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Local variation in *Rana temporaria* egg and clutch size: adaptations to pond drying?

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Egg and clutch size variation among populations of common frogs (*Rana temporaria*) were studied in 14 ponds in Sweden. Also two secondary indices, clutch mass and allocation tactics (egg size^3/clutch size) were studied. For all four characters there was a pond-wise correlation between the values across the two study years. All characters differed among ponds and all but allocation tactics differed between years. At least part of this variation must be environmental. The ponds were classified as permanent or temporary. The latter dried completely in some years, killing all tadpoles. Pond type affected all characters except egg size. Thus, clutches in shallow ponds were smaller, lighter and, correcting for clutch mass, had larger eggs (from the allocation tactics index). I suggest that this variation may be adaptive.

Introduction

Local character variation is a trait of all non-endemic animal populations. In common frogs (*Rana temporaria*), Joly (1991) and Ryser (1996) found differences in body size among local populations. My study is concerned with two other life history characters that are well covered by frog studies, namely clutch and egg size (Gibbons & McCarthy, 1986; Berven, 1988; Tejedo, 1992; Semlitsch & Schmiedhausen, 1994). However most studies are concerned with within-population variation and how this is related to individual strategies (exceptions being Joly, 1991, and Martin & Miaud, 1999). This is also the approach taken in a number of theoretical studies and reviews of life history theory (Smith & Fretwell, 1974; Roff, 1992; Ebert, 1993). The models can be used to compare optimal trait expression in different localities (Cunnington & Brooks, 2000).

The question posed in the present study is: does population variation in egg and clutch size characters exist in the study area and is this related to pond hydroperiod? Pond hydroperiod is studied because it has a profound effect on the survival of common frog tadpoles in the study ponds (Loman, 1996) as well as in other areas (Cooke, 1985; Kutenkov & Panarin, 1995). So, in temporary ponds fast developing tadpoles are at an obvious advantage. For some frogs (*Bombina orientalis*: Parichy & Kaplan, 1995; *Rana sylvatica*: Berven & Chadra, 1988), it has been shown that large eggs hatch into fast developing tadpoles. The same correlation has already been found for *Rana temporaria* in the present study area (unpubl.). This makes it particularly interesting to study if there is a relation between pond hydroperiod and egg size, one of the response variables studied here.

One reason local character variation is important to study is this. Basically, the variation may be genetic and/or due to direct environmental effects. If the variation has a genetic basis and is adaptive, this is a factor that must be taken into consideration in conservation work. Translocating individuals in order to increase diminishing populations may lead to "ecological outbreeding depression" (Schierup & Christiansen, 1996; Lardner, 2000). This is the case if the introduced genotype is not adapted to the new environment.

METHODS

The study is based on measurements on eggs and spawn clumps collected in the field and on field measurements of the length of the source pond hydroperiod.

During the breeding seasons of 1993 and 1994, spawn clumps were sampled in 14 ponds. All ponds were sampled in both years. The ponds are located in the central and southwestern part of Skåne, the southernmost province of Sweden. The southwesternmost ponds are in the vicinity of Lund, 40 km from the northeasternmost. The study ponds were classified as permanent or temporary. Temporary ponds were those eight that in some years dried out before or during the time the tadpoles metamorphosed. During the six years 1992-1997, when the ponds were monitored during the time of metamorphosis, this happened 2, 2, 2, 2, 5, 5, 5 and 6 times. The six permanent ponds always contained enough water for the entire period of tadpole life and metamorphosis. The number of spawn clumps laid in the ponds (average of 1993 and 1994) was between 19 and 310 in the temporary and between 16 and 160 in the permanent ponds.

From each pond I collected data on 6 to 22 spawn clumps. Each spawn clump was weighed and a sample of approximately 10 g was collected. The clump was then immediately returned to the pond. The sample was weighed at the pond and brought to the laboratory where the number of eggs in the sample was counted. This information was used to estimate "clutch size" (total number of eggs in the clump). The egg diameter (egg proper, excluding jelly) was measured with calipers in a sample of 15 eggs from each clump and the mean value was used as measure of "egg size". Because the ponds were visited every 5 days, I could age the spawn. Only spawn aged one to four days was used.

Two secondary indices were computed. Clutch volume was computed as the average egg volume times clutch size. "Allocation tactics index" was computed as average egg diameter^3 divided by clutch size. A large value of tactics means that a female, relative to other females that make the same total investment (same clutch mass), is more inclined to put her effort in large eggs but a smaller clutch.

Table 1. – Pearson correlation test between 1993 and 1994 values for the four indexes studied. Measurements are defined in the methods section. All tests are based on 14 ponds. The *P* values are one-tailed probabilities. A one-tailed test was used as any, hypothetical, negative correlations a priori would have been discarded as non sensical.

Index	*r*	*P*
Egg size	0.488	0.038
Clutch size	0.820	< 0.001
Clutch mass index	0.870	< 0.001
Allocation tactics index	0.580	0.015

Table 2. – Average clutch and pond values for permanent and temporary ponds. Egg size is diameter (mm), clutch size is number of eggs per clutch and clutch volume is total volume of eggs (cc). Allocation tactics is an index that is explained in the methods. *x*, mean; *s*, standard deviation; *n*, sample size.

Pond type		Egg size	Clutch size	Clutch volume	Allocation tactics
Permanent	*x*	1.88	1929	6.96	0.0040
	s	*0.12*	*347*	*1.30*	*0.0010*
	n	*6*	*6*	*6*	*6*
Temporary	*x*	1.87	1464	5.26	0.0052
	s	*0.066*	*289*	*1.51*	*0.0009*
	n	*8*	*8*	*8*	*8*

RESULTS

For all four characters (egg size, clutch size, clutch mass index and allocation tactics index), there was a significant correlation between the average value for a pond in 1993 and that in 1994 (tab. 1, fig. 1).

I also tested the independent effects of year, pond and pond type (permanent or temporary) on the four characters. Egg size was larger in 1994 than in 1993. It differed significantly among ponds but there was no effect of pond type (tab. 2-3, fig. 1). Clutch size was significantly larger in 1994 than in 1993 and it differed significantly among ponds. Clutches that were deposited in permanent ponds were larger than those in temporary ones (tab. 2-3, fig. 1). Clutch mass index showed the same pattern as clutch size; higher mass in 1994, a significant effect of pond and on average a higher mass for clutches in permanent ponds (tab. 2-3, fig. 1). The allocation tactics index differed significantly among ponds but not

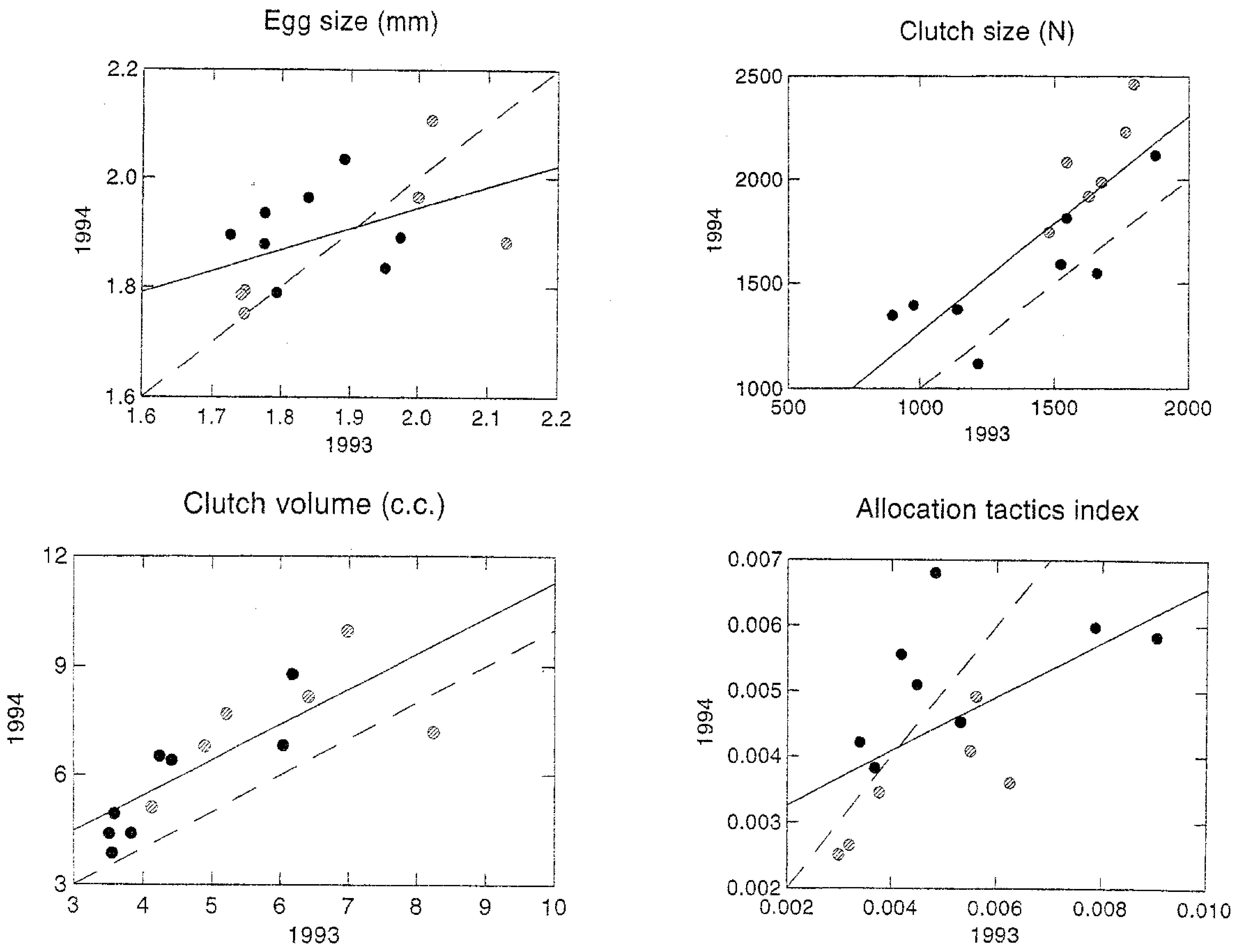

Fig. 1. – Relation between egg and clutch characters in 1993 and 1994. Permanent ponds are indicated with open circles and temporary ones with filled circles. The dashed line represents equal 1993 and 1994 values. A positive slope of the fitted linear regression (the unbroken line) indicates constancy in pond characteristics over years.

Table 3. – Effects from pond (individual pond, nested under pond hydroperiod), pond type (hydroperiod permanent or temporary) and year on egg and clutch characters, tested with a 3-way ANOVA. The interaction Pond*Year was tested but not found significant and removed for the definite test. Data are individual clutch measurements.

		Egg size	Clutch size	Clutch volume	Allocation tactics
Pond	*df*	11:222	11:224	11:221	11:221
	F	10.23	6.73	7.93	6.59
	P	< 0.001	< 0.001	< 0.001	< 0.001
Type	*df*	1:222	1:224	1:221	1:221
	F	0.015	43.0	22.7	28.3
	P	0.90	< 0.001	< 0.001	< 0.001
Year	*df*	1:222	1:224	1:221	1:221
	F	6.86	16.22	22.0	1.46
	P	0.009	< 0.001	< 0.001	0.23

years. The value was higher for temporary ponds than for permanent ones (tab. 2-3, fig. 1). This means that when correcting for the fact that females breeding in temporary ponds put less effort (a lower clutch mass index) altogether into breeding, they were more inclined to invest in larger eggs.

DISCUSSION

Indeed, there were differences in the four egg and clutch characters studied among populations breeding in different ponds. In previous studies, MARTIN & MIAUD (1999) and JOLY (1991) have detected differences in female size among neighbouring populations. In the Joly study, there were also differences in egg and clutch size between two populations. Because these variables have been shown to be correlated to female size within populations (HÖNIG, 1966; GIBBONS & MCCARTHY, 1986; JOLY, 1991; RYSER, 1996), it may well be that egg size variation between populations, both in the Joly study and in the present one, is a direct effect of among-population variation in female size. In the MARTIN & MIAUD (1998) study, the variation in egg size among populations was weak, despite among-population differences in female size.

Egg size, clutch size and clutch mass index varied between the two years. This was especially clear for clutch size; in all ponds but two were 1994 clutches larger than 1993 clutches. Also for egg size was the average pond value larger in 1994. This pond wide year effect suggests that weather factors are involved but, with only two years available for analysis, it is not possible to analyze which factors are crucial. Because the eggs are gradually formed during the preceding summer, there are numerous possibilities. Rainfall and temperature during any combination of months up to a year before spawning may potentially be involved. Again, because female size affects the clutch and egg size variables, between-year variation in female size may partly be the proximate cause, as suggested by Berven (1988) in a study of *Rana sylvatica*. Whatever the exact cues, this shows an environmental effect on egg and clutch size, either directly or indirectly through effects on female size.

Although there was a between-year variation, there was a significant year to year correlation among ponds (tab. 1); some ponds had consistently small eggs and clutches. This stresses the pond specificity. This was also manifest as a significant pond effect (tab. 3) on all variables. The pond effect was partly an effect of pond hydroperiod but note that it was still present when accounting for all effects of hydroperiod (as both factors were included in the ANOVA). These effects may be directly or indirectly (through effects on female size) due to variation in the environment and feeding conditions surrounding the ponds.

Genetic differences have been detected in allozyme studies between close populations of frogs (*Rana temporaria*: Reh & Seitz, 1990; Regnaut, 1997; Lardner, 2000; *Bufo calamita*: Sinsch, 1992). Also, frog species with a biology similar to that of *Rana temporaria* have been shown to exhibit natal pond fidelity: this includes *Bufo bufo* (Heusser, 1966; Reading et al., 1991) and *Rana sylvatica* (Berven & Grudzien, 1990). In a study of *Rana sylvatica*, Berven (1988) has shown that population differences in clutch hand egg size may have a genetic basis. Is it possible that the variation found in this study is due to microevolution? The design does not allow any definite conclusions. However, if microevolution is involved, one would expect the observed variation to be adaptive.

Was the effect of pond hydroperiod on the clutch mass index, parental investment, adaptive? Yes, possibly. The investment was less in shallow ponds. This means less stress on the female and possibly a higher survival with a better possibility to breed more times (Madsen & Shine, 2000). In shallow ponds, where breeding fails completely in some dry years, this means a higher likelihood of at least some surviving offspring during a life-time. Actually, life history theory predicts that iteroparous tendencies should be favoured in unpredictable environments (Roff, 1992).

It is also possible to find an adaptive effect of the variation in the allocation tactics. Given a fixed total investment (clutch mass), it is reasonable that it is more important to invest in large eggs (hatching into fast developing tadpoles) in temporary than in permanent ponds. Large eggs may hatch into tadpoles that develop faster than those hatched from small eggs (Berven & Chadra, 1988; Parichy & Kaplan, 1992), thus decreasing the risk of total loss of recruits in a dry summer when the pond may dry early.

It is surprising that there was no effect of pond hydroperiod directly on egg size (tab. 3). This effect could also be predicted for the same reasons I use above to argue an adaptive explanation for variation in allocation tactics index. However, egg size may (regardless of pond hydroperiod) be affected by female size (Hönig, 1966, Gibbons & McCarthy, 1986; Ryser, 1996). For this variable I have no data for the present ponds and thus I cannot control for it. If female size is not related to pond hydroperiod, such a correlation may mask possible effects of pond hydroperiod on egg size. However, the use of allocation tactics index is a method to control for variation in clutch mass, which means it may in turn control for some of the variation in female size (and other female characters that affect total clutch investment).

If the patterns recorded are indeed adaptive, it is easier to see this as an outcome of a direct, selected genetic effect than as a reaction norm (STEARNS & KOELLA, 1986; SCHLICHTING & PIGLIUCCI, 1998) or phenotypic plasticity. This means that females in all study populations may share the same genotype but this codes for different egg and clutch size strategies under the actual conditions present in the different ponds. This would lead to transgenerational phenotypic plasticity (MOUSSEAU & FOX, 1998). However, eggs are formed during the summer preceding breeding (JØRGENSEN, 1981), far ahead of the females' arrival to the breeding pond. Although complicated pathways based on breeding site fidelity are possible, such explanations seem far fetched.

To sum up, the variation recorded must at least in part be due to environmental effects. The fact that some of the variation was adaptive in ways predicted by life history theory suggests that microevolution was also involved. No direct proof for this is however available. Further studies to reveal the nature of between-pond variation in these characters need to use frogs originating in different ponds and raised in a "common garden" (FAUTH, 1998) from egg to maturity. Comparing the traits of their spawn should provide the necessary evidence.

If local adaptions are indeed involved, this also means that translocating frogs affects the genetic make-up of local target populations. In principle, this could have adverse effects (STORFER, 1999). However, the fact that evolution may have occurred in response to such a "fine grained" habitat variable as pond hydroperiod also means that this evolution may proceed quite quickly. Thus the progeny of translocated individuals are likely to adapt quickly, provided a sufficient genetical basis is provided (LARDNER, 2000).

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