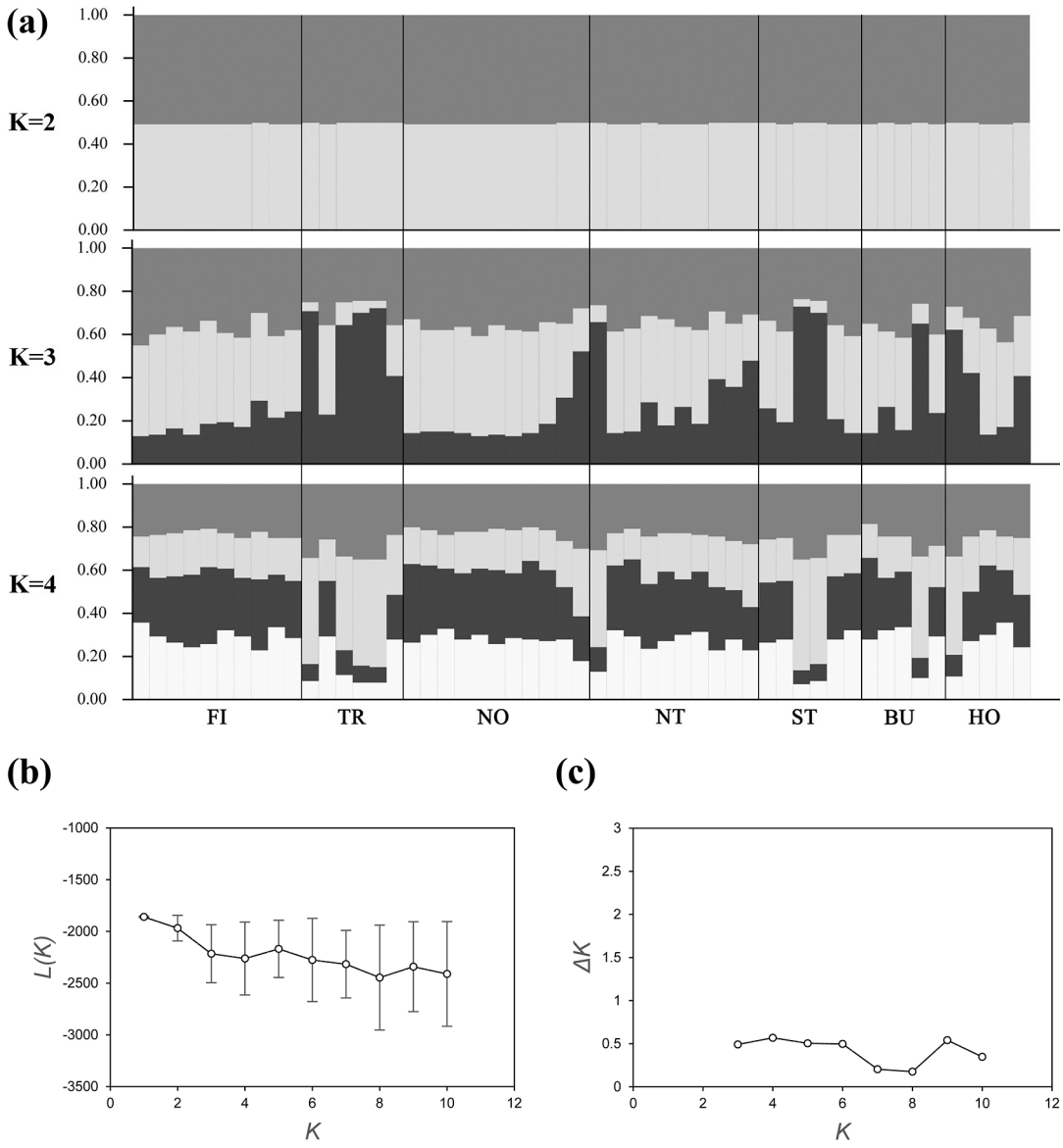

Fig. 3. Genetic structure across 59 individuals from 7 localities in Norway. (a) Bar plots showing the lack of clustering of individuals by STRUCTURE with K = 2, 3, and 4. Average probabilities ($L(K)$) (b) and ΔK (c) were calculated with K = 1–10 from 20 independent Markov chain runs. Error bars in (b) denote the standard deviation.

4. Discussion

Our results indicate that the previously suggested subspecies status of Magpies in Norway is not supported by either mitochondrial or nuclear markers. The degree of genetic differentiation among the Magpies in Norway was extremely low. AMOVA results were similar between mitochondrial sequences and microsatellite markers; most of the variance was explained by the variance within populations and the population identity and the putative subspecies border did not explain the genetic variance among the samples. Pairwise F_{ST} values calculated from mtDNA sequences indicated no differentiation. Pairwise F_{ST} values calculated from microsatellite markers indicated some

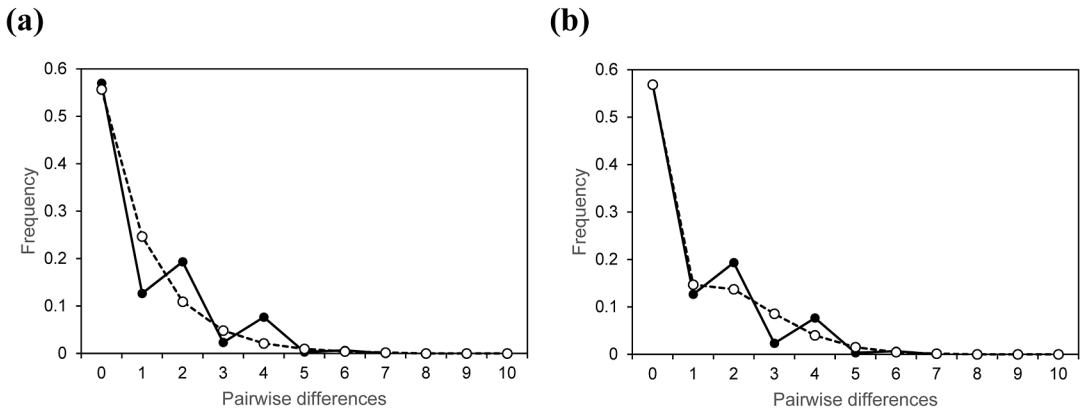

Fig. 4. Mismatch distributions showing the evidence of sudden expansion both temporally (a) and spatially (b). Observed (solid lines with closed circles) and expected (dotted lines with open circles) distributions of pairwise difference among the sequences are presented.

level of differentiation, but the pattern was neither congruent with the subspecies border nor with geographic distribution among the samples. Bayesian estimation of genetic structure among the populations did not provide sufficient evidence to reject no genetic differentiation among the samples. Taken together, these results suggest a lack of, or minimal, genetic differentiation among the Magpies in Norway.

Furthermore, the topology of the phylogenetic tree and the mismatch analysis suggested the possibility that the Norwegian Magpie populations were formed by rapid expansion. This implies that all the Magpies in Norway came from the same refugia when they followed the ice sheet northwards after the last glaciation event, and their colonization in Norway seems to have happened quickly. Investigation of other corvids such as Clark's Nutcracker (*Nucifraga columbiana*) and Spotted Nutcracker (*N. caryocatactes*) drew the same conclusions (Dohms & Burg 2013, Dohms & Burg 2014), while subspecies of Gray Jay (*Perisoreus canadensis*) probably came from different refugia judging from genetic differences between the subspecies (van Els *et al.* 2012).

If Magpies move far away from their breeding ground in the winter (Stegmann 1927), birds from different subspecies might be mixed in this analysis. However, Magpies are highly sedentary, and even natal philopatry is strong (Eden 1987, Wernham *et al*. 2002). Also, in Norway, Magpies are sedentary year round across their entire range, and even in harsh winters they remain in the northernmost areas (Collett 1921, Bakken *et al*. 2006, Husby 2006). From the first recovery of ringed Magpies in Norway in 1931, 555 have been recovered until 2006. The mean distance from the ringing location for all birds was 7 km (n = 331), and for young birds ringed in the nest it was 6 km (n = 235). The longest distance registered was 158 km (Bakken *et al*. 2006). These observations underscore that the collected birds in this analysis likely represent birds breeding in the collection locations rather than a mixture of birds breeding in different parts of Norway. In addition, most of the birds were gathered just before or just after the breeding season where they are expected to be close to their breeding ground (Husby & Slagsvold 1992).

A lack of genetic differentiation among the subspecies among European Magpies has been found in previous studies based on mitochondrial sequences (e.g., Kryukov *et al*. 2004, Haring *et al.* 2007, Zhang *et al*. 2012), but not all subspecies were considered in these studies. Moreover, it remains to be determined whether the same pattern is observed from nuclear markers. Most of the previous genetic studies of Magpies are based on mitochondrial sequences, which are useful in estimating the evolutionary history of lineages. On the other hand, inference of the demographic history of regional populations at a smaller time scale has not been done on any of the subspecies of Magpies. We believe that using nuclear markers as well as mitochondrial markers is necessary for understanding the genetic structure of regional populations of Magpies. Indeed our results with pairwise

F_{ST} suggest that the nuclear markers are more sensitive to more recent processes that lead to genetic differentiation. Thus, we suggest that future studies on the genetic differentiation among subspecies of Eurasian Magpies should include additional microsatellite markers, as it would help our understanding of more recent demographic processes. In addition, more thorough sampling not only in Norway but also throughout the Scandinavian Peninsula are needed to understand the patterns of genetic differentiation and gene flow in this region. More specifically, it would also be interesting to compare the rate and timing of expansion estimated from nuclear markers (such as microsatellites) and the rate and timing of glacial retreat, which would verify whether the Magpies' colonization indeed is related to the glacial retreat. This is important in understanding the colonization history of Magpies that is responsible for genetic differentiation among the regional populations.

Our study is the first attempt to assess the subspecies status of a regional population of a cosmopolitan species of the Eurasian Magpies. Considering that the distribution of the Eurasian Magpies is very wide and their subspecies system is based on clinal morphological characters which are notoriously difficult to use in assigning subspecies status, we suggest that genetic assessment based on nuclear markers should be conducted more rigorously in conjunction with a re-examination of morphological characters using modern multivariate statistics for character analyses. These enhanced methods will contribute to the understanding of possible hidden genetic structure among regional populations of the Eurasian Magpies.

Acknowledgements. Thanks to Jostein Aasenhus, Endre Alstad, Håkon G. Dahle, Geir Elde, Per Furuseth, Arne Johan Gravem, Tom Y. Saastad, Stein-Ole Sommerseth and Kjell S. Talle for delivering of Magpies to this project from different parts of Norway, and to the Directorate of Nature Management for the permission to shoot Magpies outside the hunting season of the species. We thank Dr. Piotr Jablonski for the help with the project and Dr. John Eimes for the linguistic help. This study was partly supported by the Ewha Global Top5 Grant 2013 of Ewha Womans University.

Genetisk granskning av skatans underartstatus i Norge

På fenotypisk basis erkänns två underarter av den euroasiatiska skatan (*Pica pica*) i Norge, med nominantrasen *P. p. pica* i söder och *P. p. fennorum* i norr. I denna studie undersöker vi om det finns genetiskt distinkta grupper av skator i Norge som underlag för diskussionen om underartstatus. Vi samlade in 61 skator från 7 olika områden i Norge och analyserade två typer av genetiska markörer, nämligen mitokondriella DNA-sekvenser och mikrosatelliter.

Den genetiska differentieringen bland skatorna var extremt låg. Merparten av den totala genetiska variationen var varians inom populationen. Populationstillhörighet eller de förmodade underartgränserna förklarade inte den genetiska variansen mellan samplen. Även om mikrosatellitmarkörerna påvisade närvaro av en viss genetisk differentiering, stämde mönstret inte överens med samplens geografiska ursprung. Våra resultat tyder på att alla skator i Norge har sitt ursprung i ett och samma refugium under den senaste istiden, och att deras kolonisering av Norge har skett mycket snabbt. Skatans status som underart i Norge kräver vidare utredning.

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Appendix. Information of the sequences retrieved from GENBANK.

Accession No_ID	Subspecies	Sample location	Source
AY701153_Ppbac4	*bactriana*	Russia: Kirov	Kryukov *et al.* 2004
AY701154_Ppbac5	*bactriana*	Russia: Invanovo reg	Kryukov *et al.* 2004
AY701155_Ppbac6	*bactriana*	Russia: Kislodovsk	Kryukov *et al.* 2004
AY701156_Pppic4	*pica*	Russia: Smolenskaya reg.	Kryukov *et al.* 2004
AY701157_Pppic5	*pica*	Russia: Smolenskaya reg.	Kryukov *et al.* 2004
AY701158_Ppleu1	*leucoptera*	Russia: Ulan Ude	Kryukov *et al.* 2004
AY701159_Ppleu2	*leucoptera*	Russia: Ulan Ude	Kryukov *et al.* 2004
AY701160_Ppleu3	*leucoptera*	Russia: Schartal	Kryukov *et al.* 2004
AY701161_Ppleu4	*leucoptera*	Russia: Ulan Ude	Kryukov *et al.* 2004
AY701162_Ppleu5	*leucoptera*	Russia: Ulan Ude	Kryukov *et al.* 2004
AY701163_Pphem1	*hemileucoptera*	Russia: Muhur-Aksy	Kryukov *et al.* 2004
AY701164_Pphem2	*hemileucoptera*	Russia: Muhur-Aksy	Kryukov *et al.* 2004
AY701165_Pppic8	*pica*	Turkey: Buyuk Camlica	Kryukov *et al.* 2004
AY701166_Pppic7	*pica*	Turkey: Buyuk Camlica	Kryukov *et al.* 2004
AY701167_Ppjan1	*jankowskii*	Russia: Ussuriland, Nadezhdinsk	Kryukov *et al.* 2004
AY701168_Ppjan2	*jankowskii*	Russia: Lower Amur, Solnechny	Kryukov *et al.* 2004
AY701169_Ppjan3	*jankowskii*	Russia: Ussuriland, Gaivoron	Kryukov *et al.* 2004
AY701170_Ppjan4	*jankowskii*	Russia: Ussuriland, Nadezhdinsk	Kryukov *et al.* 2004
AY701171_Ppjan5	*jankowskii*	Russia: Ussuriland, Nadezhdinsk	Kryukov *et al.* 2004
AY701172_Ppser1	*sericea*	South Korea: Suncheon	Kryukov *et al.* 2004
AY701173_Ppser3	*sericea*	South Korea: Daedeongri	Kryukov *et al.* 2004
EU070896_Ppicpic9	*pica*	Austria: Gars/Kamp	Haring *et al.* 2007
EU070897_Ppiccam1	*camtschatica*	Russia: Anadyr' River, Markovo Settl.	Haring *et al.* 2007