Accepted on 3 May 2007
J Zool Syst Evol Res doi: 10.1111/j.1439-0469.2007.00430.x

[1]*Zoologisches Institut, Universität Zürich-Irchel, Zürich, Switzerland;* [2]*School of Computational Science and Department of Biological Sciences, Florida State University, Tallahassee, FL, USA;* [3]*Division of Biological Sciences, University of Missouri, Columbia, MO, USA*

Hemiclone diversity in the hybridogenetic frog *Rana esculenta* outside the area of clone formation: the view from protein electrophoresis

H. Hotz[1], G.-D. Guex[1], P. Beerli[2], R. D. Semlitsch[3] and N. B. M. Pruvost[1]

Abstract

European water frog hybrids *Rana esculenta* reproduce hemiclonally, by hybridogenesis: In the germ line they exclude the genome of the parental species *Rana lessonae* and produce haploid, unrecombined gametes with a genome of the parental species *Rana ridibunda*. These hybrids coexist with and depend as sexual parasites on the host parental species *R. lessonae* (the L-E population system); matings with *R. lessonae* restore somatic hybridity in each generation of *R. esculenta*. We investigated 15 L-E system populations in northern Switzerland, which is outside *R. ridibunda*'s native range. Frequency of hybrids in samples varied from 8% in marsh ponds to 100% in gravel pits and forest ponds. Clonal diversity (variation among *R. ridibunda* genomes of hybrids), detected by six protein electrophoretic marker loci, revealed a total of eight hemiclones and locally ranged from uniclonal populations in southern parts of the survey region to six coexisting hemiclones in the north. All alleles distinguishing hemiclones occur commonly in the nearest native *R. ridibunda* populations of east-central Europe; the most probable source of clonal diversity in our samples is multiple clone formation by primary hybridizations in the sympatry area of *R. ridibunda* and *R. lessonae* and subsequent dispersal of hemiclonal lineages. A positive correlation between amount of clonal diversity and hybrid frequency, predicted by the Frozen Niche Variation (FNV) model (each hemiclone is characterized by a relatively narrow niche, coexistence is possible through niche partitioning), was not found; this contrasts with hemiclonally reproducing fish hybrids (*Poeciliopsis*). Historical factors, such as availability of different colonizing hemiclones may be strong enough to override the signal from operation of the FNV.

Key words: Allozymes – clonal coexistence – clonal reproduction – Frozen Niche Variation – genetic diversity – hybridogenesis – *Rana esculenta* complex

Introduction

A small fraction (about 0.1%) of vertebrate taxa reproduce without genetic recombination (e.g. Vrijenhoek et al. 1989). They have in common that they are of interspecies hybrid origin, are locally abundant, often have large ranges, and typically occur in disturbed or unhospitable environments (Dawley and Bogart 1989). In many cases, such hybrids form assemblages of multiple clonal lineages. This poses the question of the maintenance of such multiclonal coexistence despite the continuous possibility of stochastic or selective clone extinction. The Frozen Niche Variation model (FNV; Vrijenhoek 1979, 1984a,b; Wetherington et al. 1989) posits that in a heterogeneous environment, coexisting clones each utilize different, relatively narrow niches. This niche subdivision facilitates the coexistence of clones through the reduction of interclonal competition. Consequently, clonal mixtures have a higher average fitness than single-clone populations, and genetic clonal diversity is a prerequisite of clonal coexistence. FNV thus predicts a positive correlation between the amount of clonal diversity in hybrids and hybrid abundance relative to their parental species. An alternative explanation of the observed ecological success of hybrid clonals, the General-Purpose Genotype model (GPG; Baker 1965; Maslin 1968; Lynch 1984) assumes that single clones of hybrids are more broadly adapted than their sexual parental species because interclonal selection has favoured a generalized, environment-tolerant resource utilization and promoted a 'general-purpose' genotype with low fitness variance over time. GPG thus predicts clonal hybrids to contain a few or a single broadly adapted clone.

European water frogs of the *Rana esculenta* complex (reviewed by Graf and Polls Pelaz 1989; Günther 1990; Som et al. 2000; Plötner 2005) provide an opportunity to test the assumptions and predictions of the FNV and GPG models. *Rana esculenta* Linnaeus, 1758 (genomic composition RL) are natural hybrids between *Rana ridibunda* Pallas, 1771 (RR) and *Rana lessonae* Camerano, 1882 (LL) that reproduce hemiclonally, by a hybridogenetic (Schultz 1969) gametogenesis: In the germ line, their L genome is excluded prior to meiosis and, subsequently, the R genome is endoreduplicated (Tunner and Heppich-Tunner 1991 and literature cited therein), which is followed by a quasinormal meiosis without genetic consequences of segregation or crossing over because the two R genomes are sister chromatid-derived identical copies, and produce haploid gametes containing an unrecombined R genome. Such hybrids coexist with and reproductively depend on their sexual host parental species LL, matings with which restore somatic hybridity in each generation of *R. esculenta* (the L-E system; Uzzell and Berger 1975).

Water frog populations in Switzerland north of the Alps constitute such L-E systems; the parental species *R. ridibunda* is absent except in some regions where it was introduced by humans (Grossenbacher 1988). The nearest region where native *R. ridibunda* populations occur and new hybrid hemiclones can, therefore, be formed by primary hybridizations lies in Eastern Central Europe (Eastern Germany, Poland, Czechia, Eastern Austria and Hungary), where hybrid lineages of Switzerland north of the Alps almost certainly originated.

We here present a survey of native L-E systems in Northern Switzerland, in which we test predictions of the FNV and GPG models. We investigated population composition and clonal diversity in hybrids, using protein electrophoretic

genetic markers, and related clonal diversity with hybrid abundance.

Materials and Methods

We sampled 15 breeding localities in northern Switzerland (Fig. 1):

Northern region:

1 Äpelhusenhof: Pond near Unterstammheim (47°40′N/8°48′E; 12 km ESE Schaffhausen), Canton Zürich, Community Unterstammheim, altitude 430 m. Sampled in 1993.

2 Dätwil: Pond near Dätwil (47°36′N/8°43′E; 30 km NNE Zürich), Canton Zürich, Community Adlikon, 390 m. Sampled in 1996 and 1998.

3 Dicki: Former loam pit (47°46′N/8°39′E; 7 km N Schaffhausen), Canton Schaffhausen, Community Büttenhardt, 670 m. Sampled in 1995 and 1996.

4 Eschenriet: Pond Eschenriet (47°41′N/8°42′E; 5 km E Schaffhausen), Canton Thurgau, Community Unterschlatt, 402 m. Sampled in 2001.

5 Frauenfeld: Pools and ponds of Frauenfelder Allmend (47°35′N/8°55′E; 1 km NE Frauenfeld), Canton Thurgau, Community Frauenfeld, 390 m. Sampled in 1987 and 1991.

6 Fuchsenhölzli: Pond (former gravel pit) at Fuchsenhölzli, Andelfingen (47°35′N/8°41′E; 30 km NNE Zürich), Canton Zürich, Community Andelfingen, 385 m. Sampled in 2001.

7 Gütighausen: Pond near Gütighausen (47°35′N/8°43′E; 30 km NNE Zürich), Canton Zürich, Community Thalheim, 410 m (cf. Semlitsch et al. 1996). Sampled in 8 years (1992–1998, 2001).

8 Rheinau: Gravel pit ponds near Rheinau (47°38′N/8°38′E; 6 km S Schaffhausen), Canton Zürich, Community Rheinau, 400 m. Sampled in 1993.

9 Wagenhausen: Pond in former gravel pit near Wagenhausen (47°39′N/8°51′E; 11 km NNW Frauenfeld), Canton Thurgau, Community Wagenhausen, 430 m. Sampled in 1987 and 1993.

10 Werdhof: Pond near Werdhof (47°36′N/8°42′E; 30 km NNE Zürich), Canton Zürich, Community Kleinandelfingen, 390 m. Sampled in 1992 and 1996.

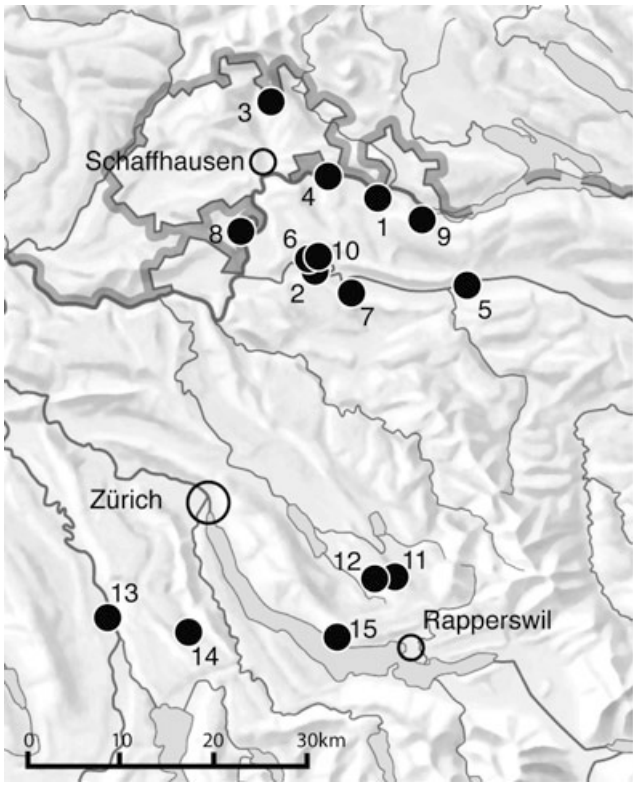

Fig. 1. Map of the survey area in northern Switzerland. Localities are keyed to the list described in Materials and Methods by numbers. Relief © 2007 Swisstopo

Southern region:

11 Hellberg: Pond near Hellberg (47°18′N/8°49′E; 1 km WSW Hinwil, 22 km ESE Zürich), Canton Zürich, Community Hinwil, 540 m (cf. Semlitsch et al. 1996). Sampled in 7 years (1992, 1994–1996, 2001–2003).

12 Hundsruggen: Swamp near Hundsruggen (47°18′N/8°48′E; 2.5 km S Wetzikon, 20 km ESE Zürich), Canton Zürich, Community Wetzikon, 540 m. Sampled in 1992.

13 Müllbach: Gravel pit near Ottenbach (47°16′N/8°24′E; 15 km SW Zürich), Canton Zürich, Community Ottenbach, 410 m. Sampled in 1995 and 1996.

14 Rifferswil: Ponds near Rifferwsil (47°14′N/8°31′E; 15 km S Zürich), Canton Zürich, Community Rifferswil, 590 m. Sampled in 1995.

15 Stäfa: Swamp near Redlikon, 2 km ENE Stäfa (47°15′N/8°45′E; 20 km SE Zürich), Canton Zürich, Community Stäfa, 520 m. Sampled in 1996.

Frogs were hand-collected at night at the breeding sites and transported to the laboratory for examination of sex and body length and for marking them. Individual frogs were uniquely marked by toe-clipping, using a numerical scheme (Twitty 1966) that omits the longest fourth toe, which is important for locomotion and skin shedding; for males, we also omitted the first finger, which is important for pair formation. Toes were frozen at −80°C for later electrophoresis. Except for some individuals used in crossing experiments, each frog was released at its collecting site.

Protein electrophoresis of the toe tissue followed standard procedures (Uzzell and Berger 1975; Hotz et al. 1997). We examined products of six allozyme loci known to vary in native central European *R. ridibunda* (Uzzell and Berger 1975; Hotz 1983; Beerli 1994; VH. Hotz and T. Uzzell, unpublished data) and sufficiently active in toe tissue for routine scoring: glucose-6-phosphate isomerase (GPI; Enzyme Commission 5.3.1.9), hexokinase (HK-1; EC 2.7.1.1), L-lactate dehydrogenase (LDH-B; EC 1.1.1.27), mannose-6-phosphate isomerase (MPI; EC 5.3.1.8), 6-phosphogluconate dehydrogenase (PGDH; EC 1.1.1.44) and phosphoglucomutase (PGM-2; EC 5.4.2.2, formerly 2.7.5.1). Alleles (Table 1) are designated by lowercase letters, following an established system (Hotz and Uzzell 1982; Hotz et al. 1997).

Eight hemiclones (multilocus *ridibunda* genome haplotypes in *R. esculenta*, designated H1-H8) were distinguished by four of the six allozyme loci used (Table 1). In previously reported performance studies (Semlitsch et al. 1996, 1997; Fioramonti et al. 1997; Rist et al. 1997; Tejedo et al. 2000a,b), these same numbers, preceded by the first three letters of the locality names, were used.

As a hemiclone diversity index, we used Parker's (1979) effective numbers of clones, $1/\Sigma(f_i^2)$, where f_i = frequency of the ith hemiclone in a population.

Data were analysed manually and using our own programs in a relational database programmed with the Fourth Dimension (ACI SA).

Table 1. Hemiclones (haplotypes of the clonally transmitted *ridibunda* genome) distinguished by protein electrophoresis of products of four loci in *Rana esculenta* lineages in northern Switzerland. Alleles are designated by lowercase letters. Two additional loci (PGDH: allele *d*; PGM-2: *d*) that vary in Central European *Rana ridibunda* were invariant in the *ridibunda* genomes of our samples

	Locus			
Hemiclone	GPI	HK-1[1]	LDH-B	MPI
H1	*a*	*c*	*c*	*c*
H2	*a*	*c*	*c*	*a*
H3	*d*	*c*	*c*	*c*
H4	*a*	*c*	*a*	*c*
H5	*a*	*b*	*a*	*a*
H6	*a*	*b*	*c*	*a*
H7	*a*	*c*	*a*	*a*
H8	*a*	*b*	*c*	*c*

[1]HK-1 *c* is a newly designated allele common in European *Rana ridibunda*; its product on continuous tris-citrate pH 6 or pH 7 and tris-EDTA-borate gels (cf. Hotz et al. 1997) moves less anodally than the *b* product.

Results

We sampled a total of 2739 water frogs in 15 breeding ponds of native L-E system populations of northern Switzerland (Fig. 1; Table 2). These consisted of 1179 (43%) *R. lessonae* and 1560 (57%) *R. esculenta*. At the breeding ponds, frequencies of hybrids relative to hosts in our samples ranged from 8% in a natural marsh area to 100% in a gravel pit and two forest ponds; mean hybrid frequency was 35% in the five uniclonal populations in the southern part of the survey area, and 70% in the 10 multiclonal populations in its northern part (Tables 2 and 3).

Multiple sampling at some localities enabled comparison of yearly differences in taxon composition. At two localities in the northern part of the survey area, the frequency of *R. esculenta* relative to *R. lessonae* increased during the survey period: At Werdhof, it nearly doubled from 46.3% in 1992 to 88.7% in 1996, and at Gütighausen there was an almost steady increase in hybrid frequency: 1992, 37.3%; 1993, 42.2%; 1994, 54.4%; 1995, 73.2%; 1996, 81.7%; 1997, 84.0%; 1998, 83.3% and 2001, 86.9%. At Hellberg, hybrid frequency fluctuated across years: 1992, 32.1%; 1994, 27.0%; 1995, 54.1%; 1996, 20.5% and 2001, 30.6%.

Four of the six allozyme loci used distinguished eight different hemiclones (*ridibunda* genome haplotypes) among a total of 1165 *R. esculenta* scored (Table 1). All the 10 localities in the northern part of the survey area (Fig. 1), in the Rhein

Table 2. Taxon composition and sex ratios in adults from natural water frog breeding ponds of northern Switzerland containing native L-E systems

			Rana lessonae					*Rana esculenta*				
Locality	Habitat	*N*	*n*	%	female	male	% /	*n*	%	female	male	% /
Northern region												
1 Äpelhusenhof	Man-made pond	61	13	21	1	12	8	48	79	5	43	10
2 Dätwil	Forest pond	88	6	7	3	3	50	82	93	63	18	78
3 Dicki	Former loam pit	133	51	38	5	46	10	82	62	24	58	29
4 Eschenriet	Forest pond	51	0	0				51	100	18	33	35
5 Frauenfeld	Woodland pools	20	17	85	3	14	18	3	15	2	1	67
6 Fuchsenhölzli	Forest pond, former gravel pit	29	0	0				29	100	26	3	90
7 Gütighausen	Man-altered forest pond	823	323	39	123	198	38	500	61	280	213	57
8 Rheinau	Gravel pit ponds	129	7	5	1	6	14	122	95	57	65	47
9 Wagenhausen	Former gravel pit pond	58	2	3	0	2	0	56	97	30	26	54
10 Werdholf	Forest marsh pond	341	108	32	36	72	33	233	68	70	163	30
Southern region												
11 Hellberg	Natural bog ponds	728	510	70	193	317	38	218	30	110	103	52
12 Hundsruggen	Marsh ponds	115	106	92	19	87	18	9	8	6	3	67
13 Müllbach	Gravel pit	59	0	0				59	100	48	11	81
14 Rifferswil	Natural bog ponds	27	11	41	10	1	91	16	59	16	0	100
15 Stäfa	Forest swamp ponds	77	25	32	4	21	16	52	68	25	24	51

Table 3. Hybrid frequency and clonal diversity in adults from natural water frog-breeding ponds of northern Switzerland containing native L-E systems

		Hybrids			Percentage of hemiclones[1]								
Locality	*N*	*n*	%	Number of hemi clones[1]	H1	H2	H3	H4	H5	H6	H7	H8	Effective number of clones[2]
Northern region													
1 Äpelhusenhof	61	48	79	2	29.5	70.5							1.713
2 Dätwil	88	82	93	3	60.0	38.6			1.4				1.965
3 Dicki	133	82	62	2	13.4	86.6							1.303
4 Eschenriet	51	51	100	$\geqslant 3$ [3]									
5 Frauenfeld	20	3	15	2		33.3	66.7						1.800
6 Fuchsenhölzli	29	29	100	$\geqslant 4$ [3]									
7 Gütighausen	823	500	61	6	66.4	22.2	8.4	2.4	0.3			0.3	2.008
8 Rheinau	129	122	95	4	86.3	11.6	1.1	1.1					1.318
9 Wagenhausen	59	57	97	3	21.6	21.6					56.9		2.402
10 Werdhof	341	233	68	6	65.9	26.2	3.1	3.1	1.3	0.4			1.978
Southern region													
11 Hellberg	728	218	30	1	100								1.000
12 Hundsruggen	115	9	8	1	100								1.000
13 Müllbach	59	59	100	1	100								1.000
14 Rifferswil	27	16	59	1	100								1.000
15 Stäfa	77	52	68	1	100								1.000

[1]Hybrid hemiclones (designated H1–H8) were determined by protein electrophoresis, using products of four of six allozyme loci that vary in *Rana ridibunda* genomes (Tables 1 and 4).
[2]Estimated following Parker (1979).
[3]For two localities (Eschenriet and Fuchsenhölzli) percentage of hemiclones could not be determined because of missing data.

and lower Thur drainages, contained multiple hemiclones, whereas all the five localities in the southern part of the survey region were uniclonal (Table 3).

Among the eight hemiclones (Table 3), H1 is the most abundant overall (67%), the only hemiclone at each uniclonal locality in the southern part of the survey area, and the most frequent across the multiclonal localities in its northern part (59%), although at four of the eight multiclonal sites, H1 was not the most abundant (0% at Frauenfeld, 13% at Dicki, 22% at Wagenhausen, 30% at Äpelhusenhof; Table 3).

The second-most abundant hemiclone was H2 (25% overall, 31% among multiclonal sites, at 7 of 8 of which it occurs); it varied locally from 12% (Rheinau) to 87% (Dicki). The remaining six hemiclones are generally rare: H3 (3.4% overall, 4.3% among multiclonal sites, locally up to 8.4% at Gütighausen); followed by H7 (2.5% overall, 3.1% among multiclonal sites), which only occurred at Wagenhausen, where it was frequent (57%); H4 (1.4% overall, 1.8% among multiclonal sites, locally up to 3.1% at Werdhof); H5 (0.4% overall, 0.5% among multiclonal sites, locally up to 3.1% at Werdhof); H6 (0.08% overall, 0.11% among multiclonal sites), confined to Werdhof (0.4%); and H8 (0.08% overall, 0.11% among multiclonal sites), confined to Gütighausen (0.3%).

Among the eight multiclonal localities for which hemiclone composition was scored, the effective number of hemiclones, a measure reflecting number and relative frequencies of hemiclones (the more hemiclones and the more evenly distributed their frequencies, the higher the effective number), had its maximal values at Wagenhausen and Gütighausen, followed by Werdhof (Table 3).

No significant differences in adult body lengths among *R. esculenta* hemiclones were detected for either sex (females: $F_{6,568} = 1.3$, $p > 0.2$; males: $F_{6,552} = 2.0$, $p > 0.05$).

The overall sex ratios (Table 2) for *R. lessonae* were 398 female, 779 male, 34% female ($G = 125.6$, df = 1, $p < 0.001$); for *R. esculenta* 780 female, 764 male, 51% female ($G = 0.2$, df = 1, $p = 0.68$). Among hemiclones of hybrids, there was a significant female excess in H1 (56%; $G = 55.5$, df = 1, $p < 0.001$) and in H3 (83%; $G = 18.4$, df = 1, $p < 0.001$), and a significant male excess in H2 (61%; $G = 13.7$, 1 df, $p < 0.001$); sex ratios did not significantly deviate from 1 : 1 in hemiclones 4 and 7. The sex ratio differences between hemiclones 1 and 2, 1 and 3, 2 and 3, 3 and 4, and 3 and 7 are significant (G tests; $p < 0.001$ for each comparison).

Among the eight hybrid hemiclones distinguished by the allozyme loci used, all *ridibunda* alleles occur as common alleles in native *R. ridibunda* populations of Eastern Central Europe (Table 4). At most loci, however, allele frequencies differ strongly between hybrid hemiclones in Switzerland and these native *R. ridibunda* populations (Table 4): Alleles *d* of GPI, *b* of HK-1, *a* of LDH-B, *e* of PGDH and *b* of PGM-2 are substantially underrepresented or not detected at all in the Swiss hybrid lineages.

Although hybrids were more frequent overall in the 10 multiclonal localities than in the five uniclonal localities (Tables 2 and 3), the correlation between hybrid frequency and clonal diversity (Fig. 2) was not strong and statistically insignificant ($r = 0.304$, $p > 0.3$). There are both populations with relatively few hybrids and moderately high clonal diversity (Frauenfeld; Table 3) and uniclonal populations with high hybrid frequency (Müllbach; Table 3).

Discussion

The large differences in local hybrid frequencies relative to the host species *R. lessonae* match earlier data on the same geographical region (Blankenhorn et al. 1973) and of L-E systems in central Poland (Berger 1977,1983; Rybacki and Berger 1994,2001). In general, *R. lessonae* are more frequent in undisturbed habitats and small water bodies, such as peat bogs and marshes, whereas *R. esculenta* is more frequent in disturbed habitats and voluminous water bodies, such as gravel pit ponds. In our samples, the highest frequencies of *R. esculenta* (all-hybrid samples) occurred in a gravel pit and two forest ponds, one of which is a former gravel pit, whereas *R. lessonae* was most abundant in marshes, peat bogs and woodland pools (Table 2). It has been shown that predator complement plays a major role in determining taxon composition of the L-E system populations (Anholt et al. 2005), *R. esculenta* being more frequent where fish are the predominant predators.

The significant male excess in *R. lessonae* may reflect a sampling bias (during the breeding period, females are more hidden, less active and do not call). The absence of a similar bias on average in *R. esculenta* (except hemiclone H2) may reflect an actual female excess in hybrid populations (visible in samples of hemiclones H1 and H3), probably caused by occasional matings between *R. lessonae* females and *R. esculenta* males that lead to all-female *R. esculenta* progeny (Berger et al. 1988). That both before ovulation and after spawning, *R. esculenta* females are less terrestrial than *R. lessonae* females may also make *R. esculenta* females more likely to be found. That *R. esculenta*, on average, did not deviate from a 1 : 1 sex ratio is a consequence of opposite deviations from 1 : 1 in hemiclones H1 and H3 (female excess) and in hemiclone H2 (male excess). In previous data from

Table 4. Allele frequencies for six variable allozyme loci in native *Rana ridibunda* populations of East-Central Europe and among *ridibunda* genomes of *Rana esculenta* in northern Switzerland

	GPI		HK-1		LDH-B		MPI		PGDH		PGM-2	
Regions	*a*	*d*	*B*	*C*	*a*	*c*	*a*	*c*	*d*	*e*	*b*	*d*
Central Poland[1]	0.56	0.44	0.58	0.42	0.78	0.22	0.55	0.45	0.43	0.57	0.10	0.90
Central Croatia[2]	0.40	0.60	0.50[3]	0.50[3]	0.58	0.42	0.49	0.51	0.64	0.36	0.20	0.80
Northern Switzerland[4]	0.97	0.03	0.01	0.99	0.04	0.96	0.28	0.72	1.00	–	–	1.00

[1]Seven populations near Poznan (81*Rana ridibunda*; H. Hotz unpublished data).
[2]Three populations near Zagreb (52*Rana ridibunda*; Hotz 1983; H. Hotz unpublished data).
[3]Based on a small subsample (three individuals).
[4]Thirteen populations of the present survey (1165 *Rana esculenta*).

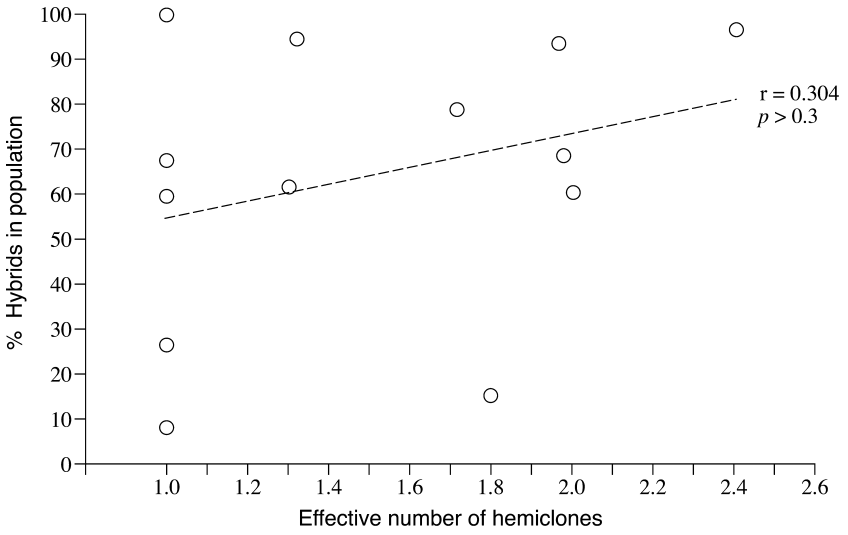

Fig. 2. Relationship between effective number (Parker 1979) of coexisting allozyme-defined hemiclones and proportion of hemiclonal hybrids in natural breeding ponds of Northern Switzerland containing native L-E systems

natural L-E systems, *R. esculenta* showed a female excess of about 60% (Berger et al. 1988) that was interpreted to result from *R. lessonae* female × *R. esculenta* male matings leading to the all-female *R. esculenta* progeny.

Clonal diversity is generally higher in regions of sympatry of *R. ridibunda* and *R. lessonae*, where new hybrid hemiclones can recurrently be formed by primary hybridizations (Hotz 1983; H. Hotz, unpublished data). Although our study region is outside the area of clone formation, our results reveal a substantial amount of clonal diversity. The estimates obviously are minimal because of the limited number of loci used for hemiclone discrimination. Moreover, sample sizes vary – from three hybrids at Frauenfeld through nine hybrids (seven scored for hemiclone) at Hundsruggen to 506 hybrids (369 scored) at Gütighausen; obviously, the probability of detecting rare hemiclones varies accordingly. Tissue grafting and mtDNA studies on *Poeciliopsis* have revealed more hemiclones than can be detected by allozymes (Angus and Schultz 1979; Moore and Eisenbrey 1979; Quattro et al. 1991). Our present allozyme data also provide conservative estimates of clonal diversity in *R. esculenta*. The high allelic variability of microsatellites, for example, provides a superior resolution (cf. Scribner et al. 1994) of clonal diversity in *ridibunda* genomes of *R. esculenta* lineages relative to allozymes: the addition of just two microsatellite loci that varied in R genomes to the allozyme data used here to discriminate between natural *R. esculenta* hemiclones has more than doubled the number of hemiclones distinguished at our Swiss study sites (Hotz et al. 2001); several allozyme-defined hemiclones consist of more than one microsatellite-defined hemiclone.

Among the eight hemiclones revealed, H1 is the most common overall and the only one found in southern parts of the survey area. This is also the one hemiclone that occurs in the central region of Switzerland north of the Alps (Vorburger 2001a). Its exclusiveness in the southern parts of the study region probably reflects historical events during the colonization process.

The hemiclones we distinguish have relatively 'shallow' genetic divergence; they differ by one to four alleles at the six loci used (Table 1). This close relationship may be related to the possibility of episodic sexual recombination in this *Rana* system (Hotz et al. 1992; Schmidt 1993; Vorburger 2001a,b; Guex et al. 2002): Occasional successful matings between two hybrids of different hemiclones, followed by meiotic recombination in the resulting female *R. ridibunda* progeny and by matings of such *R. ridibunda* with *R. lessonae* males, may lead to an array of new hybrid hemiclones, so that locally coexisting hemiclones are not independent of one another and are more closely related to one another than to allopatric hemiclones. It is not known, however, how much genetic difference there is among the eight hemiclones. Our limited set of marker loci is inadequate for assessing the genetic distance between any two of the R genomes (Guex et al. 2002).

Excepting episodic sexual recombination, the origin of clonal diversity in our study area probably consists of multiple clone formation by primary hybridizations between *R. ridibunda* and *R. lessonae* within the native range of *R. ridibunda* in East-Central Europe, followed by the dispersal of hybrid lineages to Switzerland. This is strongly supported by the presence of all *ridibunda* alleles that characterize the hemiclones in our study region as common alleles in native *R. ridibunda* populations (Table 4); the distinct underrepresentation of several common *R. ridibunda* alleles in Switzerland probably reflects stochastic (or possibly selective) loss during dispersal. We found no *ridibunda* alleles that are likely to have originated by mutations after clone formation.

Two prominent models (GPG and FNV) have been proposed to explain the ecological success of clonal hybrids that is suggested by their wide distribution and local abundance. Spontaneous heterosis of the highly heterozygous hybrids probably contributes to the success of clonal hybrids in both models (Hotz et al. 1999). Hybrid frequency related to the amount of clonal diversity and distribution of particular hemiclones across the study area can be used to test the applicability of the two models to our data. GPG predicts few or a single broadly adapted superior clone. Although H1 is the most frequent hemiclone overall and the one consistently present at all uniclonal sites, performance studies have shown that it is not generally superior to other hemiclones in fitness-related life-history traits (Semlitsch et al. 1996,1997; Rist et al. 1997; Tejedo et al. 2000a,b; H. Hotz, G.-D. Guex, R. D. Semlitsch, unpublished data); We have no data showing direct support for the GPG model (Semlitsch et al. 1997).

The Frozen Niche Variation model predicts that, if coexisting hemiclones have different adaptations and thus utilize different niches in heterogeneous environments, there should

be a correlation between local clonal diversity and local hybrid frequency. Our data are consistent with the FNV assumption of the presence of the local clonal diversity (this work). Furthermore, substantial differences between coexisting hemiclones in fitness-related life-history traits have been shown (Semlitsch et al. 1996, 1997; Fioramonti et al. 1997; Rist et al. 1997; Tejedo et al. 2000a,b; R. D. Semlitch, H. Hotz and G.-D. Guex unpublished data). Hemiclones did not differ significantly, however, in annual adult survival rates (Anholt et al. 2003). As predicted by FNV, artificial ponds with hemiclone mixtures had higher proportions of tadpoles metamorphosing than single-hemiclone ponds (Semlitsch et al. 1997). Despite matching the assumptions of the FNV model, our data, contrary to the FNV predictions, revealed no significant positive correlation between the amount of clonal diversity and the abundance of hybrids (Fig. 2). This contrasts distinctly with the data on hemiclonally reproducing Mexican fish hybrids of the genus *Poeciliopsis*, for which a significant positive correlation has been reported ($r = 0.93$, $p < 0.01$; Vrijenhoek 1979). Our results suggest that the amount of local clonal diversity observed in *Rana* hybridogens may be shaped by historical factors; it may be limited, for example, by the absence of different hemiclones as potential colonizers in neighbouring areas. We suggest that such historical effects can be strong enough to conceal the signal from local operation of the FNV model.

Acknowledgements

We thank Bradley R. Anholt, Eva Gutmann, Isabella Hegglin, Alain Pagano, Karin Rutishauser, Sabine Schmiedehausen and Christian Som for their help in collecting and marking frogs. We thank an anonymous reviewer for constructive criticisms. Collecting permits were issued by the Amt für Raumplanung, Kanton Zürich, the Planungs- und Naturschutzamt, Kanton Schaffhausen, and the Jagd- und Fischereiverwaltung, Kanton Thurgau. This research was supported by the Swiss National Fund (grants 31-37579.93, 31-59144.99 and 31-103903/1).

Zusammenfassung

Hemiklon-Diversität im hybridogenetischen Frosch Rana esculenta *ausserhalb des Areals von Klon-Bildung: die Sicht von Protein-Elektrophorese*

Europäische Wasserfrosch-Hybriden *Rana esculenta* pflanzen sich hemiklonal fort, durch Hybridogenese: In der Keimbahn schliessen sie das Genom der Elternart *Rana lessonae* aus und produzieren haploide, unrekombinierte Gameten mit einem Genom der Elternart *Rana ridibunda*. Diese Hybriden koexistieren mit und hängen als Sexualparasiten ab von der Wirts-Elternart *R. lessonae* (das L-E-Populationssystem); Paarungen mit *R. lessonae* stellen somatische Hybridität in jeder Generation von *R. esculenta* wieder her. Wir untersuchten 15 L-E-System-Populationen in der Nordschweiz, die ausserhalb des ursprünglichen *R. ridibunda*-Areals liegt. Hybrid-Frequenz in Stichproben variierte von 8% in Moortümpeln bis 100% in Kiesgruben und Waldweihern. Klonale Diversität (Variation unter *R. ridibunda*-Genomen von Hybriden), entdeckt durch Protein-Elektrophorese von 6 Marker-Loci, zeigte ein Total von 8 Hemiklonen und variierte lokal von uniklonalen Populationen in südlichen Teilen des Untersuchungsgebiets bis zu 6 koexistierenden Hemiklonen im Norden. Alle Allele, die Hemiklone unterscheiden, kommen häufig in den naheliegendsten ursprünglichen *R. ridibunda*-Populationen im östlichen Zentraleuropa vor; die wahrscheinlichste Quelle klonaler Diversität in unseren Stichproben ist multiple Klonbildung durch Primärhybridisierungen im Sympatrie-Areal von *R. ridibunda* und *R. lessonae* und nachfolgende Dispersion hemiklonaler Linien. Eine positive Korrelation zwischen dem Grad klonaler Diversität und Hybrid-Frequenz, vorhergesagt vom Frozen Niche Variation-Modell (jeder Hemiklon ist durch eine relativ enge Nische charakterisiert, Koexistenz ist möglich durch Nischen-Aufteilung), wurde nicht gefunden; das kontrastiert zu Fisch-Hybriden mit hemiklonaler Fortpflanzung (*Poeciliopsis*). Historische Faktoren wie die Verfügbarkeit verschiedener kolonisierender Hemiklone könnten stark genug sein, um das Signal vom Operieren der Frozen Niche Variation zu überdecken.

References

Angus RA, Schultz RJ (1979) Clonal diversity in the unisexual fish *Poeciliopsis monacha-lucida*: a tissue graft analysis. Evolution **33:**27–40.

Anholt BR, Hotz H, Guex G-D, Semlitsch RD (2003) Overwinter survival of *Rana lessonae* and its hemiclonal associate *Rana esculenta*. Ecology **84:**391–397.

Anholt BR, Negovetic S, Rauter C, Som C (2005) Predator complement determines the relative success of tadpoles of the *Rana esculenta* complex. Evol Ecol Res **7:**733–741.

Baker HG (1965) Characteristics and modes of origin of weeds. In: Baker HG, Stebbins GL (eds), The Genetics of Colonizing Species. Academic Press, New York, pp 147–172.

Beerli P (1994) Genetic Isolation and Calibration of an Average Protein Clock in Western Palearctic Water Frogs of the Aegean Region. PhD Dissertation, Universität Zürich, Zürich.

Berger L (1977) Systematics and hybridization in the *Rana esculenta* complex. In: Taylor DH, Guttman SI (eds), The Reproductive Biology of Amphibians. Plenum Press, New York, pp 367–388.

Berger L (1983) Western Palearctic water frogs (Amphibia, Ranidae): systematics, genetics and population compositions. Experientia **39:**127–130.

Berger L, Uzzell T, Hotz H (1988) Sex determination and sex ratios in western Palearctic water frogs: XX and XY female hybrids in the Pannonian Basin? Proc Acad Nat Sci Philadelphia **140:**220–239.

Blankenhorn HJ, Heusser H, Notter P (1973) Zur Verbreitung von *Rana esculenta* Linnaeus und *Rana lessonae* Camerano im Zürcher Oberland. Rev Suisse Zool **80:**662–666.

Dawley BM, Bogar JP (eds) (1989) Evolution and ecology of unisexual vertebrates. New York State Mus Bull 466, Albany, New York.

Fioramonti E, Semlitsch RD, Reyer H-U, Fent K (1997) Effects of triphenyltin and pH on the growth and development of *Rana lessonae* and *Rana esculenta* tadpoles. Environm Toxicol Chemi **16:**1940–1947.

Graf J-D, Polls Pelaz M (1989) Evolutionary genetics of the *Rana esculenta* complex. In: Dawley RM, Bogart JP (eds), Evolution and Ecology of Unisexual Vertebrates. New York State Mus Bull 466, Albany, New York, pp 289–302.

Grossenbacher K (1988) Verbreitungsatlas der Amphibien der Schweiz. Doc Faunistica Helv **7:**1–207.

Guex G-D, Hotz H, Semlitsch RD (2002) Deleterious alleles and differential viability in progeny of natural hemiclonal frogs. Evolution **56:**1036–1044.

Günther R (1990) Die Wasserfrösche Europas (Anura – Froschlurche). Die Neue Brehm-Bücherei 600, Wittenberg Lutherstadt.

Hotz H (1983) Genic Diversity Among Water Frog Genomes Inherited with and without Recombination. PhD Dissertation, Universität Zürich.

Hotz H, Uzzell T (1982) Biochemically detected sympatry of two water frog species: two different cases in the Adriatic Balkans (Amphibia, Ranidae). Proc Acad Nat Sci Philadelphia **134:**50–79.

Hotz H, Beerli P, Spolsky C (1992) Mitochondrial DNA reveals formation of nonhybrid frogs by natural matings between hemiclonal hybrids. Mol Biol Evol **9:**610–620.

Hotz H, Uzzell T, Berger L (1997) Linkage groups of protein-coding genes in western Palearctic water frogs reveal extensive evolutionary conservation. Genetics **147:**255–270.

Hotz H, Semlitsch RD, Gutmann E, Guex G-D, Beerli P (1999) Spontaneous heterosis in larval life-history traits of hemiclonal frog hybrids. Proc Natl Acad Sci USA **96:**2171–2176.

Hotz H, Uzzell T, Guex G-D, Alpers D, Semlitsch RD, Beerli P (2001) Microsatellites: a tool for evolutionary genetic studies of western

Palearctic water frogs. In: Plötner J, Schmeller D (eds), Third International Symposium on Genetics, Systematics and Ecology of Western Palearctic Water Frogs. Mitt Mus Naturk Berlin, Zoologische Reihe, Berlin, Vol. 77, pp 43–50.

Lynch M (1984) Destabilizing hybridization, general-purpose genotypes and geographic parthenogenesis. Quart Rev Biol **59:**257–290.

Maslin TP (1968) Taxonomic problems in parthenogenetic vertebrates. Syst Zool **17:**219–231.

Moore WS, Eisenbrey AB (1979) The population structure of an asexual vertebrate, Poeciliopsis 2 monacha-lucida (Pisces: Poeciliidae). Evolution **33:**563–578.

Parker ED (1979) Ecological implications of clonal diversity in parthenogenetic morphospecies. Am Zool **19:**753–762.

Plötner J (2005) Die westpaläarktischen Wasserfrösche – von Märtyrern der Wissenschaft zur biologischen Sensation. Laurenti-Verlag, Bielefeld.

Quattro JM, Avise JC, Vrijenhoek RC (1991) Molecular evidence for multiple origins of hybridogenetic fish clones (Poeciliidae: *Poeciliopsis*). Genetics **127:**391–398.

Rist L, Semlitsch RD, Hotz H, Reyer H-U (1997) Feeding behaviour, food consumption, and growth efficiency of hemiclonal and parental tadpoles of the *Rana esculenta* complex. Funct Ecol **11:**735–742.

Rybacki M, Berger L (1994) Distribution and ecology of water frogs in Poland. In: Ogielska M (ed), II International Symposium on Ecology and Genetics of European Water Frogs, 18–25 September 1994. Zoologica Poloniae, Wroclaw, Poland, Vol. 39, pp 293–303.

Rybacki M, Berger L (2001) Types of water frog populations (*Rana esculenta* complex) in Poland. In: Plötner J, Schmeller D (eds), 3rd International Symposium on Western Palearctic Water Frogs. Mitt Mus Naturk Berlin, Zoologische Reihe, Berlin, Vol. 77, pp 51–57.

Schmidt BR (1993) Are hybridogenetic frogs cyclical parthenogens? Trends Ecol Evol **8:**271–273.

Schultz RJ (1969) Hybridization, unisexuality, and polyploidy in the teleost *Poeciliopsis* (Poeciliidae) and other vertebrates. Am Nat **103:**605–619.

Scribner KT, Arntzen JW, Burke T (1994) Comparative analysis of intra- and interpopulation genetic diversity in *Bufo bufo*, using allozyme, single-locus microsatellite, minisatellite, and multilocus minisatellite data. Mol Biol Evol **11:**737–748.

Semlitsch RD, Schmiedehausen S, Hotz H, Beerli P (1996) Genetic compatibility between sexual and clonal genomes in local populations of the hybridogenetic *Rana esculenta* complex. Evol Ecol **10:**531–543.

Semlitsch RD, Hotz H, Guex G-D (1997) Competition among tadpoles of coexisting hemiclones of hybridogenetic *Rana esculenta*: support for the Frozen Niche Variation model. Evolution **51:** 1249–1261.

Som C, Anholt BR, Reyer H-U (2000) The effect of assortative mating on the coexistence of a hybridogenetic waterfrog and its sexual host. Am Nat **156:**34–46.

Tejedo M, Semlitsch RD, Hotz H (2000a) Covariation of morphology and jumping performance in newly metamorphosed water frogs: effects of larval growth history. Copeia **2000:**448–458.

Tejedo M, Semlitsch RD, Hotz H (2000b) Differential morphology and jumping performance of newly metamorphosed frogs of the hybridogenetic *Rana esculenta* complex. J Herpetol **34:**201–210.

Tunner HG, Heppich-Tunner S (1991) Genome exclusion and two strategies of chromosome duplication in oogenesis of a hybrid frog. Naturwissenschaften **78:**32–34.

Twitty VC (1966) Of Scientists and Salamanders. Freeman, San Francisco, CA, USA.

Uzzell T, Berger L (1975) Electrophoretic phenotypes of *Rana ridibunda*, *Rana lessonae*, and their hybridogenetic associate, *Rana esculenta*. Proc Acad Nat Sci Philadelphia **127:**13–24.

Vorburger C (2001a) Fixation of deleterious mutations in clonal lineages: evidence from hybridogenetic frogs. Evolution **55:**2319–2332.

Vorburger C (2001b) Non-hybrid offspring from matings between hemiclonal hybrid waterfrogs suggest occasional recombination between clonal genomes. Ecol Lett **4:**628–636.

Vrijenhoek RC (1979) Factors affecting clonal diversity and coexistence. Am Zool **19:**787–797.

Vrijenhoek RC (1984a) The evolution of clonal diversity in *Poeciliopsis*. In: Turner BJ (ed.), Evolutionary Genetics of Fishes. Plenum Press, New York and London, pp 399–429.

Vrijenhoek RC (1984b) Ecological differentiation among clones: the Frozen Niche Variation model. In: Wöhrmann K, Loeschcke V (eds), Population Biology and Evolution. Springer-Verlag, Heidelberg, pp 217–231.

Vrijenhoek RC, Dawley RM, Cole CJ, Bogart JP (1989) A list of the known unisexual vertebrates. In: Dawley RM, Bogart JP (eds), Evolution and ecology of unisexual vertebrates. New York State Mus Bull, 466, Albany, New York, pp 19–23.

Wetherington JD, Schenck RA, Vrijenhoek RC (1989) The origins and ecological success of unisexual *Poeciliopsis*: the Frozen Niche-Variation model. In: Meffe GK, Snelson FF (eds), Ecology and Evolution of Livebearing Fishes (Poeciliidae). Prentice Hall, Englewood Cliffs, New Jersey, pp 259–275.

Authors' addresses: Hansjürg Hotz, Zoologisches Institut, Universität Zürich-Irchel, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland. E-mail: hotz@zool.uzh.ch; Gaston-Denis Guex, Zoologisches Institut, Universität Zürich-Irchel, CH-8057 Zürich. Email: guex@zool.uzh.ch; Peter Beerli, School of Computational Sciences, Florida State University, Tallahassee, FL 32306, USA. Email: beerli@scs.fsu.edu; Raymond D. Semlitsch, Division of Biological Sciences, University of Missouri, Columbia, MD 65211, USA. Email: semlitschR@missouri.edu; Nicolas B. M. Pruvost, Zoologisches Institut, Universitat Zürich-Irchel, CH-8057 Zürich. Email: pruvost@zool.uzh.ch