

Intergradation between the Herring Gull *Larus argentatus* and the Southern Herring Gull *Larus cachinnans* in European Russia

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Abstract—Five gull populations along a 2000-km transect, running from the Barents Sea coast in the Kola Peninsula to Nizhegorodskaya oblast, were studied with respect to external morphology, vocalization, and DNA features. The transect crosses the easternmost part of the *L. argentatus* range, as well as areas of the Middle Volga basin inhabited by the gulls of *cachinnans*-like type. All the morphological and behavioral features proved to form a cline along the transect. Populations of the Barents Sea and the White Sea represent typical *L. argentatus*, while in those inhabiting the Upper and Middle Volga basin, *L. cachinnans* features predominate. The population of the Baltic Sea region is intermediate. Comparative DNA analysis by RAPD method has revealed introgression of *L. argentatus* genes southeastward, up to Nizhegorodskaya oblast, and those of *L. cachinnans* genes in the opposite direction, to the Baltic Sea. Two scenarios of introgression are discussed: via *L. cachinnans omissus* dispersal from Fennoscandia along the Volga basin and due to the expansion of the *L. c. cachinnans* range to the north. Both processes may proceed simultaneously.

INTRODUCTION

The concept of biological species prevailing in modern zoology is often difficult to apply to real situations, notwithstanding its apparent similarity and consistency. The so-called “large white-headed gull complex” represents a good example of difficulties in applying this concept to demarcation of species. This complex consists of about 20 taxa, whose range embraces almost the entire Holarctics. There is no generally accepted view on their nomenclature status. These taxa may be grouped by degree of similarity in various combinations, and the number of “good” polytypic species varies from 3 to 15 in different schemes. This is related to the fact that many taxa, well-differentiated morphologically and, hence, deserving the status of “taxonomic species,” are not closed genetic systems, because they are connected to other taxa by more or less intensive gene flows (Panov, 1989).

The pairs of forms, which are completely isolated reproductively in some areas (i.e., representing “good biological species”) but freely hybridize in others, represent the main problem for the concept of biological species. These taxa illustrate the situation of secondary “species fusion” (Mayr, 1969). The gulls *Larus argentatus* and *L. hyperboreus* provide a good example of such a type of relationships within the complex of herring gulls. These clearly differentiated species are sympatric and reproductively isolated over vast areas of the Subarctic. They hybridize intensively only in two distant regions, Iceland and northeastern Alaska (Ingolfsson, 1970, 1987; Spear, 1987).

In this work, the authors try to analyze another situation of such a kind, namely, the example of interrelationships of the herring gulls *Larus argentatus* and *L. cachinnans*; this is a situation of allopatric species gradually dispersing towards one another. This process is most evident in Western Europe. In particular, the southward dispersal of *L. argentatus* started in the late 1960s, and the northward dispersal of *L. cachinnans*, in 1976. At present, both species form mixed nesting colonies. Mixed *argentatus* × *cachinnans* pairs sometimes occur, but all attempts to nest are unsuccessful (Yesou, 1991; Yesou *et al.*, 1994).

A similar tendency toward counter-dispersal of these species takes place in Eastern Europe. Rapid expansion of *L. argentatus* southwards from Fennoscandia and in the Baltic was documented in detail in the first quarter of the 20th century (see Mierauskas *et al.*, 1991, for a review). Earlier (Monzиков and Panov, 1996a, 1996b), we provided evidence for the hypothesis of current dispersal of *L. cachinnans* upstream along the Volga basin. In general, the situation in Eastern Europe differs from that described for western France. There is no direct secondary contact between genetically pure populations of *L. argentatus* and *L. cachinnans*. However, our data allow us to suppose that allopatric autochthonous populations of these species are connected by gene flows through a chain of intermediate hybrid populations. The purpose of this work is to test this hypothesis.

MATERIALS AND METHODS

To test this hypothesis, we undertook a comparative analysis of morphobiological peculiarities of gulls in the following transect: Ainovy Islands, Barents Sea (population 1); Kandalaksha Bay of the White Sea, Murmansk oblast (population 2); Remisaar Island, the Gulf of Finland of the Baltic Sea, Leningradskaya oblast (population 3); Rybinsk Reservoir, Yaroslavl oblast (population 4); Sitniki Village, Nizhegorodskaya oblast (population 5). The transect length was ca. 2000 km.

For comparison, we used materials on the genetically pure populations of *L. cachinnans* from the Caspian Sea (population 6) and Black Sea. Phenotypic features of 78 live mature birds and 87 chicks were analyzed. Blood samples were taken for DNA analysis by modified PCR method. Adult gulls were caught from their nests by narcotization of birds using baits with α -chloralose, 33 mg per a bird weighing 800–1500 g. We visually examined phenotypic composition of all the colonies visited and data obtained on nesting biology of all populations, as well as phonograms and videos characterizing the specificity of signal behavior of the gulls of the Ainovy Islands, Kandalaksha Bay, and Rybinsk Reservoir. The sounds were recorded using Realistic and Sony TCM-121 portable tape recorders, and videos were made using a CCD-TR570E camera. We used A-PC Avisoft–Sonagraph software for the analysis of phonograms. In addition, we analyzed gull collections in the Zoological Museum of Moscow State University, totalling 276 specimens. When analyzing morphometric features, we used different methods of cluster analysis: agglomerative methods (singular and full relations, unweighted mean relation, and the Ward method, all with Euclidean distance) and iterative methods (the *k*-mean method). Calculations were based on standardized data. Due to a lack of data on males from the population from the Rybinsk Reservoir, clustering was done by female samples from all the populations studied.

The analysis of geographic variation in the “long call” acoustic signal was done on the basis of 170 phonograms using 7 independent variables: durations of the last “long syllable” in the initial part of the signal; second and fifth short syllables; pauses between “long” and the first short syllables, between the first and second and the fourth and fifth short syllables (all in ms); as well as the value of interharmonic interval, in kHz. The total sample was divided into 6 groups by the number of geographic localities, or local populations, where the phonograms were obtained. Sample size ranged from 16 to 52 phonograms. During the discriminant analysis, we obtained a classification function distinguishing two terminal groups of the transect, namely, the signals of genetically pure (by our hypothesis) gulls *L. argentatus* from Barents Sea, group 1, and *L. cachinnans* from the Caspian Sea, group 6. Using this classification function, we calculated *a posteriori* probabili-

ties of attribution of all signals from the groups 2–5 to the vocalization type of *L. argentatus* or *L. cachinnans*. In addition, we undertook discriminant analysis for six and seven groups. In the last case, it was the sample from population 5, Nizhegorodskaya oblast, which was *a priori* divided into two subgroups, including signals typical for *L. argentatus* and *L. cachinnans*, respectively. Group numbers (1–6 and 1–7) were used as an independent variable. The resulting Machalanobis distance matrix between groups was used for classification of groups with use of different methods of cluster analysis: full relations, unweighted method of mean relation, and the Ward method. In addition, we made clusterization of all 170 cases, i.e., particular phonograms, without their *a priori* classification, i.e., without preliminary attribution to one of the six or seven groups. In this analysis, we used the above-mentioned methods with different metrics (Euclidean and Pearson coefficient).

The analysis of DNA polymorphism was conducted using RAPD (modified method of polymerase chain reaction with random primer). This method allows us to compare taxon-specific sets of “bands” (patterns) obtained as products of DNA amplification with a random primer. For identification of taxa, the RAPD method was modified by us for future labeling of amplification products and their separation in polyacrylamide gel with subsequent radiography. This provides for higher resolution of this method applied to amplification products of similar lengths, owing to the better separation of fragments. The zone of differences between “pure” *L. argentatus* and *L. cachinnans* is in the area of 280–300 and 550–600 nucleotides, respectively. Our collection for genetic analysis included blood samples from 19 taxa (species and subspecies) from the family Laridae (257 specimens). We analyzed blood samples from 17 individuals from 3 autochthonous populations of *L. argentatus*, 19 individuals from the autochthonous population of *L. cachinnans* from the Sea of Azov, and 52 individuals from the Baltic region and the Volga basin.

The blood obtained from the wing vein was mixed with conservation buffer containing 50 mM EDTA, 1% NaF, and thymol and was stored without freezing at temperatures not lower than 4°C. We studied the total DNA extracted by phenol-chloroform method without preliminary sedimentation of nuclei. Each polymerase chain reaction (PCR) was performed using one randomly chosen primer. We used two 10-nucleotide primers, designated below as primer 29 (5'-CCGGCCT-TAC-3') and 45 (5'-GCCGTCCGAG-3'). The PCR procedure was made up of two steps. The first, without labeled dNTP, consisted of two cycles of incubation at 95, 38, and 72°C (5 min for each temperature), and 10 cycles with the following conditions: 95°C, 1 min; 40°, 1 min; and 72°C, 2 min. The second step, with labeled dNTP added to the reaction mixture, included 25 cycles of incubation at 95, 45, and 72°C (1 min at each temperature). The reaction mixture at the first step



Fig. 1. Distribution of different gull taxa in European Russia and adjacent territories: (a) places of data collection (1, Ainovy Islands; 2, Kandalaksha; 3, Remisaar Island in the Gulf of Finland, the Baltic Sea; 4, Rybinsk Reservoir; 5, Sitnikovskie open-cut mines in Nizhegorodskaya oblast; 6, Sivash); (b) probable directions of gene flows; and (c) range of “yellow-legged” gulls of the *omissus* type.

consisted of Taq polymerase (0.5 U per sample), primer (1 μ M), $MgCl_2$ (2.5 mM), dNTP (0.8 μ M), and working buffer. At the second step, the reaction mixture was without primer but contained labeled dNTP (1 MB per 40 samples). The amount of DNA per sample was 100 ng. Products of DNA amplification were separated in conventional 4% polyacrylamide gel, which was followed by autoradiography. In the resulting spectrum, which contained more than 90% fragments (bands) common for all studied populations, we distinguished those specific for autochthonous populations of *L. argentatus* and *L. cachinnans*. The distribution of these features was studied along the transect in the regions dividing the ranges of species.

We shall designate any stable combination of two or more bands as a “pattern,” and a particular band designated in the spectrum by its own number as the “pattern element.”

RESULTS

In European Russia, autochthonous ranges of *L. argentatus* and *L. cachinnans* are divided by vast

areas. Gull populations of unclear taxonomic position have been formed in these areas in the last few decades (Fig. 1). There is a mixture of features of several taxa, *argentatus*, *cachinnans*, and *heuglini*, in these populations. This allows us to suppose a hybrid origin of these populations (Monzиков and Panov, 1996a, 1996b). To test this hypothesis, we compared morphometric features of *L. argentatus*, *L. cachinnans*, and individuals from populations inhabiting territories between their ranges, as well as color patterns and peculiarities of signal behavior of all gulls studied.

Morphometric Features

The results of comparative analysis indicate that *L. argentatus* and *L. cachinnans* of the nominative subspecies differ significantly in general proportions (Table 1, Fig. 2a, clusters I and III). The beak is significantly shorter and more massive in *L. argentatus*; tarsus in this species is longer: differences in this feature are significant only in females ($p < 0.001$). The wing is longer in *L. cachinnans*: differences in male samples are significant at $p < 0.01$. Populations from territories

Table 1. Morphometric features of different populations of the *Larus argentatus*–*cachinnans*–*fuscus* complex in European Russia

Parameters	Taxon/Population				
	<i>argentatus</i> , Barents Sea*	Baltic Sea**	Rybinsk Reservoir	Nizhni Novgorod	<i>cachinnans</i> , Black Sea**
Males					
Wing	468.9 (0.2) 430–520 (169)	452.0 (2.4) – (16)	–	448.6 (3.7) 425–475 (15)	464.6 (1.6) – (35)
Beak length along its upper ridge	59.9 (0.3) 48–69 (169)	57.6 (0.7) – (16)	–	58.0 (0.6) 55–63 (15)	62.1 (0.4) – (34)
Beak height at the level of gonius	21.1 (0.1) 19–26 (156)	19.6 (0.2) – (16)	–	19.3 (0.3) 17–21 (15)	19.5 (0.2) – (35)
Head with beak	–	131.0 (1.0) – (16)	–	128.5 (1.6) 119–135 (10)	133.6 (0.5) – (35)
Keel	–	88.8 (1.2) – (16)	–	92.3 (0.7) 91–93 (3)	86.2 (0.5) – (35)
Tarsus	71.5 (0.3) 60–87 (169)	68.2 (1.0) – (16)	–	68.8 (0.9) 64–76 (15)	72.3 (0.4) – (35)
Females					
Wing	440 (0.9) 310–510 (262)	434.0 (1.9) – (26)	432.4 (5.1) 412–450 (7)	434.5 (3.0) 410–455 (15)	440.8 (1.0) – (71)
Beak length along its upper ridge	53.5 (0.3) 46–66 (262)	51.9 (0.4) – (26)	52.9 (0.6) 51–55 (7)	53.7 (0.2) 52–56 (15)	56.3 (0.3) – (71)
Beak height at the level of gonius	18.8 (0.6) 15–23 (248)	17.6 (0.1) (26)	18.0 (0.2) 17–19 (7)	17.9 (0.2) 17–19 (15)	17.2 (0.1) (71)
Head with beak	121.2 (1.9) 119–125 (3)	120.5 (0.6) – (26)	119.8 (1.3) 115–124 (7)	120.9 (0.9) 117–125 (9)	122.9 (0.3) – (71)
Keel	86.8 (1.6) 84–90 (3)	80.9 (0.7) – (26)	84.4 (0.8) 81–88 (7)	84.5 (3.0) 80–89 (4)	80.4 (0.4) – (71)
Tarsus	65.1 (0.3) 53–80 (264)	64.9 (0.7) – (26)	59.2 (1.2) 55–63 (7)	66.1 (0.5) 61–69 (15)	66.9 (0.3) – (71)

Note: For each feature, \bar{x} (SE), range of variation, and number of specimens studied are shown.

* According to Tatarinkova, 1975.

** According to Mierauskas *et al.*, 1991.

separating the ranges of *L. argentatus* and *L. cachinnans* (Fig. 1a, cluster II) in their proportions are closer to the former species, slightly deviating towards the latter species.

We failed to find gradual clinal variation in dimensional features upon transition from the autochthonous populations of *L. argentatus* to the populations living in northwestern and central European Russia and to autochthonous populations of *L. cachinnans*. The size of individuals seems to decrease on the interval from the Baltic to Nizhegorodskaya oblast, compared to that at the ends of the transect, in autochthonous ranges of both species. This also follows from a comparison of egg sizes of gulls from different regions (Table 2). The

results of cluster analysis indicated that “Baltic” gulls from the coast of the Gulf of Finland do not belong to genetically pure populations of *L. argentatus*, in contrast to conclusions by Mierauskas *et al.* (1991) (Fig. 2b and 2c).

Features of Coloration

In contrast to widely varying and overlapping dimensional features of *L. argentatus* and *L. cachinnans*, at least some features of coloration remain constant and species-specific throughout the ranges of both species. In particular, this concerns the specificity of the color pattern at the tips of the primaries (Fig. 3), as

well as the coloration of feet and bare rings around the eyes. In all subspecies of *L. argentatus*, the podotheca is dirty pink, and the eye rings are yellow, sometimes with an orange hue. In *L. cachinnans*, the podotheca is of different shades of yellow, the eye ring is from orange-red to carmine-red (Cramp and Simmons, 1983).

A comparative analysis of phenotype composition of gull populations living on the White Sea (Kandalaksha Bay), Baltic Sea (the Gulf of Finland), Rybinsk Reservoir (Yaroslavl oblast), and the Upper Volga basin (Nizhegorodskaya oblast) revealed that only the first of these populations is characterized by a full set of species-specific features of coloration of *L. argentatus*. At the same time, the population localized about 750 km south of the Gulf of Finland differs from both *L. argentatus* populations of the Barents Sea and the gull population of the White Sea, which are phenotypically similar, in that the former is characterized by the pronounced variability of podotheca and eye ring coloration. No specimen from the 23 studied had the pink podotheca typical for *L. argentatus*. In the majority of individuals, it was gray with a pinkish tint, often with a different admixture of yellow, or in different shades of yellow in other birds. Basically, variation in coloration of the podotheca is gradual rather than discontinuous. In a certain sense, the same concerns variation in the coloration of the eye ring, which is yellow and yellow-orange, as is in typical *L. argentatus*, while in others, it is light- or bright-orange (Table 3). Thus, it is evident that the "*L. argentatus*" population of the Gulf of Finland receives a significant gene flow from some other form, which initially has the same features that characterize autochthonous populations of *L. cachinnans*. In populations from regions located southeastward from the Gulf of Finland, the coloration of feet and the eye ring vary in a similar manner but with the prevalence of features of *L. cachinnans* (Sitnikovskie open-cut mines), or completely correspond to its species-specific characteristics (Rybinsk Reservoir) (Table 3).

The similar picture of gradual replacement of the features of *L. argentatus* by the features of *L. cachinnans* is evident also in the analysis of geographic variation in coloration of the primaries over the transect Barents Sea–Nizhegorodskaya oblast. As revealed from Fig. 3, the gulls from the Baltic Sea exhibit a notable increase in pigmentation of the primaries, as compared with those in populations of *L. argentatus* from the Barents and White seas. In the sample from Remisaar Island, 43.5% of individuals had a continuous black band on the fifth primary, which was never noted in *L. a. argentatus* from its autochthonous range. Some birds (8.7%) had a black color expanding onto the fourth covert. This concerns not only "yellow-legged" individuals, often identical with *L. cachinnans* in their coloration, but also "gray-legged" ones, which were erroneously considered by some authors as genetically pure *L. argentatus*.

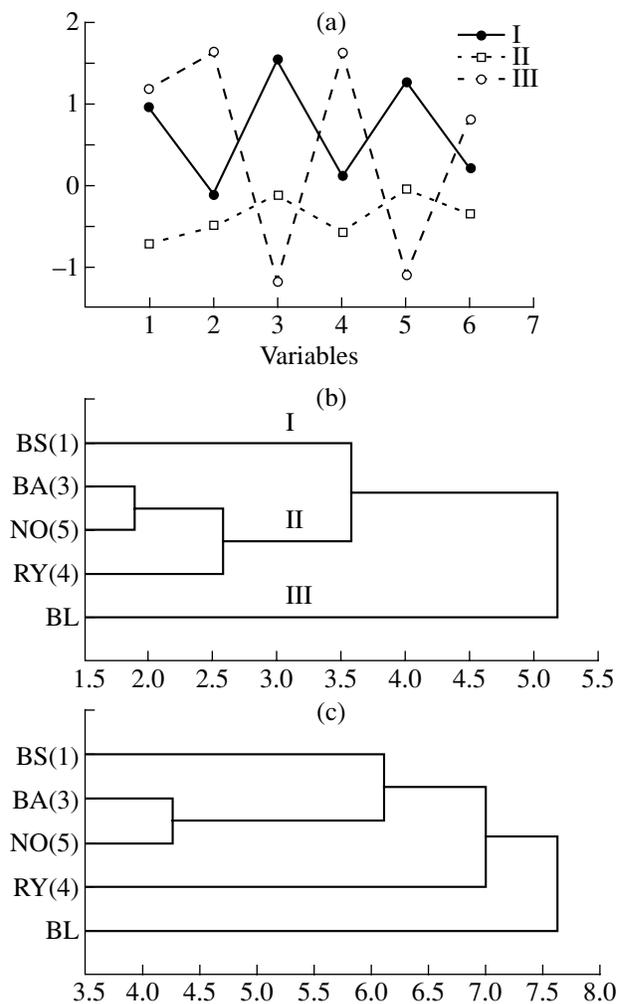


Fig. 2. Results of cluster analysis of morphometric features in the populations studied (female samples): (a) *k*-mean method. Variables: (1) wing length, (2) beak length, (3) beak height, (4) length of the head with beak, and (5) keel length, (b) tarsus length. (I) *L. argentatus* (Barents Sea, BS, population 1); (II) populations of the Baltic Sea (BA, population 3), Rybinsk Reservoir (RY, population 4), and Nizhegorodskaya oblast (NO, population 5); (III) *L. cachinnans* (Black Sea, BL). The composition of clusters as in the position b. (b) Method of full relations, Euclidean distance, standardized data. (c) Single-relation method, Euclidean distance, nonstandardized data.

About 500 km southeastward from the place of localization of the Baltic population under study, a spatially isolated gull population exists in Vologda oblast. This population began to form in the late 1960s in the Rybinsk Reservoir, which was constructed in those years (Nemtsev, 1980). By the sum of features of coloration, local gulls completely correspond to the diagnosis of *L. cachinnans*. All individuals from there had yellow-colored podotheca and an orange eye ring (Table 3). By the degree of melanization of the primaries, our sample of gulls from the Rybinsk Reservoir does not differ from the sample of *L. cachinnans* from the autochthonous range of this species (Fig. 4).

Table 2. Egg size in different populations of the *Larus argentatus*–*cachinnans*–*fuscus* complex in European Russia

Taxon/Population	Region	<i>n</i>	Length, \bar{x} (SE), range of variation	Width, \bar{x} (SE), range of variation	Source
<i>argentatus</i>	White Sea	50	74.6 (0.47) 67.1–83.2 ***	50.1 (0.23) 47.2–54.4 ***	Fil'chagov, 1991
<i>argentatus</i> x <i>omissus</i>	Baltic Sea, Gulf of Finland	57	69.8 (0.46) 61.1–80.1 **	48.4 (0.19) 44.0–51.3 ***	Our data
<i>omissus</i>	Rybinsk Reservoir	25	72.0 (0.54) 66.8–77.9 ns	49.6 (0.04) 44.7–52.8 **	Our data
<i>omissus</i> x <i>cachinnans</i> (?)	Nizhegorodskaya oblast	332	72.3 (0.20) 62.2–81.9 ***	49.9 (0.09) 44.2–55.3 ***	Monzиков and Panov, 1996
<i>cachinnans</i>	Southwestern Caspian region	126	70.1 (0.26) 63.0–78.2	48.6 (0.14) 42.1–52.3	Panov <i>et al.</i> , 1991
<i>cachinnans</i>	Black Sea	185	72.8 63.3–80.5	50.4 44.6–58.5	Kostin, 1983

Notes: Differences between *argentatus* and Caspian *cachinnans* are significant at $p < 0.001$; between *argentatus* and the gulls of Rybinsk Reservoir, at $p < 0.001$ in length and $p < 0.05$ in width. Differences between *cachinnans* and the Baltic population are nonsignificant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, differences are nonsignificant.

Table 3. Coloration of feet and the eye ring in different populations of gulls in European Russia

Featherless region	Coloration	Species/Population				
		<i>argentatus</i> , Barents Sea*	Baltic Sea (23)	Rybinsk Reservoir (10)	Nizhegorodskaya oblast (37)	<i>cachinnans</i> , Caspian Sea*
Podotheca	Pink	100.0	–	–	–	–
	Grayish, sometimes with yellow	–	69.6	100.0	8.8	–
	Yellow	–	30.4	–	91.2	100.0
Bare eye ring	Yellow, yellow-orange	100.0	39.1	–	8.1	–
	Light-orange, orange	–	60.9	100.0	83.8	–
	Orange-red, red	–	–	–	8.1	100.0

Note: Figures in brackets show the number of individuals studied.

* According to long-term observations in the colonies and the analysis of video recordings.

The phenotypic pattern of gulls from the Sitnikovskie open-cut mines, located far to the southeast and downstream the Volga River (Nizhegorodskaya oblast), was described earlier (Monzиков and Panov, 1996a, 1996b). In general, most local gulls are very similar to those from the Rybinsk Reservoir in the sum of features of coloration (Table 2, Fig. 3). The presence of numerous individuals with a red ring around the eye and hypertrophied melanization at the wing tip (black color spread onto the third primary covert) in this colony may

be associated with gene inflow from the ranges of highly pigmented *heuglingi* (Monzиков and Panov, 1996a, 1996b) and/or *barabensis*.

Therefore, there is clinal variation in the extent of melanization of coverts over the transect from the northwest to the southeast, from the southern range margin of *L. argentatus* in Fennoscandia toward the northern range margin of *L. c. cachinnans* in the lower reaches of the Volga River and the northern Caspian region.

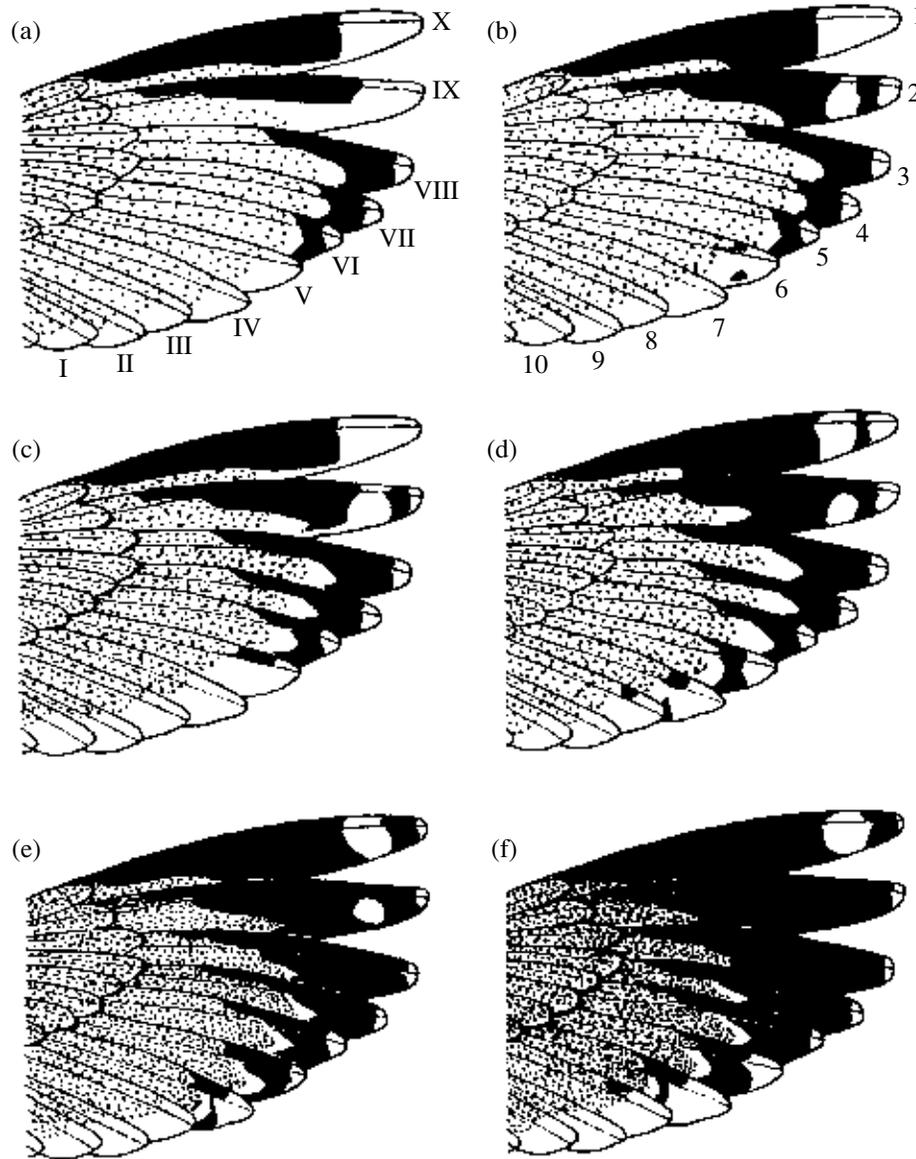


Fig. 3. Coloration of the primaries in *Larus argentatus* (a) maximum pigmentation; (b) minimal pigmentation, *L. cachinnans* (c) and (d) respectively, *L. (fuscus?) heuglingi* (e), and in the form *barabensis* (f). Roman numerals designate numbers of primaries in relation to the rules of scientific description of the wing structure, and Arabic numerals are their numbers beginning from the outer primary.

Signal Behavior

As noted earlier, signal behaviors of *L. argentatus* and *L. cachinnans* are clearly different in many parameters. This equally concerns acoustic and motor components of signalization in these three species (Panov *et al.*, 1991). All used methods of statistical analysis of geographic variation in the structure of "Long Call" over the transect provided similar results. There is a gradual change in the proportion of species-specific signals of *L. argentatus* and *L. cachinnans* from the northwest to the southeast. All 58 signals recorded in

populations 1 and 2 (Barents and White seas) are identified by the classification function as belonging to the same set of variables at $p = 1$ ("argentatus" type). In the Baltic population, the proportion of such signals is 87.5%; on the Rybinsk Reservoir, 63.1%; and in the Nizhegorodskaya oblast, 44.2%. At the same time, the proportion of signals identified as typical for *L. cachinnans* increases from 4.2%, in the Baltic population, to 31.6% in the population from Rybinsk Reservoir and 53.9% in the Nizhegorodskaya oblast. In the latter two populations, few signals are identified as intermediate: 5.3 and 1.9%, respectively. The general situation with

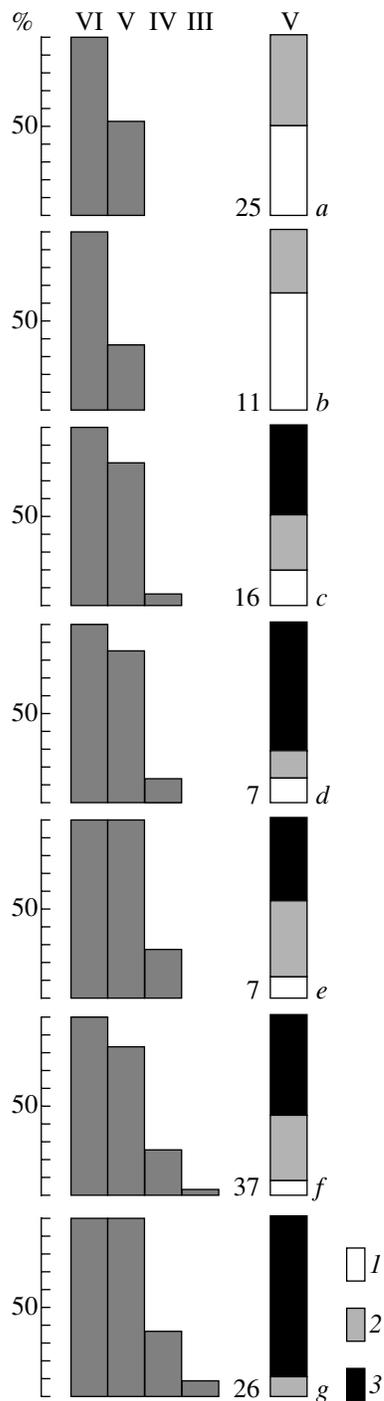


Fig. 4. Increase in the degree of pigmentation of the primaries along the Kola Peninsula–Nizhegorodskaya oblast transect and upon transition to the range of *L. cachinnans*. Proportions of individuals with black marks on the primaries from VI to III (left) and the extent of black color of the V primary (right) are shown. *a, b*—Autochthonous range of *L. argentatus* (Barents Sea and White Sea); *c, d*—Gulf of Finland of the Baltic Sea (“pink-legged” and “yellow-legged” individuals, respectively); *e*—Rybinsk Reservoir (*omissus*); *f*—Nizhegorodskaya oblast; and *g*—autochthonous populations of *L. cachinnans*. Numerals show the number of examined individuals. (1) Black mark is absent; (2) black mark present only on the outer edge of feather; and (3) continuous black band.

the degree of similarity between the populations with respect to their acoustical characteristics, as estimated by the methods of discriminant and cluster analysis, is shown in Figs. 5 and 6.

In the population from Rybinsk Reservoir, where the acoustic characteristics of the Long Call combine features of both species, We also undertook video recording of motor components of this display. It was found that all 27 videotaped demonstrations corresponded to the motor pattern typical for *L. argentatus*, although these gulls were generally similar to *L. cachinnans* in the coloration of plumage, feet, and eye ring.

In addition to the Long Call, We also recorded other signals from the acoustic repertoire of the gulls from the populations studied. A special paper will be devoted to their comparative analysis. The analysis of sonograms performed to date revealed that individuals with everyday calls typical for *L. cachinnans* prevail in the populations of Rybinsk Reservoir and Nizhegorodskaya oblast. The proportion of birds that have typical everyday calls of *L. argentatus*, according to preliminary data, decreases over the transect Gulf of Finland–Rybinsk Reservoir–Nizhegorodskaya oblast.

Comparative Analysis of DNA

Using two empirically chosen 10-nucleotide primers, I have found patterns in the distribution of DNA fragments (genetic markers) specific for the studied populations of *L. argentatus* and *L. cachinnans*. On the basis of these data, we have analyzed the occurrence of these species-specific patterns in populations whose ranges lie within areas separating the breeding ranges of *L. argentatus* and *L. cachinnans*.

Four patterns, conventionally designated as patterns A, B, C, and D, were found in isolated samples during the analysis of primer 29. Among them, pattern A is present in all populations studied. The proportion of individuals bearing this character changes from population to population irregularly, which may be explained by the relatively small sample sizes. Two patterns, B and D, were found only in the range of *L. argentatus*, and one (C) was species-specific for *L. cachinnans* (Table 4). Patterns B and D have a common element, band 3 (Table 4). The proportion of individuals with element 3 decreases from 58.5% in populations of *L. argentatus* to 17.7% in Nizhegorodskaya oblast (differences are significant at $p = 0.01$). These data are in good correspondence with the supposition about the introgression of *argentatus* genes into areas in the upper and the lower Volga. Pattern C was found in 31.6% of individuals in populations of *L. cachinnans* but, unexpectedly, proved to be absent in all the populations from the middle and upper parts of the Volga basin.

Four variants of patterns, conventionally designated as E, F, G, and H, while analyzing the distribution of

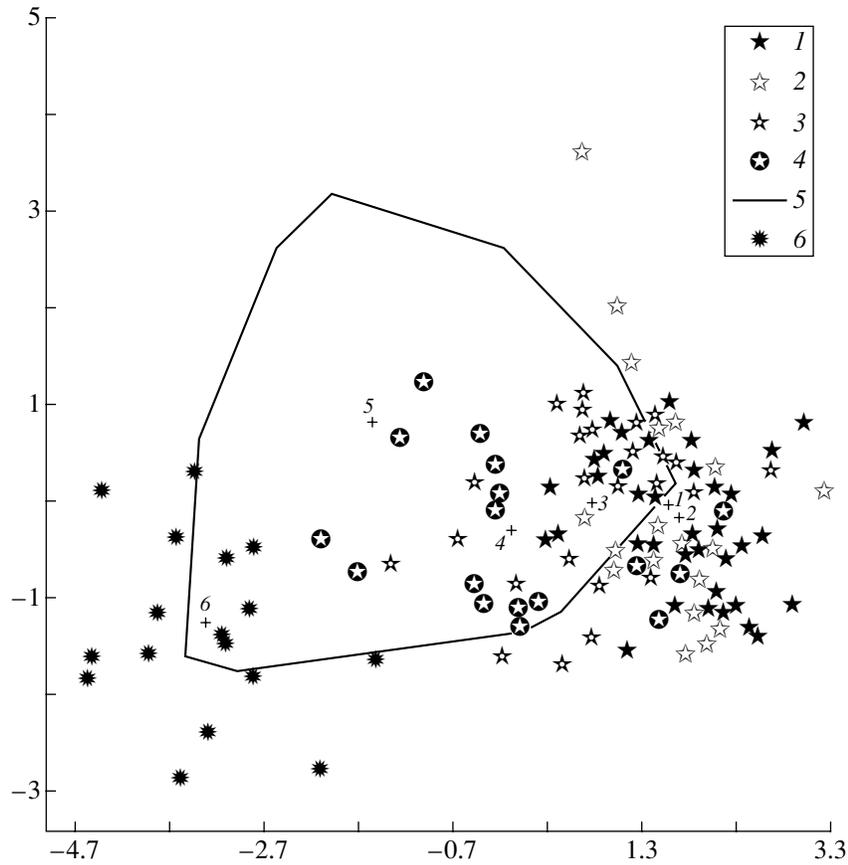


Fig. 5. Distribution of points corresponding to phonograms of the Long Call in the gulls from the populations studied in the space of the first two axes: (1, 2) autochthonous range of *L. argentatus* (Barents Sea and White Sea, respectively); (3) Gulf of Finland of the Baltic Sea; (4) Rybinsk Reservoir; (5) Nizhegorodskaya oblast (without individual points; only centroid and the range of variation are shown); and (6) autochthonous range of *L. cachinnans* (Southeastern Caspian Sea).

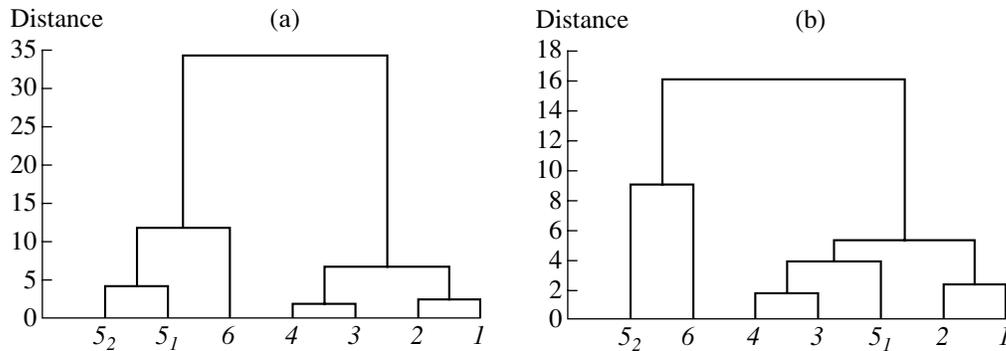


Fig. 6. Results of cluster analysis of phonograms of the Long Call of gulls from the populations studied (Machalanobis distances for the clustered groups): (a) Ward method and (b) method of unweighted mean relation. Numbers of populations (groups) as in Fig. 5. The group 5₁ is the sample from Nizhegorodskaya oblast, of *argentatus* type by its signals; 5₂ is the same, but *cachinnans* by its signals.

550–600-nucleotide DNA amplification products obtained with primer 45, were revealed (Table 5). Among them, only the first one (E) exists in all populations studied, and its occurrence is not characterized by

any distinct trends along the transect. Parameters of the frequency of pattern F also change irregularly in populations from the autochthonous range of *L. argentatus* and in gull populations from the basin of the Upper and

Table 4. Frequencies of DNA amplification patterns obtained with primer 29

Species population	Region (number of individuals studied)	Elements of pattern	Pattern variants				Proportion of elements, %
			A	B	C	D	
<i>argentatus</i>	Barents Sea (10)	no. 1	+	+	(+)		80.0(8)
		no. 2			(+)		0.0
		no. 3		+		+	50.0(5)
		Proportion of elements, % (<i>n</i>)	50.0 (5)	30.0 (3)	0.0	20.0 (2)	
	White Sea (7)	no. 1	+	+	(+)		71.4(5)
		no. 2			(+)		0.0
		no. 3		+		+	71.4(5)
		Proportion of elements, % (<i>n</i>)	28.6 (2)	42.8 (3)	0.0	28.6 (2)	
	Total (17)	no. 1	+	+	(+)		76.5(13)
		no. 2			(+)		0.0
		no. 3		+		+	58.8(10)
		Proportion of elements, % (<i>n</i>)	41.2 (7)	35.3 (6)	0.0	23.5 (4)	
Intermediate populations	Baltic Sea (15)	no. 1	+	+	(+)		100.0(15)
		no. 2			(+)		0.0
		no. 3		+		(+)	20.0(3)
		Proportion of elements, % (<i>n</i>)	80.0 (12)	20.0 (3)	0.0	0.0	
	Rybinsk Reservoir (13)	no. 1	+	+	(+)		100.0(13)
		no. 2			(+)		0.0
		no. 3		+		(+)	30.8(4)
		Proportion of elements, % (<i>n</i>)	69.2 (9)	30.8 (4)	0.0	0.0	
	Nizhegorodskaya oblast (17)	no. 1	+	+	(+)		100.0(17)
		no. 2			(+)		0.0
		no. 3		+		(+)	17.7(3)
		Proportion of elements, % (<i>n</i>)	82.3 (14)	17.7 (3)	0.0	0.0	
<i>cachinnans</i>	Sea of Azov, Caspian Sea (19)	no. 1	+	(+)	(+)		100.0(19)
		no. 2			(+)		31.6(6)
		no. 3		(+)		(+)	0.0
		Proportion of elements, % (<i>n</i>)	68.4 (13)	0.0	31.6 (6)	0.0	

Middle Volga. However, this pattern is absent in the autochthonous population of *L. cachinnans*, so it may be regarded as species-specific for *L. argentatus*. The same is true for pattern G, whose frequency gradually decreases from 21.4% to zero from the range of *L. argentatus* to the range of *L. cachinnans*. Therefore, the presence of patterns F and G in phenotypically intermediate populations may be evidence for *argentatus* gene diffusion in the basin of the middle Volga. The

characteristic of distribution of pattern H indicates the presence of an alternative process, namely, the diffusion of *cachinnans* genes into populations of this region. This pattern was found in all populations studied, except those inhabiting the Rybinsk Reservoir and localized within the autochthonous range of *L. argentatus*. The lack of this pattern in gulls from Rybinsk Reservoir may result from limited size of the sample from this area. It is also possible that here we deal with geo-

Table 5. Frequencies of DNA amplification patterns obtained with primer 45

Species population	Region (number of individuals studied)	Elements of pattern	Pattern variants				Proportion of elements, %	
			E	F	G	H		
<i>argentatus</i>	Barents Sea	no. 1				(+)	0.0	
		no. 2		+	+		62.6(5)	
		no. 3			+	(+)	37.5(3)	
		Proportion of elements, % (<i>n</i>)	37.5 (3)	25.0 (2)	37.5 (3)	0.0		
	White Sea (6)	no. 1				(+)	0.0	
		no. 2		+	(+)		50.0(3)	
		no. 3			(+)	(+)	0.0	
		Proportion of elements, % (<i>n</i>)	50.0 (3)	50.0 (3)	0.0	0.0		
	Total (14)	no. 1				(+)	0.0	
		no. 2		+	+		57.1(8)	
		no. 3			+	(+)	21.0(3)	
		Proportion of elements, % (<i>n</i>)	42.9 (6)	35.7 (5)	21.4 (3)	0.0		
	Intermediate populations	Baltic Sea (16)	no. 1				+	12.5(2)
			no. 2		+	+		25(4)
no. 3					+	+	25(4)	
Proportion of elements, % (<i>n</i>)			62.5 (10)	12.5 (2)	12.5 (2)	12.5 (2)		
Rybinsk Reservoir (12)		no. 1				(+)	0.0	
		no. 2		+	+		58.3(7)	
		no. 3			+	(+)	1.7(2)	
		Proportion of elements, % (<i>n</i>)	41.6 (5)	41.6 (5)	16.8 (2)	0.0		
Nizhegorodskaya oblast (23)		no. 1				+	21.7(5)	
		no. 2		+	+		39.1(9)	
		no. 3			+	+	39.1(9)	
		Proportion of elements, % (<i>n</i>)	39.1 (9)	26.1 (6)	13.0 (3)	21.7 (5)		
<i>cachinnans</i>	Sea of Azov	no. 1				+	18.2(2)	
		no. 2		(+)	(+)		0.0	
		no. 3			(+)	+	18.2(2)	
		Proportion of elements, % (<i>n</i>)	81.8 (9)	0.0	0.0	18.2 (2)		

graphic isolate, where the genetic processes are largely determined by the “founder effect.” Future studies will help to clarify this issue.

CONCLUSION

Thus, the areas in the Upper and Middle Volga Basin are inhabited by gulls significantly differing from

L. argentatus and *L. cachinnans*, according to diagnoses of the both species. However, by the set of features of coloration and behavior, they are much closer to *L. cachinnans* (Monzиков and Panov, 1996b). Free hybridization and intergradation of these “yellow-legged” gulls with the nominative *L. argentatus* were documented in regions positioned farther northwest from these territories, i.e., on the Baltic Sea coast

(Mierauskas *et al.*, 1991; this study). The flow of *L. argentatus* genes spreads southeastward from hybrid populations of the Baltic Sea. It was traced at least to the Nizhegorodskaya oblast. In this region, we have also found genetic features typical only for population of *L. cachinnans* studies. Therefore, we can conclude that there are two counterflows of genes from the ranges of *L. a. argentatus* and *L. c. cachinnans* through the range of “yellow-legged” gulls of the Volga River basin.

Thus, two questions arise. One concerns changes in ranges of large gulls in Eastern Europe, and another deals with their taxonomy and nomenclature. It is necessary to discuss the origin and nomenclature of “yellow-legged” gulls in the Volga River basin. Let us discuss these problems one after another.

It is generally accepted that gulls have begun to colonize the basin of the Upper and Middle Volga only in the last few decades (Nemtsev, 1980; Yudin and Firsova, 1988; Panov and Monzиков, 1996). Hypothetically, there might be at least three areas from which the gulls might penetrate this region. Firstly, the Baltic coast, inhabited by polymorphic populations formed in the first half of this century as a result of hybridization between “pink-legged” *argentatus* and “yellow-legged” *omissus* that was initially confined to water bodies of the Eastern Fennoscandia and, probably, Baltic region (Voipio, 1981; Mierauskas *et al.*, 1991). Secondly, colonization of the Volga River basin might have taken place from the south, i.e., from the range of *L. cachinnans*. Finally, the third includes the possibility of gull penetration from the populations inhabiting areas of lakes Onega and Ladoga. We hypothesize that there may be autochthonous populations of “yellow-legged” *omissus*, which were influenced by the genes of *argentatus* to a minimal extent. From the latter regions, the gulls might have colonized the Rybinsk Reservoir, which was created in the 1950s and where gull populations remain monomorphic in coloration of the podotheca. The latter would be improbable in the case of colonization of Rybinsk Reservoir by the gulls from Baltic, which are polymorphic in this feature. The gulls of the Rybinsk Reservoir differ from all other populations by a shortened tarsus (Table 1), which also needs explanation. One means of explaining this is the comparison of the populations from Rybinsk Reservoir with those from marshes of the Onega–Ladoga basin: no special studies were undertaken there. Thus, neither scenario excludes the others. So, it is possible that all three processes are parallel but have different intensities (Fig. 1).

Let us discuss the problem of the taxonomic status of “yellow-legged” gulls of the Volga Basin. This study revealed that these gulls are characterized by a distinct set of morphometric and coloration features, so they deserve distinct taxonomic status of subspecies rank. We think that these gulls should retain the existing name *omissus*. This view contradicts the opinion of

Yesou *et al.* (1994), who think that this name should not be used in future, because no clear diagnosis has been attributed to it initially, and then the name was discredited by inaccurate and often voluntary use. Assigning of this name to all gull populations of Fennoscandia, including those currently assigned to the nominative form *L. a. argentatus* (Dement'ev, 1951), serve as good example.

However, it should be taken into account that gull specialists have always applied the name *omissus* to the “yellow-legged” gulls from inland water bodies, assuming that their phenotypic and genetic peculiarities were maintained until they began to colonize habitats of maritime populations of the “pink-legged” *L. argentatus*, which made hybridization between these two forms possible (Panov and Monzиков, 1996). Data from this paper allow us to suppose that populations inhabiting the Volga basin formed as a result of southward and southeastward dispersal of gulls from the primary range of *omissus*. Until this hypothesis is disproved, we have no reasons to abandon the name *omissus* as applied to populations under consideration.

To which species does taxon under consideration belong, *L. argentatus* or *L. cachinnans*? Opinions of different authors differ significantly, and it is easy to find evidence for any alternative opinion. However, the last attempt to assign Baltic “yellow-legged” gulls of *omissus* type to the *L. argentatus* (Mierauskas *et al.*, 1991) was unsuccessful, despite the abundant empirical material used by these authors. A detailed analysis of their results provides more evidence against their hypothesis, because the *omissus* population from the marshes of Teiciu, Lithuania, in morphometric features is more similar to *L. cachinnans* than to the population from Leningradskaya oblast (*argentatus* × *omissus*), which is erroneously considered by these authors to be a standard population for *L. argentatus* (see Table 1 in their paper and Table 1 in this paper). Contrary to Mierauskas and many other authors, we think that the taxon under consideration is closest to *L. cachinnans* by the set of features analyzed. It may be considered as its subspecies, *L. cachinnans omissus*, although it has a hybrid origin.

We share the opinion of the authors who believe that *omissus* was formed in the Eastern Fennoscandia and adjacent regions as a result of old invasions of *L. cachinnans* from southern parts of its range (e.g., Voipio, 1981). There are also facts that may be used as evidence against this conclusion. In some populations from the Volga River basin, including those from the Rybinsk Reservoir, we failed to find genetic traits characterizing autochthonous populations of *L. cachinnans*. Patterns C and H serve as an example of such traits. In these populations, we also failed to observe any Long Call display with raised wings, typical for nominative *L. c. cachinnans*. However, observations from the last few years showed that different subspecies of the same gull species may reach a significant level of morphobiological

differentiation in respect of many features. In particular, this concerns *L. cachinnans*. Within this species, the nominative race and *michahelis* have diverged so significantly that some authors doubt their conspecificity. It is notable that the reaction of wing lifting during the Long Call display was described neither in *michahelis*, nor in *omissus* (Garner and Quinn, 1997).

In conclusion, the authors would like to emphasize the highly dynamic characteristic of relationships between taxa, which, according to traditions of museum taxonomy, are often regarded as definitive formed rigid entities maintaining a constant genotype and phenotypic pattern within their ranges. This typological approach is completely inapplicable to the *Larus argentatus*–*cachinnans*–*fuscus* complex, which provides a good example of microevolution proceeding before our eyes. There is a wide field for testing the spectrum of hypotheses formulated in the modern evolutionary theory, and progress is retarded only by a shortage of facts about morphobiological patterns of some taxa and their real relationships in zones of secondary contact.

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