

Differential Morphology and Jumping Performance of Newly Metamorphosed Frogs of the Hybridogenetic *Rana esculenta* Complex

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ABSTRACT.—Closely related clonal and sexual populations may coexist in spite of the theorized lower potential for the evolution of clonal genotypes. Water frogs of the *Rana esculenta* complex have hemiclinal inheritance but most populations coexist with one of the recombinant parental species. We examine whether hemiclinal lineages may counterbalance their limitations of genetic variability by the adoption of one or more non-exclusive mechanisms: the general-purpose genotype or the frozen niche-variation model. Three coexisting hemiclones of the hybrid *R. esculenta* (*GUT1*, *GUT2*, *GLIT3*) and both parental species (syntopic *R. lessonae* and allopatric *R. ridibunda*) were raised at two larval densities to examine morphological traits affecting jumping performance at the time of metamorphosis and size-independent jumping ability tested at three temperatures. Hind leg length and body mass at metamorphosis, traits that explain most of the variance in jumping performance, differed across hemiclones of *R. esculenta*. Metamorphs of hemiclone *GUT1* had the longest hindlimbs and were bigger than metamorphs of the other hemiclones at low larval density but not at high density. Size adjusted jumping performance exhibited a significant genotype by larval density interaction. Metamorphs of *GUT1* showed maximal jumping performance when raised at low larval density but at high density metamorphs of *GUT2* were the best jumpers. In addition, within particular traits, differences were found between hemiclones across densities. These results appear to be consistent with both frozen niche-variation model and the general-purpose genotype model. Comparison with parental species revealed syntopic *R. lessonae* was smaller than hemiclones at metamorphosis but conversely exhibited better size-adjusted jumping performance when raised at low larval density. Temperature affected size-adjusted jumping performance only for frogs raised at low larval density but not for those raised at high larval densities. There was no significant temperature by hemiclone interaction,

Asexual reproduction is rare as a mode of genetic inheritance among vertebrates. Only approximately 80 unisexual taxa have been described, which represent 0.1% of known vertebrate species (White, 1978; Vrijenhoek et al., 1989). Theories that attempt to explain the widespread occurrence of sexual reproduction are typically based on the assumption that asexual and sexual genotypes are fundamentally similar except for the sexual individuals' ability to produce genetically diverse progeny. It is also assumed that natural selection operates on these diverse genotypes enabling populations to evolve in response to changing environments. However, if this last assumption is discounted, asexual lineages should rapidly replace their sexual ancestors, because most escape the cost of producing males. This supplementary reproductive cost of producing males in sexuals determines the "paradox of sexuality" (Williams,

1975; Maynard Smith, 1978), and predicts that coexistence between sexuals and asexual lineages is unlikely. However, extensive genetic studies have revealed that coexistence is possible for thousands of generations, for example, in the hybridogenetic fish *Poeciliopsis* (Vrijenhoek, 1984; Quattro et al., 1992).

The central European water frog *Rana esculenta* (RL) is a natural hybrid between *Rana ridibunda* (RR) and *Rana lessonae* (LL). *Rana esculenta* reproduces by hybridogenesis where one parental genome (*L-lessonae* genome in our study area) is excluded in the germ line and the unrecombined genome of the second parental species (*R-ridibunda* genome) is clonally transmitted to progeny upon fertilization (see Graf and Polls-Pelaz, 1989; Gunther, 1990). Thus, hybridogenetic reproduction results in hemiclinal inheritance of genomes. Somatic hybridity is restored in each generation after mating parasitism of adult *R. esculenta* with the coexisting sexual host species (*Rana lessonae* in the L-E system; UzzeH and Berger, 1975). The *Rana esculenta* complex is an intriguing hemiclinal system because, contrary to other clonal vertebrates, it contains both sexes. Thus, a fundamental question is why are hemiclinal genotypes of *R. esculenta* not driven to extinction by recombinant sexual genotypes of *R. lessonae* or *R. ridibunda*?

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The short-term maintenance of the hemiclinal lineages coexisting with sexuals may occur through differential fitness advantage relative to sexuals. Two mechanisms that may not be mutually exclusive could explain the widespread occurrence of hemiclinal frogs relative to the coexisting parental *R. lessonae*. The first mechanism known as the “general-purpose” genotype hypothesis (Baker, 1965; Lynch, 1984) implies that some hemiclones have a broad tolerance to temporal and spatial heterogeneity of the environment and thus display a generalist phenotype. This mechanism has been supported in other clonal-sexual complexes (Bierzychudek, 1989; Michaels and Bazzaz, 1989; Weider, 1993; Nikiasson and Parker, 1994; Parker and Niklasson, 1995). It has also been shown that *R. esculenta* are more tolerant to severe ecological conditions than parental species (Tunner and Nopp, 1979; Berger and Berger, 1992; Semlitsch and Keyer, 1992; Semlitsch, 1995). These latter results suggest that *R. esculenta* may possess a “general-purpose” genotype with wide tolerances to environmental conditions. Yet, this mechanism does not fully explain why most clonal and hemiclinal populations consist of multiple clones (e.g., Turner et al., 1992; Hotz et al., 1994; Vrijerhoek, 1994) and why in some studies both broadly adapted and narrowly specialized clones coexist in the populations (Bierzychudek, 1989; Browne, 1992). A second alternative mechanism, consistent with clonal diversity within populations, is the resource heterogeneity model (e.g., “frozen niche-variation” model; Vrijerhoek, 1979, 1984). This model assumes that coexisting clones are narrowly specialized to different environmental conditions. Balancing selection maintains a wide array of clones in natural populations because environmental variability over time favors different clones.

During their life many amphibians face a high degree of environmental heterogeneity. They maintain a complex life cycle with a sequential occupation of aquatic and terrestrial environments that define two discrete life stages. Environmental heterogeneity exists at each life stage, especially during the larval-aquatic stage that often occurs in temporary ponds (Wilbur, 1980, 1984). Depending on the level of integration of both vital stages, environmental variability during larval period may modulate the effect of selection on metamorphosing juveniles in terrestrial environments. For example, an individual's body size at metamorphosis is largely affected by larval growth history and has long-term implications for its fitness (Berven, 1990; Bruce and Hairston, 1990; Scott 1994, Newman and Dunham, 1994). It is also shown that larger size at metamorphosis is directly re-

lated to enhanced locomotor performance in anurans (John-Alder and Morin, 1990; Coater et al., 1993). Jumping performance is probably under strong selection because it may affect survival through better antipredatory response (Heinen and Hammond, 1997) and may increase dispersal ability. Predation of amphibians is intense during metamorphosis and early juvenile life (Arnold and Wassersug, 1978; pers. obs.). Survival with predators may be a direct function of maximum jump distance displayed, especially when metamorphosed frogs living along the shoreline of a pond tend to find refuge by jumping into the pond and hiding in deep water.

At least two environmental factors may affect locomotor performance, larval growth history and temperature. Growth and developmental rates determine body size and shape at metamorphosis that would subsequently affect jumping performance (John-Alder and Moriri, 1990; Goater et al., 1993). Larval growth history may also determine variability in locomotor response, independent of size, by affecting relative muscular mass, energetic reserves, and metabolic rate. However, food deprivation and its timing did not affect energetic reserves or metabolism in an anuran species (Audo et al., 1995), thus suggesting that effects of larval history on locomotor performance would occur only through differential size and shape. Performance in anurans is also highly dependent on temperature (John-Alder et al., 1988; Bennett et al., 1989; Whitehead et al., 1989; Wine and Catten, 1992) but little is known about genetic variability in locomotor performance and the relationship between maximal performance and breadth of performance (Huey and Hertz, 1984). Furthermore, larval growth history may also modify locomotor performance across a thermal gradient.

The purpose of our study was to test the null hypothesis that clonal lineages of hybridogenetic *R. esculenta* and its syntopic parental species *R. lessonae* are equivalent both in morphological traits affecting jumping performance and in size-independent locomotor ability at the early terrestrial stage. We also examine whether the necessary clonal advantage in fitness for coexistence is achieved by adopting a mechanism of “general-purpose genotype”, or whether clones differ in performance. Consequently, the primary questions were: (1) Do hemiclones differ in morphological traits (body mass and hind-leg length) that affect performance?, (2) Do hemiclones differ in performance independent of morphology? Also, because juveniles were raised in two different larval densities and tested at three different temperatures, we also examined the question: (3) Do genotype x envi

ronmental interactions ($G \times E$) exist between hemiclones so that balancing selection might maintain the coexistence of multiple clones in a population, and (4) Is there a difference between hemiclones and the syntopic parental species *R. lessonae* such that hemiclonal lineages may be maintained in the natural ponds together with the recombining Mendelian host species?

MATERIALS AND METHODS

Population, Breeding Design and Husbandry.—Study animals were obtained from a pond near Gütighausen (Kanton Zürich, northern Switzerland, description in Semlitsch et al., 1996). During the 1993 breeding season, the frog population at Gütighausen pond contained 57% of the sexual host parental species *R. lessonae* and 43% of hybridogenetic *R. esculenta* with four different hemiclones, distinguished by *R. ridibunda* alleles at the enzyme loci GPI, MPI, and LDH-B: hemiclone *GUT1* (GPIa-MPIc-LDH-Bc; see Hotz and Uzzell, 1982 for allele designations) 68%; *GUT2* (a-a-c) 23%; *GUT3* (d-c-c) 7%, and *GUT4* (a-c-a) 2%. During the nights of 12–15 May we hand-captured >180 frogs that were then taken to the laboratory for taxon and hemiclone identification using protein electrophoresis. For comparison, we also analysed the performance of *R. ridibunda* metamorphs obtained from offspring of adults collected from native populations near Poznan, Poland. *Rana ridibunda* is not native to Switzerland and has not been observed at Gütighausen or in surrounding ponds.

Tadpoles from the three most common *R. esculenta* hemiclones (*GUT1-3*) were obtained from artificial crosses of at least four females of each hemiclone with four *R. lessonae* males. Because each individual *R. lessonae* male was crossed with each hemiclonal *R. esculenta* and also each parental *R. lessonae* female, male genetic contribution can be considered homogeneous across these genotypes. To obtain offspring of the other parental sexual species *R. ridibunda*, we made homospecific crosses with three males and three females. Female ovulation was induced by injecting an isotonic solution of the fish hormone LH-RH (H-7525, Bachem, Inc., 25 ng/g body weight). After all females initiated ovulation (approximately 24 h), sperm suspensions were prepared by crushing both testes from each male in pond water in a Petri dish. Several hundred eggs from one female at a time were stripped into the sperm suspensions. After 5 min the sperm suspension was poured into a new Petri dish and used to fertilize the eggs of the next female. The order in which females from a hemiclone were fertilized was rotated to prevent any bias. All crosses were made within 24 h on 20 May and embryos were kept in 1-liter plastic containers until hatching on 25 May.

To determine whether larval growth history affected locomotor performance in metamorphs of different genotypes, we raised tadpoles at high and low larval densities. Tadpoles within the five different genotypes (hybrid hemiclones *GUT1*, *GUT2*, *GUT3*; and both recombinant sexual lineages *LL*, *RR*) were reared in artificial ponds. The experimental design with all possible combinations of genotype \times larval density yielded 10 treatments in a 5×2 factorial design. Artificial ponds were fiberglass tanks (1.04 m wide \times 1.47 m long \times 0.80 m deep) that were positioned randomly in a rectangular array of three spatial blocks in a fenced field at the University of Zürich. Tanks were filled with 1000 l of tapwater between 29 April–2 May 1993. All tanks then received 1.0 kg of air-dried reeds (*Phragmites*) collected along the edge of a natural pond and were covered with lids made of fiberglass window screen (1 mm mesh). Tanks were inoculated with 3.0 l of concentrated plankton collected from five natural ponds on 3 and 6 May. On 29 May 1993, 360 tadpoles pooled from replicate females within each genotype were selected and carefully counted into individual cups with 30 tadpoles each. Then, we randomly assigned three cups (90 tadpoles) to those ponds with high density and one cup (30 tadpoles) to low density ponds. After the first metamorph (stage 42, Gosner 1960) was discovered, tanks were checked every other day and all metamorphs were collected and held in the laboratory for 4–5 until tail resorption was complete.

Locomotor Performance Experiments.—Metamorphs from each genotype and larval density environment were haphazardly selected for the locomotor performance experiments (2–3 d after complete tail resorption). During this period individuals were not fed because the presence of food in the stomach may affect locomotor performance (e.g., Garland and Arnold, 1983). We measured locomotor performance at three temperatures (19 C, 24 C, and 29 C) in walk-in thermal chambers. The temperatures selected cover the range of optimum locomotor performance for ranid frogs (Rome et al., 1992). Frogs were tested 12 h after being placed in the thermal chamber to acclimatize them at the experimental temperature. Locomotor performance was estimated by measuring maximum jump distance. Frogs were jumped in a linear racetrack (160 cm long, 22 cm wide and 40 cm high). The floor of the racetrack was covered by continuous-feed printer paper. Frogs were removed from their containers and placed in a small Petri dish containing neutral red dye at the starting point of the racetrack. Then, the lid was removed and frogs voluntarily jumped onto the racetrack. Frogs were gently prodded on the urostyle with

a pen to induce jumping. Neutral red blots left successive tracks allowing us to measure jump distance. Analyses were made on those individuals that made at least five consecutive jumps in a linear trajectory. Maximum jump score was the maximum recorded in two jumping series that occurred in rapid succession (<1 mm). Those individuals which jumped against lateral walls or reversed their trajectory were not included in the subsequent analyses.

The day after locomotor tests each frog was weighed to the nearest 0.1 mg (body mass, BM), after blotting them of excess water with paper towels. We also measured hind-limb length (HLL) from the urostyle to the longest toe tip, with a vernier caliper to the nearest 0.1 mm. The repeatabilities of these traits estimated with 20 individuals varied between 0.85 and 0.95 (calculated according to Lessels and Boag, 1987).

Statistical Analyses.—Hemiclinal variability in morphology was analyzed using a standard univariate ANOVA. To assess the performance of metamorphs that was independent of variation in size and thus the direct effect of locomotor ability unaffected by correlated traits such as hind-leg length, we analysed the effect of genotypes at different temperatures and larval densities using an ANCOVA. The maximum jump distance was adjusted by removing the effect of the morphological trait (HLL) that most strongly affected jumping ability. We used SAS GLM (version 6.04, SAS, 1990) to estimate expected MS and error estimates for F-tests. All factors (hemiclone, larval density and temperature) were considered fixed effects. Because of the unequal replication of our design we performed analyses using Type III sums of squares that account for unequal cell size and produce orthogonal tests of hypotheses.

Planned comparisons were used to test pairwise differences of particular hemiclones with parental *LL* and *RR* species using orthogonal contrasts. Because density and genotype treatments were applied to whole replicate tanks, all analyses consider only mean performance per tank. The block effect was negligible and therefore it was pooled into the residual term. Response variables were not transformed because their respective distribution met the assumptions of homoscedasticity and normality.

The ecological success of hemiclinal lineages coexisting with sexually recombining species may be explained by the general-purpose genotype model, namely the selection of a generalist, or by specialized genotypes, the frozen niche-variation model. The primary analytical method to contrast both models is to test for the existence of C × F interactions in fitness-related traits such as size at metamorphosis and jumping performance. This is a necessary condition

for the frozen niche-variation model, but not necessary, although possible, for the general-purpose genotype model. Later model, by contrast, will require necessarily that, under temporal environmental heterogeneity, variance among clones will exist in the geometrical mean of fitness (Lynch, 1984). Analytically this would imply a significant “hemiclone” main effect in the ANOVA. This term is interpreted as variation exists among clones in the “average” environment. Therefore, a more specific contrast of the models will require to examine not only the existence of C × E interactions main effect but also we need to check the “hemiclone” main effect. We analysed these main effects for morphological traits affecting locomotory performance across different larval densities, and for size-independent locomotory performance both across different temperatures and larval densities.

RESULTS

Variation in Morphological Traits Affecting Locomotor Performance.—A significant portion of the variance in maximum jump distance was explained by both morphological traits BM and HLL for the pooled sample (multiple R² = 0.78). A stepwise regression analysis showed that a single trait (HLL) was responsible for nearly all variability in jumping ability (partial R² = 0.77). Variance explained by BM only represented minimal variation (partial R² = 0.01).

Body mass at metamorphosis differed across different hemiclones of *R. esculenta*. This difference was, however, only found at low larval density. A post-hoc comparison showed that *GIITZ* frogs were bigger than frogs of *GUT2* and *GIIT3*, but the three hemiclones did not differ from each other at high density. A similar pattern was found for HLL, the longest hindlimbs were produced by *GIITZ* tadpoles only at low larval density (*GIITZ* > *GUT2* = *GIIT3*, Tukey *liSt* test, *P* < 0.05), but hemiclones were similar at high density (Fig. 1). Hemiclone effect was also significant implying that *GIITZ* hemiclone was bigger in body size and had longer HLL than *GUT2* at both densities. There was no significant interaction between genotype and density (Table 1).

Comparison with the two parental species showed that hybrid *R. esculenta* had intermediate values for HLL, longer than *It lessonae* and shorter than *R. ridibunda* (planned comparisons, all hemiclones vs. *R. ridibunda*, MS = 11.57, F_{1,4,49}, *p* = 0.045; all hemiclones vs. *ii. lessonae*, MS = 15.44, F_{1,4,49} = 5.99, *P* = 0.022) (Fig. 1). No significant contrasts were found for mass at metamorphosis with the exception of *GIITZ* being significantly heavier than *K. lessonae* at either

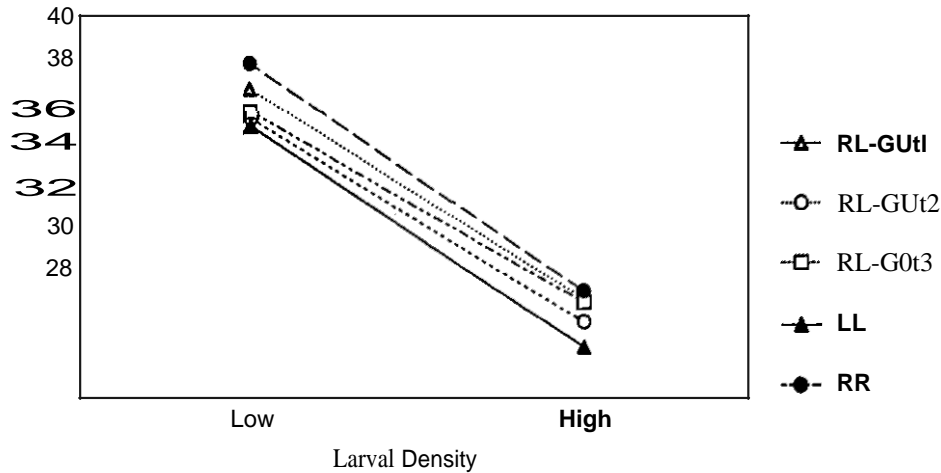


Fig. 1. Mean hind-limb length of metamorphs for different hemiclones of *R. esculenta* (RL-GUtl-3) and the parental species *It. jessonae* (LL) and *It. ridibunda* (RR) in relation to different larval densities.

larval density (MS = 0.03, $F_{1,13} = 4.65$, $P = 0.042$).

Variation in Size-independent Performance.—Comparisons across different hemiclones of *It. esculenta* showed a significant effect of the hemiclone by density interaction. At low density *GUT1* and *GUT2* frogs exhibited better performance for a similar JILL than *GUT3*. However, at high larval density *GUT2* frogs displayed greater jumping ability than frogs of either hemiclone *GUT1* or *GUT3* (Fig. 2, Table 2). Hemiclone effect was also significant implying that *CUT2* hemiclone had better jumping performance than *GUT3* for both densities.

Planned comparisons with size-adjusted means showed that hemiclones were inferior jumpers to sexual *It. lessonae* when frogs were raised at low larval densities. Pooling all hemiclones together and across both larval environments, *It. lessonae* had greater jumping ability than hemiclones as a group (Fig. 2, Table 3 and 4). Planned comparisons with allopatric *It. ridibunda* showed no variation in adjusted jumping performance of this parental species with pooled hemiclones of *It. esculenta* for both larval environments (Table 4).

Temperature positively affected jumping performance; higher temperatures increased maxi-

mum jump length relative to lower temperatures. A significant density by temperature interaction indicated that the thermal effect on jumping performance was not parallel at both densities (Fig. 3). At high density metamorphs were insensitive to temperature variation whereas its effect on jumping performance was positive and linear for individuals reared at low larval density (adjusted jumping performance: 29 C > 24 C > 19 C, Tukey HSD test, $P < 0.05$). Orthogonal contrasts revealed this linear increase, with MS twice as large as that of deviation contrasts (Deviation: MS = 4748.94, $df = 2$, $P = 0.0001$; Lineal: MS = 9488.36, $df = 2$, $P < 0.00001$). Otherwise, temperature by density interaction reflects a divergent effect of larval density on adjusted jumping performance across temperature. At lower temperatures (19 C and 24 C) adjusted jumping performance did not vary between larval densities. However, at 29 C frogs raised at low density jumped significantly longer than high density frogs ($F_{1,29} = 4.95$, $P = 0.034$, Fig. 3). Absence of a significant genotype by temperature interaction revealed that locomotory performance at different temperatures did not vary among hemiclones, nor between hemiclones and either parental species (Table 2 and 3).

TABLE 1. Table of ANOVA of different hemiclones of *Rana esculenta* and larval density for mass at metamorphosis and hind-leg length.

Source	Mass at metamorphosis				Hind-leg length				
	df	Type III MS	F value	P	df	Type III MS	F value	P	
Hemiclone	2	0.038	4.404	0.034	2	13.062	4.586	0.031	
Density	1	4.275	7.936	<0.001	1	1459.5	512.5	<0.001	
Hclone × Den	2	0.006	0.805	0.468	2	0.506	0.177	0.839	
Error	13	0.008			13	2.848			
Model: $F_{6,13} = 37.57$, $P = 0.0001$, R-Square = 0.9455					Model: $F_{6,13} = 40.01$, $P = 0.0001$; R-Square = 0.9486				

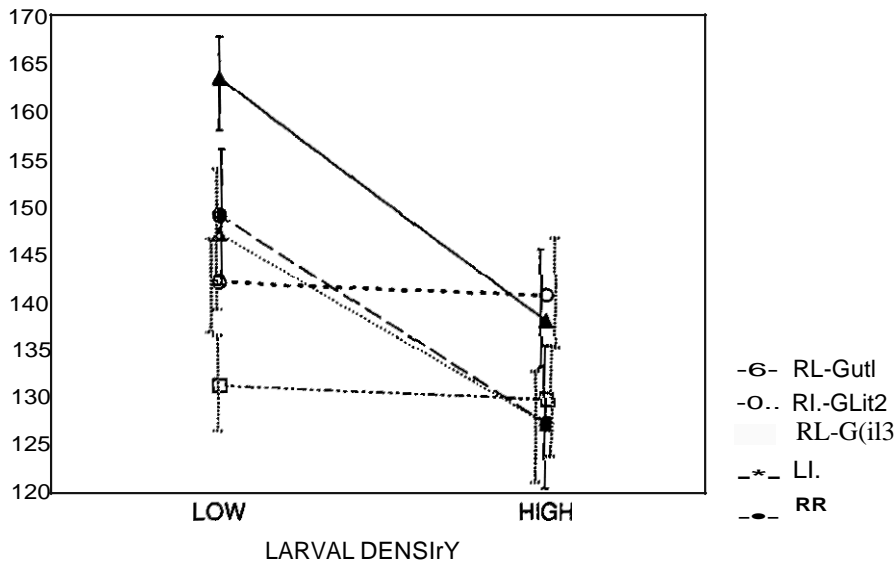


FIG. 2. Least-squares means (± 1 SE) for maximum jump distance for different hemiclones of *FL esculenta* (RL-Güt1-3) and the parental species *k. kssonae* (LL) and *R. ridibunda* (RR) in relation to different larval densities.

Discussion

Our results showed that *Rana esculenta* hemiclones differed in morphological traits affecting jumping performance. Hemiclone *GLITZ* was bigger in body mass and had longer hind-leg length than the other hemiclones. However, this

TABLE 2. Table of ANCOVA of different hemiclones of *Rana esculenta*, larval density and temperature with hind-leg length (TILL) as covariate for maximum jump distance. Multiple comparisons table of LS means is also shown; values below diagonal are for high larval density, above are for low larval density. Based on PDIF options of the LSMEANS statement of GLM procedure in SAS 6.04.

		Source		
		df	Type III MS	F value
Hemiclone Density	2	621.988	3.69	0.0336
Temperature	1	5.279	0.03	0.8604
Hemiclone x Density	2	1921.328	11.40	0.0001
Hemiclone K Temperature	2	595.167	3.531	0.0384
Density K Temperature	4	73.640	0.44	0.7832
TILL	1	10,567.59	62.69	0.0001
Error	41	168.557		
Model: $ISAJ = 51.59$; $p = 0.0001$; R-Square = 0.961				
Pr> ITI HOt LSnean(i) = Lsmear(j)*				
	i/j	GUT1	GUT2	GUT3
	GUT1		0.791	0.019
	GUT2	0.014		0.034
	GUT3	0.546	0.047	

difference was only found at low larval density. In addition, size-adjusted jumping performance exhibited a significant genotype by larval density interaction. Metamorphs of *GLITZ* and *GUT2* showed better jumping performance than *GUT3* at low larval density but at high density frogs of *GUT2* were the best jumpers than the other hemiclones. This interaction is interesting, since if we had studied body size and hind leg length at metamorphosis only, we might have concluded that *GLITZ* hemiclone is a broadly specialized, general-purpose genotype. If, on the other hand, we had studied relative jumping performance only, we might have concluded that *GLITZ* is narrowly adapted to low larval

TABLE 3. Table of ANCOVA of larval density and temperature with hind-leg length (HLL) as covariate

for maximum jump distance for *R. esculenta* hemiclones and parental species, syntopic *K. tessonne* and allotypic *R. ridibunda*. Model: $F_3 = 42.22$; $P = 0.0001$; R-Square = 0.949.

Source	df	Type III MS	F value	P
Genotype	4	515.040	2.55	0.0471
Density	1	452.278	2.24	0.1394
Genotype K Density	4	552.856	2.73	0.0359
Genotype K Temperature	8	108.685	0.54	0.8242
Temperature K Density	2	1670.560	8.26	0.0006
Gen K Den K Tern	8	172.776	0.85	0.5589
HLL	1	14,435.682	71.39	0.0001
Error	68	202.220		

TABLE 4. a priori-planned comparisons using orthogonal contrast to test for differences in adjusted means of maximum jumping distance of *R. esculenta* hemiclones and parental species, syntopic *R. lessonae* (*LL*) and allopatric *R. ridibunda* (*RI*?) as a function of larval density.

	cli	MS	P	df	MS	P
All hemiclones and densities vs. <i>LL</i>	1	1024.555	0.028			
All hemiclones and densities vs. <i>RI</i> ?	1	4.341	0.884			
		Low larval density			High larval density	
All hemiclones vs. <i>LL</i>	1	2092.298	0.001	1	1.568	0.931
<i>GUT1</i> vs. <i>LL</i>	1	597.263	0.090			
<i>GUT2</i> vs. <i>LL</i>	1	1093.959	0.023			
<i>GUT3</i> vs. <i>U</i>	1	3179.018	0.001			
All hemiclones vs. <i>RI</i> ?	1	203.404	0.319	1	157.684	0.381
Error	68	202.221				

density environments whereas *GUT2* hemiclone would be a broadly-adapted genotype. Therefore, results seem to be consistent with the predictions of both the general-purpose genotype model and the frozen niche-variation model. *Rana esculenta* hemiclones appear to be a combination of generalized and specialized traits affecting life history, physiological performance, and competitive ability (Semlitsch et al., 1997). This pattern is similar to that found in other clonal species such as *Poeciliopsis* (Vrijenhoek and Pfeiler, 1997; Vrijenhoek, 1998) and suggest that the complexity in life history and physiological traits of clones may lead to erroneous conclusions about the generalist or specialist nature of clones if only a few fitness related traits or environments are examined.

Although size at metamorphosis alone has been shown to affect survival and other components of fitness in amphibians (see references in the Introduction), enhanced jumping performance also may influence higher survival rates on the hazardous pond margin or affect important life-history traits such as likelihood of dispersion and location of suitable microhabitats.

Some tests of whether size-corrected locomotor performance affects survival have been made in reptiles (Jayne and Bennett, 1990; Janzen 1993). However, estimates of selection on locomotor performance are still lacking in amphibians. Studies on selection on recombining species require detailed individual estimates of lifetime reproductive success to establish the proper selection gradients for particular traits (Arnold, 1983). Analysis of selection in clonal individuals has the logistic advantage that the unit of selection changes from the individual to the clone (Templeton, 1982). The selection process does not operate on single genes nor on groups of genes and their phenotypes, but on adaptive complexes consisting of entire genomes (Schultz, 1982). Therefore, an analysis of clonal selection in *R. esculenta* would basically require an estimate of clonal frequencies over time. The frequency distribution of clones at the Gtitzhausen population (Semlitsch et al., 1996, 1997; H. Hötzel, unpubl.) seems to fit well with variability both in morphology and size-corrected performance, thus suggesting that selection may be acting directly on these traits. *GUT1*, the

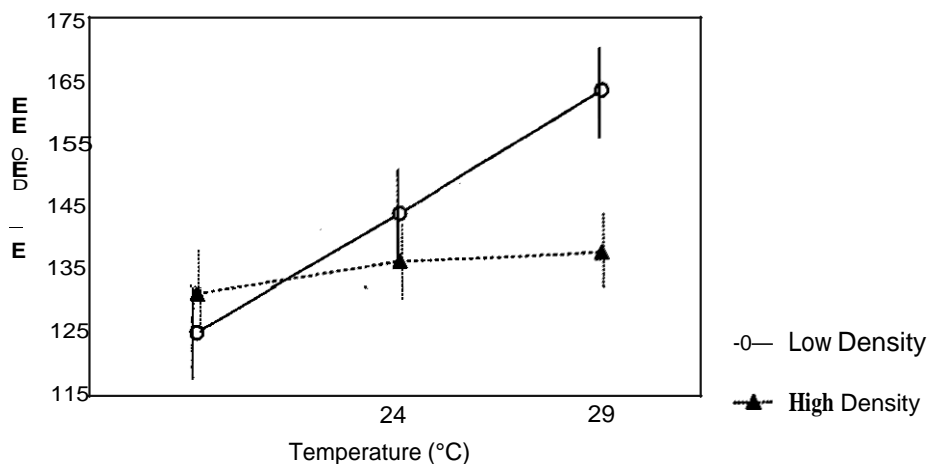


FIG. 3. Least-squares means (± 1 SE) for maximum jump distance of water frog metamorphs for different experimental temperatures in relation to different larval densities

most frequent clone (68%), also gave the largest metamorphs in low density ponds. Its size-corrected jumping ability was higher than that of *GUT3* at low density and lower than that of *GUT2* at high larval density. *GUT3* was the least frequent hemiclone in the pond (7%), but was not different in mass at metamorphosis than the more frequent *GUT2* (23%). However, size-independent performance was better for *GUT2* at high larval densities. Differences in other fitness-related traits during the larval period may also explain clonal frequency pattern. *GUT3* tadpoles exhibited the shortest larval period and highest percentage of metamorphs, but *GUT3* showed better larval performance than the more common *GUT2* (Semlitsch et al., 1997). The better size-adjusted locomotor performance of *GUT2* metamorphs when compared to *GUT3* metamorphs may further explain its differential ecological success.

The parental species *R. lessonae* is more frequent (57%) than hemiclones (43%) at the Ciitighausen pond. The prevalence of parental species relative to hemiclones is more difficult to explain based on the presumed influence of morphological and performance traits on fitness. The locomotor performance of *R. lessonae* metamorphs was better relative to the hemiclones when tadpoles were reared at low densities and they exhibited no different jumping ability when tadpoles were reared at high larval densities. The Gutighausen pond is nearly permanent and it is possible that the level of larval competition is low, yielding better relative performance and higher success of *R. lessonae* relative to the hemiclones. Other life-history characteristics during the larval stage may be responsible for the observed higher frequency of sexuals relative to hemiclones. Under favorable conditions *R. lessonae* produced more metamorph than hemiclones in experimental ponds that were not subjected to declining water level and where larval competition was low (Semlitsch and Reyer, 1992).

We found that locomotor performance of waterfrog metamorphs was highly dependent on temperature, as was generally found in other locomotor studies in anurans (Whitehead et al., 1989; Wine and Gatten, 1992). Jumping distance is linearly proportional to peak power output generated by hindlimb musculature that is quite temperature-sensitive (Rome et al., 1992). However, this thermal sensitivity was only found at low larval density but contrary to expectations, not observed for those frogs raised at high larval densities. This is clear evidence that larval growth history does modulate important juvenile life-history traits such as temperature-sensitivity of locomotor performance. Future studies should be conducted to analyze proximate

physiological mechanisms that determine this divergent performance response as influenced by historical effects and consequently its potential implication at the ecological level.

Our understanding of the long-term fitness consequences of hemiclonal reproduction in *R. esculenta* is still incomplete, and the proximate causes explaining its relative success will require additional studies which compare fitness during the terrestrial-juvenile stage such as juvenile growth rate, survival, desiccation tolerance, migration ability, and age of sexual maturity to make meaningful predictions about the evolutionary success of this hybridogenetic system.

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