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SHORT COMMUNICATIONS

Frequency of Iris Depigmentation in Urban Populations of *Rana arvalis* Frogs

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The absence of normal pigmentation of the iris is widespread in many amphibian species, both anuran (Cain and Utesch, 1976) and urodelean (Dubois *et al.*, 1973; Engelmann and Obst, 1976). This anomaly apparently results from various nonhomologous mutations, including somatic mutations (Rostand, 1958; Dubois, 1979). In some species, such as axolotl and *Hyla arborea*, its manifestation may be determined by two different mutations (Humphrey and Bagnara, 1967; Benjamin, 1970; Dalton and Hoerter, 1974; Nishioka and Ueda, 1985). In *Rana pipiens*, for example, one mutation is dominant and the other is recessive; the former occurs also in *R. calamitans* (Vorps, 1976), and the latter, in *R. nigromaculata* (Richards *et al.*, 1969). Hybridization experiments with *Rana esculenta* (Rostand and Darre, 1970) have shown that this anomaly is determined by a recessive mutation (Rostand, 1953) and may accompany albinism (Tunner, 1979). Significant differences in pigmentation of aberrant individuals suggest that this trait may be polygenic (Dubois and Vachard, 1971) or semidominant and dependent on a modifier gene, as in *R. pipiens* (Browder, 1968). Dubois (1968) has supposed that this anomaly is determined by one recessive gene, and its unilateral manifestation is a consequence of mosaicism. It has also been described in the brown frogs *R. temporaria* (Rostand, 1953) and *R. arvalis* (Vershinin, 1988).

Thus, we deal with an anomaly that is manifested in the absence of normal iris pigmentation in one or both eyes. This trait has been observed in many amphibian species, but its genetic background may differ even in different populations of the same species (Dubois, 1979). In fact, it may be regarded as partial albinism.

The groups of *Rana arvalis* Nilss. frogs from a large urban agglomeration (the city of Yekaterinburg) and a rural area beyond its boundaries were analyzed for the frequency of iris depigmentation (Fig. 1, table). On the whole, 14 233 postmetamorphic juveniles (1980–1998) and 1570 adult frogs (1976–1998) were examined. Based on the criteria of zoning and the results of hydrochemical analysis used in previous studies (Vershinin, 1980, 1983), the urban area was divided into zones differing in the degree of anthropogenic transformation: zone II, areas with multistory buildings; zone III, areas with low buildings; zone IV, the park-forest belt; and C, the control zone. Genetic analysis of the frogs has not been performed, because this anomaly is mainly observed among postmetamorphic juveniles, as in the case of *R. esculenta* complex (Dubois, 1979).

During the observation period, 10 953 postmetamorphic juveniles from the urban populations were examined. Among them, iris depigmentation in only one eye was revealed in 147 individuals (1.39%), and in both eyes, in 30 individuals (0.274%); manifestation of this trait on the left and right body sides was observed in 22 and 19 frogs (0.6 and 0.5%), respectively. In the rural population (n = 3306), eight juveniles (0.242%) had iris depigmentation in one eye, six in the left and two in the right (0.242 and 0.181%, respectively), and five juveniles (0.151%) had bilateral depigmentation. Thus, unilateral anomalies of iris pigmentation occurred more frequently. Note that skin color in all aberrant frogs was normal.

Relative to the total number of anomalies occurring in frog populations inhabiting different zones within city limits (Vershinin, 1995) and in the rural population, the proportion of iris depigmentation varied from 38.7 to 74.4%, but a significant difference in this parameter was revealed only between zones IV (park-forest belt) and II ($\chi^2 = 8.42$, $p < 0.01$). On the whole, iris depigmentation accounted for 52.5% of all morphological aberrations in juveniles from the urban area and 43.5% of aberrations in juveniles from the rural population.

Among the adult frogs from the urban populations examined between 1976 and 1998 (n = 826), three individuals with this anomaly were revealed (0.363%): an immature frog with iris depigmentation in the right eye in 1987, an immature frog with bilateral iris depigmentation in 1991, and a mature male with iris depigmentation in the left eye in 1992). In the rural population (n = 648), only one immature frog with this anomaly (0.15%) was revealed in 1989.

The frequencies of iris depigmentation among juvenile frogs from the urban and rural populations differed

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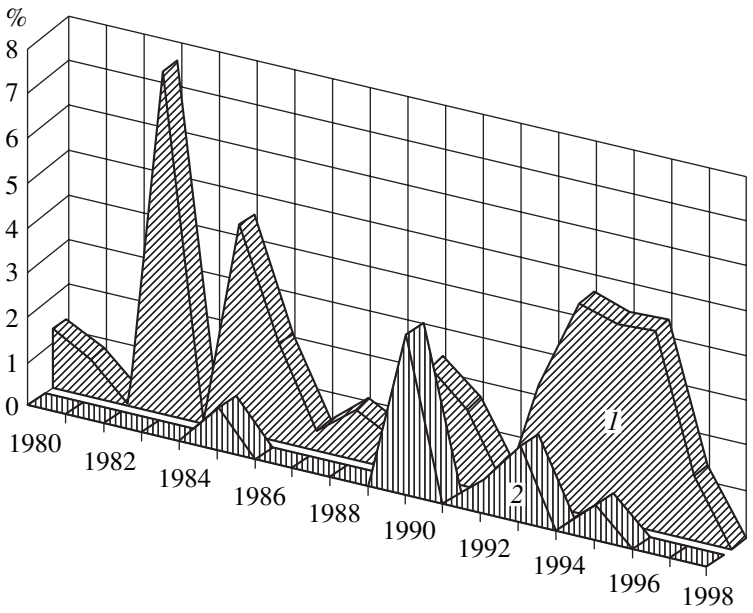

Fig. 1. Frequencies of iris depigmentation in (*1*) urban groups and (*2*) rural population of *R. arvalis.*

significantly (χ^2 = 28.99, $p \ll 0.001$), whereas those among mature frogs did not (χ^2 = 0.585).

Data on the occurrence of this anomaly in postmetamorphic juvenile *R. esculenta* frogs from central France over 11 years (Dubois, 1979) indicate that its frequency varies in different years from 0.2 to 0.7%, being generally closer to 0.2%. This value is similar to the frequency of iris depigmentation in the rural

Frequency of iris depigmentation in juvenile frogs from the urban area

Year	Total number of frogs	Bilateral depigmentation		Unilateral depigmentation		Number of anomalous frogs	Proportion of the total number, %
		n	%	*n*	%		
1980	387	0	0	5	1.3	5	1.3
1981	352	0	0	3	0.85	3	0.85
1983	13	0	0	1	7.7	1	7.7
1984	243	0	0	0	0	0	0
1985	627	9	1.44	20	3.19	29	4.4
1986	414	2	0.48	7	1.69	9	2.17
1987	1534	2	0.13	5	0.33	7	0.46
1988	1480	4	0.27	13	0.88	17	1.15
1989	1423	1	0.07	9	0.63	10	0.70
1990	1459	5	0.34	31	2.12	36	2.47
1991	959	4	0.42	12	1.25	16	1.67
1992	532	0	0	1	0.19	1	0.19
1993	285	1	0.35	7	2.46	8	2.81
1994	343	2	0.58	14	4.08	16	4.66
1995	227	0	0	10	4.41	10	4.41
1996	67	0	0	3	4.48	3	4.48
1997	420	0	0	6	1.43	6	1.43
1998	188	0	0	0	0	0	0
Total	10927	30	0.274	147	1.39	177	1.62

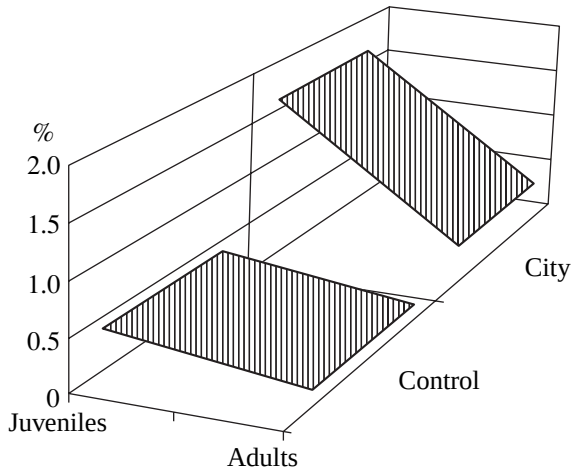

Fig. 2. Total proportions of individuals with iris depigmentation among juvenile and adult frogs.

R. arvalis population. Among adult frogs, the corresponding frequencies are also similar: 0.15% in the rural *R. arvalis* population and 0.07% in the *R. esculenta* population studied by Dubois (1979). Comparison of the frequencies of iris depigmentation in juvenile *R. arvalis* from the urban and rural populations with those in *R. esculenta* (according to Dubois, 1979) has shown that the difference is highly significant in the former case ($\chi^2 = 58.28$, $p \ll 0.001$) and not significant in the latter ($\chi^2 = 3.59$).

As follows from Fig. 2, the frequencies of this anomaly in juvenile and adult frogs differed by a factor of 4.4 in the urban populations ($\chi^2 = 8.04$, $p < 0.01$) and by a factor of 2.6 in the rural population ($\chi^2 = 0.876$, the difference is not significant). The total frequency of iris depigmentation was 4.9 times higher among juveniles than among adult frogs ($\chi^2 = 12.39$, $p < 0.001$). This fact suggests that the absence of iris pigmentation in juvenile frogs may be somehow related to the probability of them living to an age of maturity. Although there are no apparent reasons for a decrease in animal viability because of this anomaly, such a relationship has been noted by other researchers in frogs of the *R. esculenta* complex (Rostand, 1953; Dubois, 1979).

As mentioned above, iris depigmentation may be either unilateral or bilateral. If this anomaly results from disturbances of individual development, it is logical to assume that its manifestation on one body side is independent of that on the opposite side. In such cases, the probability of its unilateral manifestation is calculated by dividing the number of depigmented eyes by the doubled number of animals studied. Therefore, the probability of bilateral depigmentation (under conditions of its independent manifestation on each body side) is equal to the squared probability of unilateral depigmentation, and the theoretically possible number of bilateral variants in the sample is equal to the total number of animals multiplied by the calculated probability of bilateral depigmentation. This theoretical number was calculated for juvenile frogs and compared

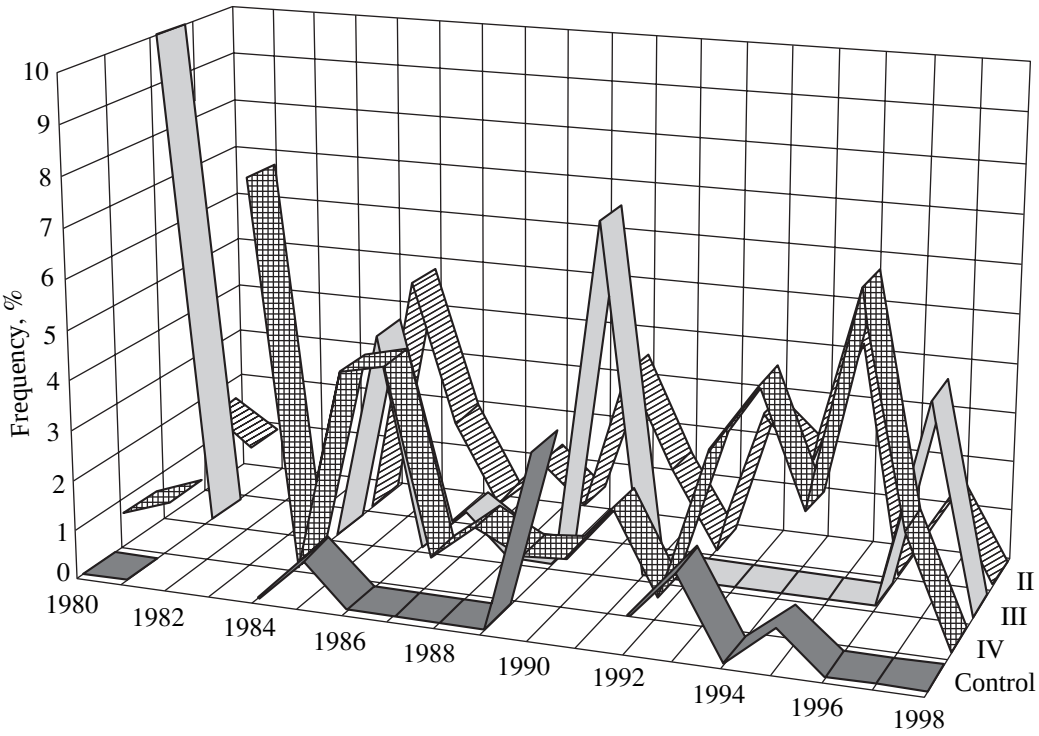

Fig. 3. Dynamics of the frequency of iris depigmentation in frogs from different city zones (II–IV) and in the control.

with the experimental data, and the actual probability of bilateral depigmentation proved to be two orders of magnitude higher than the calculated probability: 0.00246 vs. 0.0000625 ($\chi^2 = 32.45$, $p \ll 0.001$). This result contradicts the hypothesis of independent iris depigmentation on the left and right body sides in the bilateral variant and provides evidence for a hereditary nature of this anomaly.

Iris depigmentation is probably determined by genetic factors, as it has been proven for all other anomalies with a similar phenotypic manifestation. Apparently, there is a complex of recessive traits that, being in the homozygous state, may provide for a high frequency of individuals with such a phenotype; on the other hand, they may be responsible for a low rate of their survival due to inbreeding depression in the urban isolates formed due to fragmentation of natural biotopes and reduction of the reproductive part of the population (Simberloff, 1983).

Among the probable factors responsible for fluctuations in the frequency of this anomaly, attention should be focused on ambient temperature in the period of spawning and embryonic development: in the years characterized by the absence of frogs with iris depigmentation, no frosts occurred in this period. Apparently, cold spells in spring promote the phenotypic manifestation of this anomaly, as temperature is a factor influencing the probability of anomalies in amphibians (Voitkevich, 1938, 1961; Obukhova, 1984; Van Valen, 1974). In the brown frog *R. temporaria*, a species closely related to *R. arvalis*, iris depigmentation was revealed in only one out of 2534 postmetamorphic juveniles (0.00039%) and one out of 491 adult frogs (0.2%) from the urban area and never occurred in the rural population during the observation period. Such a low frequency of this anomaly in *R. temporaria* may be explained by a greater role of the hereditary component in its ontogeny, compared to *R. arvalis*, as well as by its high ecological plasticity (Surova, 1988) and cold hardiness (Hertwig, 1898; Terent'ev, 1950).

The frequencies of iris depigmentation in isolated groups of *R. arvalis* frogs changed from year to year in a similar way (Fig. 3). In 1984 and 1998, for example, no frogs with this anomaly were found in urban groups (table); in 1985, 1990, 1994, and 1996, the frequency of iris depigmentation in these groups reached 4.4–4.66%, and one frog with this anomaly was found in the rural population (0.9%, $n = 108$). Apparently, the conditions promoting the manifestation of this anomaly (in contrast to other aberrations) are similar throughout the urban agglomeration and its vicinities. On the other hand, its total frequency in the urban groups of frogs is more than four times higher than in the control rural population, although the long-term dynamics of this parameter are similar. This may be evidence that isolation of urban groups has an effect on the frequency of manifestation of this anomaly and that the intensity of the mutation process in the city is higher.

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