

## SPECIFIC RESPONSES OF SEXUAL AND HYBRIDOGENETIC EUROPEAN WATERFROG TADPOLES TO TEMPERATURE

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**Abstract.** The European waterfrog, *Rana esculenta*, is a hemiclinal hybrid that must coexist with the parental species *Rana lessonae* in order to reproduce. It is not clear what allows the two morphologically, genetically, and ecologically similar forms to coexist, but differential success of the hybrid and its sexual host among environments suggests that these frogs may differ in their adaptive abilities, and that ecology plays an important role in determining the relative frequencies of the two related species. The objective of this study was to identify factors that may promote coexistence. We investigated the effect of temperature, food level, and food quality on a variety of life history traits in a laboratory experiment. Our results indicated that tadpoles of the two forms respond differently to temperature. Probability of metamorphosis and survival of *R. lessonae* were higher at 24°C, while the hybrid, *R. esculenta*, had a better survival rate and a much larger body mass at metamorphosis at 18°C. We then tested the results of our laboratory experiment by assessing the distribution of the hybridogen and the parental species in natural populations as a function of temperature, and found that the relative frequency of *R. esculenta* tadpoles declined with increasing temperature. We use these results to evaluate the applicability of the generalist and frozen niche variation models that had been proposed earlier as explanations for the coexistence of the sexual parental species, *R. lessonae*, and the hybridogen, *R. esculenta*.

**Key words:** amphibian; clonal species; coexistence; frozen niche variation model; general-purpose genotype; habitat segregation; hybridogenesis; *Rana esculenta*; *Rana lessonae*; temperature and life history traits.

### INTRODUCTION

Most unisexual fish and amphibia are interspecific hybrids that require sperm of a related sexual species to reproduce. In gynogenetic systems, sperm is required to activate cleavage, but eggs develop without recombination into genetically identical (clonal) offspring. In contrast, in hybridogenetic systems, only the maternal genome is transmitted without recombination to the egg, while the paternal genome is replaced each generation by sperm from the sexual parental species, restoring the hybrid condition (Schultz 1969, reviewed in Schlosser et al. 1998). In the absence of the sexual species, hybridogens are expected to go extinct, and unlike parthenogenetic reptiles that can exist independently of their parental species (Clanton 1934, Darvsky et al. 1985), the geographic distribution of hybridogenetic species is intimately linked to that of the parental sexual species.

The hybridogenetic and sexual species may share considerable genetic and ecological similarity, sug-

gesting that they experience resource competition as well as competition for matings with males of the sexual species (e.g., Moore and McKay 1971, Wilbur 1971, Thibault 1978, reviewed in Beukeboom and Vrijenhoek 1998). Thus, what allows the two forms to coexist is a fundamental question in the population ecology of gynogens or hybridogens and their sexual host (Beukeboom and Vrijenhoek 1998).

Two general explanations have been put forward to explain the coexistence of hybridogens with sexual forms. The widespread distributions of some unisexual vertebrates suggest that they are broadly adapted generalists with wide ecological tolerances and phenotypic plasticity, but that competitive interactions will confine their success to underutilized resources and areas to which the sexuals are poorly adapted. The generalist model (Baker 1965, Lynch 1984) argues that successful single clones of asexually reproducing organisms are more broadly adapted than their sexual relatives because, via interclonal selection, they have evolved more generalized resource utilization. The clones with the highest geometric mean fitness should have a broad tolerance to relevant physical, chemical and biotic gradients. Several studies have identified such “general-purpose” genotypes that appear broadly adapted and tolerant of a wide range of environmental conditions (for a review see Lynch 1984, Bierzychudek 1989, Schlosser et al. 1998).

However, clonal diversity can be high, and in various

Manuscript received 9 August 1999; revised 29 February 2000; accepted 1 March 2000.

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taxa, different clones have been found to coexist in sympatry. This observation inspired the frozen niche variation hypothesis (Vrijenhoek 1979, 1984) which argues that polyphyletic origins of clones will lead to an array of different clones, each of which possesses a sample of the phenotypic and ecological variation present in the parental sexual population. In a heterogeneous environment, selection will then eliminate poor competitors that are ecologically similar to other clones, leading to an equilibrium where the remaining clones occupy different ecological niches. Each clone would express only a fraction of the total niche use observed in the sexual population. If the frozen niche variation hypothesis applies, the coexistence of unisexual forms with their sexual ancestors as well as the coexistence of various clones should occur because of narrow fundamental niches of clones relative to the sexuals (Vrijenhoek 1979, 1984, Case and Taper 1986). The conditions necessary for stable coexistence between gynogens or hybridogens and their sexual host are thought to be highly restrictive (Stenseth et al. 1985). However, reduced niche overlap as invoked by the frozen niche model may be a primary factor for stabilizing sperm-dependent parthenogenetic complexes (Kirkendall and Stenseth 1990).

Thus, local environmental conditions may determine which particular genotypes or clones prevail at any one site. Nonrandom distribution of clones between local habitat patches has been found in a number of invertebrate and vertebrate taxa. Such spatial segregation of phenotypically similar clones may serve to reduce direct competition and be a result of different ecological requirements and resource uses (e.g., Booij and Guldemond 1984, Harshman and Futuyma 1985, Schenk and Vrijenhoek 1986, 1989, Browne and Hoopes 1990, Case 1990, Weider 1993, Bolger and Case 1994, reviewed in Vrijenhoek 1998).

Similar to other unisexual vertebrate systems, the European water frog, *Rana esculenta*, is a diploid hybrid possessing one genome from each of the parental species, *Rana lessonae*, and *Rana ridibunda*. Within the most common breeding system, the *R. lessonae*-*R. esculenta* (L-E) complex of central Europe, the only sympatric parental species is *R. lessonae*. *R. esculenta* has an unusual altered gametogenic mechanism that transmits the ancestral *R. ridibunda* genome without recombination to its haploid gametes. Matings with the parental host species *R. lessonae* produce *R. esculenta* progeny (Berger 1973).

*R. esculenta* has a more elaborate clonal system than that found in other unisexual vertebrates. Although this complex shows most of the other characteristics of unisexuals, males and females are common, i.e., *R. esculenta* is bisexual. The breeding system can nevertheless be regarded as effectively unisexual, as in many populations only females produce the next generation (Graf and Polls-Pellaz 1989).

Several studies have shown a wider environmental

tolerance of *R. esculenta* tadpoles relative to its sexual host *R. lessonae*, especially in harsh environments such as drying ponds, low food, high larval density, interspecific competition, and agricultural chemicals (Semlitsch and Reyer 1992, Semlitsch 1993, Fioramonti et al. 1997). These findings suggest that *R. esculenta* might possess a general-purpose genotype (Lynch 1984) with broad tolerances to environmental extremes. However, most of these investigations have treated *R. esculenta* as a single genotype, so that the observed broad environmental tolerance could result from an array of different hemiclones, each adapted to a different narrow range of resources along an environmental gradient. When three different hemiclones were separately tested, there were significant differences in performance along environmental gradients such as larval density and food level (Semlitsch et al. 1997). Such differences in performance could lead to niche partitioning, as suggested by the frozen niche variation hypothesis (Vrijenhoek 1979, 1984). The specific niches partitioned among clones are still unknown. However, the relative success of the two taxa across habitats could depend on food type, food abundance, position in the water column, diel pattern of activity, and thermal gradients (Semlitsch et al. 1997).

The objective of this study was to identify factors that promote coexistence in populations of the hybridogenetic *R. esculenta* complex. We compared variation in the performance of the hybrid *R. esculenta* with that of the parental species *R. lessonae* under a range of environmental factors. Our results indicate that the two forms have different temperature optima. We then tested the results of our laboratory experiment by assessing the distribution of the hybridogen and the parental species in natural populations as a function of temperature. We use these results to evaluate the applicability of the generalist and frozen niche variation models as explanations for the coexistence of the sexual parental species, *R. lessonae*, and the hybridogen, *R. esculenta*.

## MATERIALS AND METHODS

### *Laboratory study*

We collected adult frogs on 25 and 29 May 1994 from a pond near Hellberg, Kanton Zurich, Switzerland. The frog population at this pond was 64% *R. lessonae* and 36% *R. esculenta* comprising a single hemiclone (Semlitsch et al. 1996). We determined the genotype of each frog by protein electrophoresis (Uzzell and Berger 1975). We artificially crossed four male *R. lessonae* with two *R. lessonae* females and two *R. esculenta* females on 4-5 June following the methodology of Berger et al. (1994). Sperm suspensions from each male were used to fertilize each female, resulting in a total of 16 families. Thus, offspring from the parental species and hybrid differed only by the maternal half of their genome (sexually produced in *R.*

*lessonae* and clonally produced in *R. esculenta*), and its interaction with the paternal contribution. For the hybrid crosses, we crossed male *R. lessonae* with female *R. esculenta*, as opposed to male *R. esculenta* with female *R. lessonae*, because this is the most frequent mating combination in natural populations (Blankenhorn 1977).

We reared *R. lessonae* and *R. esculenta* tadpoles at all combinations of two temperatures, two food levels, and three different diets, resulting in 24 treatment combinations, which were all replicated eight times. The two temperatures were 18°C and 24°C (with alarms at temperatures  $\pm 2^\circ\text{C}$  which were never activated), which are the upper and lower limits for the tadpoles to metamorphose in the lab without inducing a high mortality (S. Negovetic and R. D. Semlitsch, unpublished data), and are well within the range of temperatures measured in ponds with successful reproduction (G. Abt, personal communication). We set the high food ration at the maximum a tadpole could eat without polluting the water with excess food, and the low ration at one third of the high ration. We increased food rations as the tadpoles gained mass, and adjusted them according to consumption every three days at the high food level. Starting food rations were 5 mg/d at low food level and 15 mg/d at high food level. The final rations reached 20 mg/d at low and 60 mg/d at high food level. The diets consisted of dried, pulverized nettle powder and freeze-dried, pulverized *Tubifex* worms, provided in three ratios (0.8:0.2, 0.5:0.5, and 0.2:0.8). Thus, we created variable protein:carbohydrate ratios (low, intermediate, high) that were independent of changes in the total mass of food (Steinwascher and Travis 1983).

When tadpoles were free swimming, we randomly assigned animals of each taxon to a treatment and reared them individually in plastic boxes ( $20 \times 11.5 \times 7.5$  cm) filled with 1.0 L of aged tap water. We arranged boxes into two randomized complete blocks in each temperature chamber. Except for the first nine days, we fed the tadpoles and changed water every 72 h.

The experiment lasted for four months, without completion of metamorphosis in all treatments. By this time, the remaining tadpoles had stopped their development. We used survival, metamorphosis (forelimb emergence; stage 42, Gosner 1960), mass at metamorphosis, and mass  $\pm 0.1$  mg at day 30 (mean  $\pm 1$  SE) as response variables. We weighed tadpoles immediately after the emergence of the first forelimb, in case of mortality during tail resorption. Although mass at tail resorption has been shown to be the best estimate of body size at metamorphosis (Travis 1984), our data indicated that mass at forelimb emergence and tail resorption were strongly correlated in any case ( $r = 0.962$ ,  $n = 25$ ). The time period of the first 30 d was the best estimate of tadpole growth because it was not affected by metamorphosis, which began on day 51.

TABLE 1. Number of individuals surviving the experiment.

Species	High temp. (24°C)		Low temp. (18°C)	
	High food	Low food	High food	Low food
<i>Rana esculenta</i>	19	4	23	14
<i>R. lessonae</i>	20	8	18	4

We did not separately analyze early development rate because the correlation between mass at day 30 and developmental stage (Gosner 1960) was very high ( $r = 0.921$ ,  $df = 188$ ).

We analyzed the probability of surviving and the probability of metamorphosis among treatments using a Generalized Linear Model with binomial errors using the `glm()` function of S-Plus 4.5. We assessed significance using likelihood ratio tests that are asymptotically  $\chi^2$  distributed. We analyzed the timing of metamorphosis as a Cox proportional hazard model using the `coxph()` function of S-Plus 4.5. Mass at metamorphosis and at d 30 were logarithmically transformed before analysis to reduce skewness and meet the assumptions of normally distributed errors. We tested for differences in treatment effects and their interactions with ANOVA using the GLM procedure of SPSS statistical software (SPSS Incorporated, Chicago, Illinois, USA).

#### Field study

In 1996, we sampled the pond at Hellberg that was used for the lab experiment as well as two additional ponds in northern Switzerland, separated from Hellberg by up to 100 km. These two ponds contained two additional hemiclones not found at Hellberg. These three hemiclones are by far the most common of those identified in northern Switzerland (Semlitsch et al. 1996). We sampled using minnow traps placed in a variety of habitats. We measured water temperature at the opening of the traps at the time they were placed. We collected the traps after 1–2 h and transferred the tadpoles to labeled plastic bags before returning them to the laboratory, where we euthanized the animals in an excess of Tricaine methanesulfate (Bachem Incorporated, Basel, Switzerland) and froze them for later identification by gel electrophoresis, using the same methods as for the adults. We analyzed the proportion of *R. esculenta* caught in a minnow trap as a function of trap temperature and pond as a generalized linear model using the `glm()` function of S-Plus 4.5 (Stat-Sci Incorporated 1998) with binomial errors and the logit link.

## RESULTS

### Laboratory experiment

Survival was highest at high food level, and when tadpoles were fed the intermediate protein diet (Tables 1 and 2). There was no significant overall genotype or temperature effect, but there was a strong genotype by

TABLE 2. Analysis of deviance for the probability of survival.

Treatment	df	$\Delta$ deviance	<i>P</i>
Genotype (G)	1	2.13	0.14
Temperature (T)	1	1.38	0.24
Food level (F)	1	57.69	<0.0001
Diet (D)	2	98.68	<0.0001
G $\times$ T	1	27.01	<0.0001
G $\times$ F	1	0.06	0.81
T $\times$ F	1	0.20	0.65
G $\times$ T $\times$ F	1	7.02	0.008

Notes: Changes in deviance are asymptotically chi-square distributed. All remaining higher order interactions were not significant.

temperature interaction term. *Rana esculenta* survived better at low temperature, while *Rana lessonae* survived better at high temperature. This effect was even larger at low food level (Tables 1 and 2).

More tadpoles reached metamorphosis at high food level than at low food level, and the intermediate protein diet produced the highest proportion of metamorphs (Tables 3 and 4). More *R. lessonae* than *R. esculenta* reached metamorphosis, and overall, more tadpoles metamorphosed at both high temperature and high food level. However, both these effects were in the neighborhood of alpha and there was no significant interaction (Tables 3 and 4). The significant three-way interaction term indicates that the effect of low food level on the number of metamorphs at low temperature is stronger for *R. lessonae* than it is for *R. esculenta*.

Tadpoles raised at high food levels and at high temperature had a shorter larval period, and *R. lessonae* metamorphosed earlier than *R. esculenta* (Fig. 1, Table 5). Tadpoles raised on the intermediate protein diet also emerged earlier than those raised on the other diets tested (Table 5). Unlike the probability of metamorphosis, there was no significant interaction between genotype and temperature for the timing of metamorphosis. Excluding the low food level treatments where few individuals metamorphosed did not affect the significance of the interaction term ( $z = -1.22, P = 0.22$ ). However, further subdividing the data to look within each temperature treatment at the high food level showed that the difference between the two species was significant at 18°C ( $z = -4.00, P < 0.0001$ ) but not at 24°C ( $z = -1.09, P = 0.28$ ). Parameter estimates for other interactions are not available because the maximum likelihood function failed to converge, due to few metamorphs at low food levels.

*R. esculenta* was heavier at metamorphosis than *R. lessonae* across treatments, but the difference in mass at metamorphosis was largest at low temperature, where *R. esculenta* was 400 mg heavier (Table 6, Fig. 2). Although food level and diet both affected survival, time to metamorphosis, and the probability of metamorphosis, they did not affect the mass at metamorphosis (Table 6).

TABLE 3. Number of individuals reaching metamorphosis before the end of the experiment.

Species	High temp. (24°C)		Low temp. (18°C)	
	High food	Low food	High food	Low food
<i>Rana esculenta</i>	18	0	12	1
<i>R. lessonae</i>	20	6	18	0

In the first 30 d, *R. lessonae* grew faster (9.1 mg/d) than *R. esculenta* (8.5 mg/d) (Fig. 3, Table 6). Growth rate was almost twice as high when the tadpoles were provided with more food and kept at a higher temperature (Fig. 3, Table 6). At low temperature, growth rate of *R. lessonae* was more strongly affected by food level (low: 4.3 mg/d, high: 9.6 mg/d) than was that of *R. esculenta* (low: 4.0 mg/d, high: 6.5 mg/d) (Fig. 3, Table 6). Body mass was also higher when tadpoles were fed with the intermediate protein diet.

Field study

The relative proportion of *R. esculenta* declined with increasing temperature ( $z = -4.4, P < 0.0001$ , Fig. 4) independent of the significant variation in the frequency of *R. esculenta* among the three ponds, which ranged from 7% to 64% ( $z = 3.65, P = 0.0003, z = -4.01, P < 0.0001$ ). The date of collection had no effect on the proportion of *R. esculenta* collected in the traps ( $z = 0.22, P = 0.58$ )

DISCUSSION

The most pronounced differences in the performance of *R. lessonae* and *R. esculenta* tadpoles in our laboratory experiment were caused by temperature. The parental sexual species, *R. lessonae*, performed better at high temperature, *R. esculenta* at low temperature. This result predicted the outcome of our field study, where the spatial variation in abundances of *R. lessonae* and *R. esculenta*, within and between ponds, indicated habitat partitioning on the basis of thermal microhabitat preferences, thereby providing at least one basis for coexistence.

In the laboratory, tadpoles exhibited significant phe-

TABLE 4. Analysis of deviance for the probability of metamorphosis.

Treatment	df	$\Delta$ deviance	<i>P</i>
Genotype (G)	1	3.71	0.054
Temperature (T)	1	3.79	0.052
Food level (F)	1	96.78	<0.0001
Diet (D)	2	7.98	0.018
G $\times$ T	1	0.59	0.44
G $\times$ F	1	0.56	0.55
T $\times$ F	1	0.71	0.40
G $\times$ T $\times$ F	1	6.72	0.01

Notes: Changes in deviance are asymptotically chi-square distributed. All remaining higher order interactions were not significant.

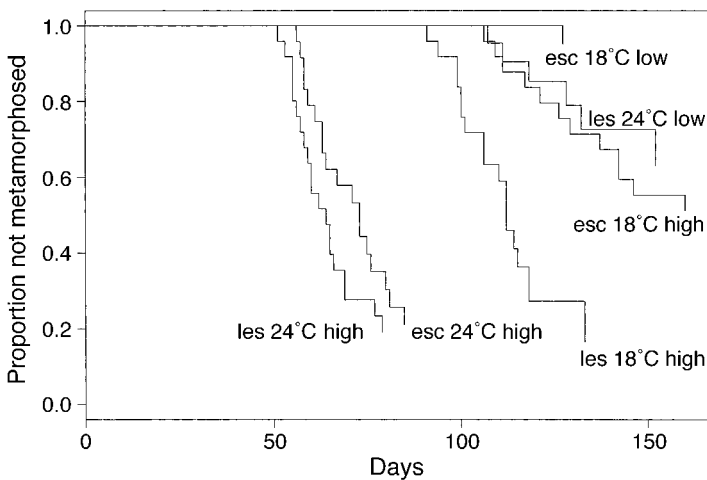


FIG. 1. The proportion of larvae that have not yet reached metamorphosis as a function of genotype, temperature, and food level. Two treatment combinations produced no metamorphosis. The plot accounts for loss of animals through death. Abbreviations: esc = *R. esculenta*, les = *R. lessonae*.

notypic variation in response to temperature in several traits directly related to fitness. *R. lessonae* had higher survival at 24°C than *R. esculenta*, and also produced more metamorphs, especially at the low food level. In contrast, *R. esculenta* survived better than the parental species at 18°C. This effect was again more pronounced at the low food level. While in most treatments *R. lessonae* had a higher growth rate, it performed most poorly at 18°C at low food level, where it also had a much lower mass at metamorphosis than *R. esculenta*. This suggests that tadpoles of *R. lessonae* may be better adapted to the high temperature, and *R. esculenta* to the low temperature, where they are each better able to exploit scarce resources. *R. lessonae* may lack the ability to adjust feeding rate, digestive efficiency or developmental rates at cold temperature, which would compensate for the unfavorable environment (see Rist et al. 1997). While *R. esculenta* thrived much better at low temperature, both at scarce and abundant food resources, *R. lessonae* apparently invested more of its energy in development and less in growth, in order to

escape the poor conditions at the minimal possible size (Werner 1986). This was also suggested by the result for the length of the larval period, as *R. lessonae* metamorphosed significantly earlier and at a smaller body size than *R. esculenta*. However, this effect was not modified by temperature. At high food levels almost all of the tadpoles had reached metamorphosis within two to four months which is the range of development times observed in the field (Günther 1990; G. Abt, *personal communication*).

Differential thermal behavior has also been reported for adults of the two parental species *R. lessonae* and *R. ridibunda* (Sinsch 1984). The temperature preference of *R. ridibunda* lies clearly below that of *R. lessonae*. Thus, *R. esculenta* might be expected to show an intermediate preference, which is lower than that of *R. lessonae*. The preference of *R. lessonae* to chorus in the warmer parts of the pond (Sjögren et al. 1988) may reflect a strategy to maximize growth and development of the offspring.

As long as a habitat is thermally diverse, competitive exclusion between the two closely related taxa is unlikely to occur. If the hybridogen spends most of its time in a different thermal zone than its sexual progenitor, then competition might be reduced and resources divided. If such responses are sensitive enough, then the frequency of genotypes could, in part, depend on the amount of thermally optimal habitat available on a temporal and spatial basis (Schultz and Fielding 1989). The contrasting responses to temperature in various life history traits of *R. lessonae* and *R. esculenta* suggest a basis for dividing the habitat in a way that enhances coexistence by differential resource use, which was confirmed in our field study.

Although the lab study was based on only two matrilines in the sexual species, *R. lessonae*, it allowed us to predict the distribution of tadpoles in the field. Therefore, we have more confidence in the the generality of the results, especially because the field study

TABLE 5. Effect of experimental treatments on the instantaneous probability of metamorphosis estimated by Cox proportional hazards.

Treatment effect	Relative <i>P</i>	<i>z</i> †	<i>P</i>
Genotype (G)	0.592	-4.03	<0.0001
Temperature (T)	0.346	-6.81	<0.0001
Food level (F)	0.129	-8.77	<0.0001
Diet 1 (intermediate vs. low protein)	2.392	5.00	<0.0001
Diet 2 (high vs. low protein)	1.120	1.29	0.2
G × T	0.923	-0.65	0.51

Notes: Animals that died are censored at the date of death; animals that failed to metamorphose are censored at the end of the experiment. Parameter estimates for more complex models are not available because the maximum likelihood function failed to converge.

† Standard deviations away from zero in a normal distribution.

TABLE 6. Summary of the univariate analysis of variance (ANOVA) of mass at metamorphosis (mg) and at day 30 of *R. lessonae* and the hybridogen *R. esculenta* reared at two temperatures, two food levels, and three diets.

Response variable	Effect	MS	df	F ratio	P
Mass at metamorphosis	Genotype (G)	0.57	1	4.55	0.037
	Temperature (T)	2.21	1	17.78	<0.001
	Food level (F)	0.02	1	0.15	0.700
	Diets (D)	0.21	2	1.67	0.197
	G × T	0.54	1	4.33	0.041
	G × F	0.04	1	0.33	0.570
	G × D	0.10	2	0.77	0.467
	Error		0.12	65	
Mass at day 30	Genotype (G)	0.39	1	5.64	0.019
	Temperature (T)	14.59	1	209	<0.001
	Food Level (F)	28.25	1	405	<0.001
	Diets (D)	0.45	2	6.49	0.002
	G × T	0.22	1	3.22	0.075
	G × F	0.01	1	0.01	0.989
	G × D	0.07	2	1.04	0.356
	T × F	0.18	1	2.63	0.107
	T × D	0.74	2	10.57	<0.001
	F × D	0.07	2	0.95	0.389
	G × T × F	0.43	1	6.21	0.014
	G × T × D	0.01	2	0.08	0.962
	G × F × D	0.25	2	3.57	0.030
	T × F × D	0.05	2	0.71	0.495
	G × T × F × D	0.01	2	0.15	0.860
Error		0.07	166		

Note: Because of empty cells, we only tested the main effects and the two-way interactions with genotype for mass at metamorphosis.

encompassed sites that were widely dispersed from each other and contained two more *R. esculenta* hemiclones. Within-species or matriline variation in performance is less of a problem in the hybrid, as the two matrilines were the same hemiclone. They differed from the sexual form only by the inherited maternal genome, because every matriline was mated to the same set of males. Thus, variation due to the paternal component was controlled.

Comparative analyses of the thermal biology of hybridogenetic clones are rare (but see Bulger and Schultz 1981, Vrijenhoek and Pfeiler 1997), despite the profound influence of thermal environment on the ecology of ectothermic organisms. For example, in a desert stream-dwelling poeciliid fish, coexisting clonal and sexual forms differed dramatically in survival during

heat or hypoxic stress (Vrijenhoek and Pfeiler 1997). In tadpoles of various taxa, temperature has been shown to be an important proximate factor controlling daily and seasonal activity patterns, spatial distribution, development, and physiological processes, as well as social interactions and all aspects of resource use (e.g., Beiswenger 1977, Berven and Gill 1983, Griffiths 1985, Browne et al. 1988, Warkentin 1992). Further, changes in temperature can have pronounced effects on the expression of life history traits, such as the maturation period, length of the reproductive period, total lifespan, brood size, brood numbers, interbrood interval, and total offspring production in a variety of taxa (e.g., Murphy et al. 1983, Baldwin and Dingle 1986, Browne et al. 1988, Huey and Kingsolver 1993).

In general, increasing temperature accelerates larval

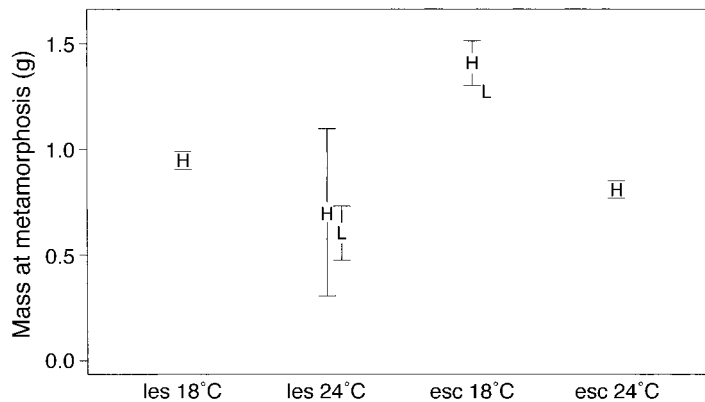


FIG. 2. Mass (mean  $\pm$  1 SE) of larvae at metamorphosis (Stage 42; Gosner 1960). Food level treatments are denoted with H for high and L for low levels. At low food level, *R. lessonae* produced no metamorphs at low temperature, while *R. esculenta* produced no metamorphs at high temperature and only a single metamorph at low temperature. Abbreviations: esc = *R. esculenta*, les = *R. lessonae*.

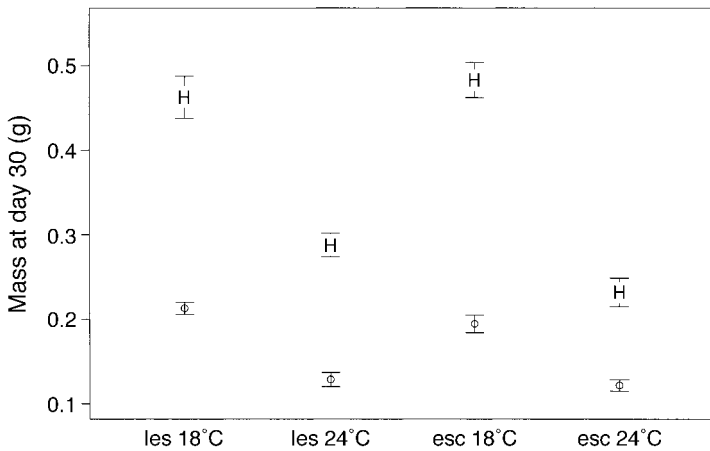


FIG. 3. Mass (mean  $\pm$  1 SE) of larvae at day 30. The high food level treatment is denoted with an H. Abbreviations: esc = *R. esculenta*, les = *R. lessonae*.

differentiation relatively faster than larval growth. Developmental patterns change from delayed metamorphosis at a large size under cool conditions to early metamorphosis at a small size under warm conditions (Smith-Gill and Berven 1979). Our data are consistent with this pattern. However, we also observed a strong genotype effect. At low temperature, *R. esculenta* grew to be much larger and metamorphosed later than *R. lessonae*, while there was no difference in size between the two genotypes at high temperature, where *R. lessonae* metamorphosed slightly but not significantly earlier.

Results for *R. lessonae* indicate that phenotypic variation in length of the larval period and size at metamorphosis are underlain by genetic variation, and individuals that are growing most rapidly complete metamorphosis early regardless of size, because they also differentiate rapidly (Smith-Gill and Berven 1979). Semlitsch (1993) found that the success of *R. esculenta* is likely due to its ability to metamorphose earlier than *R. lessonae* under resource-limited conditions. Our data

only partly confirm this finding. We show that superior performance of *R. esculenta* at low temperature will be enhanced when resources are scarce, but the success of *R. lessonae* at high temperature will also be more pronounced under the same conditions.

The differential responses of the two species to temperature in our laboratory experiment and the observed habitat segregation in our field study demonstrate the potential for differential resource use. The results thus favor the frozen niche variation model over the general-purpose genotype model as an explanation for coexistence within the *R. esculenta* complex. The expectation of *R. esculenta* possessing a general-purpose genotype is not supported by the data in our laboratory experiment. The hybridogen did not show a wider environmental tolerance, and the variance in performance across treatments was similar to, if not higher than that of the sexual form. These results are in accordance with the findings of Semlitsch et al. (1997), who found large performance differences among three different hemi-

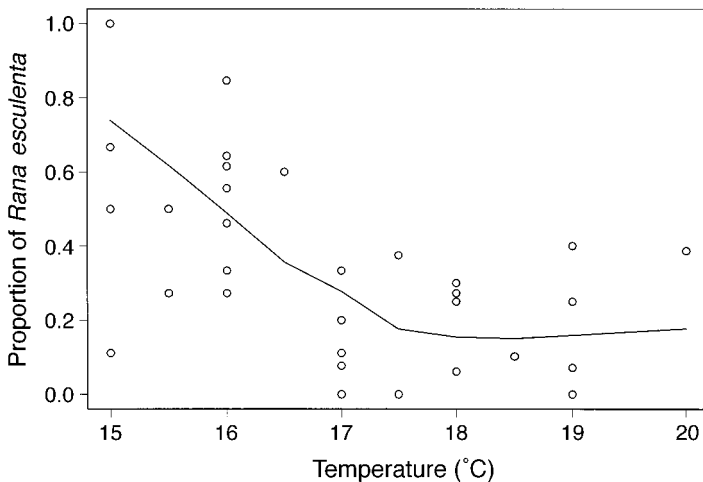


FIG. 4. Proportion of *Rana esculenta* tadpoles caught in minnow traps as a function of microhabitat temperature. The central trend line is a lowest local regression smoother of the data (Hastie and Tibshirani 1990).

clones of *R. esculenta* along a realistic environmental gradient such as larval density and food level.

Despite the genetic and morphological similarities, it is not surprising that these two closely related species show differential responses to temperature in a number of relevant life history traits, and thus should have different ecological requirements, especially because *R. esculenta* is a hybrid whose parental forms show different thermal ecologies. The preference of *R. esculenta* for lower temperatures could promote coexistence with *R. lessonae* and affect the structure of mixed populations. If different ponds are predominantly cool or warm, this would favour one species over the other and could lead to different equilibria. The composition of *R. esculenta*-*R. lessonae* populations is highly variable among localities, and the proportion of *R. esculenta* in an assemblage can vary from 7% in forest or natural marshes to 98% in gravel pits (Blankenhorn 1973, Berger 1983). There is significant phenotypic variation in several life history traits of naturally coexisting hemiclones that could mediate the outcome of interclonal competition, and hence coexistence (Semlitsch et al. 1997), but the specific niches partitioned among clones are unknown. Our study demonstrates the potential for thermal niche segregation in different *R. esculenta* hemiclones, because up to three hemiclones were present at some field sites. Further, the hemiclone investigated in the laboratory did not show a broad tolerance to temperature. However, we do not dismiss the general-purpose genotype model as an explanation for the success and widespread distribution of some hemiclones, as the generalist and the clonal specialist hypotheses are not mutually exclusive (Vrijenhoek 1979, 1998, Semlitsch et al. 1997, Vrijenhoek and Pfeiler 1997, Schlosser 1998). Selection may favor a generalist strategy for one suite of traits, while another suite of traits might be disruptively selected as predicted by the frozen niche variation hypothesis. Yet, if differences in the physical environment related to resource use are temporally stable, or if tadpoles can track environments across landscapes, specialized clones will clearly be at an advantage over generalists.

#### ACKNOWLEDGMENTS

We thank H. Hotz, G. Guex, and N. Pruvost for help with the crosses and electrophoresis, R. Muheim, C. Som, and C. Rauter for assistance in the field, and B. Schmidt for comments on early versions of the manuscript. Comments by D. Pfennig, R. Vrijenhoek, and an anonymous reviewer greatly improved the final manuscript. This study was supported by Swiss National Science Foundation grants NF31-28568.90 to R. Semlitsch and NF31-37579.93 to B. Anholt. Preparation of the manuscript was supported by a Natural Sciences and Engineering Research Council of Canada research grant to B. Anholt.

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