Factors contributing to the maintenance of the genetic polymorphism at the locus LDH-B in the pool frog, *Rana lessonae*

Benedikt R. Schmidt, Hansjürg Hotz, Bradley R. Anholt, Gaston-Denis Guex, and Raymond D. Semlitsch

Abstract: We tested for environmental factors that may lead to balancing selection and to the maintenance of a genetic polymorphism at the enzyme locus lactate dehydrogenase B (LDH-B) in the pool frog, *Rana lessonae*. We raised tadpoles individually in a factorial experiment in which we manipulated temperature, food level, and food quality. The only statistically significant difference among LDH-B genotypes was in growth rate, with the heterozygote performing best. Although the difference was not significant, heterozygotes also tended to perform best for size at metamorphosis. However, heterozygotes did not perform best in terms of other traits (age at metamorphosis and rates of survival and metamorphosis), where differences among LDH-B genotypes were also not significant. The size of the effect of LDH-B genotype depended on the environment, which suggests that the locus may be selectively neutral in some environments. There were no genotype–environment interactions in the sense that reaction norms along environmental gradients did not cross. When we raised tadpoles in groups, *e/e* homozygotes had a significantly higher body mass and developed at the significantly highest rate. In addition, there may be a trade-off between larval and adult performance: adult frogs show a different ranking in performance of LDH-B genotypes than tadpoles do. These results suggest that this genetic polymorphism is maintained through heterozygote advantage, possibly in conjunction with antagonistic pleiotropy.

Résumé : Nous avons tenté de déterminer quels facteurs écologiques peuvent contribuer à une sélection d'équilibre et au maintien du polymorphisme génétique au locus lactate dyshydrogénase B (LDH-B) chez la grenouille *Rana lessonae*. Nous avons élevé des têtards individuellement au cours d'une expérience de type factoriel où la température, la quantité de nourriture et la qualité de la nourriture étaient prédéterminées. Le taux de croissance s'est avéré la seule variable à différer significativement d'un génotype LDH-B à l'autre et ce sont les hétérozygotes qui avaient la meilleure performance. De même, ce sont les hétérozygotes qui avaient la taille la plus élevée à la métamorphose, mais la différence avec les autres génotypes n'était pas significative. Cependant, les hétérozygotes n'étaient pas favorisés quant aux autres variables (âge à la métamorphose, survie, taux de métamorphose) puisque les différences entre les divers génotypes LDH-B n'étaient pas significatives. L'importance de l'influence du génotype dépend de l'environnement, ce qui semble indiquer que le locus peut n'avoir aucun effet sélectif en certains milieux. Il n'y a pas d'interactions génotype–environnement dans la mesure où les normes de réaction le long de gradients environnementaux ne s'entrecroisent pas. Au cours d'expériences d'élevage en groupe, les têtards homozygotes se sont avérés avoir une masse significativement plus élevée et se sont développés à un rythme significativement plus rapide. De plus, il se peut qu'il y ait un compromis entre les performances larvaire et adulte : chez les grenouilles adultes, la performance des divers génotypes LDH-B n'est pas la même que chez les têtards. Ces résultats indiquent que ce polymorphisme génétique se maintient grâce à la performance supérieure des hétérozygotes, peut-être combinée à la pleiotropie antagoniste.
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B.R. Schmidt[1] and B.R. Anholt,[2] Zoologisches Institut, Universität Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland.
H. Hotz and G.-D. Guex. Zoologisches Museum, Universität Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland.
R.D. Semlitsch. Division of Biological Sciences, University of Missouri, Columbia, MO 65211, U.S.A.

[1] Author to whom all correspondence should be sent at the following address: Zoologisches Institut, Universität Basel, Rheinsprung 9, CH-4051 Basel, Switzerland (e-mail: schmidtb@ubaclu.unibas.ch).
[2] Present address: Department of Biology, University of Victoria, P.O. Box 3200, Victoria, BC V8W 3N5, Canada.

Introduction

While quantitative genetic variation may persist under directional selection (mutation–selection balance; Lande 1975), there is little possibility of the maintenance of genetic polymorphism at individual loci under directional selection. Allozymes often differ in their kinetic properties and thermostability, which are often inversely related (Watt 1991, 1994). These biochemical differences are associated with differences in physiology, behaviour, or life history (Watt et al. 1983; Powers et al. 1993), and this offers the potential for natural selection to operate on allozymes (Gillespie 1991; Powers et al. 1993; Watt 1994). Thus, these enzyme polymorphisms can be maintained by heterozygote advantage or balancing selection (for reviews see Stearns 1992; Falconer and Mackay 1996).

Table 1. Breeding design used to produce eight families and replication.

	e/e		*e/b*				*b/b*	
	F1	F3	F2	F4	F5	F7*	F6	F8†
Cross								
Dam								
1, *e/e*	×		×					
2, *e/e*		×		×				
3, *b/b*					×		×	
4, *e/b*						×		×
Sire								
5, *e/e*	×				×			
6, *e/e*		×				×		
7, *b/b*			×				×	
8, *b/b*				×				×
Replication (pooled over treatments)								
n	36	36	24	24	24	48	36	26

Note: *e/e*, *e/b*, and *b/b* indicate LDH-B genotypes of parents and offspring; F1–F8 are families; *n* shows the number of replicates (i.e., "correct" tadpoles). See the text for explanation.

*In this family, half of the tadpoles had LDH-B genotype *e/e*. Some of these tadpoles were raised but not used in the statistical analyses.

†In this family, half of the tadpoles had LDH-B genotype *e/b*. Some of these tadpoles were raised but not used in the statistical analyses.

A particularly well-studied enzyme is lactate dehydrogenase (LDH; EC 1.1.1.27), which catalyses the interconversion of pyruvate and lactate, and is thus involved in both the catabolism and anabolism of carbohydrates. During anaerobic glycolysis (which occurs in larval amphibians (Weigmann and Altig 1975; but see Gatten et al. 1984)), the conversion of pyruvate to lactate by LDH is essential for continued ATP production. LDH may also convert lactate to pyruvate, which in turn may be used in gluconeogenesis or in the generation of ATP by means of aerobic metabolism (Holbrook et al. 1975; Powers et al. 1991, 1993). In most vertebrates, the LDH gene family (e.g., Markert et al. 1975; Quattro et al. 1993; Tsuji et al. 1994) consists of at least two independent loci, LDH-A and LDH-B; several groups have independently evolved an additional tissue-specific third locus, LDH-C. Frogs of the genus *Rana* have two LDH loci, of which LDH-A is primarily expressed in skeletal muscle and LDH-B primarily in heart muscle, and both loci are about equally expressed in some other tissues such as liver (e.g., Wright and Moyer 1966; Vogel 1977; Hotz 1983).

The pool frog, *Rana lessonae* Camerano, 1882, is polymorphic for two common alleles at the locus LDH-B through most of its range.[3] Genetic variation at the locus LDH-B, as at other loci, is low or absent at the northern edge of the distributional range of *R. lessonae* (Sjögren 1991). In *R. lessonae* in Switzerland, LDH-B has the two common alleles, *e* and *b*, in the ratio 2:1; there is also a rare third allele.[3] LDH-B is sex-linked in *R. lessonae*; it is also linked to three other enzyme loci (Hotz et al. 1997), two of which are usually monomorphic in *R. lessonae*. When competing in artificial ponds with the hybridogenetic associate *Rana esculenta* (Graf and Polls Pelaz 1989; Schmidt 1993; the performance of *R. lessonae* tadpoles is generally negatively affected by the presence of *R. esculenta* (Semlitsch 1993*a*)), *e/e* homozygotes of *R. lessonae* had the highest body masses, the shortest development period, and the highest proportion of metamorphs among survivors, and *b/b* homozygotes had the lowest masses, the longest development period, and the lowest proportion of metamorphs among survivors. Heterozygotes had intermediate values for all three traits. This result depended on density: *e/e* and *e/b* were very similar in performance at low density, but *b/b* always performed worst. Hybridogenetic *R. esculenta* had higher masses at metamorphosis and a shorter development period at both densities when they had received the *e* allele from their *R. lessonae* parent.[3] In sum, the LDH-B genotype (or a closely linked locus) appears to influence important life-history traits that affect fitness (e.g., Smith 1983, 1987; Semlitsch et al. 1988; Goater 1994; Golay 1996) and, therefore, is not selectively neutral. Still, the *b* allele, which performed worst under all conditions used at the larval stage, is apparently maintained within populations and is widespread among populations.

We tested for the effects of temperature, food level, and food quality on the performance of LDH-B genotypes of *R. lessonae* to better understand the results obtained by Hotz and Semlitsch[3] and the maintenance of this polymorphism. Temperature and the amount of food have repeatedly been shown to be environmental gradients along which reaction norms of genotypes may cross (e.g., Powers et al. 1993 for LDH-B) and life histories and their quantitative genetics may vary according to temperature and food level (e.g., Berven et al. 1979; Gebhardt and Stearns 1992; Ebert et al. 1993; Semlitsch 1993*b*; Newman 1994). Because LDH-B is involved in the metabolism of carbohydrates, and food composition may affect tadpole life histories (Steinwascher and Travis 1983; Kupferberg et al. 1994), we also manipulated the carbohydrate content of the food. The experimental design allowed us to test for heterozygote advantage, genotype–environment interactions, and the possibility of selective neutrality.

Materials and methods

Breeding design

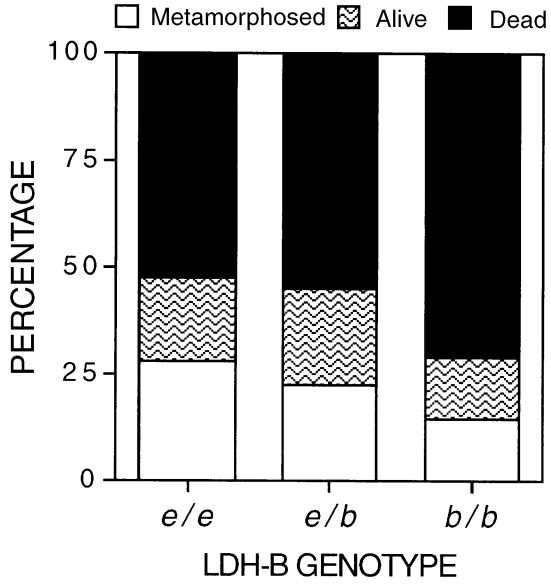
Adult *R. lessonae* were collected in May and June 1994 at a pond near Hellberg, Switzerland. The LDH-B genotype of each frog was determined by starch-gel electrophoresis prior to artificial breeding. Four female *R. lessonae* were crossed with four male *R. lessonae* (for procedures see Semlitsch 1993*b*; Berger et al. 1994). Each female was crossed with two males and each male was crossed with two females (Table 1). Because we could catch only one *b/b* female that was ready to ovulate, we used a heterozygous female instead. Consequently, the offspring of that female (families 7 and 8) are of two LDH-B genotypes (Table 1). Their LDH-B genotype was determined at the end of the experiment. Only individuals with the appropriate genotype (F7: *e/b*; F8: *b/b*) were used in the statistical analyses.

Experimental design

We measured the effects of three LDH-B genotypes, two temperatures, two food levels, and three food qualities in a completely randomised factorial experiment. We used three LDH-B genotypes, each

[3] H. Hotz and R.D. Semlitsch. 1998. Differential performance among LDH-B genotypes in *Rana lessonae* tadpoles. Submitted for publication.

Fig. 1. Effect of the LDH-B genotype on frequencies of death and metamorphosis. "Alive" denotes tadpoles that were alive on day 175 but had not metamorphosed.



of which was represented by two or four families (see "Breeding design"). Families were nested within genotypes. We raised tadpoles at the upper (24°C) and lower (19°C) thermal limit for long-term survival of *R. lessonae* tadpoles (S. Negovetic and R.D. Semlitsch, unpublished data) in two walk-in environmental chambers. The low food ration was the amount of food a tadpole could consume in 3 days; the high food level was three times greater. The amount of food was increased stepwise during the experiment, but the high:low ratio was always 3:1. Tadpoles were fed the same amount of food at both temperatures. Food consisted of finely ground dried nettle leaves and freeze-dried tubifex worms mixed in three ratios, 8:2, 5:5, and 2:8.

These factors were combined to create 12 treatment combinations. Replication is shown in Table 1. In total, 348 tadpoles were raised. Of these, 255 had the "correct" genotype for the experimental design (i.e., not all tadpoles of families 7 and 8 were used).

Experimental procedures

After hatching, tadpoles were randomly assigned to individual plastic dishpans (20 cm long × 11.5 cm wide × 7.5 cm deep) filled with 1.0 L of aged tap water. After the first 8 days, the water in each container was changed every 3 days, immediately before the tadpoles were fed. The experiment was terminated after 175 days.

Tadpoles that were not used in the experiment were kept at 19°C in groups of about 50 in plastic dishpans (30 cm long × 20 cm wide × 11 cm deep) filled with 3.5 L of aged tap water. Families were kept separate in two or three replicate dishpans. The water was changed when necessary (usually twice a week) and food was provided ad libitum. After 175 days we took a haphazard sample (72) of these tadpoles and used them to test whether the LDH-B genotype had an effect on tadpoles kept in groups. We expected similar results to those of Hotz and Semlitsch (see footnote 3).

Response variables and statistical analyses

Growth and development rates were determined after 30 days of the experiment. Metamorphosis was defined as emergence of at least one forelimb (stage 42; Gosner 1960). Metamorphs were weighed and the total number of days from the start of the experiment was recorded. For tadpoles raised in groups, we measured body mass and development stage after 175 days.

The probabilities of survival and metamorphosis were analysed as a general linear model with binomially distributed errors and the logit link using GLIM 3.77. Significance was tested by comparing the goodness of fit of nested models (that is, comparing two models, one with parameters estimating the treatment effect and one without these parameters). The change in goodness of fit is a likelihood-ratio test that is asymptotically χ^2 distributed, with degrees of freedom equal to the change in degrees of freedom (Crawley 1993). The daily probabilities of metamorphosis and death were analysed using the Cox regression with Poisson and Weibull distributed errors, respectively, in GLIM 3.77, to account for right-censoring of the data.

Analyses of growth and development were carried out on log-transformed data using type III sums of squares in PROC GLM in SAS version 6.08. Families were a random effect nested within LDH-B genotypes. At the low food level, no tadpoles metamorphosed. Therefore, all ANOVAs for traits at metamorphosis were carried out without tadpoles raised at the low food level to avoid missing cells and the food-level treatment was dropped from the analysis. After ANOVA, we tested for effects between pairs of allelic genotypes using the Tukey Studentised range test regardless of whether the main effect was significant (Zar 1984, p. 186; all $\alpha = 0.05$), because for natural selection to occur it is sufficient that one allelic genotype differ from the others. Combining all of the analyses into a single multivariate ANOVA did not provide any additional insight. We omit these analyses for the sake of brevity.

Our nested ANOVA assumes that each family is an independent unit. Indeed, each family is an unique combination of maternal and paternal genomes (see "Breeding design" and Table 1). However, each maternal and paternal genome is represented in two LDH-B genotypes. Thus, they may be considered non-independent and consequently nesting is not optimal. We treat each family as unique and use nested ANOVA. Shared maternal or paternal genomes make LDH-B genotypes more similar than they would have been if we had used completely independent families. Thus, nested ANOVA is a conservative test of differences among genotypes.

We also performed an additional analysis to overcome the problem of non-independence. We calculated ANOVA using families and then calculated linear orthogonal contrasts. First, we tested families 1 and 3 against families 2 and 4. This contrast tests for effects of LDH-B but is confounded with sire effects (see "Breeding design" and Table 1). We then tested families 1 and 3 against families 5 and 7. This contrast also tests for effects of LDH-B but is confounded with dam effects. SAS cannot estimate contrasts if there are missing cells. It can estimate contrasts if the involved interactions or main effects are not specified in the model (Littell et al. 1991). Thus, we removed interactions from the models that contained missing cells. No removed interaction was significant.

Results

Survival

LDH-B genotype *e/e* produced the most metamorphs (27.8% of all *e/e* tadpoles metamorphosed) and *b/b* the fewest (14.5%; Fig. 1). The *e/e* homozygotes also had the lowest mortality rates (52.8%) and *b/b* the highest (71.0%). The heterozygote was intermediate (metamorphosed: 22.5%; died: 55.0%). However, the LDH-B genotype did not significantly affect the probability of metamorphosis or survival, nor were there significant genotype–environment interactions (Table 2).

Growth and development rates

The heterozygote (*e/b*) had the highest growth rate (6.0 ± 0.29 mg/day (mean ± SE)). The two homozygotes were similar in performance (*e/e*: 5.4 ± 0.31 mg/day; *b/b*: 5.2 ± 0.36 mg/day; Fig. 2). There was a significant difference between *e/b* and *b/b* (Tukey test) but no overall genotype effect (Table 3). Although there was no significant genotype × food

Table 2. Summary of generalised linear models (GLM) on rates of metamorphosis and death.

Effect	Scaled deviance	df	*P*	Test	Model No. and effect tested	Model	Scaled deviance (df)
				Frequency of metamorphosis			
GENO	2.70	2	0.2592	Model 4*a* – model 3*a*	1, full model	G + T + Q + L + G × T + G × Q × G + L + T × Q + T × L + Q × L + three-way I + four-way I	120.54 (218)
TEMP	22.39	2	<0.001	Model 4*c* – model 3*a*	2, three- and four-way I	G + T + Q + L + G × T + G × Q + G × L + T × Q + T × L + Q × L	127.66 (234)
QUAL	5.26	2	0.0720	Model 4*d* – model 3*c*	3*a*, two-way I other than T × Q	G + T + Q + L + T × Q	137.09 (245)
LEV	107.7	1	<0.001	Model 4*b* – model 3*a*	3*b*, T × Q I	G + T + Q + L + G × T + G × Q + G × L + T × L + Q × L	133.46 (236)
T × Q	5.8	2	0.0550	Model 3*b* – model 2	3*c*, main effects	G + T + Q + L	142.97 (247)
Two-way I except T × Q	9.43	11	0.5822	Model 3*a* – model 2	4*a*, GENO	T + Q + L + T × Q	139.79 (247)
Three- and four-way I	7.12	16	0.9708	Model 2 – model 1	4*b*, LEV	G + T + Q + T × Q	244.78 (246)
					4*c*, TEMP	G + Q + L	165.36 (248)
					4*d*, QUAL	G + T + L	148.23 (249)
				Daily probability of metamorphosis			
GENO	1.45	2	0.4843	Model 4*a* – model 3*a*	1, full model	G + T + Q + L + G × T + G × Q + G × L + T × Q + T × L + Q × L + three-way I + four-way I	76.67 (218)
TEMP	22.79	1	<0.001	Model 4*c* – model 3*a*	2, three- and four-way I	G + T + Q + L + G × T + G × Q + G × L + T × Q + T × L + Q × L	80.44 (234)
QUAL	3.05	2	0.2176	Model 4*d* – model 3*c*	3*a*, two-way I other than T × Q	G + T + Q + L + T × Q	85.77 (245
LEV	85.1	1	<0.001	Model 4*b* – model 3*a*	3*b*, T × Q I	G + T + Q + L + G × T + G × Q + G × L + T × L + Q × L	85.41 (236)
T × Q	4.97	2	0.0833	Model 3*b* – model 2	3*c*, main effects	G + T + Q + L	90.62 (247)
Two-way I except T × Q	5.33	11	0.9141	Model 3*a* – model 2	4*a*, GENO	T + Q + L + T × Q	87.22 (247)
Three- and four-way I	3.77	16	0.9992	Model 2 – model 1	4*b*, LEV	G + T + Q + T × Q	170.92 (246)
					4*c*, TEMP	G + Q + L	113.41 (248)
					4*d*, QUAL	G + T + L	93.67 (249)
				Frequency of death			
GENO	3.7	2	0.1572	Model 3*a* – model 2	1, full model	G + T + Q + L + two-way I + three-way I + four-way I	233.41 (218)

Table 2 (*concluded*).

Effect	Scaled deviance	df	*P*	Test	Model No. and effect tested	Model	Scaled deviance (df)
TEMP	3.5	1	0.0613	Model 3*b* – model 2	2, two-, three-, and four-way I	G + T + Q + L	264.53 (247)
QUAL	3	2	0.2231	Model 3*c* – model 2	3*a*, GENO	T + Q + L	268.19 (249)
LEV	71.5	1	<0.001	Model 3*d* – model 2	3*b*, TEMP	G + Q + L	267.99 (248)
Two-, three-, and four-way I	31.8	29	0.3287	Model 2 – model 1	3*c*, QUAL	G + T + L	267.54 (249)
					3*d*, LEV	G + T × Q	336.04 (248)
Daily probability of death							
GENO	1.7	2	0.4274	Model 3*a* – model 2	1, full model	G + T + Q + L + two-way I + three-way I + four-way I	350.48 (218)
TEMP	0.46	1	0.4976	Model 3*b* – model 2	2, two-, three-, and four-way I	G + T + Q + L	362.32 (247)
QUAL	1.43	2	0.4891	Model 3*c* – model 2	3*a*, GENO	T + Q + L	364.02 (249)
LEV	33.47	1	<0.001	Model 3*d* – model 2	3*b*, TEMP	G + Q + L	362.78 (248)
Two-, three-, and four-way I	11.48	29	0.9979	Model 2 – model 1	3*c*, QUAL	G + T + L	363.75 (249)
					3*d*, LEV	G + T + Q	395.79 (248)

Note: "Frequency" denotes GLM with binomial errors on the frequency of metamorphosis and death and "daily probability" denotes GLM on the daily probability of metamorphosis (Poisson distributed error) or death (Weibull distributed error). The removal of interaction terms is shown in condensed form because no interaction term was significant. TEMP × QUAL is shown because it was marginally nonsignificant. GENO (G), the LDH-B genotype; TEMP (T), temperature; QUAL (Q), food quality; LEV (L), food level; I, interaction.

Fig. 2. Effect of the LDH-B genotype on growth rates. Food levels are shown separately to demonstrate their differential effects on the performance of genotypes. Values are given as the mean and standard error. Solid symbols represent high food levels and open symbols represent low food levels.

level interaction (Table 3), a Tukey test revealed differences only at the high food level, not at the low food level. At the high food level, heterozygotes had a growth rate of 7.78 ± 0.44 mg/day. There was almost no difference between the homozygotes (*e/e*: 6.56 ± 0.49 mg/day; *b/b*: 6.59 ± 0.71 mg/day; Fig. 2). At the low food level, there was almost no difference among the three genotypes (*e/e*: 4.25 ± 0.24 mg/day; *e/b*: 4.22 ± 0.20 mg/day; *b/b*: 4.21 ± 0.24 mg/day).

The ANOVA preceding the contrasts showed significant effects of family on growth rates (Table 4; see also Table 3). One out of two contrasts comparing the heterozygous families with *b/b* families was significant (Table 4). The apparently superior performance of *e/b* families 5 and 7 is likely to have been due to LDH-B effects, because it appeared both in comparisons confounded with dam effects and in those confounded with sire effects. When all heterozygous families were tested together against homozygous families, the contrasts showed that *e/b* > *b/b* and thus confirmed the results of the Tukey test. Excluding tadpoles raised at the low food level did not change the results qualitatively.

All LDH-B genotypes developed at almost the same rate (*e/b*: 0.92 ± 0.006 stages/day; *e/e*: 0.91 ± 0.007 stages/day; *b/b*: 0.91 ± 0.008 stages/day). The effect was not significant, and there were no genotype–environment interactions (Table 3).

Age and size at metamorphosis

Heterozygotes had the greatest mass at metamorphosis (889 ± 34 mg). Both homozygotes had lower masses (*b/b*: 858 ± 68 mg; *e/e*: 811 ± 35 mg; Fig. 3). However, the LDH-B genotype had no significant effect on mass at metamorphosis and there were no genotype–environment interactions (Table 5).

The *e/e* homozygote had the shortest larval period (99.1 ± 7.34 days); *e/b* and *b/b* had longer larval periods of similar length (*e/b*: 103.7 ± 5.87 days; *b/b*: 103.0 ± 9.69 days;

Table 3. Summary of the nested ANOVA for growth rates and development rates.

		Growth rate			Development rate		
Source of variation	df	Type III mean square	*F*	*P*	Type III mean square	*F*	*P*
GENO	2	0.068	0.25	0.7835	0.002	0.85	0.4817
FAM	5	0.264	2.24	0.0510	0.002	1.44	0.2100
TEMP	1	13.018	110.43	0.0001	0.246	130.37	0.0001
QUAL	2	0.060	0.51	0.5986	0.002	1.49	0.2263
LEV	1	10.651	90.35	0.0001	0.179	94.90	0.0001
G × T	2	0.100	0.85	0.4278	0.002	1.16	0.3147
G × Q	4	0.251	2.13	0.0783	0.003	1.68	0.1556
G × L	2	0.128	1.08	0.3382	0.001	0.52	0.5943
T × Q	2	0.001	0.01	0.9879	0.002	1.08	0.3399
T × L	1	1.942	16.48	0.0001	0.046	24.50	0.0001
Q × L	2	0.062	0.53	0.5885	0.005	3.08	0.0477
Three- and four-way interactions	16	0.3473	2.93	all >0.2500	0.004	2.39	all >0.5000
Residual	208	0.117			0.001		

Note: GENO (G), LDH-B genotype; FAM, family (nested within GENO); TEMP (T), temperature; QUAL (Q), diet; LEV (L), food level.

Fig. 3), but the effect of the LDH-B genotype was not significant and there were no genotype–environment interactions (Table 5).

Orthogonal contrasts yielded no additional insight (Table 6). There were no significant effects of families, nor were any contrasts significant as would be anticipated from the results of the nested ANOVA and Tukey test.

Tadpoles raised in groups

When raised in groups, *e/e* homozygotes had the highest mass (741 ± 48 mg) and *b/b* the lowest (513 ± 40 mg). Heterozygotes were intermediate (666 ± 37 mg; ANOVA, genotype effect: $F_{[2,69]} = 9.80$, $P = 0.0002$; Fig. 3). A Tukey test showed a significant difference between *e/e* and *b/b*.

The *e/e* homozygotes were the most developed (35.3 ± 0.52 Gosner stages) and *b/b* the least (32.5 ± 0.57 Gosner stages; ANOVA, genotype effect: $F_{[2,69]} = 7.30$, $P = 0.0013$; Fig. 3). A Tukey test indicated that *e/e* > *b/b*; *e/b* were intermediate (33.8 ± 0.46 Gosner stages) and reached significantly higher development stages than *b/b* (Tukey test).

Discussion

This experiment tested for two mechanisms that could maintain genetic variation: heterozygote advantage and genotype–environment interactions. Three main results emerged: (1) heterozygotes grew at the highest rate, whereas there were no significant differences for other traits, (2) the size of the genotype effect depended on the environment, and (3) there were no genotype–environment interactions in the sense that reaction norms along environmental gradients did not cross. Additionally, we suggest that heterozygote advantage may maintain genetic variation jointly with antagonistic pleiotropy. We cannot exclude the possibility that an anonymous linked locus, rather than LDH-B, caused the effects. However, the results of various studies suggest that LDH-B may

Table 4. Summary of ANOVA for growth rate and linear orthogonal contrasts comparing families (see the text for explanation).

Source of variation	df	Type III mean square	*F*	*P*
FAM	7	0.306	2.74	0.0102
TEMP	1	11.901	106.43	0.0001
QUAL	2	0.161	1.44	0.2393
LEV	1	9.979	89.24	0.0001
F × T	7	0.157	1.41	0.2063
F × Q	14	0.135	1.21	0.2732
F × L	7	0.193	1.73	0.1056
T × Q	2	0.045	0.41	0.6676
T × L	1	1.653	14.79	0.0002
Q × L	2	0.227	2.03	0.1341
F × T × Q	14	0.107	0.96	0.4934
T × T × L	7	0.079	0.71	0.6634
F × Q × L	14	0.090	0.81	0.6550
T × Q × L	2	0.340	3.05	0.0502
Residual	167	0.111		

Contrast	df	Contrast sum of squares	*F*	*P*
F1, F3 vs. F2, F4	1	0.008	0.07	0.7892
F1, F3 vs. F5, F7	1	0.329	2.94	0.0881
F1, F3, vs. F6, F8	1	0.191	1.71	0.1924
F6, F8 vs. F2, F4	1	0.107	0.96	0.3279
F6, F8 vs. F5, F7	1	0.847	7.57	0.0066
F1, F3 vs. F2, F4, F5, F7	1	0.073	0.66	0.4187
F6, F8 vs. F2, F4, F5, F7	1	0.478	4.28	0.0401

Note: The four-way interaction was excluded to avoid missing cells. FAM (F), family; TEMP (T), temperature; QUAL (Q), diet; LEV (L), food level.

Fig. 3. Effect of the LDH-B genotype on mass and age at metamorphosis of tadpoles raised individually (A) and mass and development stage at day 175 of tadpoles raised in groups (B). Values are given as the mean and standard error.

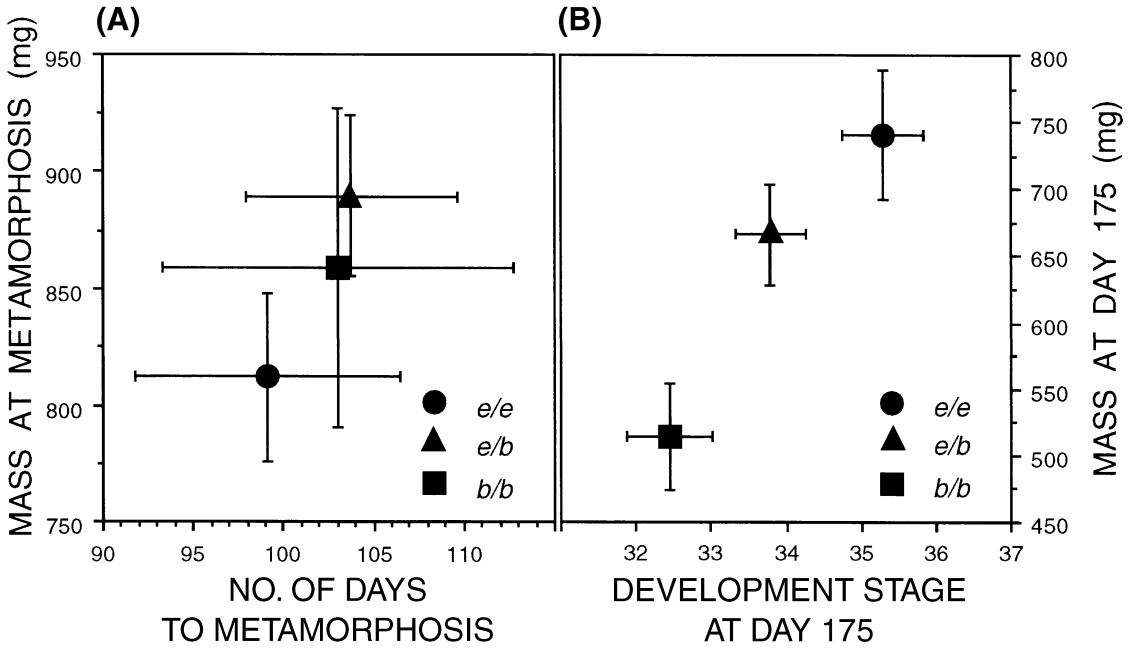


Table 5. Summary of the nested ANOVA for mass at metamorphosis and number of days to metamorphosis.

	Mass at metamorphosis				Days to metamorphosis		
Source of variation	df	Type III mean square	*F*	*P*	Type III mean square	*F*	*P*
GENO	2	0.048	1.96	0.1991	0.028	0.90	0.4409
FAM	5	0.025	1.02	0.4169	0.031	1.03	0.4131
TEMP	1	0.623	25.56	0.0001	1.984	64.29	0.0001
QUAL	2	0.003	0.15	0.8532	0.062	2.03	0.1456
G × T	2	0.007	0.30	0.7392	0.006	0.19	0.8213
G × Q	4	0.002	0.12	0.9744	0.022	0.74	0.5680
T × Q	2	0.047	1.92	0.1596	0.007	0.23	0.7887
G × T × Q	1	0.007	0.32	0.5731	0.006	0.22	0.6410
Residual	37	0.024			0.030		

Note: To avoid missing cells, tadpoles from the low food level treatment were excluded from the analysis. GENO (G), LDH-B genotype; FAM, family (nested within GENO); TEMP (T), temperature; QUAL (Q), diet.

be causally involved in the differences we found (e.g., Powers et al. 1991, 1993; Pierce and Crawford 1997). Effects of LDH-B on performance were also found by Hotz and Semlitsch (see footnote 3) when they used frogs from a different population more than 35 km away from Hellberg (our study site). Also, the genetic background or nongenetic maternal effects (which are strong in *R. lessonae* (Semlitsch 1993*b*)), as expressed in the effects of family on growth rate, may affect the effect of the LDH-B genotype on phenotypes.

Heterozygote advantage

There are a few clear examples of single-locus heterozygosity associated with better performance in plants and animals (Endler 1986). In this study, LDH-B heterozygotes grew at the significantly highest rate, and the homozygotes were similar to each other in growth rate (Fig. 2). The growth-rate differences were small, but differences of this magnitude may alter competitive interactions among larval and juvenile amphibians (Werner and Anholt 1996; Peacor and Werner 1997; Woodward and Travis 1991). This difference in growth rate would probably have been accentuated in a more natural environment (Travis 1983). In the laboratory, however, heterozygotes had the highest mass at metamorphosis (Fig. 3), but the difference was not significant. This suggests that heterozygote advantage, if present, interacted with other factors in maintaining this polymorphism.

Table 6. Summary of ANOVA for growth rate and linear orthogonal constrasts comparing families (see the text for explanation)

		Mass at metamorphosis			Days to metamorphosis		
Source of variation	df	Type III mean square	*F*	*P*	Type III mean square	*F*	*P*
FAM	7	0.033	1.46	0.2051	0.031	1.12	0.3674
TEMP	1	0.626	27.38	0.0001	2.545	91.85	0.0001
QUAL	2	0.051	2.23	0.1188	0.116	4.22	0.0207
Residual	46	0.022			0.027		
Contrast	df	Contrast sum of squares	*F*	*P*	Contrast sum of squares	*F*	*P*
F1, F3 vs. F2, F4	1	0.030	1.33	0.2554	>0.001	0.01	0.9395
F1, F3 vs. F5, F7	1	0.034	1.50	0.2262	0.032	1.17	0.2852
F1, F3 vs. F6, F8	1	0.059	2.61	0.1132	0.067	2.42	0.1264
F6, F8 vs. F2, F4	1	0.004	0.18	0.6711	0.057	2.06	0.1576
F6, F8 vs. F5, F7	1	0.006	0.29	0.5925	0.010	0.36	0.5489
F1, F3 vs. F2, F4, F5, F7	1	0.045	2.00	0.1636	0.008	0.31	0.5816
F6, F8 vs. F2, F4, F5, F7	1	0.006	0.29	0.5901	0.038	1.38	0.2466

Note: All interactions were excluded to avoid missing cells. FAM (F), family; TEMP, temperature; QUAL, diet.

Environment-dependent selective neutrality of LDH-B genotypes

The effects of allozymes may depend on the environment. This may explain why some studies show selection on allozymes and some do not. Powers et al. (1993) showed that LDH-B genotypes of *Fundulus heteroclitus* differ in swimming speed at 10°C but not at 25°C, a result that is consistent with the results of biochemical investigations. When competing with the hybridogenetic associate *R. esculenta*, heterozygotes performed best but similarly to *e/e* homozygotes in mass at metamorphosis at low density (see footnote 3) (which would correspond to a high food level per individual), whereas the absolute difference among LDH-B genotypes was small at high density. The present study showed a difference among genotypes at the high food level but not at the low food level (Fig. 2), therefore selection on LDH-B genotypes is likely to vary in time and space, and may sometimes be near neutral. However, one should note that growth rates of *R. lessonae* tadpoles are generally much higher in the laboratory and large artificial ponds than they were in this laboratory experiment (three- to four-fold; Semlitsch 1993*a*, 1993*b*). Because the differences among LDH-B genotypes increase with food level (Fig. 2), LDH-B may be under selection in most environments. Given a high mortality rate at the low food level compared with other studies on *R. lessonae* (Semlitsch 1993*a*, 1993*b*), the low food level used in this study may be considered the lower limit of the food-level gradient.

Lack of significant genotype–environment interactions

Balancing selection due to genotype–environment interactions can maintain both quantitative genetic variation and polymorphisms at enzyme loci (Gillespie and Turelli 1989; but see Hoekstra et al. 1985; Kreitman and Akashi 1995). Differential performance of allelic genotypes maintains genetic polymorphisms in two particularly well studied cases, the PGI polymorphism in *Colias* butterflies (Watt 1977, 1983; Watt et al. 1983) and the LDH-B polymorphism in the fish *Fundulus heteroclitus* (Powers et al. 1993). In the present study, performance varied across environmental gradients but genotypes did not change ranks.

The biotic environment may also maintain genetic variation.The performance of genotypes may depend on the competitive environment in which they are raised. Additive genetic correlations between performance in distinct competitive environments may be negative (Shaw et al. 1995). We found that *R. lessonae* tadpoles raised in groups showed a different ranking from tadpoles raised individually, and the effect of the LDH-B genotype was much stronger and statistically significant. The ranking of LDH-B genotypes of tadpoles raised in groups was the same as that of LDH-B genotypes found previously (see footnote 3) when tadpoles from a population 35 km away from our study site were raised in artificial ponds under competition from *R. esculenta*. Although we are not aware of a mechanistic explanation, the effect of biotic factors on genotype performance should be studied in more detail.

Is there antagonistic pleiotropy between larval and adult performance?

The genotypes of both tadpoles raised in artificial ponds under competition from *R. esculenta* (see footnote 3) and the tadpoles raised in groups in this study showed the ranking *e/e* > *e/b* > *b/b*. Adult male frogs caught in the wild showed exactly the opposite ranking of LDH-B genotypes: *b/b* > *e/b* > *e/e* for body length (*e/e*: 52.6 ± 0.41 mm; *e/b*: 54.1 ± 0.40 mm; *b/b*: 54.6 ± 0.74 mm (H. Hotz, G.-D. Guex, and

P. Beerli, unpublished data); ANOVA, $F_{[2,166]} = 4.16$, $P = 0.0173$; Tukey test, *b/b* > *e/e*). Clearly, more detailed analyses of adult reproduction and survival are needed, but the data presented here suggest that there is a trade-off between larval and adult performance. Thus, antagonistic pleiotropy (Rose 1982) may be involved in the maintenance of the polymorphism at locus LDH-B in *R. lessonae*. However, theoretical models suggest that the conditions for this are very restrictive (Curtsinger et al. 1994).

Conclusions

The results of this study suggest that heterozygote advantage is involved in the maintenance of the genetic polymorphism at the locus LDH-B in *R. lessonae*. However, the locus seems not to be always under selection. When conditions for growth were unfavourable, LDH-B genotypes did not differ in performance. Otherwise the abiotic environments in which they were tested do not seem to be important for the maintenance of this polymorphism, because there were no genotype–environment interactions. The biotic environment may affect the performance of genotypes. There may also be a trade-off between larval and adult performance that interacts with heterozygote advantage to maintain the LDH-B polymorphism in *R. lessonae*.

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