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Geographical and within-population variation of larval life-history traits in *Rana temporaria* and *R. arvalis*

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Abstract. The variance components of larval traits of two brown frog species in two widely separated populations were estimated using full-sib/half-sib breeding design and raising the tadpoles under laboratory conditions. The means of length of larval period (LP) in two species were nearly identical in each of two localities. The counter-gradient variation was revealed in size at metamorphosis (SM), LP and growth rate (GR) in *R. temporaria* and in LP and GR in *R. arvalis*. In *R. temporaria* from both localities, the heritability in SM was lower than in LP. In *R. arvalis*, the heritability in SM was higher than in LP. In both species, the heritability in GR in northern and southern populations was similar or some higher in northern population. In *R. temporaria*, the stronger (than in *R. arvalis*) influence of parental body length (including maternal effects) on SM, LP and GR reflects the importance of these traits for fitness at postmetamorphic stages of life cycle.

Introduction

The variation in larval life-history traits in temperate zone anurans depends on: (1) adaptation to local ecological conditions; (2) climatic factors, primarily the length of activity season and ambient temperatures in a given locality; (3) parental influences, including genetic and non-genetic effects. The magnitude of these three groups of factors differs in geographically remote populations (Laurila et al., 2002; Laugen et al., 2003). In addition, the dependence of larval traits on parental body size is connected with the fact that adult body size is subject to natural and sexual selection (Lyapkov et al., 2004 a, b). Therefore, in related species that differ in adult body size, this dependence can be subject to change. A comparison between two species of the brown frogs, *Rana temporaria* and *R. arvalis* permits us to test this possibility: while being different in their adult body size (Kabardina, 2002), these species are quite similar in duration of the embryonic and larval development and in body size at metamorphosis (Lyapkov, 1995).

The aim of the present study is to compare two geographically separated populations of *R. temporaria* and *R. arvalis*, with separating the components of phenotypic variation in larval life-history traits and evaluating the effects of parental body size within each population.

Materials and Methods

The adult amplectant *R. temporaria* and *R. arvalis* were collected in populations from two localities: in Moscow province, near Zvenigorod Biological Station of Moscow State University, 55 km westward of Moscow (55º44′ N, 36º51′ E, hereafter called "ZBS"); and in Kirov province, near Kipenevshchina village, 40 km westward of Kirov (58º40′ N, 49º5′ E, hereafter called "Kirov"). An incomplete factorial breeding design (Laurila et al., 2002; Sommer, Pearman, 2003) was used to separate additive genetic variation (V_A) from non-additive genetic variation (V_N), maternal effects (V_M) and environmental variation (V_E). The males and females were anesthetized and then males' testes were removed and crushed in amphibian Ringer solution (after Laugen et al., 2002). Eggs from each of two females were fertilized by sperm suspension from two males. The tadpoles obtained from artificial fertilizations were raised in common garden experiment. Each full-sib group was reared in two aquaria (replications). The standard laboratory conditions included the density of 20 tadpoles per 18 l aquarium, feeding *ad libitum* and raising by constant temperature of 20ºC until metamorphosis.

In each species and each of populations, different numbers of males (and correspondingly females) were used for mating. Unfortunately, the *R. temporaria* progeny of some matings died before hatching and therefore the numbers of successful mating "matrices" (i.e., progeny of 2 females mated by 2 males) were low: one mating "matrix" from ZBS and one from Kirov population in *R. temporaria*, 2 "matrices" from ZBS and 1 "matrix" from Kirov population in *R. arvalis*. For these full schemes, the cross-classified 3-way ANOVA design (with factors "females", "males" and "replications") was used in each species and each population. In addition, in *R. temporaria*, the whole data set (with excluding at random the progeny of one of two males in the cases of full "matrices") were used in nested 3-way ANOVA design ("females" were nested in "males"). In this nested design, the data on progeny of 5 males and 10 females from ZBS population (i.e. 10 full-sib groups), and on progeny of 3 males and 6 females from Kirov population (6 full-sib groups) were used. The causal components of variance in each larval trait were obtained by the restricted maximum-likelihood (REML) options in module VARCOMP in STATISTICA 6.0 (Statsoft Inc.). The standard interpretations (Falconer, Mackay, 1996) are used for results of both ANOVA-designs for estimating components of phenotypic variation, with residual causal variation component consisting of residual variance ($\sigma^2_{residual}$) and replication variance (σ^2_{repl}).

The genetic correlations were estimated by calculating product-moment correlations among male means (Laurila et al., 2002). The significance

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of differences of product-moment correlations from zero was determined directly from correlation coefficients values and sample sizes.

The influence of parental body size on larval traits was estimated by cross-classified 2-way ANOVA (factors "female size" and "male size", both with 2 levels: "large" and "small"), separately for each population of each species (*R. temporaria*: altogether 440 metamorphs from ZBS and 318 from Kirov; *R. arvalis*: 326 and 229, respectively). Body length was determined in each individual with ~~caliper~~ to the nearest 0.1 mm. In each female, the egg diameter was determined by measuring 20-30 eggs with eyepiece micrometer, to the nearest 0.05 mm.

In each species, the data on metamorphs raised in laboratory conditions were compared with data of those from natural ponds within the same populations (Lyapkov, 1995 and unpubl. data).

Results and Discussion

Metamorphs from lab vs. those from natural ponds

In metamorphs from experimental crosses, the mean length of embryonic and larval periods (from fertilization up to the end of metamorphosis, **LP,** days) in two species was nearly identical in each locality (Table 1, upper part). In *R. temporaria*, the mean size (body length) at metamorphosis (**SM,** mm) was higher in metamorphs from Kirov as compared to those from ZBS. The reverse differences between localities were revealed in LP. As a result the mean growth rate (**GR,** mm/day) in metamorphs from Kirov was much higher than in those from ZBS. In *R. arvalis*, mean SM and LP were lower in metamorphs from Kirov than in those from ZBS, while mean GR was higher. These differences between two localities within each species indicate on countergradient variation in all three traits in *R. temporaria* and in LP and GR – in *R. arvalis*.

In *R. temporaria* (in both localities), mean SM and GR in metamorphs from natural ponds were lower (and mean LP – higher) than in those raised under laboratory conditions (Table 1). In *R. arvalis* from ZBS, the mean LP in metamorphs from natural ponds were higher (and the mean GR – lower) than in those from laboratory. These results revealed the physiological constraints

Locality	Trait	Species:	*Rana temporaria*		*Rana arvalis*	
		Origin:	Lab	Nat	Lab	Nat
ZBS	SM		**15.77**	**13.37**	**15.50**	15.90
	LP		**58.83**	**77.22**	**58.14**	78.28
	GR		**0.269**	0.173	**0.268**	0.203
Kirov	SM		**16.51**	**16.34**	**14.48**	
	LP		**51.20**	**55.0**	**51.67**	
	GR		**0.323**	0.297	**0.282**	
		Female body size:	small	large	small	large
ZBS	SM	Male body size				
		small	**15.22**	**15.68**	15.93	15.14
	LP	large	**16.07**	**16.85**	15.83	15.22
		small	**58.16**	58.56	**60.03**	59.01
	GR	large	**60.10**	59.07	**56.17**	58.96
		small	0.262	**0.269**	**0.267**	0.257
Kirov	SM	large	0.268	**0.285**	**0.282**	0.259
		small	16.74	**16.10**	14.47	**13.60**
	LP	large	16.93	**16.48**	15.02	**14.65**
		small	52.06	50.39	**58.36**	51.50
	GR	large	51.80	50.40	**49.64**	51.24
		small	0.322	0.320	**0.248**	**0.265**
		large	0.328	0.328	**0.303**	**0.287**

Table 1. The mean values of larval traits from laboratory crosses (Lab) and natural breeding ponds (Nat) (upper part of the table), and the effects of parental body size on mean values of progeny larval traits (lower part of the table).

Notes. Within each species, the significant (P<0.05) differences between metamorphs from Lab and Nat are shown by underlining and the differences between localities are shown by bold typing. The significant (P<0.05) differences between progeny of small and large females are shown by underlining, and the differences between progeny of small and large males are shownby bold typing. See text for traits designation.

Table 2. The significance of factors' effects (upper part of the table); the proportions (%) of components of phenotypic variation in SM, LP and GR (median part of the table); and the genetic (above diagonal) and phenotypic (below diagonal) correlations among larval traits (lower part of the table).
Notes. The significant ($P<0.05$) influence of factors are shown by "+", non-significant – by "–". The significant variation components and correlation coefficients are underlined. See text for traits and variation components designation.

Species		Locality:		ZBS			Kirov	
		Trait:	SM	LP	GR	SM	LP	GR
	ANOVA design	Factor						
R. t.	Cross-classified	males (m)	–	–	–	–	–	–
		m × f	+	–	+	+	–	+
		females (f)	–	–	–	–	–	–
		replications	–	–	–	–	–	–
	Nested	males	–	–	–	+	+	+
		females	+	+	+	–	+	–
		replications	+	–	+	–	–	–
R. a.	Cross-classified	males (m)	–	–	–	–	–	–
		m × f	–	+	+	–	+	+
		females (f)	–	–	–	–	–	–
		replications	–	–	–	–	–	–
		Variation component						
R. t.	Cross-classified	V_A	0	46.38	0	64.81	99.07	0
		V_N	59.99	0	65.80	35.19	0.93	32.56
		V_M	25.42	0	19.37	0	0	11.13
		V_E	14.60	53.62	14.83	0	0	56.31
	Nested	V_A	47.18	100	60.10	57.77	79.44	53.56
		V_N	52.82	0	39.90	0	0	0
		V_M	0	0	0	0	0	0
		V_E	0	0	0	42.23	20.56	46.44
R. a.	Cross-classified	V_A	80.99	8.71	25.82	49.53	16.36	58.21
		V_N	10.03	82.02	58.96	0	83.64	41.79
		V_M	0	4.58	4.53	0	0	0
		V_E	8.98	4.69	10.69	50.47	0	0
		Trait						
R. t.	Correlation coefficients:	**SM**	×	~~0.561~~	~~0.832~~	×	~~-0.972~~	~~0.993~~
		LP	~~0.064~~	×	~~0.008~~	~~-0.035~~	×	~~-0.993~~
		GR	~~0.789~~	~~-0.557~~	×	~~0.822~~	~~-0.594~~	×
R. a.	Correlation coefficients:	**SM**	×	0.561	0.832	×	-0.972	0.993
		LP	0.064	×	0.008	-0.035	×	-0.993
		GR	0.789	-0.557	×	0.822	-0.594	×

imposed on SM, LP and GR in both species and within each of two localities.

Variance components in larval traits and correlations between traits

In *R. temporaria* (both in ZBS and Kirov), the proportion of V_A (i.e. heritability) in SM and in LP (Table 2, median part) was relatively high (including results of nested ANOVA). At both localities, the heritability in SM was lower than in LP. The results of nested ANOVA confirmed that the heritability in GR was also high in both localities.

In *R. arvalis* (unlike in *R. temporaria*), the heritability in SM was higher than in LP, both at ZBS and Kirov. The proportion of V_N in LP was high and the proportion of V_E in all traits was relatively low. At each locality, the heritability in LP was lower in *R. arvalis* than in *R. temporaria*. The heritability in SM was higher in *R. arvalis* than in *R. temporaria* (in ZBS) or similar in both species (in Kirov).The heritability in GR was high at both localities (as in *R. temporaria*). This indicates that additive variation in GR did not deplete under strong selection pressure in northern populations of both species.

In *R. temporaria*, genetic and phenotypic correlations coincided in each locality (Table 2, lower part). Both types of correlations between SM and GR were positive, while those between LP and GR were negative. The correlations between SM and LP were non-significant. In *R. arvalis*, both genetic and phenotypic correlations were similar with those in *R. temporaria*, except that the genetic correlation between LP and GR was non-significant in both localities. In the cases of negative or non-significant genetic correlations between SM and LP the directional selection can operate on both traits increasing the fitness of metamorphs. This possibility exists in both species and both localities. The significant positive (however weak) phenotypic correlation between SM and LP was revealed only in *R. temporaria* in Kirov, indicating the environmental constrains in northern populations. It is worth mentioning that in both species from natural ponds of ZBS the phenotypic correlations between LP and GR were generally positive (Lyapkov, 1995).

The non-genetic parental influence on larval traits

In *R. temporaria* (Table 1, lower part), the progeny of large males and large females (in ZBS) was characterized by higher mean values of SM and GR and by lower mean values of LP. In Kirov the same differences were revealed in progeny of large and small females in LP and in progeny of large and small males in SM. In *R. arvalis*, the similar effects were revealed in progeny of large and small males (in LP and in GR) but not in progeny of females (with only one exception – in GR, within small males, in Kirov). The stronger influence of parental body length on SM, LP and GR coincide with a stronger dependence of these traits on fitness at postmetamorphic stages of life cycle (both in juveniles and adults) in *R. temporaria* as compared to *R. arvalis*. In *R. temporaria*, these larval traits determine the body size of juvenile and immature individuals before the 1st and 2nd winterings, and thus – the survivorship during wintering (Lyapkov, 1997). Furthermore, larger males have higher breeding success while larger females have higher fecundity and reproductive effort (Lyapkov et al., 2004 a, b). The between-species differences in heritability in SM (see above) also coincided with the more tight interrelation between SM and fitness in *R. temporaria*.

The relatively high proportion of V_M in SM and GR was revealed in *R. temporaria* from ZBS only (Table 2). In all other cases, the proportion of V_M was low. The positive correlation between the egg size and SM in *R. temporaria* from ZBS ($r=+0.165$; $n= 440$; $P<0.001$) fitted in well with this result, but in the Kirov population the correlation among these traits was negative ($r=-0.297$; $n= 318$; $P<0.001$). The correlation between the egg size and LP was negative at Kirov ($r=-0.523$; $P<0.001$) but positive at ZBS ($r=+0.122$; $P<0.02$). The correlation between the egg size and GR was weakly positive (ZBS: $r=+0.087$; $P=0.069$; Kirov: $r=+0.139$; $P<0.02$). Surprisingly, in *R. arvalis* (both in ZBS and Kirov), the correlations between the egg size and SM (ZBS: $r=-0.114$; $n=326$; $P<0.05$; Kirov: $r=-0.204$; $n=229$; $P<0.01$) and between the egg size and GR (ZBS: $r=-0.305$; $p<0.001$; Kirov: $r=-0.110$; $P=0.097$) were negative, while the egg size positively correlated with LP (ZBS: $r=+0.312$; $P<0.001$; Kirov: $r=-0.122$; $P=0.065$). In general, the effects of egg size (on SM – at ZBS; on LP – at Kirov, and on GR – at both localities) were stronger in *R. temporaria* than in *R. arvalis*. Therefore, the maternal effects related to egg size appear to be stronger in *R. temporaria*.

The relatively low mean value of LP and high mean values of SM and GR revealed in common garden experiments at northern populations, the relatively high heritability, and the low proportion of maternal effects in all traits fit in well to the data obtained for Swedish populations of *R. temporaria* (Laurila et al., 2002; Laugen et al., 2002; Laugen et al., 2003) and *R. arvalis* (Räsänen et al., 2005) from different latitudes. The same trends were revealed in SM (but not in LP) in two Alpine populations from low and high elevation (Sommer, Pearman, 2003). But the other results of my study (the average values of SM, LP and GR, the between-population differences in proportion of variation components, in genetic and phenotypic correlations) do not coincide with the data mentioned above, indicating the distinctness among these widely separated populations. Apparently, the source of differences between southern and northern populations, in both species, concerns not only the relative contribution of variation components to single traits, but also the patterns of their interaction.

Conclusions

(1)The results of common garden experiment indicated the counter-gradient variation in SM, LP and GR in *R. temporaria* and in LP and GR in *R. arvalis*. The physiological constraints on SM, LP and GR were revealed in both species and within samples from each of two localities.

(2) At both localities, the heritability in LP was lower in *R. arvalis* than in *R. temporaria*. In the southern population, the heritability in SM was higher in *R. arvalis*, while in the northern population it was similar in both species.

(3) In *R. temporaria*, at both localities, the heritability in SM was lower than in LP. In *R. arvalis*, the heritability in SM was higher than in LP.

(4) In both species, the heritability in GR in northern and southern populations was similar or somewhat higher in the northern population. This contradicts the hypothesis that the stronger selection pressure in northern populations can deplete the additive genetic variation in GR.

(5) In *R. temporaria*, the stronger (as compared to *R. arvalis*) influence of parental body length (including maternal effects) on SM, LP and GR reflects the importance of these traits for fitness at postmetamorphic stages of life cycle (both juveniles and adults).

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