

The evolutionary history of the white wagtail species complex, (Passeriformes: Motacillidae: *Motacilla alba*)

Maliheh Pirayesh Shirazinejad

Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran

Mansour Aliabadian

Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran

Research Department of Zoological Innovations, Institute of Applied Zoology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran

aliabadi@um.ac.ir

Omid Mirshamsi

Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran

Research Department of Zoological Innovations, Institute of Applied Zoology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran

Abstract

The white wagtail (*Motacilla alba*) species complex with its distinctive plumage in separate geographical areas can serve as a model to test evolutionary hypotheses. Its extensive variety in plumage, despite the genetic similarity between taxa, and the evolutionary events connected to this variety are poorly understood. Therefore we sampled in the breeding range of the white wagtail: 338 individuals were analyzed from 74 areas in the Palearctic and Mediterranean. We studied the white wagtail complex based on two mitochondrial DNA markers to make inferences about the evolutionary history. Our phylogenetic trees highlight mtDNA sequences (ND2, CR), and one nuclear marker (CHD1Z), which partly correspond to earlier described clades: the northern Palearctic (clade N); eastern and central Asia (clade SE); southwestern Asia west to the British Isles (clade SW); and Morocco (clade M). The divergence of all clades occurred during the Pleistocene. We also used ecological niche modelling for three genetic lineages (excluding clade M); results showed congruence between niche and phylogenetic divergence in these clades. The results of the white wagtail ancestral area reconstruction showed the influence of dispersal on the distribution and divergence of this complex species. The most important vicariance event for the white wagtail complex may have been caused by the Gobi and Taklamakan deserts. We conclude that the ancestral area of the white wagtail complex was probably in the Mediterranean, with its geography having a considerable effect on speciation processes.

Keywords

ancestral area – dispersal – historical biogeography – niche divergence – vicariance – white wagtail complex

Introduction

The primary function of the Pleistocene in shaping the phylogeographic patterns of many birds has been recognised (Avice, 1998). Hence, the last period of the ice ages is considered the potential driver for population divergence and speciation processes (Hewitt, 1996; Avice, 1998). Dispersal events and climatic changes also cause population separations, which can ultimately lead to speciation (Smith et al., 2014). During the Last Glacial Maximum (LGM), the Mediterranean zone had an impressive effect on speciation processes because it was not influenced by the dominant climatic conditions (Otto-Bliesner et al., 2006). Therefore, relict populations remained alive and the Mediterranean zone was a shelter for species to persist in (Taberlet et al., 1998; Hewitt, 2000, 2004) and later recolonized the Northern Hemisphere (Tzedakis et al., 2002; Petit et al., 2003), shaping the phylogeographic patterns of many species (Médail & Diadema, 2009).

The white wagtail (*Motacilla alba*) is an adaptable species found in much of Europe, Asia and parts of North Africa that undertakes long-distance migration but is a year-round resident in the southern Palearctic (Alström et al., 2003). The white wagtail has attracted considerable attention because of genetic similarity among taxa despite a variety in plumage, and many different opinions have been presented to explain the morphological divergence occurring in this species complex. The white wagtail complex with different plumage pattern of black, white and grey colour on the

different regions of body is completely related to geographical distribution (Stepanyan, 2003). Cramp (1988) defined four subspecies group based on the head pattern of adult male in the breeding season: 1) *M. a. personata*, *M. a. alboides* 2) *M. a. ocularis*, *M. a. lugens* (3) *M. a. alba*, *M. a. dukhunensis*, *M. a. yarelli* (4) *M. a. baicalensis*, *M. a. leucopsis*. Other researchers represent the white wagtail as a single polytypic species with fourteen subspecies (Dementev & Gladkov, 1954); or nine subspecies (Dickinson & Christidis, 2014). Nevertheless, Sangster et al. (1999) presented nine species. Stepanyan (2003) considered *M. personata*, *M. lugens*, and *M. alba* to be different species. Ödeen (2001) by comparing two mitochondrial DNA sequences, introduced two subspecies groups, *alba* and *alboides*, for this complex. Furthermore, Pavlova et al. (2005) showed a clear incongruence between molecular and morphological results. They suggested a single species for the species complex of *M. alba*.

Voelker (2002), using mtDNA, reconstructed an ancestral area of the genus *Motacilla* and suggested the eastern Palearctic as the origin area and other two important colonization areas in parts of Africa at 4.5 and 2.9 Mya. The author concluded that dispersal events play a key role in the distribution patterns of the existing species, to the extent that even the North Atlantic Ocean and Beringia could not prevent the distribution of the species of this genus. Furthermore, Voelker (2002) suggested that the cold climate and the resulting climatic changes may have acted as a driving force for speciation processes during the ice ages.

Li et al. (2015) inferred four clades based on a phylogeographic study: the northern Palearctic (clade N), south-western Asia and the British Isles (clade SW), central and eastern Asia (clade SE), and Morocco (clade M). The divergence time of the species complex was estimated to be the Pleistocene. Based on niche modelling, the authors also revealed that eastern parts of Asia, southern parts of Europe and western regions of Asia would have been well suited for *M. alba* during the LGM. They proposed a late expansion in white wagtail clades and a mixing of genetic material along the edges of distribution of each clade. Additionally, the authors professed that the south-western (SW) clade could have persisted in the southern parts of the Black Sea during the LGM. Because of the lack of samples from the Mediterranean region, they could not fully support the hypothesis that clade SW had been replaced by clade N. Moreover, Pavlova et al. (2005) and Li et al. (2015) claimed that the ancestor of the northern populations (northern Palearctic) came from the eastern Palearctic and extended to the northern Palearctic after the Pleistocene. Pirayesh et al. (2017) introduced nine separated ecological niches for white wagtail species complex. Their results indicated different taxa are clearly divergent not only in plumage but also in ecological niche.

The wide geographic sampling of the species needs to be determined, its ancestral area be clarified, and evolutionary events specified. Pavlova et al. (2005) and Li et al. (2015) included samples from northern and south-eastern populations of the white wagtail complex in the Palearctic. But because of the lack of data from south-western Asia and Iran, the ancestral area and historical events of the white wagtail complex need to be verified. Therefore, with additional data from south-western Asia and Iran, we examined different scenarios of the spatio-temporal routes

of colonization and the level of diversification among geographical regions. In sum, this study including all eleven nominal subspecies of the white wagtail complex will test (a) whether south-west clade of *M. alba* complex in Iran is present or replaced by northern clade (Li et al., 2015), (b) the probability of the far-eastern parts of Eurasia as a glacial refugium for the ancestor of the northern clade of wagtails during the Pleistocene (Pavlova et al., 2005; Li et al., 2015), and (c) any congruence between niche differentiation and the divergence of clades. This study will also elucidate how Pleistocene climatic conditions influenced dispersal rates, vicariance events, speciation and the ancestral area of the white wagtail.

Materials and methods

Sampling and specimen records

A total of 65 specimens of white wagtail (blood, feather and muscle) from 29 localities in Iran (south-western Asia) during the breeding season (April–September) were collected. All samples were housed in the Zoological Museum of Ferdowsi University of Mashhad (ZMFUM). Muscle and blood samples were maintained in 99% ethanol at -20°C and Queen's buffer, respectively. A total of 273 sequences from 45 different geographic localities in Eurasia were retrieved from GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) (supplementary table S1).

DNA sequencing

For all specimens, two mitochondrial DNA regions (ND2: NADH dehydrogenase subunit 2 and CR: control region) and one nuclear (CHD1Z: chromo-helicase-DNA binding protein) intron were amplified using primers L5215 (Hackett, 1996), H1064 (Drovetski et al., 2004), LCR4, H1248 (Tarr, 1995), 2550F and 2718R (Fridolfsson & Ellegren, 1999),

respectively. The PCR protocol for each pair of mtDNA and nDNA primers was retrieved from Pavlova et al. (2005) and Borge et al. (2005), respectively.

Phylogenetic and phylogeographic analyses

The sequences were aligned by ClustalW using MAFFT software v.7 (obtained from <http://mafft.cbrc.jp/alignment/software>). The final total analyzed length was 920 bp of ND2, 377 bp of CR, and 461 bp of CHD1Z. We performed separate phylogenetic analyses for each DNA marker, the combined mitochondrial DNA marker and for a combination of all DNA markers (mtDNA+CHD1Z). PartitionFinder v1.1.1 (Lanfear et al., 2012) was used to find the best model for the tree as TrN+I+G. MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) was used for Bayesian inference of phylogenetic trees with the Markov Chain Monte Carlo (MCMC) set at six million generations. The convergence of two parallel runs was tested using Tracer v. 1.4.1 (Rambaut & Drummond, 2007) and verified ($\text{ESS} \geq 7750$). Maximum likelihood (ML) analysis was conducted using PhyML v.3.0 (Guindon et al., 2010), while the algorithm of the searching tree was set on Subtree-Pruning-Regrafting (SPR).

BEAST v.2.4.4 software (Bouckaert et al., 2014) was used to study the divergence time with the molecular clock rate set at 0.0135 substitutions/site/million years (Li et al., 2015). In addition, based on the Modeltest 3.7 (Posada & Buckley, 2004), I and G were set at 0.5722 and 1.2362, respectively. Different prior trees, including the Yule model, Birth-Death model, Coalescent Constant Population and Coalescent Exponential Population, with an MCMC set on 100 million generations were run. Log file results were analysed using Tracer ver. 1.4.1, and the Birth-Death Model was selected based on the highest values of ESS (1357) and the Bayes factor (5089).

The condensed tree drawn in the BEAST software was also applied to the Reconstruct Ancestral State in Phylogenies (RASP) software RASP v.3.2 (Yu et al., 2015). This program used for inferring historical biogeography through reconstructing ancestral geographic distributions on phylogenetic trees. RASP utilizes the Statistical Dispersal-Vicariance Analysis (S-DIVA) (Yu et al., 2010), LAGRANGE (Ree & Smith, 2008) and BayArea (Landis et al., 2013) analysis. Furthermore, S-DIVA, LAGRANGE and BayArea analyses were applied to identify the ancestral area, dispersal-variance events and the number of speciation events based on three different approaches including parsimony, likelihood and Bayesian, respectively. The output of all three methods can be displayed and compared with each other. The distribution pattern based on zoogeographical classification was divided into four regions a) eastern Palearctic, b) western Palearctic, c) Mediterranean and d) Oriental (Smith, 1983) and were indexed as A to D in the RASP. The graphical viewer of phylogenetic trees with pie colours as the origin of each clade is design by FigTree (<http://tree.bio.ed.ac.uk/software/figtree/>) (fig. 4).

Population genetic structure analyses and demography

The genetic distances of white wagtail complex species were calculated in three genes ND2, CR, and CHD1Z using the Tamura-Nei (TrN) parameter as implemented in MEGA version 7 (Kumar et al., 2016). Inter- and intra-clade distances were calculated using ExcaliBAR software (Aliabadian et al., 2014). We used DnaSp v.5 (Librado & Rozas, 2009) to calculate nucleotide diversity (π), haplotype diversity (h) and the number of polymorphic sites (S). Furthermore, Arlequin 3.5 (Excoffier & Lischer, 2010) is used for calculating Tajima's D test, Fu's F_s test and the mismatch distribution test. All the tests were conducted

to identify the expansion of the clades. A unimodal plot of mismatch distribution analysis, negative and significant amounts of Tajima's D and Fu's F_s test were interpreted to be an expansion event. The nearer the peak to the Y axis, the more recent the expansion. Conversely, a bimodal or jagged plot shows stable populations. Tajima's D is the difference between different measures of the site-frequency spectrum and deviation from the null hypothesis can indicate selection/contraction or population selective sweep/expansion. The null hypothesis, neutrality, is confirmed when $D = 0$, while $D > 2$ and $D < -2$ represent a balancing selection and a selective sweep, respectively. The equation $t = \tau/2\mu k$ was applied to calculate the time of expansion for each clade, where t is the time of expansion, K is the nucleotide number, μ is the mutation rate, and τ is calculated from the mismatch distribution analysis (Rogers & Harpending, 1992). The white wagtail haplotype network and visualization of genealogical relationships was constructed using PopART 1.7 (<http://popart.otago.ac.nz>) based on a minimum spanning network (Bandelt et al., 1999).

Present and past ecological niche modelling (ENM)

We examined whether the congruence of niche and phylogenetic divergence among the three clades is present or not. Therefore, we extracted the geo-referenced coordinates for all of the sequences used in the present study from the literatures. Then, 19 climatic layers (2.5 arc-minute resolution), were downloaded from WorldClim (<http://www.worldclim.org>) for three periods; the present, the LGM, and the Holocene (6000–7000 years ago). The correlation between the climatic variables was tested using Pearson's correlation coefficient ($r \leq \pm 0.85$) with 2000 random points. Finally, seven bioclimatic variables were selected,

including bio3 = isotherm, bio4 = temperature seasonality, bio5 = max temperature of warmest month, bio7 = temperature annual range, bio8 = mean temperature of wettest quarter, bio13 = precipitation of wettest month, bio14 = precipitation of driest month, and bio15 = precipitation seasonality. To generate the niche model for each clade, we applied MaxEnt 3.3.3 (Phillips & Dudík, 2008) using geo-referenced points and the selected climatic variables. The area under the curve (AUC) was also applied to confirm the accuracy of the resultant ENMs. AUC >0.9, >0.8, and >0.7 show a correct prediction for the model with 'very good', 'good' and 'useful' differentiation abilities, respectively (Swets, 1988). The ENMtools software package (Warren et al., 2010) was used to measure niche overlap and to run an identity test to confirm the niche divergences. The niche overlap among the three clades (SE, SW and N) was calculated based on a pairwise comparison the I statistic (Warren et al., 2008) and Schoener's D (Schoener, 1968). The niche overlap for D and I is variable between 0–1, and the closer the value to 1, the greater the similarity and overlap of niches. The results obtained from niche overlap and the identity test for the present were compared to confirm niche divergence. If the identity test rejects the null hypothesis, the clades based on ecological niche are significantly separated. In this study, data associated with clade M were omitted from the ENM analysis due to the low number of samples (2).

Results

Genetic diversity and neutrality test

The differences in the average of the TrN genetic distances of the ND2 gene were low, except for clade M (table 1). The results of the 1758 bp ND2, CR, and CHD1Z markers showed

TABLE 1 The average of the TrN genetic distances based on the ND2 gene (interclade: bold; intraclade: non-bold).

Clades	N	SE	SW	M
N	0.00093			
SE	0.011	0.002902		
SW	0.011	0.01	0.002095	
M	0.024	0.027	0.027	0.0011

few haplotype numbers and polymorphic sites (not shown). Moreover, the results of the 1297 bp ND2 and CR genes for the 338 samples showed 134 polymorphic sites (including 76 informative parsimony and 58 singleton variable sites) and 127 haplotypes with a haplotype diversity of 0.8489 and a nucleotide diversity of 0.005. However, the largest number of haplotypes (69) was in clade N, which includes 216 samples. The greatest haplotype diversity, with 27 haplotypes, was in both clades SE and SW. The results of DnaSp for all clades are shown in table 2, but clade M is excluded because of low sample size. The haplotype network showed that clade M is separated from the rest of the clades by 27 steps, while clade N is separated from SE by five steps and clade SE from SW by seven steps. However, clade SW and N were not linked together (fig. 1). The mismatch distribution of the N and SW clades was unimodal, whereas it was bimodal for clade SE, which occurred after the addition of the Iranian samples (fig. 1). All values of Tajima's *D* and Fu's *F*_s test were significantly negative (table 2).

Estimating distribution and niche comparisons

Using Maxent, three distribution maps for clades N, SE and SW based on seven bioclimatic variables were obtained in the past and present, where a warmer colour represents more suitable habitat for the population (fig. 2). The values of the training and test data

of the AUC for clades N, SE and SW are from 0.961 to 0.998, which represent the accuracy of the models obtained using Maxent. The percentage contribution of bioclimatic layers in the present for clades N (mean temperature of wettest quarter = 27.5%), SW (mean temperature of wettest quarter = 44.6%), and SE (temperature seasonality = 51%); in the Holocene for clades N (precipitation of wettest month = 21.3%, temperature seasonality = 20.4%), SW (precipitation seasonality = 28.2, temperature seasonality = 25.8%) and SE (isotherm = 24.4, temperature seasonality = 19.8); and at the LGM for clades N (temperature seasonality = 38.8%), SW (temperature seasonality = 27.1%) and SE (temperature seasonality = 27.9) were obtained.

In the present, the measured niche overlap between N-SW clade (*I* = 0.46 and *D* = 0.16), N-SE clade (*I* = 0.48 and *D* = 0.22) and SE-SW clade (*I* = 0.57 and *D* = 0.41) represented that the most and the least niche overlaps are between SE-SW clade and N-SW clade, respectively. The niche overlap in the Holocene between N-SW clade (*I* = 0.41 and *D* = 0.13), N-SE clade (*I* = 0.42 and *D* = 0.16) and SE-SW clade (*I* = 0.54 and *D* = 0.39) showed the same results for the most and the least niche overlaps with the present. On the other hand, the niche overlap in the LGM between N-SW clade (*I* = 0.39 and *D* = 0.11), N-SE clade (*I* = 0.41 and *D* = 0.14) and SE-SW clade (*I* = 0.51 and *D* = 0.32) indicated that the most and the least niche overlaps are the same as the present and in compliance with the Holocene results (table 3).

The true niche overlapping values between N and SE clade (*D* = 0.22 and *I* = 0.48) is compared to identity test (*I* = 0.865 ± 0.026 and *D* = 0.835 ± 0.014), which is significant. As well, the true niche overlap value between N and SW clades are *D* = 0.16 and *I* = 0.46, which is significant compared to Identity test with

TABLE 2 Results of Arlequin and DnaSP. *p ≤ 0.05, **p ≤ 0.01.

	n	h	Hd	π	Peak	SSD	Tajima 'D	Fu's F _s	τ	Expansion (years ago)
SE	59	27	0.909	0.00294	Bimodal	0.003	-1.51*	-15.69**	4.727	134,000
SW	61	27	0.840	0.00214	Unimodal	0.004	-1.88**	-20.66**	0.656	19,000
N	216	69	0.649	0.00091	Unimodal	0.005	-2.73**	-29.54**	1.00	28,000

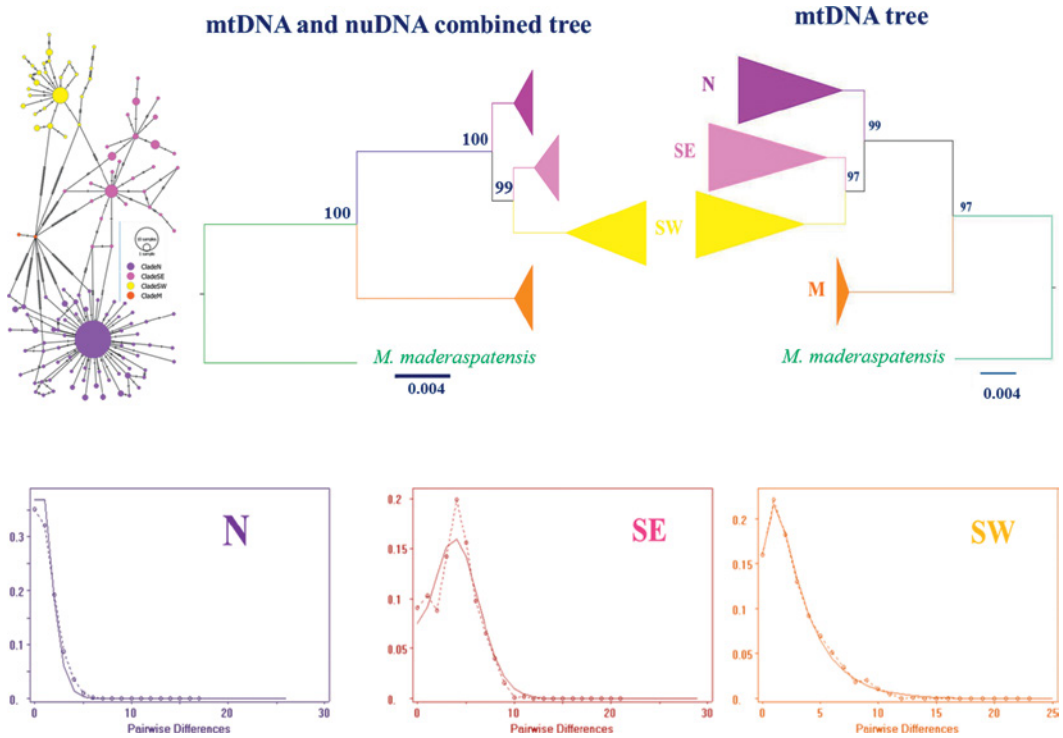


FIGURE 1 Minimum spanning haplotype network (right), MrBayes trees (left), and mismatch distribution of three clades of white wagtail (below).

$I = 0.881 \pm 0.028$ and $D = 0.853 \pm 0.019$. Finally, the true niche overlap value between SE and SW clades $D = 0.41$ and $I = 0.57$, which is significant compared to Identity test with $I = 0.898 \pm 0.031$ and $D = 0.885 \pm 0.019$. The results of comparing the niche overlap and identity test in the present were significant; the null hypothesis of niche identity is rejected and indicates distinct niche divergence in all three clades (fig. 3).

Phylogeny trees and the phylogeographic pattern

The results of MrBayes, PhyML and BEAST based on the combined sequences of 338 samples from 74 localities showed the same topology of four clades, including N, SE, SW and M (Figs. 1, 4, supplementary fig. S1). Europe includes clades SW and N, North Africa (Morocco) includes clades M and N, East Asia includes clades N and SE, and North Asia only

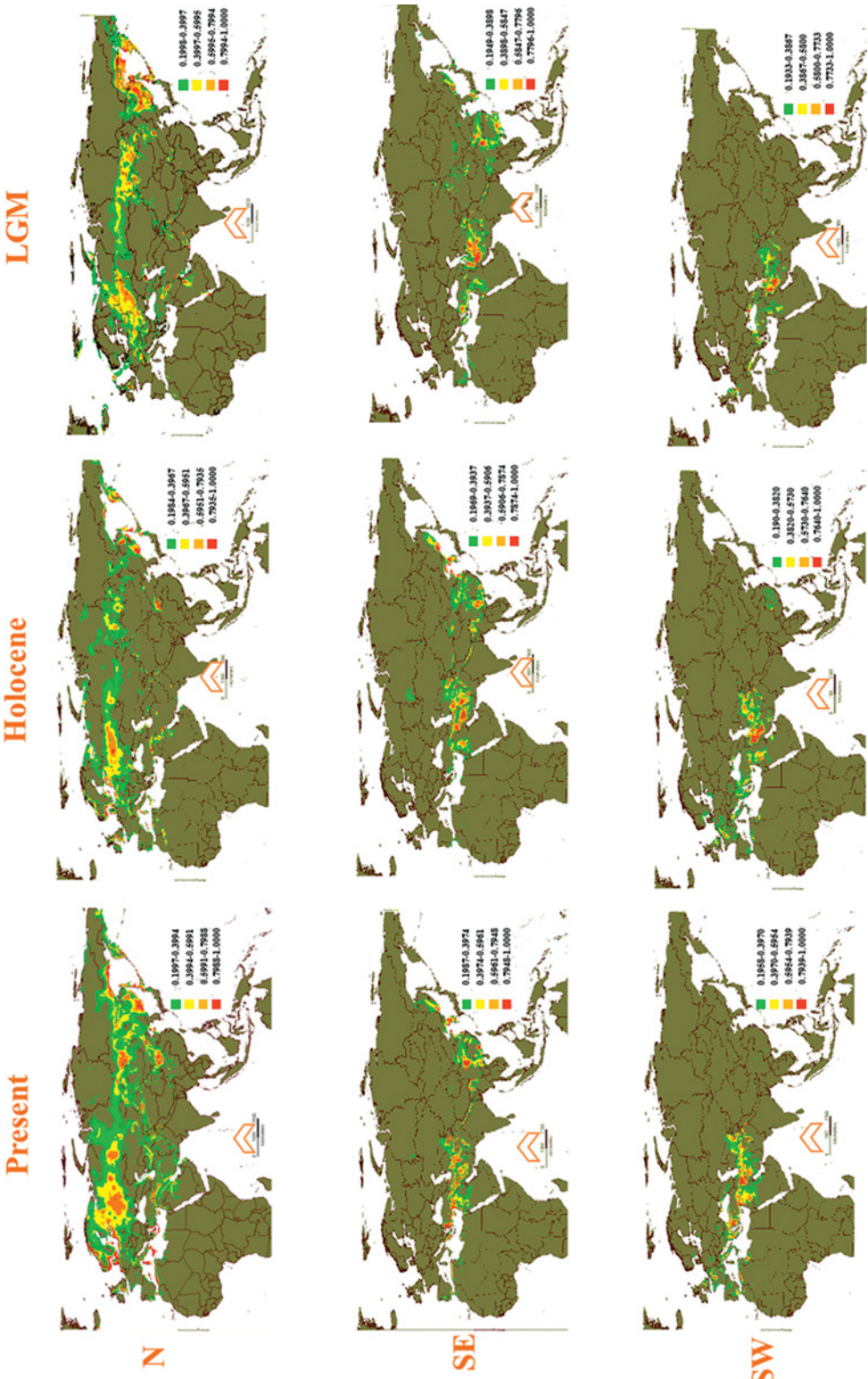


FIGURE 2 Ecological niche modelling pattern of white wagtail during the present, Holocene, and LGM. Red, orange and yellow colours represent more suitable areas for the species; green indicates less suitable areas.

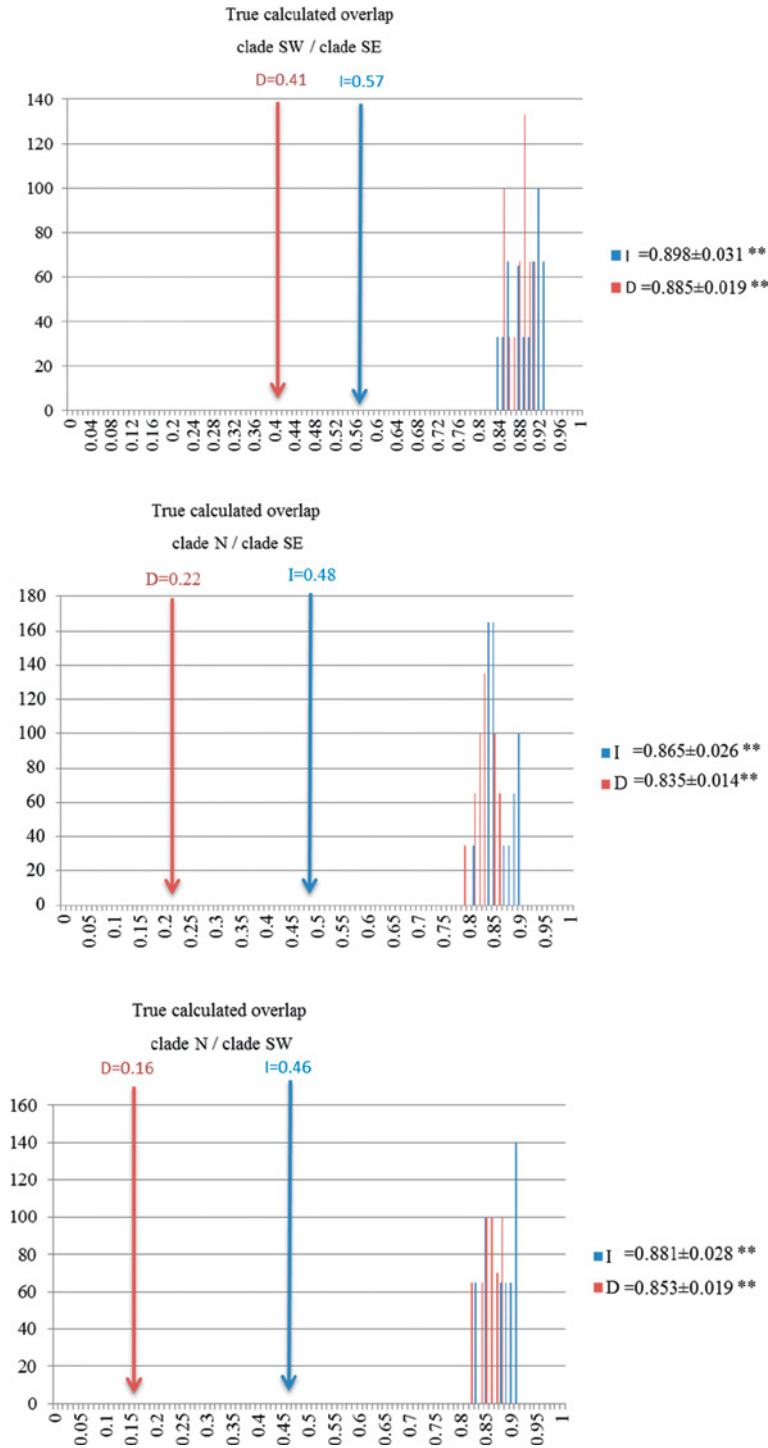


FIGURE 3 Results of the identity test. Red and blue arrows show the true calculated niche overlap results. Columns represent the amount of niche overlap in the replicates of the identity tests. The true calculated overlap values (I and D) are far outside the 99.9% confidence intervals of the identity test results and thus highly significant (indicated by two asterisks**).

includes clade N; finally, the three clades SE, SW and N are in South West Asia (fig. 5A). All clades diverged during the Pleistocene; in detail, the divergence of clade M from the others was at 0.92 Mya, and the divergence of the clades SE-SW and clade N occurred at 0.4 Mya and 0.22 Mya, respectively (fig. 4).

In all analyses using the RASP v.3.2 software, the values of dispersal were much higher than those of vicariance events. Based on LAGRANGE analysis, 124 dispersal, 75 vicariance, and 2 extinction events occurred. The number of speciation events within areas included A: 123, B: 50, C: 81, and D: 18. S-DIVA inferred 97 dispersal, 78 vicariance, and 2 extinction events. Finally, the results of BAY-AREA showed 213 dispersal, 70 vicariance, and 5 extinction events. In LAGRANGE analysis, the highest value for dispersal occurred at 0.03 Mya, for vicariance at 0.01 Mya and for extinction at 0.48 Mya (fig. 6B, table 4). The expansion time estimated by the mismatch distribution for clade N was 0.028 Mya,

that for clade SE was 0.134 Mya and that for clade SW was 0.019 Mya (results are shown in table 2). On the other hand, the mtDNA has a smaller effective population size and is also maternally inherited. Thus, the nuclear marker overcomes these biases. The results of mtDNA and mito-nuclear trees indicated the same topology (fig. 1).

Discussion

Genetic relations between clades

In agreement with previous studies (Pavlova et al., 2005; Li et al., 2015), this species complex showed very low genetic distance between clades. The greatest TrN genetic distances of all the clades was in clade M, and the shortest distance was in clades SE-SW. However, the largest number of haplotypes was in the clade N, and the most varied haplotypes were in clades SE and SW. The graph obtained from the mismatch distribution was unimodal for

TABLE 3 Niche overlap of clades in the white wagtail complex during the present, Holocene, and LGM. (D: bold; I: non-bold).

Time	Present			Holocene			LGM		
Clades	N	SE	SW	N	SE	SW	N	SE	SW
N		0.48	0.46		0.42	0.41		0.41	0.39
SE	0.22		0.57	0.16		0.54	0.14		0.51
SW	0.16	0.41		0.13	0.39		0.11	0.32	0

TABLE 4 Ancestral area reconstruction based on three historical biogeography analyses.

Historical biogeography analysis	Area Division				Events		
	A	B	C	D	Dispersal	Vicariance	Extension
S-DIVA	0.25	0	0.75	0	97	78	2
LAGRANGE	0	0	1	0	124	75	2
BayArea	0.26	0.1	0.64	0	213	70	5

* A) eastern Palearctic, B) western Palearctic, C) Mediterranean and D) Oriental

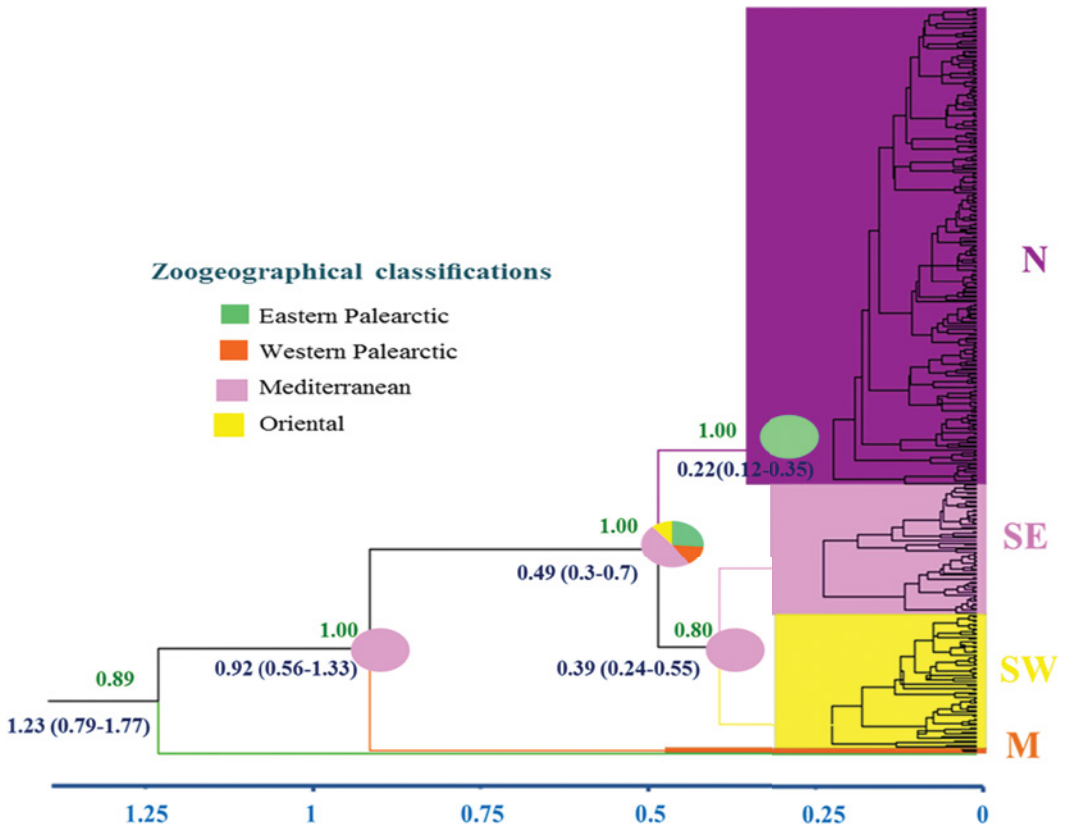


FIGURE 4 Estimates of phylogenetic tree, divergence times and LAGRANGE ancestral area reconstructions of the *M. alba* complex. The chronogram tree is based on BEAST analysis of the combined dataset. Colour pies indicate the origin of a given node based on four zoogeographical areas followed by LAGRANGE analysis.

clades N and SW, which shows the recent expansion of these clades in the past; in disagreement with (Li et al., 2015), our results suggest that clade SE was bimodal and subject to the addition of Iranian samples, which shows that populations are stable. The value of Tajima's *D* and value of Fu's *F_s* test were significantly different at $P \leq 0.05$ for the N, SE and SW clades, which is almost -2 or more, so, populations included in these clades may have experienced a mitochondrial selective sweep. Furthermore, this is indicative of a population expansion, which is obviously consistent with a model of survival and recolonization from a refugium.

Niche occupancy profiles and niche divergence

Based on the ecological niche modelling during the present, the most important environmental variable affecting the populations of clades N and SW was the mean temperature of the wettest quarter. Clade N showed a geographic distribution in western and south eastern Palearctic regions, and clade SW was distributed in Iran, Turkmenistan, Uzbekistan, Afghanistan, Iraq, Syria, Lebanon, peripheral Mediterranean areas, and parts of southern Europe and North Africa. Based on ENM, the geographical distribution of clade SE included most areas of Iran except for the northern

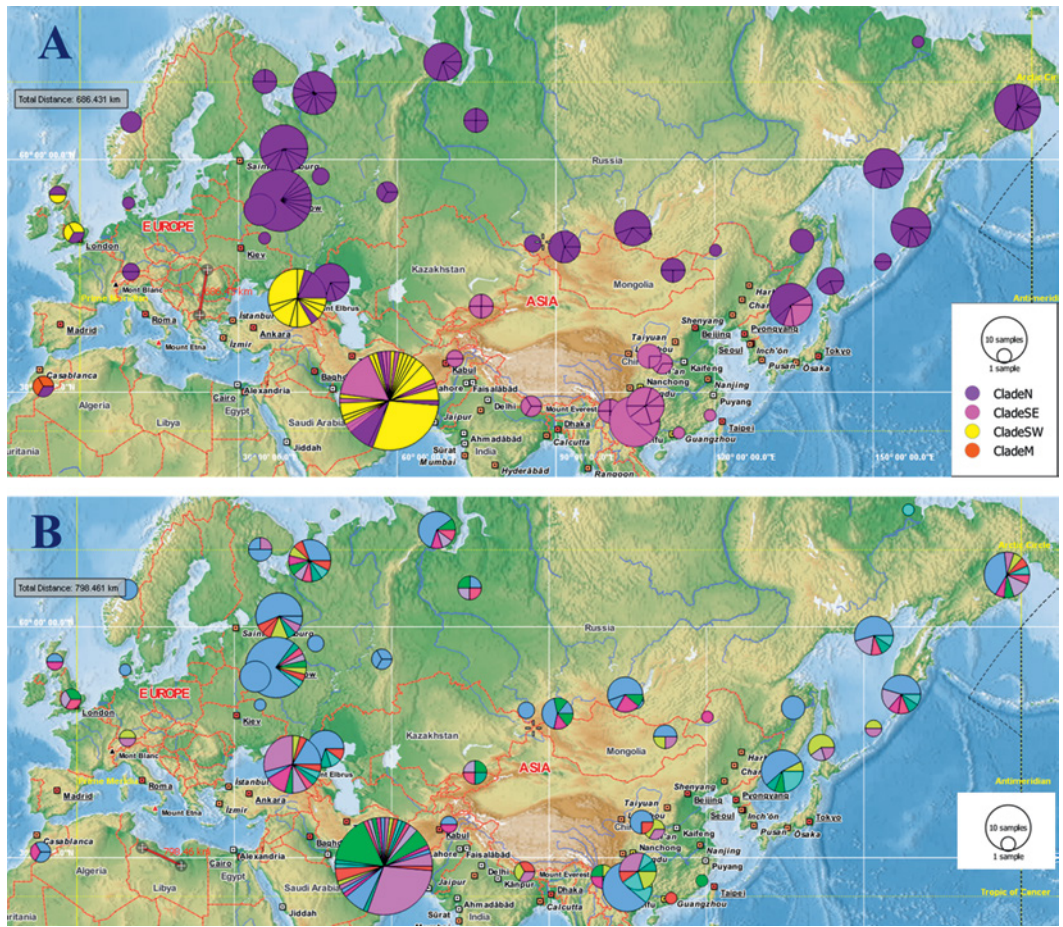


FIGURE 5 A) Geographical distribution of haplotypes based on clades. B) Geographical distribution of haplotypes based on haplotypes diversity in PopART 1.7.

and southern regions, Nepal, Syria, Lebanon, peripheral areas of the Mediterranean basin, Korea, and parts of China and North Africa. The most important environmental variable for clade SE was temperature seasonality.

The geographical distribution during the Holocene for clade N was eastern Europe; for clade SE, it was Iran, Afghanistan and South-east China; and for SW, it was Iran. One of the most important environmental variables determining the distribution pattern that was common in all clades was temperature

seasonality, which was ranked second; the most important environmental variables in clades N, SE, and SW were precipitation of the wettest month, isotherm and precipitation seasonality, respectively.

Based on Maxent analysis, during the LGM, temperature seasonality played the most important role in the distribution of all clades, and the distribution pattern for clade N was Eastern Europe, Kamchatka and Khabarovsk; for clade SE, it was Iran, Turkey, and south-central China; and for clade SW, it was Iran

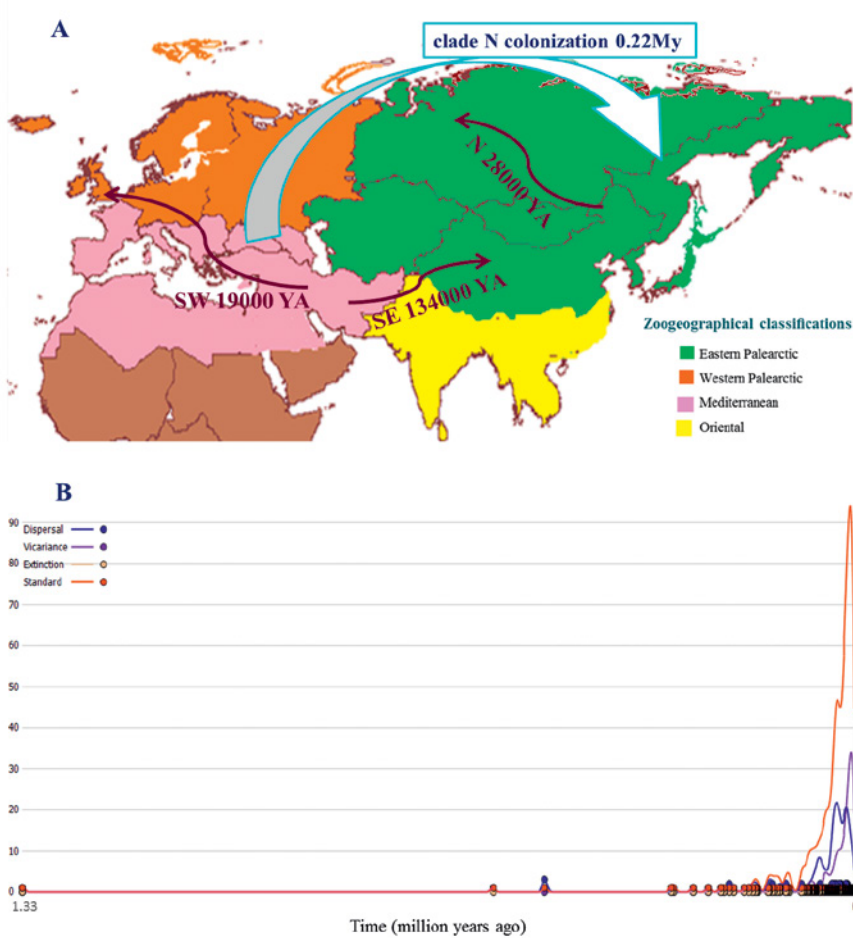


FIGURE 6 A) Zoogeographical classifications of the white wagtail complex and the time of expansion. B) The time-events curve (including dispersal, vicariance, extinction event and the total number of biogeographic events).

and the North Caucasus (fig. 2). There were more variations in potentially favorable areas across time for clade N than for its southern counterparts. According to fig. 2, it appears that Far East Asia was very favorable to clade N during the LGM.

Integrating analysis of molecular and ecological niche data has become more common recently (Raxworthy et al., 2007; Rissler & Apodaca, 2007; Flanders et al., 2011; Zhao et al., 2012), and this approach provides the best interpretation for phylogeographic and

distribution patterns. It is argued that climatic conditions play an important role in ecological speciation (Svensson, 2012). It is particularly notable when natural selection causes divergence in traits and affects reproduction. Therefore, it is an impediment to the gene flow between lineages that have adapted to different environments (Dobzhansky, 1937; Mayr, 1942; Schluter, 2000), and as a result, the ecological speciation occurs. In this study, the divergence of niches was completely consistent with the divergence of the clades in the

molecular data. Moving from the LGM to the present, niche overlapping decreases and niche divergence increases; this pattern indicates the separation of niches in the past. It may be concluded that niche separation resulted in separation of the clades during the Pleistocene, however, it must be noticed that the niche separation could also be just have been driven by geographical isolation as a result of climatic shifts and then subsequent niche adaptation (table 3).

Historical biogeography of white wagtails

In a previous study (Li et al., 2015), the clades N and SE were considered a sister group, whereas in the present study with adding Iranian samples, the clades SE and SW are considered a sister group. Iran is the only area where three clades (SE, SW and N) exist. Note that clade SE was divided into two sub-clades (fig. 4), in which the Iranian Plateau and Nepal samples were placed in one sub-clade. Based on historical biogeography analysis, vicariance may play an important role in separating these two sub-clades. This geographical barrier is probably the Gobi Desert and the Taklimakan Desert. During the Pleistocene, water sources in the Gobi Desert and the intensity of rainfall decreased (Yang, Ding & Ding, 2006); furthermore, the Himalayas created a barrier for the monsoon winds and exacerbated this issue (Rea et al., 1998; Yang et al., 2006). The Gobi Desert and Taklimakan Desert are also responsible for the separation of populations of Brown-cheeked Rail and Water Rail (Tavares et al., 2010).

The processes of expansion and contraction caused by climatic changes during the Pleistocene induced a change in distribution range; this can result in significantly changing the genome (Hewitt, 1996). According to the data presented here, the divergence in all

clades of the white wagtail occurred during the Pleistocene and dates back up to 0.92 Mya. The oldest expansion time occurred in clade SE 134 thousand years ago, during Marine Isotope Stage (MIS) 6. The expansion time of clade N goes back to 28 thousand years ago and that for clade SW to 19 thousand years ago (MIS2).

The results of RASP software showed congruent in all analysis (S-DIVA, LAGRANGE and BayArea) that dispersal may have played an important role in the historical development of white wagtail complex distributions and the Mediterranean as the most plausible ancestral area. Voelker (2002), based on S-DIVA, located the ancestral area of the genus *Motacilla* in the eastern Palearctic and inferred crucial role dispersal in distribution patterns of *Motacilla* species. The oldest fossils discovered for the genus *Motacilla* are related to the Mediterranean zone (Hungary during the late Miocene (Jánossy, 1995) and Bulgaria during the late Pliocene (Boev, 1996). At present, the Mediterranean region is one of the hotspots of biodiversity in the world (Cuttelod et al., 2008). The Mediterranean region served as a refugium during the ice age and a place of speciation in plants and mammals (Quézel, 1978, 1985; Cheylan, 1990). In addition, Iran, as part of the Mediterranean based on the zoogeographical classifications, is the only region that includes three clades and has the highest haplotype diversity (fig. 5B). Iran can be regarded as the ancestral area for the white wagtail; the climate in Iran was colder during the ice age than the present, and a decrease in evaporation made it wetter than it now is (Krinsley, 1970). Geological studies have shown that during the LGM and Holocene, southern Iran and Yazd had a dry climate (Thomas et al., 1997; Kehl et al., 2005). In other studies, the temperatures estimated for the last period of glaciation for different parts of Iran include those higher than 5°C in the mountains

of Alborz and Zagros (Bobek, 1963), from 5–8°C in central Iran (Krinsley, 1970), and between 8 and 10°C in southern Iran (Frenzel et al., 1992). In addition, during the Late Glacial and Lower Holocene, western Iran and eastern Turkey experienced relatively high rainfall (Goodfriend & Magaritz, 1988; Roberts & Wright Jr, 1993), while southern Zagros and southern Iran experienced a dry climate (Kehl et al., 2009). Palaeopedological evidence suggests that desert and semi-desert areas in central Iran experienced increased moisture during the Late Quaternary (Khademi et al., 1997; Khademi & Mermut, 1999; Farpoor et al., 2004), and the oldest of the three clades SE, N and SW is clade SW, which covered most of Iran and even semi-desert regions.

In Morocco, the British Isles, Krasnodar, Primore and Iran, the combination of two clades could be due to Pleistocene climate oscillations. Pleistocene climate changes could have caused expansions and contractions in the distribution range of species due to shifts from dry to humid periods (Le Houérou, 1997), which resulted in secondary contact zones in the Mediterranean region and western Palearctic. Haffer (1977) introduced northern Iran as one of the most important secondary contact zones of birds in the Mediterranean region. In this case, geological investigations of the soil composition north of Iran in Babolsar (Antoine et al., 2006), Neka and Agh Band during the LGM or the Late Glacial (Frechen et al., 2009) show that Babolsar was wetter than the two other regions, which indicates the presence of a wet slope in the north of Iran and south of the Caspian Sea. Further research is required in these areas, where clades SE and SW have secondary contact.

The results obtained from the historical biogeography analysis showed that dispersal plays a more important role in separation of

clades than vicariance, and molecular evidence showed the role of migration in speciation of the Old World species of Motacillidae (Outlaw & Voelker, 2006). Clade N colonized the eastern Palearctic approximately 220 thousand years ago, and expansion occurred 28 thousand years ago; this timeline was confirmed in a previous study (Pavlova et al., 2005; Li et al., 2015). All dispersal routes and the colonization of clade N started in the Mediterranean region and then spread to other areas as shown in fig. 6A. Considering the long migrations of the white wagtail complex, there is no limitation in flying, and vicariance plays a lesser role in the formation of the speciation of this species complex. Ecological separation in the past, as well as dispersal, played important roles in the historical biogeography and the present distribution of this species complex.

The colour-polymorphic birds show a greater speciation rate than the monomorphic species (Hugall & Stuart-Fox, 2012), and speciation events are faster among individuals with different colouration (Gray & McKinnon, 2007). In this case, plumage may differ among different ecological or environmental areas (Roulin, 2004; McKinnon & Pierotti, 2010) and can cause ecological or behavioural isolation. Finally, as expected, divergence of plumage among populations represents an important reproductive barrier (sexual selection). Therefore, the nine distinctive morphological taxa of the white wagtail complex can be considered newly derived species because of a narrow hybrid zone (reproductive barrier and natural selection versus hybridization) (Pavlova et al., 2005) and niche divergence. According to the results, it seems that the best way to confirm separation of the populations of the white wagtail complex is to study epigenetic changes (methylation patterns of DNA) or gene expression.

Conclusions

The results of the biogeographic analyses confirm that the eastern Palearctic may be regarded as a refugium for the N clade. Furthermore, the most important factor in the distribution and divergence of the clades in the white wagtail complex is dispersal events. Clade SE showed a different structure and vicariance event as a major role in a divergence of two sub-clades considering the Iranian samples. Note that clade SW covers widespread distribution in the north and center of Iran; and have not been replaced by the expanding haplotype group N. Therefore, hotspot speciation may have occurred in the Mediterranean region, especially on the Iranian Plateau. The Pleistocene climatic conditions have probably made Iran the centre of speciation and the ancestral area with high haplotype diversity and the existence of three clades (SE, SW and N).

Acknowledgements

Many thanks to the HPC centre of Ferdowsi University of Mashhad for performing the MrBayes and BEAST analyses. Financial support was provided by Ferdowsi University of Mashhad (project no. 3/29692). We appreciate the reviewers for providing us with their valuable comments.

Supplementary material

Supplementary material is available online at: <https://doi.org/10.6084/m9.figshare.8858072>

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RECEIVED: 27 NOVEMBER 2017 | REVISED AND

ACCEPTED: 17 APRIL 2019

EDITOR: V. NIJMAN