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EMBRYOLOGY

Comparison of Variability of *Rana temporaria* (Amphibia, Anura) Gastrula from Different Populations Developing under the Conditions of Antropogenic Pollution

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Abstract—The gastrulation of *Rana temporaria* embryos was studied at the natural spawning sites of Moscow city and Moscow district by means of morphometric analysis. We demonstrated that anthropogenic pollution plays an important role in morphogenesis of Anura. The measurements of embryos from the Moscow ponds made it possible to show an increased variability of gastrulation. Moreover, the level of gastrulation variability depends on the degree of pollution of a spawning pond and is coupled with increased correlations between the morphological features. This increase of correlations could be due to: (1) an increased number of correlating features (appearance of new correlations) and (2) strengthening of the existing correlations. The first way makes the gastrulation process more coordinated, and the second one causes the appearance of morphological structures, which are normally formed only at the next developmental stages.

Key words: variability, gastrulation, *Rana temporaria,* adaptation.

Studies of the effect of anthropogenic pollution on various invertebrates and vertebrates are one of the most promising trends of investigations at the interface of zoology, ecology, and embryology. These studies are mostly aimed at identification of permissible concentrations of various drugs, pollutants, affecting the early development of animals. Laboratory animals or animals from natural populations, whose development has been extensively studied and whose use is advantageous economically are usually chosen as test-objects (Umweltchemikalien, 1980; Dumpert and Zietz, 1984; Ha; and Hemry, 1992). The South African clawed toad *Xenopus laevis* is one of such objects and has given origin to the well know program FETAX (Frog Embryo Teratogenesis Assay—*Xenopus*) (Dawson *et al.*, 1985). Field studies are carried out on the most widespread amphibians, for example *Rana pipiens* (Allran and Karasov, 2001, 2002), *R. arvalis* (Andren *et al.*, 1989), *R. temporaria* (Dunson *et al.*, 1992; Leont'eva and Semenov, 1997; Johansson *et al.*, 2001), *Bufo americanus* (Hecnar, 1995), etc. As a result, 211 pollutants were tested on 45 different amphibian species by 1992 (Hall and Henry, 1992).

Unfortunately, there are practically no studies of the reaction of natural populations to environmental contamination, rather than of the effect of this factor on development. Only few studies describe a higher resistance to pollutants in amphibians from populations occurring in water bodies contaminated with organic pollutants (Hecnar, 1995; Johansson *et al.*, 2001; Severtsova, 2002) or in markedly acidified water bodies (Andren *et al.*, 1989). These studies were carried out, as a rule, either on adults (Vershinin, 1997), or on tadpoles, i.e., at the aqueous stage, which allows estimation of the degree and pattern of water body pollution (Freda, 1986; Horne and Dunson, 1995). There are practically no studies of the mechanisms underlying possible adaptation of amphibians at the earliest developmental stages, for example, at the gastrula stage, although it was shown that these stages are more sensitive (Dumpert, 1987; Carey and Bryant, 1995; Severtsova, 2002).

The aim of this work was to study possible mechanisms of adaptation of gastrulation processes in the common frog embryos under the conditions of anthropogenic pollution of spawning water bodies.

MATERIALS AND METHODS

Studies of the effect of anthropogenic pollution of water bodies on variations of early embryogenesis of the common frog *Rana temporaria* L. were carried out near the Zvenigorod Biological Station (ZBS), Moscow State University, and three municipal regions of South-West Moscow, Brateevo (South Administrative District), Vostryakovo, and Matveevskoe (West Administrative District) for five years from 1997 to 2001, In the region Brateevo, three shallow (no deeper than 50 cm) ponds with clay bottom, 400, 920, and 120 m^2 were examined, which were located on the well illuminated open Gorodnya river bank. In the region Vostryakovo, four examined water bodies are located along the Vostryakovo cemetery fence. Water body 1 is located in 100 m from the Moscow Ring Freeway and represents a deep (more than 2 m) ditch grown with grass and willow by edges. Water body 2 is a large pond, ca. 10 000 m^2 in area, with clay-sandy bottom and steep banks. Shallow water bodies 3 and 4, 3600 and 500 m^2 in area, are strongly willowed and, as a result, their bottom is covered by decaying foliage. In addition, water body 4 contains a lot of garbage from the cemetery, including metal rails. In the region Matveevskoe, a water body, 750 m^2 in area, is located in the Setun' river flood plain and contains a lot of decaying foliage from willows growing along the banks.

In the region of ZBS, three spawning grounds were examined. The first represents a plant-filled pond, 2400 m^2 in area, on the open well warmed Moskva river bank. The second is a water body, 790 m^2 in area, located on the second terrace of Moskva river, in the forest. The third is a water body, ca. 850 m^2 in area, located in a shadowy artificially dammed ravine.

Chemical analysis of water from the examined water bodies was performed in the Laboratory of Sources of Water Supply, Institute "Vodokanal". Unfortunately, these analyses were performed only in spring 1999, 2000, and 2001 because of the lack of funds.

Water composition was estimated by the following indices: odor (in points), turbidity (mg/l), color (degree), pH, and total hardiness (mg eq/l); concentration was also determined for Ca^{2+} and Mg^{2+} (mg eq/l), Cl^-, SO_4^{2-}, Al^{3+}, Zn^{2+}, Pb^{2+}, and Fe^{3+} (mg/l), HCO_3^- (mg eq/l), and oil products (mg/l).

All fixed embryos at the stage of middle-late gastrula were subjected to morphometric analysis using the method proposed by Cherdantsev and Scobeyeva (1994). All membranes, including the yolk membrane, were removed, sagittal fractures were made using the standard method, and the following parameters were measured under a dissection microscope using an ocular micrometer to a precision of 50 μm (Fig. 1): D_1, total gastrula diameter; D_2, yolk plug diameter; *ArthG*, archenteron roof height; *vh*, maximum height of yolk column; *LbalD*, depth of dorsal blastopore lip invagination; *LbalV*, depth of ventral blastopore lip invagination; *G*, distance between the points of maximum depth of dorsal and ventral blastopore lip invagination; *preg*, distance between gastrocoel and blastocoel cavities; *ArthB*, blastocoel roof height.

These parameters ensure the fullest description of gastrulation, one of the most important stages of embryogenesis (Slack *et al.*, 1992; Gilbert, 1993; Cherdantsev and Scobeyeva, 1994). The parameters, such as *LbalD, LbalV*, and *G* are the main indices of progress of gastrulation, since they reflect the degree and pattern of formation of the blastopore lips.

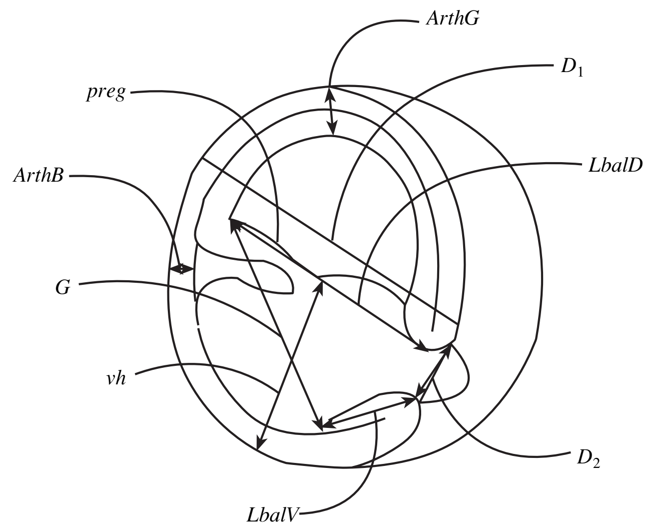

Fig. 1. Studied morphometric parameters of the common frog (*Rana temporaria*) embryos at the stage of middle-late gastrula. For designations see text.

ArthG characterizes the formation of chordamesoderm at the studied developmental stage. *preg* and *ArthB* are closely related to the preceding stage of embryogenesis, blastulation, since they mark the position of reducing blastocoel and, hence, can serve, together with *ArthG* as an index of embryo's "looseness." "Loose" gastrulas are usually defined as gastrulas either with loose cell junctions, or with abnormally large cells, which do not allow the formation of cavities or even continuation of development because of the physical features of morphogenesis.

According to the normal tables (Dabagyan and Sleptsova, 1975, 1991), gastrulation of anuran amphibians lasts from stage 11 until stage 19. We chose stage 17 for morphometric analysis of early development, which is characterized by a small yolk plug and definitive dorsal and ventral blastopore lips. However, the fixed materials could not be uniform due to asynchronous development of embryos even from the same batch. Therefore gastrulas from all studied populations were fixed during the period from stage 16 until stage 20 and morphometry was statistically processed for the sample as a whole and for embryos fixed at stage 17. In addition, a study of variation in D_1 has shown statistical significance of interpopulation differences by this parameter, which makes it difficult comparison of the absolute values of other parameters characterizing gastrulation and related to the total egg diameter. Therefore, for a more correct investigation of morphometric variability of the early development, analysis was carried out by both absolute and relative values of parameters standardized with reference to D_1.

The results obtained were processed using Excel and Statistica, 5.0, software.

Table 1. Results of Chemical analysis of water from water bodies of the studied regions of Moscow and Moscow District

Indices	LAC*	Moscow District	Brateevo	Vostryakovo	Matveevskoe	Ramenki
Odor, points	2	3.0	3.0	4.5	5.0	5.0
Turbidity, mg/l	1.5	6.50	6.75	9.00	19.56	8.30
Color, degrees	20	–	28.5	40.5	44.0	34.0
pH	7–9	6.79	6.92	6.79	7.08	7.15
Rigidity, mg eq/l	7	1.83	1.68	1.79	4.05	4.20
Content, mg/l:						
oil products	0.3	0.025	0. 220	0.144	0. 137	0.141
calcium	9	1.175	1.050	1.213	2.600	3.000
magnesium	3.3	0.65	0.71	0.47	1.29	1.07
sulfates	500	–	5.25	9.13	7.33	35.70
chlorides	350	–	23.10	20.77	29.83	69.35
zinc	5	0.110	0.166	0.536	0.360	0.213
lead	30	3.09	4.32	5.75	7.43	3.43
iron	0.3	0.373	0. 452	1.015	2.040	1.466
aluminum	0.5	0.25	0.22	0.34	0.25	0.35

Note: ^ Mean interannual and interwaterbody values are given. * LAC (limiting accessible concentrations) are given in accordance to GOST 28-74-82, SanPiN 4630-88.

RESULTS AND DISCUSSION

The results of water analysis (Table 1) suggest that in all samples, pH varied from 6.8 to 7.2, which markedly exceeds the lethal or teratogenic concentrations of H^+ for the studied stages of amphibian development (Schlichter, 1981; Freda, 1986; Sadinski and Dunson, 1992; Surova, 2001), but is within the limits of permissible concentrations (LPC). Some of the studied physical parameters of water exceeded, on average, the LPCs. Thus, the most significant excess of LPCs for odor, which are estimated in points, were observed in Matveevskoe and Moscow District water bodies with great amounts of decaying foliage from willows growing on their banks. The excess of LPCs for water turbidity was more than fourfold in the Moscow District and more than 13-fold in Matveevskoe. The water from the Moscow District water bodies was not estimated by color, while in all city water bodies, LPCs for this parameter were exceeded 1.5- to 2-fold.

Among the studied chemical parameters, such as content of oil products, ions of some metals (Ca^{2+}, Mg^{2+}, Zn^{2+}, Pb^{2+}, and Al^{3+}), chlorides, and sulfates, the concentration of iron ions alone exceeded LPCs. This was especially noticeable in Matveevskoe and, to a lesser extent, in Vostryakovo.

All other parameters of the chemical composition of water in the city water bodies exceeded those for the Moscow District water body, but did not exceed LPCs. For example, the content of oil products exceeded by one order of magnitude that in the Moscow District water body and was especially high in Brateevo, which could be explained by the close proximity of Kapotnya Oil Refinery. The contents of Ca^{2+} and Mg^{2+} in Matveevskoe and of Zn^{2+} and Pb^{2+}, Al^{3+} in Vostryakovo were 2–2.5 and 2-4 times those in the Moscow District, respectively. The contents of chlorides and sulfates used for road cleaning in winter and washed by meltwater into city water bodies also exceeded those in the Moscow District. The content of one of the main city pollutants, Pb^{2+}, in the city water bodies was much higher, 1.1- to 2.4-fold, due to the close proximity of autoroutes.

Thus in all studied city water bodies, all parameters of water composition exceeded those for the Moscow District water body. These data agree with the results of many-year monitoring of the chemical composition of small Moscow rivers by MosvodokanalNIIproekt (*Proekty razvitiya*…, 2001). As a result, all Moscow rivers were divided in four groups: moderately polluted, polluted, dirty, and very dirty (Romanova *et al.*, 2001). According to this classification, the Gorodnya river, in the flood plain of which the Brateevo water bodies are located, is moderately polluted, while Setun' is polluted. The main pollutants in the Gorodnya river are chlorides and iron ions, while in the Setun' river, oil products and heavy metals, as well as surface active substances (*Gosudarstvennyi doklad*…, 1994). Thus it can be stated that the water bodies under study are moderately polluted.

Interpopulation comparison of the mean values of the characters "developmental stage" and "egg diameter" has shown that in the Moscow District, as well as in Vostryakovo, samples of large eggs were fixed, which were at the most advanced stage of development (Table 2). The eggs from other studied regions of Mos-

Table 2. Statistical indices of the studied parameters of the common frog (*Rana temporaria*) embryos

Parameters		Regions	$x \pm SE$	Lim	σ^2	As	Es
Sample as a whole	*Stad*	Moscow District	18.23 ± 0.03	16–20	1.17	–0.23	–0.85
		Moscow	17.71 ± 0.02	16–21	1.07	0.47	–0.54
		Brateevo	17.28 ± 0.04	16–20	0.66	1.20	2.29
		Vostryakovo	18.14 ± 0.03	16–21	1.05	0.21	–0.92
		Matveevskoe	17.46 ± 0.04	16–20	0.97	0.32	–0.92
	D_1	Moscow District	37.35 ± 0.08	28–45	9.55	–0.35	–0.08
		Moscow	36.20 ± 0.06	28–45	6.77	0.20	–0.07
		Brateevo	35.59 ± 0.07	30–40	2.98	–0.36	–0.08
		Vostryakovo	36.42 ± 0.09	30–44	6.34	0.02	–0.53
		Matveevskoe	36.45 ± 0.14	28–45	10.59	0.15	–0.59
Stage 17	D_1	Moscow District	35.71 ± 0.17	28–43	8.97	–0.36	–0.74
		Moscow	35.76 ± 0.08	30–45	4.91	–0.05	0.47
		Brateevo	35.70 ± 0.10	30–39	2.99	–0.64	0.16
		Vostryakovo	36.49 ± 0.13	30–41	4.88	–0.35	–0.37
		Matveevskoe	35.04 ± 0.16	30–45	6.38	0.57	1.47
Sample as a whole	*vh*	Moscow District	19.99 ± 0.07	12–32	6.76	–0.29	0.96
		Moscow	19.27 ± 0.05	9–30	5.32	0.37	2.26
		Brateevo	18.56 ± 0.08	9–24	3.08	–0.26	1.14
		Vostryakovo	18.69 ± 0.07	11–28	4.26	–0.50	1.01
		Matveevskoe	20.85 ± 0.10	14–30	5.62	0.92	2.20
	vh/D_1	Moscow District	0.54 ± 0.002	0.32–0.82	0.004	0.13	1.05
		Moscow	0.53 ± 0.001	0.28–0.90	0.004	0.33	1.82
		Brateevo	0.52 ± 0.002	0.28–0.77	0.003	0.13	1.61
		Vostryakovo	0.51 ± 0.002	0.32–0.90	0.003	0.08	3.44
		Matveevskoe	0.57 ± 0.002	0.42–0.81	0.003	0.70	0.63
Stage 17	*vh*	Moscow District	19.82 ± 0.17	13–32	8.83	0.23	0.47
		Moscow	19.53 ± 0.07	9–32	3.65	0.86	4.88
		Brateevo	19.05 ± 0.09	9–24	2.58	–0.70	4.35
		Vostryakovo	19.28 ± 0.09	11–24	2.03	–0.24	0.39
		Matveevskoe	20.42 ± 0.15	14–30	5.66	1.17	2.71
	vh/D_1	Moscow District	0.55 ± 0.004	0.35–0.82	0.004	0.26	1.33
		Moscow	0.55 ± 0.002	0.28–0.77	0.003	0.40	1.91
		Brateevo	0.54 ± 0.003	0.28–0.77	0.002	0.07	3.66
		Vostryakovo	0.53 ± 0.002	0.38–0.63	0.002	–0.36	0.56
		Matveevskoe	0.58 ± 0.003	0.47–0.75	0.003	0.68	0.42
Sample as a whole	D_2	Moscow District	4.21 ± 0.10	0–16	13.29	0.78	0.27
		Moscow	5.29 ± 0.07	0–16	10.58	0.08	–0.21
		Brateevo	6.45 ± 0.12	0–15	7.78	–0.10	0.48
		Vostryakovo	3.86 ± 0.10	0–13	8.49	0.04	–0.83
		Matveevskoe	6.40 ± 0.14	0–16	11.09	0.19	–0.21
	D_2/D_1	Moscow District	0.12 ± 0.003	0–0.46	0.010	0.75	0.14
		Moscow	0.15 ± 0.002	0–0.45	0.009	0.17	–0.14
		Brateevo	0.18 ± 0.003	0–0.44	0.006	0.10	0.81
		Vostryakovo	0.11 ± 0.003	0–0.37	0.006	0.08	–0.75
		Matveevskoe	0.18 ± 0.004	0–0.45	0.009	0.17	–0.36

Table 2. (Contd.)

Parameters		Regions	$x \pm SE$	Lim	σ^2	As	Es
Stage 17	D_2	Moscow District	7.74 ± 0.13	4–16	5.86	0.80	0.16
		Moscow	7.08 ± 0.09	2–15	2.95	0.99	1.43
		Brateevo	7.12 ± 0.09	2–11	2.02	0.23	0.38
		Vostryakovo	6.58 ± 0.08	4–13	1.96	1.07	2.01
		Matveevskoe	7.59 ± 0.14	4–15	4.73	0.86	0.17
	D_2/D_1	Moscow District	0.22 ± 0.003	0.11–0.4	0.004	0.71	0.18
		Moscow	0.20 ± 0.002	0.06–0.45	0.002	1.05	1.90
		Brateevo	0.20 ± 0.002	0.06–0.33	0.002	0.45	1.14
		Vostryakovo	0.18 ± 0.002	0.10–0.37	0.002	1.14	2.31
		Matveevskoe	0.22 ± 0.004	0.11–0.45	0.004	0.88	0.63
Sample as a whole	*ArthG*	Moscow District	3.22 ± 0.03	1–10	0.89	1.21	3.47
		Moscow	2.97 ± 0.02	2–7	0.58	0.98	2.66
		Brateevo	2.93 ± 0.03	2–7	0.56	0.96	2.41
		Vostryakovo	2.99 ± 0.03	2–6	0.58	0.62	0.53
		Matveevskoe	2.97 ± 0.03	2–7	0.60	1.55	6.08
	$ArthG/D_1$	Moscow District	0.090 ± 0.001	0.05–0.29	0.0010	1.38	4.52
		Moscow	0.08 ± 0.0005	0.05–0.21	0.0005	0.85	1.58
		Brateevo	0.082 ± 0.001	0.05–0.21	0.0005	1.07	3.09
		Vostryakovo	0.085 ± 0.001	0.05–0.19	0.0005	0.79	1.07
		Matveevskoe	0.082 ± 0.001	0.05–0.17	0.0005	0.74	1.09
Stage 17	*ArthG*	Moscow District	3.09 ± 0.06	2–6	0.997	0.89	0.48
		Moscow	2.86 ± 0.02	2–7	0.497	0.90	2.49
		Brateevo	2.88 ± 0.04	2–7	0.586	1.24	3.66
		Vostryakovo	2.83 ± 0.04	2–5	0.421	0.34	0.01
		Matveevskoe	2.89 ± 0.04	2–6	0.466	0.75	1.81
	$ArthG/D_1$	Moscow District	0.09 ± 0.001	0.05–0.19	0.0010	0.84	0.60
		Moscow	0.08 ± 0.001	0.05–0.21	0.0004	0.96	2.71
		Brateevo	0.08 ± 0.001	0.05–0.21	0.0005	1.40	4.29
		Vostryakovo	0.08 ± 0.001	0.05–0.14	0.0003	0.44	0.11
		Matveevskoe	0.08 ± 0.001	0.05–0.15	0.0004	0.43	0.36
Sample as a whole	*LbalD*	Moscow District	30.72 ± 0.08	7–40	9.03	–0.99	3.39
		Moscow	29.99 ± 0.06	15–38	8.05	–0.51	1.71
		Brateevo	29.56 ± 0.09	22–34	4.14	–0.43	–0.19
		Vostryakovo	30.52 ± 0.10	15–38	8.81	–0.84	2.91
		Matveevskoe	29.57 ± 0.13	18–37	9.83	–0.26	0.42
	$LbalD/D_1$	Moscow District	0.82 ± 0.002	0.43–0.97	0.003	–2.56	20.39
		Moscow	0.83 ± 0.001	0.44–0.97	0.003	–1.74	8.30
		Brateevo	0.83 ± 0.002	0.66–0.94	0.002	–0.78	1.00
		Vostryakovo	0.84 ± 0.002	0.44–0.97	0.003	–2.72	14.75
		Matveevskoe	0.81 ± 0.002	0.60–0.95	0.002	–0.73	1.25
Stage 17	*LbalD*	Moscow District	29.54 ± 0.15	21–35	7.66	–0.61	–0.14
		Moscow	30.02 ± 0.08	18–37	5.87	–0.35	1.07
		Brateevo	30.12 ± 0.11	22–34	3.73	–0.98	1.33
		Vostryakovo	30.99 ± 0.15	23–36	6.06	–0.24	–0.13
		Matveevskoe	28.83 ± 0.15	18–37	6.03	–0.17	2.53
	$LbalD/D_1$	Moscow District	0.83 ± 0.002	0.56–0.95	0.002	–1.07	4.52
		Moscow	0.84 ± 0.001	0.60–0.97	0.002	–0.94	3.31
		Brateevo	0.84 ± 0.002	0.71–0.94	0.001	–0.80	1.55
		Vostryakovo	0.85 ± 0.002	0.65–0.97	0.002	–0.76	3.97
		Matveevskoe	0.82 ± 0.003	0.60–0.95	0.002	–1.00	2.99

Table 2. (Contd.)

Parameters		Regions	$x \pm SE$	Lim	σ^2	As	Es
Sample as a whole	*LbalV*	Moscow District	8.88 ± 0.06	1–19	4.33	–0.05	1.58
		Moscow	7.76 ± 0.05	1–18	5.37	–0.65	1.02
		Brateevo	6.98 ± 0.10	1–12	5.13	–0.99	0.60
		Vostryakovo	8.67 ± 0.06	2–18	3.19	0.28	2.35
		Matveevskoe	7.08 ± 0.11	1–12	6.7	–0.51	–0.67
	$LbalV/D_1$	Moscow District	0.24 ± 0.001	0.03–0.50	0.003	–0.15	2.07
		Moscow	0.21 ± 0.001	0.03–0.55	0.004	–0.72	1.60
		Brateevo	0.19 ± 0.001	0.03–0.40	0.004	–0.94	0.86
		Vostryakovo	0.24 ± 0.001	0.05–0.55	0.002	0.43	3.75
		Matveevskoe	0.19 ± 0.001	0.03–0.33	0.004	–0.74	–0.45
Stage 17	*LbalV*	Moscow District	7.75 ± 0.09	3–15	2.95	0.29	0.88
		Moscow	7.06 ± 0.06	1–12	3.38	–0.88	1.45
		Brateevo	6.86 ± 0.11	1–12	4.12	–1.30	2.01
		Vostryakovo	7.62 ± 0.09	2–11	2.20	–0.37	0.38
		Matveevskoe	6.71 ± 0.11	2–12	3.20	–0.14	–0.29
	$LbalV/D_1$	Moscow District	0.22 ± 0.003	0.09–0.39	0.002	0.23	0.48
		Moscow	0.20 ± 0.002	0.03–0.40	0.002	–1.09	2.25
		Brateevo	0.19 ± 0.003	0.03–0.40	0.003	–1.31	2.68
		Vostryakovo	0.21 ± 0.002	0.05–0.29	0.001	–0.57	0.84
		Matveevskoe	0.19 ± 0.003	0.06–0.29	0.002	–0.42	–0.63
Sample as a whole	*G*	Moscow District	21.70 ± 0.07	12–37	5.98	0.03	1.11
		Moscow	21.82 ± 0.06	15–31	6.36	0.50	0.09
		Brateevo	22.05 ± 0.10	17–30	5.75	0.62	0.35
		Vostryakovo	21.37 ± 0.08	15–30	5.91	0.31	–0.34
		Matveevskoe	22.32 ± 0.11	16–31	7.06	0.61	0.02
	G/D_1	Moscow District	0.58 ± 0.002	0.31–0.88	0.003	0.32	2.35
		Moscow	0.60 ± 0.002	0.44–0.88	0.005	0.69	0.23
		Brateevo	0.62 ± 0.003	0.45–0.79	0.004	0.46	–0.26
		Vostryakovo	0.59 ± 0.002	0.44–0.83	0.003	0.62	0.71
		Matveevskoe	0.62 ± 0.003	0.45–0.88	0.006	0.64	–0.35
Stage 17	*G*	Moscow District	21.46 ± 0.14	12–29	6.79	–0.10	0.18
		Moscow	22.19 ± 0.08	16–30	5.32	0.42	0.10
		Brateevo	22.31 ± 0.11	17–30	4.46	0.64	0.66
		Vostryakovo	22.33 ± 0.14	16–29	5.40	–0.01	–0.20
		Matveevskoe	21.89 ± 0.16	17–30	6.26	0.70	0.11
	G/D_1	Moscow District	0.60 ± 0.003	0.32–0.77	0.003	–0.44	2.81
		Moscow	0.62 ± 0.002	0.47–0.88	0.003	0.46	0.21
		Brateevo	0.63 ± 0.003	0.50–0.79	0.003	0.37	0.01
		Vostryakovo	0.61 ± 0.004	0.47–0.83	0.004	0.52	0.26
		Matveevskoe	0.63 ± 0.004	0.47–0.88	0.004	0.52	0.25

Table 2. (Contd.)

Parameters		Regions	$x \pm SE$	Lim	σ^2	As	Es
Sample as a whole	*preg*	Moscow District	1.74 ± 0.09	1–8	1.37	2.28	6.92
		Moscow	1.66 ± 0.05	0–8	1.68	1.59	3.01
		Brateevo	1.60 ± 0.07	0–5	1.17	1.21	1.09
		Vostryakovo	1.12 ± 0.08	0–6	0.91	2.24	8.13
		Matveevskoe	2.08 ± 0.10	0–8	2.41	1.32	1.62
	$preg/D_1$	Moscow District	0.05 ± 0.003	0–0.21	0.001	2.04	5.20
		Moscow	0.05 ± 0.001	0–0.22	0.001	1.55	2.71
		Brateevo	0.05 ± 0.002	0–0.14	0.001	1.18	0.99
		Vostryakovo	0.03 ± 0.002	0–0.16	0.001	2.45	8.79
		Matveevskoe	0.06 ± 0.003	0–0.22	0.002	1.27	1.24
Stage 17	*preg*	Moscow District	1.69 ± 0.13	1–8	1.58	2.71	9.27
		Moscow	1.68 ± 0.06	0–8	1.56	1.79	4.13
		Brateevo	1.56 ± 0.07	0–5	0.94	1.40	1.68
		Vostryakovo	1.26 ± 0.12	0–6	1.26	2.05	5.61
		Matveevskoe	2.15 ± 0.13	0–8	2.33	1.55	2.58
	$preg/D_1$	Moscow District	0.05 ± 0.003	0.02–0.21	0.001	2.48	7.65
		Moscow	0.05 ± 0.001	0–0.22	0.001	1.73	3.75
		Brateevo	0.04 ± 0.002	0–0.14	0.001	1.29	1.36
		Vostryakovo	0.04 ± 0.004	0–0.16	0.001	2.19	5.78
		Matveevskoe	0.06 ± 0.004	0–0.22	0.002	1.48	2.24
Sample as a whole	*ArthB*	Moscow District	2.39 ± 0.17	0–12	4.51	2.62	7.93
		Moscow	3.47 ± 0.10	1–13	7.04	1.15	0.43
		Brateevo	3.28 ± 0.16	1–11	7.11	1.28	0.42
		Vostryakovo	4.44 ± 0.21	1–11	6.49	0.49	–0.88
		Matveevskoe	3.06 ± 0.17	1–13	6.54	1.64	2.57
	$ArthB/D_1$	Moscow District	0.07 ± 0.005	0–0.51	0.004	3.47	16.36
		Moscow	0.10 ± 0.003	0–0.37	0.006	1.18	0.56
		Brateevo	0.09 ± 0.005	0.03–0.33	0.006	1.34	0.63
		Vostryakovo	0.12 ± 0.006	0–0.28	0.005	0.50	–0.87
		Matveevskoe	0.09 ± 0.005	0.03–0.37	0.006	1.59	2.31
Stage 17	*ArthB*	Moscow District	2.30 ± 0.23	0–12	4.96	2.56	6.94
		Moscow	3.88 ± 0.14	1–13	7.92	0.98	–0.01
		Brateevo	3.78 ± 0.21	1–11	8.26	0.99	–0.37
		Vostryakovo	4.56 ± 0.25	1–10	5.58	0.33	–0.96
		Matveevskoe	3.50 ± 0.26	1–13	8.72	1.44	1.46
	$ArthB/D_1$	Moscow District	0.07 ± 0.007	0.03–0.33	0.004	2.58	6.89
		Moscow	0.10 ± 0.004	0–0.37	0.007	1.03	0.14
		Brateevo	0.11 ± 0.006	0.03–0.33	0.007	1.05	–0.22
		Vostryakovo	0.12 ± 0.006	0–0.28	0.004	0.39	–0.71
		Matveevskoe	0.10 ± 0.008	0.03–0.37	0.007	1.35	1.17

Note: $x \pm SE$, mean and standard error; Lim, limits; σ^2, dispersion values; As, coefficient of asymmetry; Es, coefficient of excess.

Table 3. Coefficients of variation of the studied relative parameters of the common frog embryos at the gastrula stage in the sample as a whole and fixed only at stage 17

Regions	Studied parameters									
	Stad	D_1	D_2/D_1	$ArthG/D_1$	vh/D_1	$LbalD/D_1$	G/D_1	$LbalV/D_1$	$preg/D_1$	$ArthB/D_1$
	Sample as a whole									
Moscow District	5.93	8.27	86.98	29.86	11.80	6.63	9.49	21.55	96.57	65.99
Moscow	5.84	7.19	62.62	26.42	11.25	6.16	11.47	28.47	76.98	74.31
Brateevo	4.70	4.85	43.18	25.93	9.84	5.09	10.55	32.19	83.62	63.49
Vostryakovo	5.62	6.91	75.89	26.91	10.61	6.54	10.04	19.56	56.90	88.67
Matveevskoe	5.64	8.93	53.44	26.16	9.81	5.96	13.16	33.53	83.59	67.06
	State 17									
Moscow District		8.39	27.56	30.95	11.66	5.14	9.01	21.96	95.81	69.86
Moscow		6.19	24.65	25.27	9.61	4.97	9.44	24.72	74.18	73.02
Brateevo		4.85	19.77	27.78	9.15	4.08	8.52	29.21	78.61	60.63
Vostryakovo		6.06	22.46	22.73	7.56	4.84	9.82	17.45	52.38	93.19
Matveevskoe		7.21	27.89	23.92	8.92	5.62	10.05	25.20	84.33	66.31

cow were significantly smaller and, approximately, by one stage younger, although there were significant differences between these Moscow populations. If the Brateevo sample was relatively uniform, with predominance of batches having relative low D_1 values, the Matveevskoe sample (Table 2) contained a rather large number of eggs with a diameter of 1.7–1.8 mm, as well as ca. 2 mm gastrulae. As a result the variations of most morphometric parameters of gastrulae from Matveevskoe were higher than in other common frog populations we studied.

Significant interpopulation differences in the egg diameter called for comparative analysis by relative values of other parameters characterizing gastrulation and related to the egg size, rather than by their absolute values. In addition, the analysis of gastrulae was carried out at stage 17 only in order to reveal the differences determined by different rates of morphogenesis, above all, because the analyzed parameters are well expressed at the gastrula stage. In addition, stage 17 in all studied common frog populations was represented by the greatest number of embryos, which allowed a correct statistical processing of morphometric parameters.

The significant increase of gastrula parameters in the Matveevskoe population correlated also with the increased mean absolute and relative values of D_2, *vh*, *G. preg*, and *ArthB* (Table 2). However, the values of *LbalV* and *LbalD* were low, since the embryos from this region were characterized by a relatively high amount of yolk cells, which was especially noticeable when the embryos fixed at stage 17 were analyzed. During analysis of the entire sample, this effect was strengthened also by an earlier fixation of gastrulae as compared to other analyzed common frog populations.

The gastrulae of the Brateevo population proved, on the contrary, to be the smallest and, hence, the mean values of their morphometric parameters and level of variation were rather low (Table 2). Thus, the absolute and relative reserves of nutrients in embryos of this population were low, which was also expressed in mean values of *LbalD* and *LbalV.*

Comparative analysis of the parameters characterizing morphogenetic changes of common frog gastrulation suggests that the eggs of the Vostryakovo population were the closest to those from the Moscow District (Table 2). The differences in the mean absolute and relative values of D_2, *ArthG, LbalV, G*, and *preg* were not significant, although dispersion and coefficients of asymmetry were in the latter significantly higher than in the former. The differences between these populations were more noticeable when *LbalD, ArthB*, and *vh* were analyzed, i.e., those parameters, which are related to the amount of yolk in the egg, which was also reliably higher in the Moscow District population.

Analysis of coefficients of variation of the studied parameters in all regions (Table 3) made it possible to isolate three groups of parameters. The first group includes D_2, *ArthB*, and *preg* related to passive processes occurring during embryonic development, such as yolk plug closure (D_2) determined by cell epiboly and reduction of blastocoel characterized by changes in the blastocoel roof height (*ArthB*) and distance between gastrocoel and blastocoel (*preg*).

The parameters *ArthG* and *LbalV* comprising the second group proved to be less variable. *ArthG* is a marker of still starting cell ordering and neural plate formation, while the high level of *LbalV* variation is related to the activity of this process, which decreased

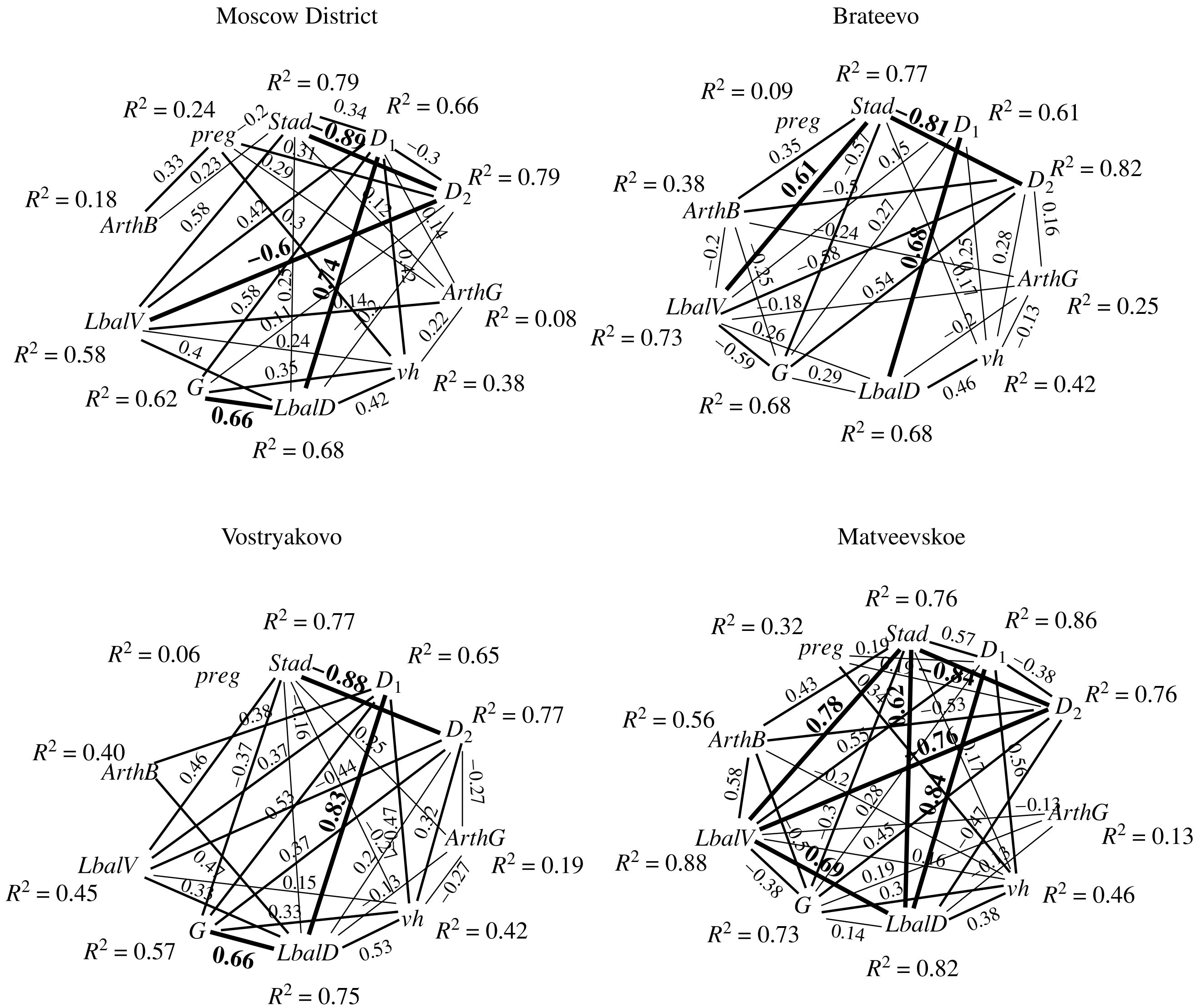

Fig. 2. Results of correlation analysis of morphometric parameters of the common frog (*Rana temporaria*) embryos at the stage of middle-late gastrula; only significant Spearman coefficients of correlation and multiple coefficients of correlation R^2 : (▬) $R^2 > 0.6$ are indicated.

as development proceeded. The parameters *Stad*, D_1, *LbalD*, *G*, and *vh*, related to a certain extent to the egg size, were the least variable and comprised the third group.

Interregional comparison of common frog gastrulae by these three groups of parameters has shown that both highly variable (group 1) and least variable parameters (group 3) made significant contributions to variability of the Moscow District population. In the Vostryakovo population, gastrulation proved to be least variable, while egg size and parameters characterizing the yolk plug closure and blastocoel reduction were more variable. In the Brateevo population, on the contrary, the gastrulation parameters proved to be the most variable, while the coefficients of variation of group 1 and 3 parameters were the lowest. Thus, in the Brateevo population, the variations of egg size were rather low, while the blastopore ventral lip invagination and archenteron roof thinning were highly variable. In the Matveevskoe population, the gastrulation variability was high, as well as egg size parameters.

Interaction of the analyzed parameters at the gastrula stage was estimated by calculation of the Spearman coefficient and multiple coefficients of correlation for each analyzed parameter. These data suggest that the number of significant coefficients of correlation was the highest in the Matveevskoe population (71.1%), while in the Brateevo and Vostryakovo populations (53% in each), it was lower than in the Moscow District (64.4%) (Fig. 2). Analysis of the coefficients of correlation has shown that in the studied Moscow regions, the percentage of rigidly bound interrelated parameters

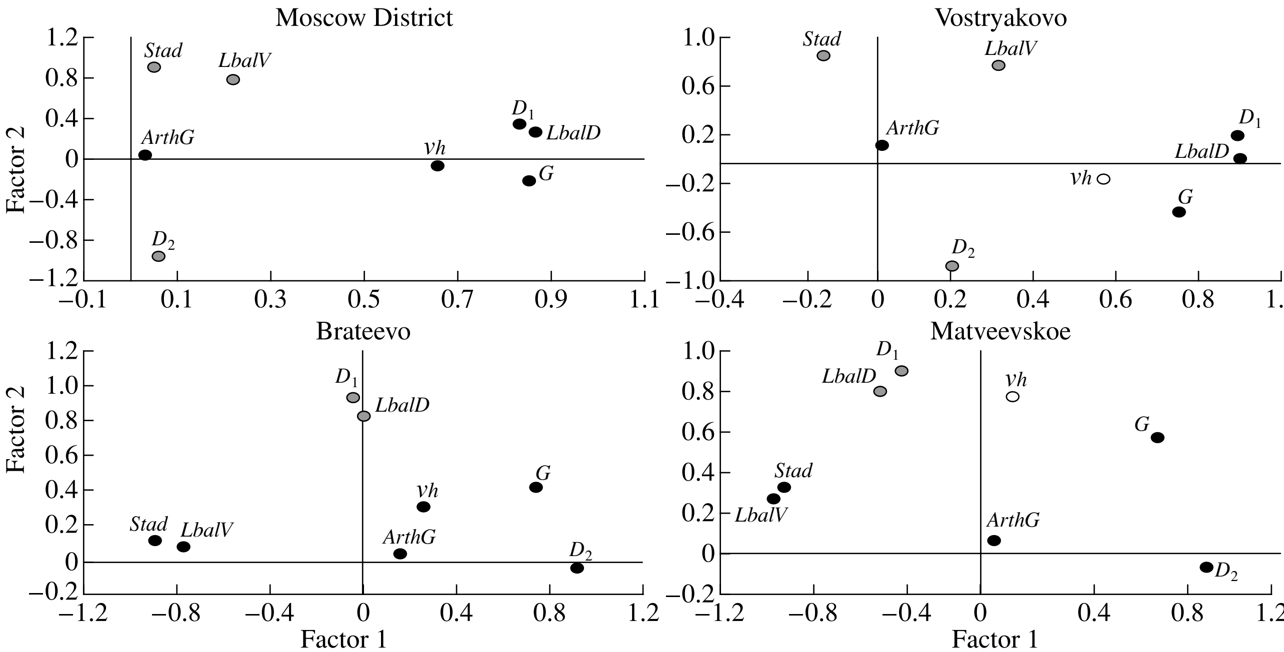

Fig. 3. Results of factor analysis of parameters of the common frog (*Rana temporaries*) embryos at the gastrula stage.

(with the coefficient of correlation >0.6) increased: 18.8, 12.5, and 10.3%, respectively (Fig. 2). However, if in the Vostryakovo population the set of highly correlated parameters was similar to that in the Moscow District population, while the egg size parameters played a significant role, in the Brateevo population the egg diameter was related to other morphometric parameters by very weak coefficients of correlation (Fig. 2). The correlation of parameters was the highest in the Matveevskoe population and both developmental stage parameters and egg size parameters were rigidly bound. Note that in the Brateevo region, as well as in the Moscow District, the highest percentage of pairs with weakly interrelated parameters (with the coefficient of correlation < 0.3) was observed: 58.3 and 51.7%, respectively. In the Vostryakovo and Matveevskoe region, the percentage of such pairs was less: 33.3 and 40.6%, respectively.

Factor analysis performed by the method of principal components using normalizing varimax rotation has shown that the highest proportion of the observed dispersion of parameters was accounted for by three factors (Fig. 3). Factor 3 was common for all studied common frog population (not shown in Fig. 3) and its effect was related to the preparation for the next developmental stage, neurulation, with the highest load on the parameter *ArthG*.

The effects of two other factors were also common for all studied common frog populations but their effects on gastrulation differed in different populations. The parameters related to egg size (D_1, *LbalD*, *vh*, and *G*) had the highest load for factor 1 in the Moscow District and Vostryakovo populations, while the rate of development played a lesser role: D_2, *LbalV*, and *Stad* had the highest load for factor 2. In the Brateevo and Matveevskoe populations, gastrulation changes were more important, since D_2, *Stad*, *G*, and *LbalV* had the highest load for factor 1 (Fig. 3). The egg size parameters were less important, but in the Matveevskoe population, unlike in the Brateevo population, the amount of nutrients was essential.

Thus, according to the analysis of mean morphometric parameters of the common frog embryos during gastrulation, the Vostryakovo and Moscow District populations proved to be most close in egg size parameters. The differences between these populations concerned only the reserve of nutrients; In the Vostryakovo population, the relative height of yolk column was lower, which is confirmed by our earlier data (Severtsova *et al.*, 2001). This did not practically affect the general pattern of gastrulation, but determined the significance of differences in the mean values of the studied morphometric parameters.

As follows from the above results, gastrulation changes played the most important role in the Brateevo population. The total coordination of morphogenetic processes during gastrulation in this population was the lowest, as compared to all other studied populations of the common frog. Note that the highest coefficients of correlation were observed between highly variable parameters formed during gastrulation.

Gastrulation in the Matveevskoe population seems the most interesting. Like in the Brateevo population, an increased variability of gastrulation processes and related increase of correlation of the morphogenetic parameters were observed and the coefficients of correlation were even higher than in the Brateevo population. Significant and rigid (>0.6) coefficients of correlation were recorded only in the Matveevskoe population. Hence, it can be proposed that, when combined with a high variability of egg size parameters, the high correlation of developmental may provide for integrity of the embryo during development.

The above presented data suggest that the correlation of morphogenetic processes can be increased in two ways: as a result of an increased number of interrelated parameters or as a result of elevated coefficients of correlation for a group of parameters. In the first case, the integrity of embryonic development is preserved and maintained, which allows a more distinct differentiation between developing structures. In the second case, development is accelerated due to rigid correlation inside a group of parameters, which characterize, as a rule, the development of a certain structure at the studied stage.

Both ways were described in the common frog population we studied. In the Matveevskoe and Vostryakovo populations, the general correlation of development increased due to the formation of a great number of weak coefficients of correlation and led to a delayed development at the studied stage. Enhanced correlations within a small group of parameters were noted in the Brateevo population, which led, apparently, to accelerated development. In the embryos from this population at the gastrula stage, parameters were formed, which were characteristic for the neurula stage, while gastrulation was not yet terminated, i.e., heterochronies appeared. Such an obliteration of interstage boundaries, not characteristic for the normal development, was, apparently, possible only for those structures, which are not involved in direct interaction at the studied developmental stage, e.g., blastopore ventral lip and archenteron roof. However, enhanced heterochrony and disturbed self-organization processes of early morphogenesis (Cherdantsev, 2000) lead to increased mortality rate and amount of defective embryos.

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