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## DIVERGENCE OF POPULATIONS OF YELLOW WAGTAIL, *MOTACILLA FLAVA*, AND CITRINE WAGTAIL, *MOTACILLA CITREOLA* (MOTACILLIDAE, PASSERIFORMES), IN THE MIDDLE VOLGA OF RUSSIA

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**Divergence of Populations of Yellow Wagtail, *Motacilla flava*, and Citrine wagtail, *Motacilla citreola* (Motacillidae, Passeriformes), in the Middle Volga of Russia.** Artemieva, E. A., Mishchenko, A. V., Makarov, D. K. — Blood samples of “yellow” wagtails collected in the areas geographically representing the Middle Volga breeding populations of these species were investigated. After isolation of mtDNA barkoding of studied “yellow” wagtails species was conducted. Amplification of the subunit of cytochrome oxidase I gene used as a genetic marker for the comparison of the samples was carried out. After sequencing and sequence alignment of gene cytochrome c-oxidase I, based on the comparison of genetic distances between individuals of the studied species using Jalview phylogenetic trees of populations of species *Motacilla flava* Linnaeus, 1758 and *Motacilla citreola* Pallas, 1776 were constructed.

Key words: phenotype, genotype, population, mtDNA barkoding, wagtails, Middle Volga.

### Introduction

Among the most controversial in the taxonomic terms groupings passerine bird occupies a special place polytypical complex *Motacilla flava* in sensu lato (Gladkov, 1954; Portenko, 1960; Stepanyan, 1990; Sotnikov, 2006; Artemyeva, Muraviev, 2012 b), forms the group’s inherent very complex individual and geographical variability (Zarudny 1891; Beregovoy, 1970; Bakhtadze, 1987; Grichik, 1992; Babenko, 1981; Red’kin, 2001 a, 2001 b; Muraviev et al., 2014; Cramp, 1988; Artemieva, et al., 2013). In addition to environmental factors, and ethological reproductive isolation among sympatric species of birds can play an insulating role and molecular genetic features of species. To determine the actual family relationships within the taxa requires an integrated approach that combines assessment of the variability of phenotypic and genotypic evidence of specific forms, including molecular genetic (Artemieva, Muraviev, 2012 a; Pavlova et al., 2003; Vili et al., 2009).

The objective of the work is to study and to identify phenotypic and genotypic divergence in populations of yellow wagtail *Motacilla flava* and citrine wagtail *Motacilla citreola* under sympatric in the territory of the Middle Volga region of Russia.

### Material and methods

As an object of genetic research the species of the “yellow” wagtails group *Motacilla flava* Linnaeus, 1758 and *Motacilla citreola* Pallas, 1776 (Passeriformes, Motacillidae) of the subgenus *Budytes* Cuvier, 1817 were selected. We used 11 dried blood samples *M. flava* on filter paper from 6–9.05.2012, and 9 dry blood samples *M. citreola* on filter paper from 5–9.05.2012 collected in the surroundings of Nizhniy Novgorod (the Volga, Oka floodplains, wastewater treatment plants, meadows). Sampling for genetic investigations was carried out by the non-invasive method. For blood sampling bird were caught with mist net, morphometry indices and plumage characteristics were taken, banding was performed.

Original scale gradation of morphometry indices, as well as color and patterns of birds feather (tables 1 and 2) were worked out (tables 1 and 2). V. K. Ryabitshev’s system of color features and plumage patterns was used (2001).

Blood samples were taken from the birds’ brachial vein during the ringing using IsoCode STIX™ paper with special impregnation. It can be easily used in the field, what significantly reduces the effects of stress, as only 1–2 sampled blood drops (equal to 10–12 µl) is sufficient for genetic expertise.

Isolation of DNA from dried blood samples on paper (CosmoBio, Schleicher & SchuellBiosciences) was performed using a set of GeneJET Whole Blood Genomic DNA Purification Mini Kit (Thermo Scientific). Samples were cut from paper, homogenised and placed for incubation lysis solution (Lysis Solution, Thermo Scientific), containing proteinase K (56 °C, 15 min). Further, the precipitation of DNA was carried out with 96 % ethanol and recovering on silicon columns (GeneJET Genomic DNA Purification Columns, Thermo Scientific).

As the genetic marker gene fragment of cytochrome c oxidase I (COI) was used. For amplification of the needed area the following composition of PCR mixture (20 µl): dNTP (250 µl), the primers (0.5 µl), the buffer (1X), taq-polymerase (10 units), DNA template (1 µl) deionized water (to a final volume) was used. Polymerase chain reaction was carried out using a thermal cycler FlexCycler (Analytik Jena) at the following temperature settings: DNA denaturation — 94 °C, 2 min; 30 cycles under the conditions: DNA denaturation — 94 °C, 30 sec, primer annealing — 55 °C, 30 sec, elongation — 72 °C, 40 sec; completion chain — 72 °C, 5 min. The separation of fragments was carried out in a 1 % analytical agarose gel, then the preparative gel was used for isolation and purification of the needed fragment (using a set GeneJET Gel Extraction Kit (Thermo Scientific)).

The purified amplification products of the same length were sequenced using capillary genetic analyzer ABI PRISM 3500 (Life Technologies) (with preliminary sequence reaction with fluorescently-labeled deoxyribonucleotides (ddNTP) and subsequent purification of terminated fragments kit). The obtained sequences were analyzed and adjusted using program 2 Sequence Scanner software (Life Technologies Corporation) [<http://www.lifetechnologies.com>]; the resulting sequence of the gene cytochrome c-oxidase I was compared with available ones in GenBank [<http://www.ncbi.nlm.nih.gov/genbank>] and aligned using the program ClustalW2 (EMBL-EBI), genetic distances between individuals were determined using MEGA 4 (method of nearest neighbors (Neighbor-joining)).

## Results and discussion

Morphometry according to standard procedures was carried out; the basic features of plumage of males and females yellow wagtail *M. flava* and citrine wagtail *M. citreola* were defined, pairwise correlation analysis of signs of coloration and pattern of feather and morphometry were held; and then the most informative features (uncorrelated with each other and with any one of the other signs) were identified. For *M. flava* they were tarsus length, body length, painting of “a mustache”; for *M. citreola* — tarsus length, body length, “necklace” (tables 1–4).

**Table 1. Morphometric parameters of *Motacilla flava***

Ordinal number	Series number of rings	Date of gear	Time of fishing	Sex (P1)	Age (P2)	Fat (P3)	Weight (P4)	Length of wing (P5)	Length of tail (P6)	Length of tarsus (P7)	Cul1 (P8)	Body length (P9)
fl1	XH 51036	6.05	18–20	1 m	1 ad	0	20.0	84.0	68.0	32.0	11.0	16.3
fl2	XH 51039	6.05	19–45	1 m	1 ad	0	17.0	82.0	76.0	30.0	11.0	16.0
fl3	XH 51046	7.05	9–05	1 m	1 ad	3	16,6	81,0	72,0	23,1	9,3	16,7
fl4	XH 51048	7.05	10–30	1 m	1 ad	2	17.1	81.0	76.0	23.5	9.0	17.2
fl5	XH 51051	7.05	20–40	2 f	1 ad	0	15,0	75,0	71,0	23,43	10,0	14,5
fl6	XH 51052	7.05	20–40	1 m	1 ad	0	18,5	83,0	75,0	24,91	11,0	16,1
fl7	XH 51053	8.05	6–45	1 m	1 ad	0	16,5	82,0	71,0	23,3	9/7	16,5
fl8	XH 51058	8.05	10–20	1 m	1 ad	1	17,3	86,0	74,0	23,0	9,5	16,3
fl9	XH 51060	8.05	12–30	1 m	1 ad	0	16,7	83,0	71,0	24,9	9,3	15,4
fl10	XH 51067	9.05	8–10	1 m	1 ad	1	17,5	84,0	68,0	23,8	9,5	15,5
fl11	XH 51073	9.05	21–40	2 f	1 ad	0	16,4	78,0	70,0	23,8	8,7	14,0

Signs of coloration and pattern of feather of *Motacilla flava*, expressed in points:

fl1, XH 51036 (series and number of the ring), the date of catch — 6.05, while catch — 18–20, floor (1 m), a subspecies *thunbergi*, white mustache (P10) — 1 point, the crown of dark gray (P11) — 2 points, brow tight (P12) — 1 point, chin white (P13) — 1 point, ear coverts dark gray (P14) — 2 points, back olive-green (P15) — 2 points, chest bright yellow (P16) — 3 points.

fl2, XH 51039 (series and number of the ring), the date of catch — 6.05, while catch — 19–45, floor (1 m), a subspecies *thunbergi*, white mustache (P10) — 1 point, the crown of dark gray (P11) — 2 points, brow tight (P12) — 1 point, chin yellow (P13) — 3 points, ear coverts dark gray (P14) — 2 points, back olive-green (P15) — 2 points, chest bright yellow (P16) — 3 points.

fl3, XH 51046 (series and number of the ring), the date of catch — 7.05, while fishing — 9–05, floor (1 m), a subspecies *flava*, white mustache (P10) — 1 point, the crown gray (P11) — 1 point eyebrow wide (P12) — 2 points, chin white (P13) — 1 point, ear coverts gray (P14) — 1 point, back olive-green (P15) — 2 points, chest bright yellow (P16) — 3 points.

fl4, XH 51048 (series and number of the ring), the date of catch — 7.05, while catch — 10–30, floor (1 m), a subspecies *flava*, mustache half white (P10) — 2 points, the crown gray (P11) — 1 score eyebrow wide (P12) — 2 points, chin white (P13) — 1 point, ear coverts gray (P14) — 1 point, back olive-green (P15) — 2 points, chest bright yellow (P16) — 3 points.

fl5, XH 51051 (series and number of the ring), the date of catch — 7.05, while catch — 20–40, the floor (2 f), a subspecies *flava*, grayish mustache (P10) — 3 points, the crown gray (P11) — 1 point eyebrow wide (P12) — 2 points, chin whitish-yellow (P13) — 2 points, ear coverts gray (P14) — 1 point, back grayish olive (P15) — 1 point, chest grayish-yellowish (P16) — 1 score.

fl6, XH 51052 (series and number of the ring), the date of catch — 7.05, while catch — 20–40, floor (1 m), a subspecies *thunbergi*, mustache half white (P10) — 2 points, the crown of dark gray (P11) — 2 points, brow tight (P12) — 1 point, chin yellow (P13) — 3 points, ear coverts dark gray (P14) — 2 points, back olive-green (P15) — 2 points, chest bright yellow (P16) — 3 points.

fl7, XH 51053 (series and number of the ring), the date of catch — 8.05, while fishing — 6–45, floor (1 m), a subspecies *flava*, white mustache (P10) — 1 point, the crown gray (P11) — 1 point eyebrow wide (P12) — 2 points, chin white (P13) — 1 point, ear coverts gray (P14) — 1 point, back olive-green (P15) — 2 points, chest, yellow with dark spots (P16) — 2 points.

fl8, XH 51058 (series and number of the ring), the date of catch — 8.05, while catch — 10–20, floor (1 m), a subspecies *thunbergi*, mustache half white (P10) — 2 points, the crown of dark gray (P11) — 2 points, eyebrows practically no (P12) — 0 points, chin white (P13) — 1 point, ear coverts dark gray (P14) — 2 points, back olive-green (P15) — 2 points, chest yellow with dark stained (P16) — 2 points.

fl9, XH 51060 (series and number of the ring), the date of catch — 8.05, while catch — 12–30, floor (1 m), a subspecies *thunbergi*, yellow mustache (P10) — 4 points, the crown of dark gray (P11) — 2 points, brow tight (P12) — 1 point, chin yellow (P13) — 3 points, ear coverts dark gray (P14) — 2 points, back olive-green (P15) — 2 points, chest bright yellow (P16) — 3 points.

fl10, XH 51067 (series and number of the ring), the date of catch — 9.05, while fishing — 8–10, floor (1 m), a subspecies *flava*, mustache gray-yellow (P10) — 5 points, the crown gray (P11) — 1 score eyebrow wide (P12) — 2 points, chin, yellow (P13) — 3 points, ear coverts gray (P14) — 1 point, back olive-green (P15) — 2 points, chest, yellow with dark spots (P16) — 2 points.

fl11 (no blood sample), XH 51073 (series and number of the ring), the date of catch — 9.05, while catch — 21–40, the floor (2 f), a subspecies *flava*, grayish mustache (P10) — 3 points, the crown gray (P11) — 1 point, eyebrow wide (P12) — 2 points, chin whitish-yellow (P13) — 2 points, ear coverts gray (P14) — 1 point, back grayish olive (P15) — 1 point, chest grayish-yellowish (P16) — 1 point.

**Table 2.** Morphometric parameters of *Motacilla citreola*

Ordinal number	Series number of rings	Date of gear	Time of fishing	Sex (P1)	Age (P2)	Fat (P3)	Weight (P4)	Length of wing (P5)	Length of tail (P6)	Length of tarsus (P7)	Cull (P8)	Body length (P9)
1	2	3	4	5	6	7	8	9	10	11	12	13
ct1	XH 47691	5.05	17–20	1 m	1 ad	0	25.0	80.0	65.0	27.0	11.0	15.0
ct2	XH 51041	7.05	6–15	2 f	1 ad	0	15.0	77.0	71.0	23.8	13.9	14.8
ct3	XH 51042	7.05	6–15	2 f	1 ad	0	16.0	79.0	72.5	24.5	9.9	16.0
ct4	XH 51043	7.05	6–15	1 m	1 ad	0	16.0	85.0	75.0	23.56	9.7	16.0

Table 2. Continued

1	2	3	4	5	6	7	8	9	10	11	12	13
ct5	XH 51044	7.05	6–15	1 m	1 ad	0	16.0	83.0	72.0	23.77	9.5	16.0
ct6	XH 51045	7.05	9–05	2 f	1 ad	0	16.7	79.0	69.0	23.16	10.4	15.5
ct7	XH 51047	7.05	9–50	1 m	1 ad	1	16.1	79.0	72.0	24.6	9.7	16.0
ct8	XH 51049	7.05	10–30	1 m	1 ad	1	15.6	82.0	71.0	24.3	9.9	15.2
ct9	XH 51068 hybrid	9.05	8–10	1 m	1 ad	1	18.3	83.0	73.0	24.8	9.9	16.8
ct10	XH 50736 overfishing 2011 hybrid	9.05	9–20	2 f	0	1	17.9	76.0	65.0	24.0	10.1	14.9

Signs of coloration and pattern of feather of *Motacilla citreola*, expressed in points:

ct1, XH 47691 (series and number of the ring), the date of catch — 5.05, floor (2 f), a subspecies *citreola*, no eyebrows (P10) — 0 points, collar (nape) black wide (P11) — 2 points, lemon-crown yellow (P12) — 4 points, the back is dark gray (P13) — 3 points, chest lemon-yellow (P14) — 4 points, at the back small black spot (P15) — 3 points, stripes on the wing wide (P16) — 2 points, no necklaces (P17) — 0 points, throat lemon-yellow (P18) — 5 points.

ct2, XH 51041 (series and number of the ring), the date of catch — 7.05, floor (2 f), a subspecies *werae*, eyebrow yellowish (P10) — 1 point, the collar (nape) is absent (P11) — 0 points, the crown pale yellowish (P12) — 3 points, back light gray (P13) — 1 point, chest dirty-gray (P14) — 1 point, neck gray (P15) — 1 point, stripes on the wing tight (P16) — 1 point, necklaces no (P17) — 0 points, throat yellowish (P18) — 1 point.

ct3, XH 51 042 (series and number of the ring), the date of catch — 7.05, floor (2 f), a subspecies *citreola*, eyebrow yellow (P10) — 2 points, collar (nape) is absent (P11) — 0 points, the crown gray yellow (P12) — 2 points, back gray (P13) — 2 points, chest yellowish (P14) — 2 points, neck dark gray (P15) — 2 points, stripes on the wing tight (P16) — 1 point, a necklace there (P17) — 1 point, throat yellow-ocher (P18) — 3 points.

ct4, XH 51043 (series and number of the ring), the date of catch — 7.05, floor (1 m), a subspecies *werae*, eyebrows not (P10) — 0 points, collar (nape) narrow dark gray (P11) — 2 points, the crown Lemon yellow (P12) — 4 points, back gray (P13) — 2 points, chest pale lemon-yellow (P14) — 3 points, the nape gray (P15) — 1 point, stripes on the wing wide (P16) — 2 points, necklace there (P17) — 0 points, throat lemon-yellow (P18) — 5 points.

ct5, XH 51044 (series and number of the ring), the date of catch — 7.05, floor (1 m), a subspecies *citreola*, no eyebrows (P10) — 0 points, collar (nape) black wide (P11) — 2 points, lemon-crown yellow (P12) — 4 points, the back is dark gray (P13) — 3 points, chest lemon-yellow (P14) — 4 points, his head without black spots (P15) — 5 points, stripes on the wing wide (P16) — 2 points, necklace there (P17) — 1 point, lemon-yellow throat (P18) — 5 points.

ct6, XH 51045 (series and number of the ring), the date of catch — 7.05, floor (2 f), a subspecies *citreola*, eyebrow yellow (P10) — 2 points, collar (nape) is absent (P11) — 0 points, the crown gray yellow (P12) — 3 points, back light gray (P13) — 1 point, chest yellowish (P14) — 2 points, the nape gray (P15) — 1 point, stripes on the wing tight (P16) — 1 point, necklaces no (P17) — 0 points, throat yellow (P18) — 2 points.

ct7, XH 51047 (series and number of the ring), the date of catch — 7.05, floor (1 m), a subspecies *werae*, eyebrows not (P10) — 0 points, collar (nape) narrow dark gray (P11) — 2 points, the crown Lemon yellow (P12) — 4 points, back gray (P13) — 2 points, chest pale lemon-yellow (P14) — 3 points, the nape gray (P15) — 1 point, stripes on the wing wide (P16) — 2 points, necklace there (P17) — 0 points, throat lemon-yellow (P18) — 5 points.

ct8, XH 51049 (series and number of the ring), the date of catch — 7.05, floor (1 m), a subspecies *werae*, eyebrows not (P10) — 0 points, collar (nape) narrow dark gray (P11) — 2 points, the crown Lemon yellow (P12) — 4 points, back gray (P13) — 2 points, chest lemon-yellow (P14) — 4 points back of the head with a large black spot (P15) — 4 points, stripes on the wing wide (P16) — 2 points, necklaces no (P17) — 0 points, throat lemon-yellow (P18) — 5 points.

ct9, XH 51068 (series and number of the ring), hybrid, date gear — 9.05, floor (1 m), a subspecies *werae*, eyebrows not (P10) — 0 points, collar (nape) narrow dark gray (P11) — 2 points, crown lemon-yellow (P12) — 4 points, back gray (P13) — 2 points, chest lemon-yellow (P14) — 4 points back of the head with a large black spot (P15) — 4 points, stripes on the wing wide (P16) — 2 points, necklaces no (P17) — 0 points, throat lemon-yellow (P18) — 5 points.

ct10, XH 51736 (series and number of the ring), hybrid, overfishing 2011, the date of catch — 9.05, floor (2 f), a subspecies *citreola*, eyebrow ocher-yellow (P10) — 3 points, collar (nape) is absent (P11) — 0 points, the crown gray (P12) — 1 point, back gray (P13) — 2 points, chest yellowish (P14) — 2 points, the nape gray

(P15) — 1 point, stripes on the wing tight (P16) — 1 score a necklace there (P17) — 1 point, throat buffy-yellowish (P18) — 4 points.

**Table 3.** The results of correlation analysis of signs morphometry and coloration of feather *Motacilla flava*, received after the standardization of data

Char-acters	P1	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16
P1	1.000	-0.306	-0.561	<b>-0.851</b>	-0.256	-0.237	-0.279	<b>-0.840</b>	0.267	-0.430	0.392	0.048	-0.430	<b>-1.000</b>	<b>-0.800</b>
P3	-0.306	1.000	-0.093	0.093	0.202	-0.397	-0.449	0.539	-0.138	-0.407	0.258	-0.450	-0.407	0.306	0.383
P4	-0.561	-0.093	1.000	<b>0.686</b>	-0.122	<b>0.644</b>	0.540	0.420	-0.247	0.567	-0.448	-0.054	0.567	0.561	0.456
P5	<b>-0.851</b>	0.093	<b>0.686</b>	1.000	0.057	0.248	0.224	0.572	-0.078	0.591	<b>-0.653</b>	-0.010	0.591	<b>0.851</b>	0.537
P6	-0.256	0.202	-0.122	0.057	1.000	-0.088	0.150	0.439	-0.409	0.264	-0.301	0.037	0.264	0.256	0.263
<b>P7</b>	-0.237	-0.397	<b>0.644</b>	0.248	-0.088	1.000	<b>0.739</b>	0.105	-0.371	0.598	-0.355	0.132	0.598	0.237	0.464
P8	-0.279	-0.449	0.540	0.224	0.150	<b>0.739</b>	1.000	0.178	-0.416	<b>0.625</b>	-0.419	0.270	<b>0.625</b>	0.279	0.221
<b>P9</b>	<b>-0.840</b>	0.539	0.420	0.572	0.439	0.105	0.178	1.000	-0.569	0.158	-0.186	-0.461	0.158	<b>0.840</b>	<b>0.738</b>
<b>P10</b>	0.267	-0.138	-0.247	-0.078	-0.409	-0.371	-0.416	-0.569	1.000	-0.194	0.177	0.571	-0.194	-0.267	-0.360
P11	-0.430	-0.407	0.567	0.591	0.264	0.598	<b>0.625</b>	0.158	-0.194	1.000	<b>-0.911</b>	0.295	<b>1.000</b>	0.430	0.399
P12	0.392	0.258	-0.448	<b>-0.653</b>	-0.301	-0.355	-0.419	-0.186	0.177	<b>-0.911</b>	1.000	-0.084	<b>-0.911</b>	-0.392	-0.252
P13	0.048	-0.450	-0.054	-0.010	0.037	0.132	0.270	-0.461	0.571	0.295	-0.084	1.000	0.295	-0.048	-0.098
P14	-0.430	-0.407	0.567	0.591	0.264	0.598	<b>0.625</b>	0.158	-0.194	<b>1.000</b>	<b>-0.911</b>	0.295	1.000	0.430	0.399
P15	<b>-1.000</b>	0.306	0.561	<b>0.851</b>	0.256	0.237	0.279	<b>0.840</b>	-0.267	0.430	-0.392	-0.048	0.430	1.000	<b>0.800</b>
P16	<b>-0.800</b>	0.383	0.456	0.537	0.263	0.464	0.221	<b>0.738</b>	-0.360	0.399	-0.252	-0.098	0.399	<b>0.800</b>	1.000

Note. Marked correlations and characters are significant at  $p < 0.050$ ;  $N = 11$  (casewise deletion of missing data).

On the basis of the obtained data of pairwise correlation analysis of features plumage and morphometry using Ward's cluster analysis the dendrogram of the investigated characters was built and their clusters were allocated (fig. 1).

On the basis of the obtained data of pairwise correlation analysis of features plumage and morphometry using Ward's cluster analysis the dendrograms of the investigated characters was built and their clusters were allocated (fig. 2).

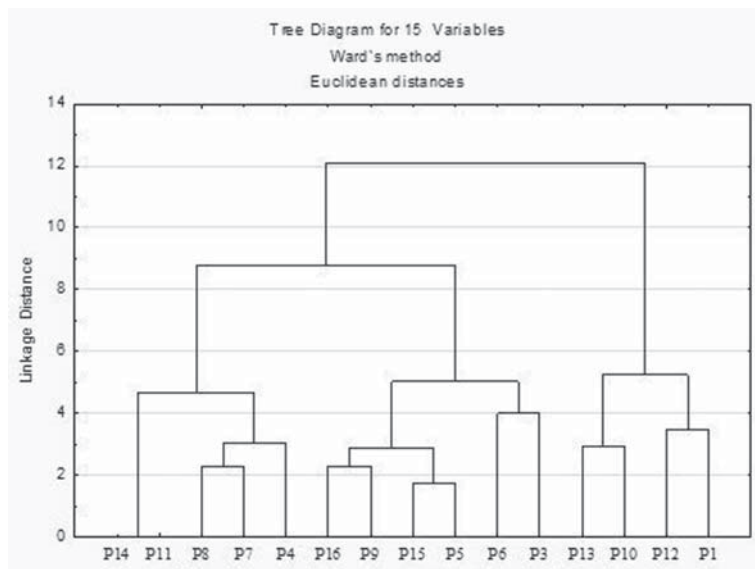


Fig. 1. Clusters signs of coloration of feather and morphometry of *Motacilla flava*, obtained by Ward method.

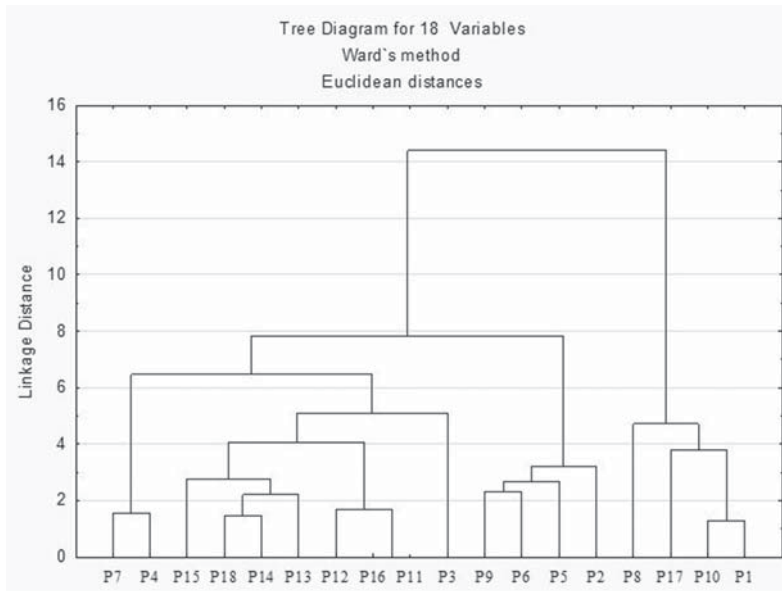


Fig. 2. Clusters signs of coloration of feather and morphometry of *Motacilla citreola*, obtained by Ward method.

The method of clustering the results of the pairwise correlation analysis of plumage color characteristics and morphometry by Ward identified three clusters of samples of the yellow wagtails *M. flava*, what was associated with the presence in samples the individuals of the subspecies *M. f. flava* and *M. f. thunbergi*, one individual was a hybrid; as well as three clusters of samples of the citrine wagtail *M. citreola*, what was associated with the presence in samples the individuals of subspecies of *M. c. citreola* and *M. c. werae*, two individuals were hybrid (fig. 3, 4).

The sequences of the gene cytochrome c-oxidase I were obtained in sequencing of the amplified DNA fragments of *M. flava* blood samples, and construction of phylogenetic trees by the nearest neighbors (Neighbor-joining) method was held. The sequences of the gene of interest were different in all dry samples taken from particular animals, indicating genetic heterogeneity of *M. flava* and *M. citreola* populations in the studied area. The sequences are aligned using ClustalW2, then with the use of JalView phylogenetic trees of all species

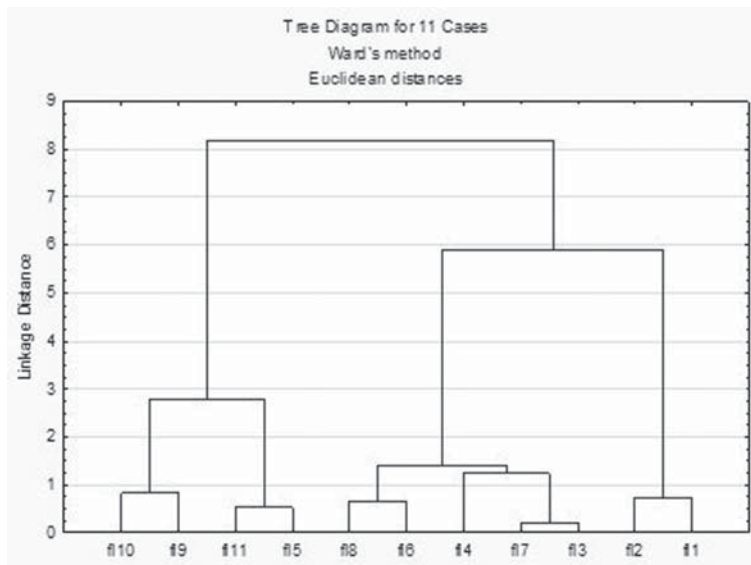


Fig. 3. Clusters of *Motacilla flava* in the space of informative features, obtained by Ward method.

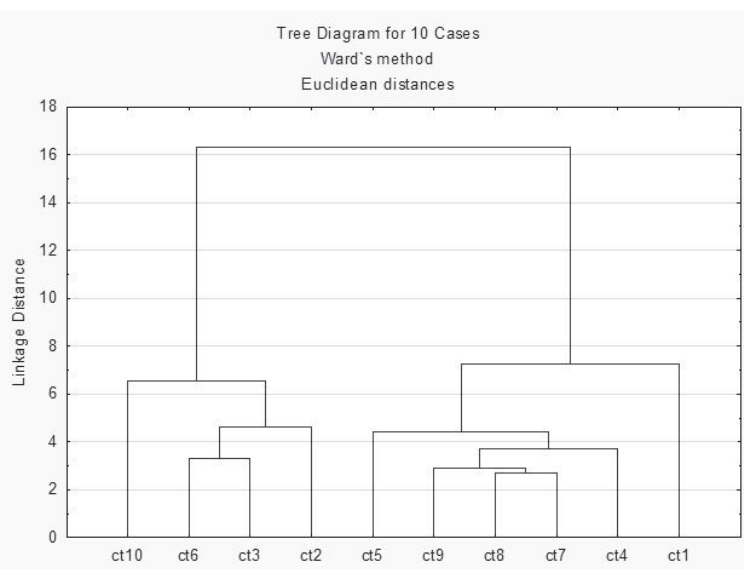


Fig. 4. Clusters of *Motacilla citreola* in the space of informative features, obtained by Ward method.

studied populations of *M. flava* and *M. citreola* were constructed with indicating genetic distances (tables 5 and 6; fig. 5, 6).

In phylogeographic analysis of nucleotide sequences of mitochondrial genes in the Middle Volga populations of traditionally recognized species *M. flava* and *M. citreola* the existence of the lines, respectively, distributed in the European part of Russia and neighboring countries that meet subspecies *M. f. flava* and *M. f. thunbergi* were discovered; the third line is related to the presence of hybrid individuals in the studied populations. Within the species *M. citreola* the existence of three lines, corresponded to subspecies *M. c. citreola* and *M. c. werae* was also revealed. This third line also corresponds to the presence in the hybrid population of the Middle Volga.

Phenotypic and genotypic population structure of *M. flava*

Coloration of the “whiskers” (the most informative indication of feather color) representatives of the subspecies *M. flava* varies from yellow to white in *M. f. thunbergi* and from gray to yellow-gray in *M. f. flava*. According to the genetic research five individuals belonged to the subspecies *M. f. thunbergi*, six — to the subspecies



Fig. 5. Phylogenetic tree species *Motacilla flava*, built on the basis of genetic analysis of the gene sequences of cytochrome c oxidase I in the program JalView, method Average Distance (weighted average).

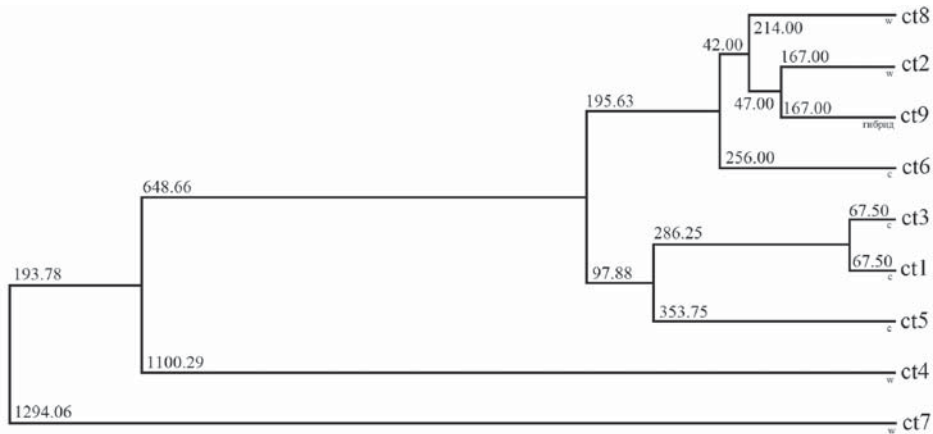


Fig. 6. Phylogenetic tree species *Motacilla citreola*, built on the basis of genetic analysis of the gene sequences of cytochrome c oxidase I in the program JalView, method Average Distance (weighted average).

*M. f. flava*. In the representatives of the subspecies *M. f. thunbergi* there are several variants of “mustache” plumage coloration: specimen with yellow “mustache” (1 individual), half yellow-half white “mustache” (2 individuals), and white “mustache” (2 individuals). The representatives of the subspecies *M. f. flava* also have different “mustache” plumage coloration: with a gray-yellow “mustache” (1 individual), with gray “mustache” (2 individuals), one individual with a half-white “mustache” and 2 individuals with white “mustache”. The overall phenotype ratio is 4 white : 3 yellow half (half white): 2 gray : 1 yellow : 1 gray-yellow. Probably, this feature can be inherited by the type of complementarity or incomplete dominance.

#### Phenotypic and genotypic population structure of *M. citreola*

The presence of “necklace” (the most informative indication of feather color) in the representatives of *M. citreola* subspecies varies discretely. It presents in the representatives of *M. c. citreola* subspecies but absents in the representatives of *M. c. werae* subspecies. Hybrid individuals also have two similar phenotype states by the presence of “necklaces”. Molecular genetic study of 10 *M. citreola* individuals revealed that 4 individuals belonged to the subspecies *M. c. werae*, 4 individuals — to subspecies of *M. c. citreola*, two individuals were hybrid. In representatives of the subspecies *M. c. citreola* phenotypic splitting on the basis of the existence of “necklace” was in 2 animals developed a necklace, from 2 individuals “necklace” is missing. At 4 representatives of the subspecies *M. c. werae* «Necklace” is missing. Ratio for a given trait phenotypes — 7 : 3 (2 : 1). Probably, this feature can be inherited as follows test cross: allele Aa (lack of “necklaces”) — genotype of *M. c. werae*, AA allele (presence of “necklaces”) — genotype of *M. c. citreola*. Then, when crossed will be a splitting of 2 Aa (*werae*): 1 aa (*citreola*).

In spite of this given subspecies on a number of futures in their morphology biology and ecology, generally are quite different, what evidenced by the comparative analysis of mitochondrial DNA and allows us to esteem these forms as different species. Certain ratios of genotypes and phenotypes in the space of the habitat can mark the areas of hybridization of the phenotypes.

#### Conclusions

Genetic structure of the Middle Volga populations of Yellow Wagtail *M. flava* and citrine wagtail *M. citreola* is uniform. Subpopulations with dominated subspecies *M. f. flava* and *M. f. thunbergi*, subspecies of *M. c. citreola* and *M. c. werae*, were revealed; besides there were also hybrid birds.



**Table 4.** The results of correlation analysis of signs morphometry and coloration of feather *Motacilla citreola*. received after the standardization of data. Marked correlations and characters are significant at  $p < .010$ ; N = 10 (casewise deletion of missing data)

Character	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18
P2	-0.408	1.000	-0.408	-0.077	0.526	0.590	0.115	0.081	0.392	-0.680	0.408	0.680	0.000	0.287	0.291	0.408	-0.509	-0.000
P3	-0.250	-0.408	1.000	-0.086	-0.090	-0.078	0.061	-0.330	0.140	-0.037	0.250	0.037	0.000	0.273	0.109	0.250	-0.089	0.433
P4	-0.253	-0.077	-0.086	1.000	-0.042	-0.655	<b>0.865</b>	0.009	-0.196	-0.132	0.253	0.132	0.531	0.401	0.177	0.253	-0.150	0.321
P5	<b>-0.764</b>	0.526	-0.090	1.000	1.000	0.631	-0.021	-0.499	0.627	-0.729	<b>0.764</b>	0.729	0.406	0.714	0.546	<b>0.764</b>	-0.232	0.623
P6	-0.306	0.590	-0.078	0.631	0.631	1.000	-0.437	-0.205	0.704	-0.456	0.306	0.456	-0.151	0.093	0.114	0.306	-0.149	0.101
<b>P7</b>	-0.392	0.115	0.061	-0.021	-0.021	-0.437	1.000	0.031	-0.099	-0.339	0.392	0.339	0.599	0.472	0.290	0.392	-0.168	0.408
P8	0.446	0.081	-0.330	-0.499	-0.499	-0.205	0.031	1.000	-0.592	0.120	-0.446	-0.120	-0.486	-0.581	-0.321	-0.446	-0.301	-0.727
<b>P9</b>	-0.427	0.392	0.140	0.627	0.627	0.704	-0.099	-0.592	1.000	-0.373	0.427	0.373	0.181	0.410	0.323	0.427	0.014	0.416
P10	<b>0.909</b>	-0.680	-0.037	-0.729	-0.729	-0.456	-0.339	0.120	-0.373	1.000	<b>-0.909</b>	<b>-1.000</b>	-0.440	-0.729	-0.524	<b>-0.909</b>	0.526	-0.590
P11	1.000	0.408	0.250	<b>0.764</b>	<b>0.764</b>	0.306	0.392	-0.446	0.427	<b>-0.909</b>	1.000	<b>0.909</b>	0.645	<b>0.899</b>	0.576	1.000	-0.356	<b>0.866</b>
P12	<b>-0.909</b>	0.680	0.037	0.729	0.729	0.456	0.339	-0.120	0.373	-1.000	<b>0.909</b>	1.000	0.440	0.729	0.524	<b>0.909</b>	-0.526	0.590
P13	-0.645	0.000	0.000	0.406	0.406	-0.151	0.599	-0.486	0.181	-0.440	0.645	0.440	1.000	0.757	0.638	0.645	0.345	<b>0.782</b>
P14	<b>-0.899</b>	0.287	0.273	0.714	0.714	0.093	0.472	-0.581	0.410	-0.729	<b>0.899</b>	0.729	0.757	1.000	<b>0.792</b>	<b>0.899</b>	-0.146	<b>0.880</b>
P15	-0.576	0.291	0.109	0.546	0.546	0.114	0.290	-0.321	0.323	-0.524	0.576	0.524	0.638	<b>0.792</b>	1.000	0.576	0.161	0.523
P16	1.000	0.408	0.250	<b>0.764</b>	<b>0.764</b>	0.306	0.392	-0.446	0.427	<b>-0.909</b>	1.000	<b>0.909</b>	0.645	<b>0.899</b>	0.576	1.000	-0.356	<b>0.866</b>
<b>P17</b>	0.356	-0.509	-0.089	-0.232	-0.232	-0.149	-0.168	-0.300	0.014	0.526	-0.356	-0.526	0.345	-0.146	0.161	-0.356	1.000	0.000
P18	<b>-0.866</b>	0.000	0.433	0.623	0.623	0.101	0.408	-0.727	0.416	-0.590	<b>0.866</b>	0.590	<b>0.782</b>	<b>0.880</b>	0.523	<b>0.866</b>	0.000	1.000

**Table 5. Genetic distances between the studied samples of individuals of *Motacilla flava* in the Middle Volga populations obtained using MEGA 4**

Series, number of rings	XH 51067	XH 51060	XH 51058	XH 51053	XH 51052	XH 51051	XH 51048	XH 51046	XH 51039	XH 51036	XH 50736
XH 51067	–	0.009	0.009	0.009	0.009	0.009	0.009	0.003	0.009	0.009	0.014
XH 51060	0.009	–	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.006
XH 51058	0.009	0.000	–	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.006
XH 51053	0.009	0.000	0.000	–	0.000	0.000	0.000	0.006	0.000	0.000	0.006
XH 51052	0.009	0.000	0.000	0.000	–	0.000	0.000	0.006	0.000	0.000	0.006
XH 51051	0.009	0.000	0.000	0.000	0.000	–	0.000	0.006	0.000	0.000	0.006
XH 51048	0.009	0.000	0.000	0.000	0.000	0.000	–	0.006	0.000	0.000	0.006
XH 51046	0.003	0.006	0.006	0.006	0.006	0.006	0.006	–	0.006	0.006	0.011
XH 51039	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.006	–	0.000	0.006
XH 51036	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000	–	0.006
XH 50736	0.014	0.006	0.006	0.006	0.006	0.006	0.006	0.011	0.006	0.006	–

**Table 6. Genetic distances between the studied samples of individuals of *Motacilla citreola* in the Middle Volga populations obtained using MEGA 4**

Series, number of rings	XH 51047	XH 51049	XH 51041	XH 51068	XH 51045	XH 51042	XH 47691	XH 51043	XH 51044
XH 51047	–	0.011	0.007	0.007	0.011	0.004	0.004	0.004	0.011
XH 51049	0.011	–	0.004	0.004	0.015	0.007	0.007	0.007	0.015
XH 51041	0.007	0.004	–	0.000	0.011	0.004	0.004	0.004	0.011
XH 51068	0.007	0.004	0.000	–	0.011	0.004	0.004	0.004	0.011
XH 51045	0.011	0.015	0.011	0.011	–	0.007	0.007	0.007	0.015
XH 51042	0.004	0.007	0.004	0.004	0.007	–	0.000	0.000	0.007
XH 47691	0.004	0.007	0.004	0.004	0.007	0.000	–	0.000	0.007
XH 51043	0.004	0.007	0.004	0.004	0.007	0.000	0.000	–	0.007
XH 51044	0.011	0.015	0.011	0.011	0.015	0.007	0.007	0.007	–

At the same time individuals like *M. f. flava* and *M. f. thunbergi*, and *M. c. citreola* and *M. c. werae* genetically well differentiated with maximum genetic distance equal to 524,30 and 1100,29, 1294,06 suitability to meet at *M. flava* subspecies level, while *M. citreola* species level differences. The most informative signs of morphometric and feather coloration in *M. flava* — tarsus length, length and color of the “whiskers”, and in *M. citreola* — as the length of the tarsus and the length of the body other than these symptoms — the presence of “necklaces” were revealed.

Subspecies forms of *M. flava* — *M. f. flava*, *M. f. thunbergi* are included in the Western complex of *M. flava* forms; subspecies form of *M. citreola* — *M. c. citreola*, *M. c. werae* constitute a separate branch of the genetic group of polytypical *M. flava* s. 1. (Red'kin, 2001 b). In the North-Western and Northern Europe form of *M. f. thunbergi* is common; there mixed populations of *M. f. flava* and *M. f. thunbergi* are also found at nesting. Throughout its habitat males of *M. f. thunbergi* dwell sympatricly with white “eyebrows” form of *M. f. flava*, and form all variants of transitions between these forms by hybridization. The spectrum of autogenetic processes in populations of *M. flava* and *M. citreola* throughout species habitat in conditions of wide sympatry reflects the mechanisms of reproductive isolation forms of specific and subspecies rank and is the result of microevolution of the group — polytypic complex of *M. flava*.

Thus, the possibility of genetic separation between sympatric breeding populations of the yellow wagtail, *M. flava* and citrine wagtail *M. citreola* in the Middle Volga was shown.

Phylogeographic analysis of the nucleotide sequences of the mitochondrial gene cytochrome oxidase I in the studied populations of “yellow” wagtails revealed the existence of separate lines distributed in the European part of Russia and neighboring countries and linked with the presence of subspecies of *M. f. flava*, *M. f. thunbergi* and *M. c. citreola*, *M. c. werae* respectively.

Thus the forms of *M. c. citreola* and *M. c. werae* owing to the significant genetic distances deserve the status of species.

Despite wide sympatry in nesting places, there is a selective mating between males and females of each studied species, what prevents the free crossing and supports isolating mechanisms in populations.

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