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GENETIC DIVERGENCE OF THE SPECIES OF THE YELLOW WAGTAILS GROUP (PASSERIFORMES, MOTACILLIDAE) IN EUROPEAN TERRITORY OF RUSSIA

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Genetic Divergence of the Species of the Yellow Wagtails Group (Passeriformes, Motacillidae) in European Territory of Russia. Artemieva, E. A., Mishchenko, A. V., Makarov, D. K. — Phenotypic and genotypic diversity of populations of species of the yellow wagtails in the space of their ranges in a wide sympatry reflects the mechanisms of reproductive isolation of species and forms of subspecies rank and features of microevolution group — polytypic complex *Motacilla flava* Linnaeus, 1758. The distribution of species of the yellow wagtails in the European part of Russia is sympatric. The purpose of this study was to investigate whether sympatric settlement led to any genetic separation between the populations of these species. 20 blood samples and 2 samples of eggs collected in areas geographically representing the MID and the southern Russian breeding populations of these species, including all juvenile ringed in 2012, were used. After the polymerase chain reaction (PCR) 4 types of the yellow wagtails group *Motacilla flava* L., 1758; *M. feldegg* Michahelles, 1830; *M. lutea* (S. G. Gmelin, 1774); *M. cilreola* Pallas, 1776 (Passeriformes, Motacillidae) were sequenced. After aligning the sequences of the gene cytochrome oxidase I, based on the comparison of genetic distances between these species phylogenetic tree of genus *Motacilla* was constructed. These results suggest that, despite the broad sympatry in nesting places, there is a selective mating between males and females of each species studied in spite of the free crossing and insulating mechanisms in populations.

Key words: phenotype, genotype, population, mtDNA, barcoding, wagtails, European territory of Russia.

Introduction

Phenotypical and genotypical diversity of populations of species of the group of the yellow wagtails in wide sympatric conditions represents the mechanisms of reproductive isolation of the forms of species and subspecies rank and features of microevolution. In spite of the wide sympatry in nesting area there is selective mating between males and females of each of the studied species which inhibits free crossing and supports insulating mechanisms in populations. (Zarudny, 1891; Beregovoy, 1970; Babenko, 1981; Bakhtadze, 1987; Grichik, 1992; Red'kin, 2001 a, 2001 b; Sotnikov, 2006; Artemieva, Muraviev, 2012 a; Pavlova et al., 2003; Kerr et al., 2009).

The objective of the research was to identify genotypic divergence in species of the yellow wagtails group under sympatric conditions in the European territory of Russia.

Material and methods

Species of the yellow wagtails group of subgenus *Budytes* Cuiv. 1817, in sensu lato were selected for molecular-genetic research: their morpho-ecological features are typical for genus *Motacilla* and family Motacillidae; the species are polymorphic (more than 20 species and subspecies forms were described to date); they inhabit extremely various ecological and geographical conditions and belong to transpaleoartic polytypical group. The studied group of the yellow wagtails is represented by the species *Motacilla flava* Linnaeus, 1758; *M. feldegg* Michahelles, 1830; *M. lutea* (S. G. Gmelin, 1774); *M. cilreola* Pallas, 1776.

Yellow wagtails are the nesting migratory species of the birds with Eurasian areals. They are widely spread in the European part of Russia. Yellow and citrine wagtails rise up to highest latitude of Arctic and nest in tundra and forest tundra. Citrine and black-headed wagtails spread more locally and restricted with latitudinal temperature factor (forest-steppe and steppe and desert area) are the rarest (Gladkov, 1954; Portenko, 1960; Stepanyan, 1993; Sotnikov, 2006; Artemieva, Muraviev, 2012b; Muraviev, Artemieva, Beme, 2014; Crampe, 1988).

The yellow wagtail species are confined to the floodplain landscapes and inhabit all native zones of the European part of Russia. Stations suitable for nesting belong to the open habitats such as meadow, meadow-marsh, meadow-steppe and steppe of various humidity — hygrophilic to xerophilous, but the most mesophilic. They build their nests on the ground, under the cover of grass, bumps, etc. There are from 5 to 7 eggs in the masonry. After hatching the

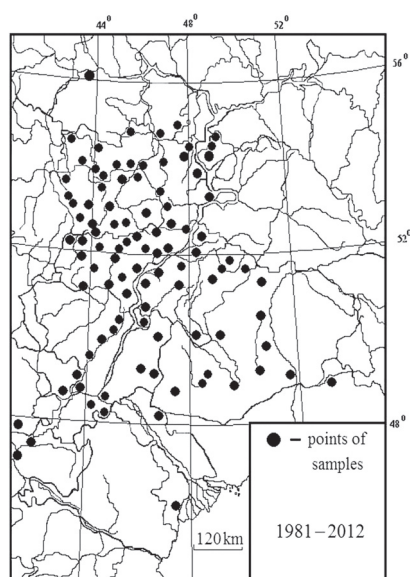


Fig. 1. Sample points of yellow wagtail species on the European territory of Russia (Artemieva, Muraviev, 2012).

gataattcacaatcctgg (Kerr et al., 2009). Quality of the PCR product was detected with analytical gel-electrophoresis.

Obtained PCR-products (from 400 to 550 bp) were purified and sequence reaction with fluorescent-labeled deoxyribonucleotides (ddNTP) followed by purification of the set of terminated fragments was performed. As a result of sequencing of that fragment of the cytochrome oxidase I gene nucleotide sequence was obtained and processed with bioinformatic methods. Sequences were aligned with ClustalW2 software (EMBL-EBI-URL: <http://www.ebi.ac.uk/Tools/msa/clustalw220>) and phylogenetic tree for the studied species of *Motacilla* genus was constructed using JalView software (Jalview-URL: <http://www.jalview.org>) and genetic distances were defined.

Results and discussion

As a result of molecular genetic studies the nucleotide sequences of the cytochrome oxidase I gene fragments were obtained for four species of the *Motacilla* genus. After research of 4 species of the yellow wagtails of the genus *Motacilla* (*M. flava*, *M. feldegg*, *M. lutea* and *M. citreola*) it was shown that nucleotide sequences of the cytochrome oxidase I gene were not the same in all dried samples from studied species of yellow wagtails. This confirms that all the populations are genetically heterogeneous and belong to different species of the yellow wagtails, but to the same genus *Motacilla*. On the base of obtained data phylogenetic tree for four species of the genus *Motacilla* was constructed and genetic distances were defined (table 1, fig. 2, 3).

These species living together in the European Russia are associated with floodplain intrazonal cenoses of the forest and forest-steppe as well as steppe and semi-desert complexes. It should be noted that species *M. lutea* and *M. feldegg* are the closest to each other (genetic distance is 1.33). On the opposite species *M. flava* is the farthest from the others (genetic distance is 2.83) that may indicate its hybrid origin. Perhaps existence of hybrids of yellow wagtail with citrine and black-headed in natural populations in the area of wide sympatry of these species points at the same fact. But only diagnostics using nuclear markers can confirm this suggestion. Citrine

chicks develop for 9–14 days and leave the nest. Adult birds and chicks feed with a variety of insects. Strength of populations of yellow and citrine wagtail in the European part of Russia is quite high and stable, strength of citrine and especially black-headed wagtails is reduced due to a violation of the habitats suitable to nesting (Gladkov, 1954; Portenko, 1960; Stepanyan, 1993; Sotnikov, 2006; Cramp, 1988; Artemieva, Muraviev, Beme, 2013) (fig. 1).

Limiting factors of nesting are the next: reduction of biotopes suitable for nesting and feeding biotopes, anthropogenic factor (Gladkov, 1954; Portenko, 1960; Stepanyan, 1993; Sotnikov, 2006; Artemieva, Muraviev, 20126; Muraviev, Artemieva, Beme, 2014).

For the molecular-genetic research of the species of the yellow wagtails the next probes were used: 11 dried blood samples on the filter paper *M. flava* (from 6–9.05.2012, Nizhniy Novgorod suburbs); 3 dried shell samples (from 20.05.2012, 25.05.2013, Ulyanovsk Region, Cherdakly, Peschanoe lake) and 1 egg content *M. lutea* (from 7.07.2013, Ulyanovsk Region, Cherdakly, Peschanoe lake); 1 dried sample of egg shell *M. feldegg* (from 16.06.2012, Rostov Region, Azovsky District, suburbs of Kagal'nik village).

DNA was isolated from the dried blood samples of the birds of *Motacilla* genus with the help of GeneJET Whole Blood Genomic DNA Purification Mini Kit and from the dried shell scraps with the help of GeneJET Genomic DNA Purification Kit. Quality of the isolated DNA was checked with the spectrophotometer.

Cytochrome C oxidase I gene site was amplified using forward primer — ttctccaaccacaagacattggcac and reverse primer — acgtggga-

Table 1. Genetic distances between studied species of the genus *Motacilla* (defined using MEGA 4 software)

Species	<i>M. flava</i>	<i>M. feldegg</i>	<i>M. lutea</i>	<i>M. citreola</i>
<i>M. flava</i>	–	0.104	0.100	0.093
<i>M. feldegg</i>	0.104	–	0.024	0.045
<i>M. lutea</i>	0.100	0.024	–	0.042
<i>M. citreola</i>	0.093	0.045	0.42	–

wagtail does not represent an independent genetic branch of the, as is commonly believed, and is sufficiently removed from all other groups of species (genetic distance — 1.67).

All studied species and subspecies of yellow wagtails group show high level of genetic diversity (genetic distances from 695.00 to 1119.38). You can see two big clusters on the dendrogram — of citrine wagtail and of the other studied species of that group. The first cluster is rather uniform and consists of only *M. citreola* specimens belonging to pure subspecies *M. c. citreola* (genetic distance 724.16) and *M. c. werae* (genetic distance 1175.79). At that in this mixed *M. c. citreola* cluster we can select *M. c. werae* group possibly presented with hybrids with nominative subspecies *M. c. citreola*. It is interesting that one of the specimens of small citrine wagtail *M. c. werae* also presents in the second big cluster including the rest studied species of yellow wagtails (genetic distance 849.62). It may indicate the hybrid nature of this subspecies taxon. In general, we can conclude that genetic branch associated with citrine wagtail is relatively independent, but not as much as it is considered to be at the moment. Thus pure line of nominative subspecies *M. c. citreola* with probable hybridization with nominative subspecies of yellow wagtail *M. f. flava* and citrine wagtail *M. lutea* probably give subspecies form of small citrine wagtail *M. c. werae*.

The second large cluster is rather heterogeneous and consists of species of yellow wagtail *M. flava* (genetic distances 654.33–1119.38), black-headed wagtail *M. feldegg* (genetic distance 695.50) and citrine wagtail *M. lutea* (genetic distances 695.50–895.00). Inside the cluster the complex group of yellow wagtails subspecies — nominative *M. f. flava* and crossbred *M. f. thunbergi* (genetic distance 654.33–717.17), group of black-headed wagtail *M. feldegg* (genetic distance 695.50) and hybrid citrine wagtail *M. lutea* (genetic distances 695.50–749.00), group of pure citrine wagtail (genetic distance 895.00) can be allocated. Nominative subspecies of gray-headed wagtail *M. f. thunbergi* (genetic distance 1119.38) is the farthest from others. Group of *M. flava* unites pure specimens of nominative subspecies *M. f. flava* (genetic distances 576.50–654.33), hybrid specimens of *M. f. flava* (genetic distances 667.36–677.13), hybrid specimens of *M. f. thunbergi* (genetic distances 580.00–607.33) and pure *M. f. thunbergi* (genetic distance 717.17).

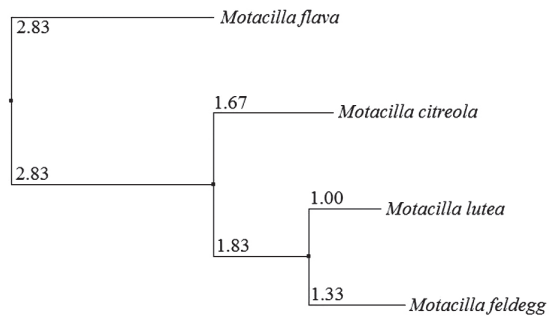


Fig. 2. Phylogenetic dendrogram of the species of the genus *Motacilla* built after alignment of sequences of the cytochrome oxidase I gene using JalView software (average version).

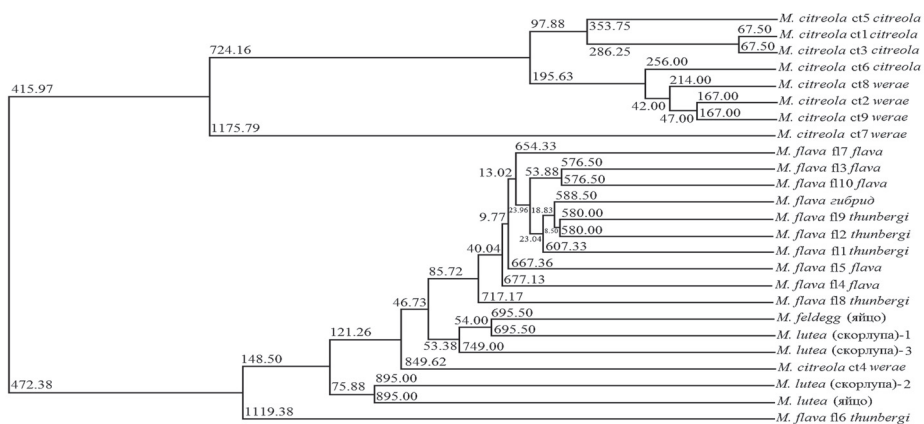


Fig. 3. Phylogenetic dendrogram of the species of the genus *Motacilla* built after alignment of sequences of the cytochrome oxidase I gene using JalView software (full version).

Conclusion

1. Species *M. lutea* and *M. feldegg* are the most closely related in the investigated complex of the yellow wagtails.
2. Confirmation of hybrid origin of species *M. flava* requires the use of nuclear markers.
3. Species *M. citreola* is not completely independent genetic branch, as is commonly believed. It is a subordinate link between *M. lutea*, *M. feldegg* and *M. flava*, and integrated into the overall genetic system of genus *Motacilla*.

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