RESEARCH LETTER

Distribution and habitat use of water frog hybrid complexes in France

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ABSTRACT

Hybrid zones are either distributed along clines or in a mosaic of patches. This distribution may depend upon variation in taxon habitat use. Habitat use and distribution of diverse taxa of water frogs (*Rana ridibunda*, *R. lessonae*, *R. perezi*, *R.* kl. *grafi* and *R.* kl. *esculenta*) in France are analysed to determine whether water frog complexes conform to the mosaic or clinal model. Biogeographical scenarios may be invoked in order to explain the distribution of water frogs. However, the distribution of *R. perezi* and *R.* kl. *grafi*, being restricted to regions characterized by Mediterranean or Oceanic climatic conditions, suggests that these frogs do not endure cold winters. *R. ridibunda* is widespread in Southern France and its distribution suggests multiple introductions. It is concluded that water frogs conform to the mosaic zone model rather than to the tension zone model because: (i) taxa exhibited differences in habitat use, (ii) pure parental species were documented and (iii) hybrids are not unfit relative to parental species.

Key words Amphibians, France, hybrid zone, mosaic zone model, *Rana* species, tension zone model, water frogs.

INTRODUCTION

Several models, including the tension zone and the mosaic zone models (Barton & Hewitt, 1985; Hewitt, 1988; Harrisson, 1990; Howard *et al.*, 1993; Arnold, 1997), have been proposed either to explain or to predict the distribution of taxa involved in a hybrid zone. With regard to spatial structures, hybrid zones conform either to clinal distribution (according to the tension zone model; Barton & Hewitt, 1985) or to a mosaic of patches (Harrisson, 1986, 1990). The first model predicts an environment-independent distribution of the taxa involved in the hybrid zone. The second model considers that environmental heterogeneity affects taxonomic composition (and thus, hybridization events) through variation in habitat use among the different taxa of a complex. In fact, the validation of one model requires the establishment of the distribution of each taxon and its specific ecological requirements. In another respect, these models predict hybridization events that are expected to be more numerous in the case of a mosaic zone. Thus, such models allow inference as to the population dynamics and evolution of the hybrid zone (Harrisson, 1990).

European water frogs (*Amphibia: Ranidae*) are characterized by the widespread and abundant natural occurrence of interspecific hybridization (review in Graf & Polls-Pelaz, 1989). Some of these hybridization events give rise to stable hybrid lineages characterized by a hemiclonal reproduction

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mode called hybridogenesis (Schultz, 1969). The most widespread complex, involving the parental species *R. ridibunda* Pallas 1771 and *R. lessonae* Camerano 1882 and the hybridogen *Rana* kl. *esculenta* Linnaeus 1758, occurs in Central and Eastern Europe (Graf & Polls-Pelaz, 1989; Dubois & Ohler, 1995). The hybridogen generally co-exists, as a sexual parasite, with one of the parental species on which depends the restoration of hybrid lineages. The most frequent situation in this complex is the L–E hybridogenetic system, i.e. the co-existence between the parental host species *R. lessonae* and the hybridogen (Uzzell & Berger, 1975). Another hybridogen (*R.* kl. *grafi* Crochet *et al.*, 1995) occurs in Southern France and Spain (Graf *et al.*, 1977; Uzzell & Tunner, 1983; Graf & Polls-Pelaz, 1989; Arano *et al.*, 1995a; Hotz *et al.*, 1995). In the P–G system (which is analogous to the L–E system; Graf & Polls-Pelaz, 1989), hybrid lineages are restored at each generation through mating of the hybridogen *R.* kl *grafi* with the parental host species *R. perezi* Seoane 1885.

Differences in the distributional ranges of these water frog complexes have been explained by a biogeographical postglacial scenario. *R. ridibunda* has probably expanded from a Balkan refugium, *R. lessonae* from an Italian refugium and *R. perezi* from the Iberian refugium (Uzzell, 1982). The origin of the hybridogens is likely to be the result of these expansions although hybridogens may have expanded their range with their parental host species. However, contemporary taxon expansion following ecological or evolutionary changes may also have occurred. If so, this could be evidenced by a comparison of older distribution maps with more recent ones. However, frog introductions by man also have to be taken into consideration because they may have contributed to the modification of water frog distributions (Grossenbacher, 1988; Pagano *et al.*, 1997). Thus, assessing water frog distribution is an important goal because it should allow: (i) inferences as to historical and/or ecological determinants of distribution patterns and (ii) inferences and predictions concerning water frog evolution.

In the *esculenta* complex, habitat preferences of each taxon have been described (e.g. Günther, 1974; Wijnands, 1977; Lada *et al.*, 1995; Morand & Joly, 1995; Rybacki & Berger, 1995; Plénet *et al.*, 1998; Plénet *et al.*, 2000). *R. ridibunda* preferentially

inhabits sites under strong river influence with well-oxygenated waters, such as dead arms near the active channel. In contrast, frogs of the L–E system avoid running waters and tolerate ponds with hypoxic water (Lada *et al.*, 1995; Plénet *et al.*, 2000). As far as we are aware, the ecology and habitat use of frogs of the P–G system have not yet been described. We hypothesize that *R. perezi* is a vicariant species of *R. ridibunda* and that *R.* kl. *grafi* predominates in the same habitat types as *R. perezi*. Moreover, we expect that *R.* kl. *grafi* should have a greater ability to inhabit different habitat types because of 'heterozygositic advantage'.

In regard to clinal structure and/or patchiness of distribution, our study aims at specifying whether water frog complexes conform to a tension zone or to a mosaic zone model. After genetic identification of each frog using allozymic specific markers, the five distinct taxa involved in the *grafi* and *esculenta* complexes were recorded in the area of our study. Thus, the first goal of our investigation was to specify taxon distribution and its determinants and the second to specify the main habitat types occupied by each water frog taxon, especially those in the P–G system.

MATERIAL AND METHODS

Altogether, 819 frogs were sampled randomly in 80 aquatic sites belonging to distinct habitat types (Tables 1 and 2). Sampling effort was similar at each site, catching frogs by hand, by night for 2 h per site. Sample size varied according to frog density.

In order to determine the taxonomic composition at each site, each frog was identified using a combination of specific allozymic markers, specifically lactate dehydrogenase (LDH-B; E.C. 1.1.1.27), glycerol-3-phosphate dehydrogenase (αGDH; E.C. 1.1.1.8), s-adenosyl-l-homocysteine hydrolase (AHH; E.C. 3.3.1.1), mannose-phosphate-isomerase (MPI, E.C. 5.3.1.8) and phosphoglucomutase (PGM-2, E.C. 2.7.5.1). Tissue homogenization, buffer composition, starch gels and staining solutions were prepared following standard procedures (Murphy *et al.*, 1996). Migration was performed on 12% Tris citrate pH 6 starch gels at 12 V/cm for 3.5 h.

For our description of taxonomic composition, we took into account only the 44 sites in which $n \geq 5$ because sampling size varied between

Table 1 List of sites of the present study. Sample size is reported in the third column

No.	Sites	*N*	Habitat type	Latitude & longitude
1	Delta de la Dranse	11	River or dead arm	46.23/6.35
2	Etournelles	15	Gravel pit	46.15/5.56
3	Chautagnes	63	River or dead arm	46.03/5.53
4	Lavours	119	Marsh	46.01/5.48
5	Morte de la Barre	47	Gravel pit	45.56/5.36
6	Verots	15	Marsh	45.59/5.04
7	Jons	32	Gravel pit	45.48/5.06
8	Cleppé (Forez)	4	Gravel pit	46.00/4.04
9	Pierre Bénite	31	River or dead arm	45.38/4.48
10	Platière	19	Gravel pit	45.20/4.46
11	Crolles	5	River or dead arm	45.36/6.10
12	St Jean de Moirans	1	Marsh	45.14/5.47
13	Poliénas	5	Large pond	45.11/5.14
14	Printegarde	20	Marsh	45.06/4.54
15	Bourg Saint Andéol	18	River or dead arm	44.24/4.39
16	Rochegude	12	River or dead arm	44.15/4.18
17	Goudargues 1	9	Large pond	44.13/4.20
18	Goudargues 2	22	River or dead arm	44.13/4.20
19	Auzon	12	River or dead arm	44.09/4.14
20	Bouquet	26	River or dead arm	44.07/4.18
21	Lussan Concluses	8	River or dead arm	44.08/4.24
22	Lussan l'aiguillon	23	River or dead arm	44.07/4.23
23	St Just Vacquières	17	River or dead arm	44.06/4.14
24	Gorges du Gardon	1	River or dead arm	44.02/4.45
25	Canevere	3	Marsh	43.47/4.21
26	Puy Ste Réparade	1	River or dead arm	43.46/5.47
27	Camargue S–E	6	Marsh	43.42/4.33
28	Camargue S	18	Marsh	43.28/4.41
29	Astouin	3	Marsh	43.36/4.27
30	St Mathieu Tréviers	7	Gravel pit	43.56/4.03
31	Gabriac-Notre Dame Londres	10	Shallow pond	43.56/3.59
32	Grès	1	Marsh	43.52/4.05
33	Montpellier	13	River or dead arm	43.43/4.06
34	Argelliers-La Boissière	3	Large pond	43.43/4.02
35	Pissevache	7	Marsh	43.12/3.10
36	Fabrègues	1	River or dead arm	43.22/2.43
37	Capestang	1	River or dead arm	43.24/2.30
38	Ornaisons	2	River or dead arm	43.18/2.37
39	Le Canet	1	Shallow pond	42.35/3.03
40	Marcays	2	Shallow pond	43.18/2.22
41	Les Peyrets	3	River or dead arm	43.22/2.22
42	St Lieux lès Lavaur–St Sulpice	6	Gravel pit	43.28/2.18
43	Buzet-Bessières	4	Gravel pit	43.52/1.47
44	Vacquiers	1	Large pond	43.59/1.11
45	Toulouse	3	Shallow pond	43.32/1.28
46	La Loubère	2	River or dead arm	42.54/0.11
47	Los Certales	12	Large pond	42.27/0.30
48	Chalemera	3	River or dead arm	41.55/0.41
49	Deltebre	14	Marsh	40.46/0.39
50	Tabar	6	River or dead arm	42.25/–1.43
51	Aos	3	Gravel-pit	42.40/–1.22
52	Uroz	7	Gravel-pit	42.41/–1.40

Table 1 *continued.*

No.	Sites	*N*	Habitat type	Latitude & longitude
53	Muez	3	Gravel-pit	42.39/–1.5
54	Anna	3	River or dead arm	42.55/–2.09
55	Saint Palais	1	River or dead arm	43.13/–0.46
56	Moachtia	2	River or dead arm	43.25/–1.07
57	Larrehouroua	3	River or dead arm	43.27/–1.32
58	Ondres	2	Large pond	43.42/–1.17
59	Bayonne	6	Shallow pond	43.41/–1.25
60	Tarnos	11	Marsh	43.50/–1.19
61	Agos	2	River or dead arm	44.02/–0.30
62	Peyrehourtic	4	Large pond	44.19/–0.20
63	Langon	4	Shallow pond	44.35/–0.17
64	Izon	6	Marsh	44.56/–0.22
65	Etauliers	4	Marsh	45.34/–0.43
66	Chatenet	2	River or dead arm	45.38/–0.06
67	St Symphorien	5	Marsh	45.53/–1.00
68	Yves	9	Marsh	46.01/–0.36
69	Albe	2	Marsh	46.12/–1.01
70	Népoux	7	Shallow pond	46.34/0.56
71	Le Linaud	6	Marsh	46.39/–0.47
72	Rouches	12	Marsh	46.39/–1.47
73	Sables d'Olonne	2	Large pond	46.56/–2.12
74	Saint Pardoux	9	Large pond	46.52/–0.35
75	Bressuire	5	Large pond	47.00/–0.35
76	Chiffonière	8	Marsh	47.02/–1.06
77	Petit Mars	4	Marsh	47.48/–1.12
78	Guenrouët	4	Large pond	47.51/–1.12
79	Fégréac	7	Large pond	47.48/–1.54
80	Sévérac	4	Large pond	47.34/–2.18

Table 2 Criteria of habitat typology

Habitat type	Flow or connectivity with the main channel	Gravel content of substratum	Variation of water level	Depth	Surface of the site
River, dead arm	High	High	High	Variable	Variable
Gravel-pit	Intermediate	High to intermediate	Intermediate	Variable	Variable
Marsh	Low	Low	Intermediate	Low	Variable
Large pond	Low	Low	Low	Deep	> 10 m²
Small pond	Low	Low	Low	Low	< 10 m²

sites. In contrast, we used the whole sample for investigating habitat use, habitat preferences, and distribution.

For each species, the hypothesis of ubiquitous distribution was tested using χ^2 for comparing the number of frogs observed in each habitat type with the expected number (i.e. the same number of individuals in each habitat type).

RESULTS

The 819 individuals were identified taxonomically using allozymic variation (Table 3). The total sample was composed of 43.35% *R. ridibunda* (*n* = 355 frogs), 21.73% *R.* kl. *esculenta* (*n* = 178), 15.63% *R. perezi* (*n* = 128), 10.13% *R.* kl. *grafi* (*n* = 83) and 9.16% *R. lessonae* (*n* = 75).

Table 3 Allele variation detected at five enzymatic loci evidenced specific markers that allowed taxonomic identification of the five water frog taxa. The first three loci allowed discrimination between *R. ridibunda*, *R. lessonae* and *R.* kl. *esculenta*, while the last two loci discriminated the *perezi* genome from others thus allowing identification of *R. perezi* and *R.* kl. *grafi*

LDH-B	MPI	PGM-2	α-GDH	AHH	Species
Allozyme a or c	Allozyme a or c	Allozyme b or d	Fast	Fast	*R. ridibunda*
Allozyme b or e	Allozyme h	Allozyme c	Fast	Fast	*R. lessonae*
Genotype ae, ce, ab, bc	Genotype ah, ch	Genotype cd	Fast	Fast	*R.* kl. *esculenta*
Genotype ai, ci, ad, cd		Genotype dd or bd	Fast–slow	Fast–slow	*R.* kl. *grafi*
Allozyme i or d	Allozyme l or m	Allozyme d	Slow	Slow	*R. perezi*

Among the 44 sites where sample size was ≥ 5 frogs, the taxonomic composition was:
• Pure *R. ridibunda* population in 14 ponds (31.82%);
• L–E system (*R. lessonae* + *R.* kl. *esculenta*) in six ponds (13.64%);
• Pure *R. perezi* population in five ponds (11.36%);
• Pure *R.* kl. *esculenta* population in five ponds (11.36%);
• P–G system (*R. perezi* + *R.* kl. *grafi*) in five ponds (11.36%);
• Pure *R.* kl. *grafi* population in two ponds (4.54%);
• R–G assemblage (*R. ridibunda* + *R.* kl. *grafi*) in two ponds (4.54%);
• R–E assemblage (*R. ridibunda* + *R.* kl. *esculenta*) in two ponds (4.54%);
• Pure *R. lessonae* population in one pond (2.27%);
• P–R–G assemblage (*R. ridibunda* + *R. perezi* + *R.* kl. *grafi*) in one pond (2.27%); and
• L–P assemblage (*R. perezi* + *R. lessonae*) in one pond (2.27%).

The taxa of the P–G system (*R. perezi* and *R.* kl. *grafi*) were restricted to the south and west of France and to Spain within the zone of mild winters. The taxa of the L–E system (*R. lessonae* and *R.* kl. *esculenta*) were found mainly in Western and Eastern France but also marginally in the south (sites 40 and 41; Fig. 1). *R. ridibunda* occurred in all areas, although it was not common in Western France.

Each species significantly differed from the theoretical ubiquitous distribution (*R.* kl. *esculenta* χ^2, 2 d.f. = 116, $P < 0.0001$; *R. lessonae* χ^2, 4 d.f. = 57, $P < 0.0001$; *R.* kl. *grafi* χ^2, 4 d.f. = 19, $P = 0.0007$; *R. perezi* χ^2, 4 d.f. = 59, $P < 0.0001$; *R. ridibunda* χ^2, 4 d.f. = 183, $P < 0.0001$). Thus, Fig. 2 highlights a strong preference of *R. ridibunda* for rivers and gravel-pits, of *R.* kl. *esculenta* and *R. lessonae* for marshes, of *R. perezi* for rivers and dead arms. *R.* kl. *grafi* was predominant in marshes, rivers and dead arms, although it was less specialized to one main habitat type than the former species.

Like the two hybridogens, *R. ridibunda* was found in all habitat types. *R. perezi* was absent from gravel-pits in our samples. *R. lessonae* was not found in gravel-pits, rivers and dead arms. The comparison between hybridogens and their respective parental species suggested a 'heterozygositic advantage' in respect of an ability to occupy diverse habitat types. *R.* kl. *esculenta* was very often present in marshes in contrast with *R. ridibunda*. *R.* kl. *esculenta* was present in rivers, dead arms and gravel-pits, while *R. lessonae* was absent from these habitats. *R.* kl. *grafi* occupied gravel-pits, from which *R. perezi* was absent, and marshes in contrast with *R. ridibunda*.

DISCUSSION

Taxon distribution

Our results confirm that *R. perezi* and its associated hybrid *R. grafi* are restricted to southern parts of France, in agreement with the published range of these taxa (Iberian Peninsula and Southern France; Graf & Polls-Pelaz, 1989). However, *R. perezi* was identified in sites 66 and 73. This suggests either that its range extends further north than thought previously or that these two populations were introduced by man. The distributional limits of these two taxa can be explained in two ways. First, they may still be extending their range following a postglacial expansion from Spain, which constituted a *refugium* during the Würm glacial period. Secondly, these taxa may be limited by ecological requirements that prevent further
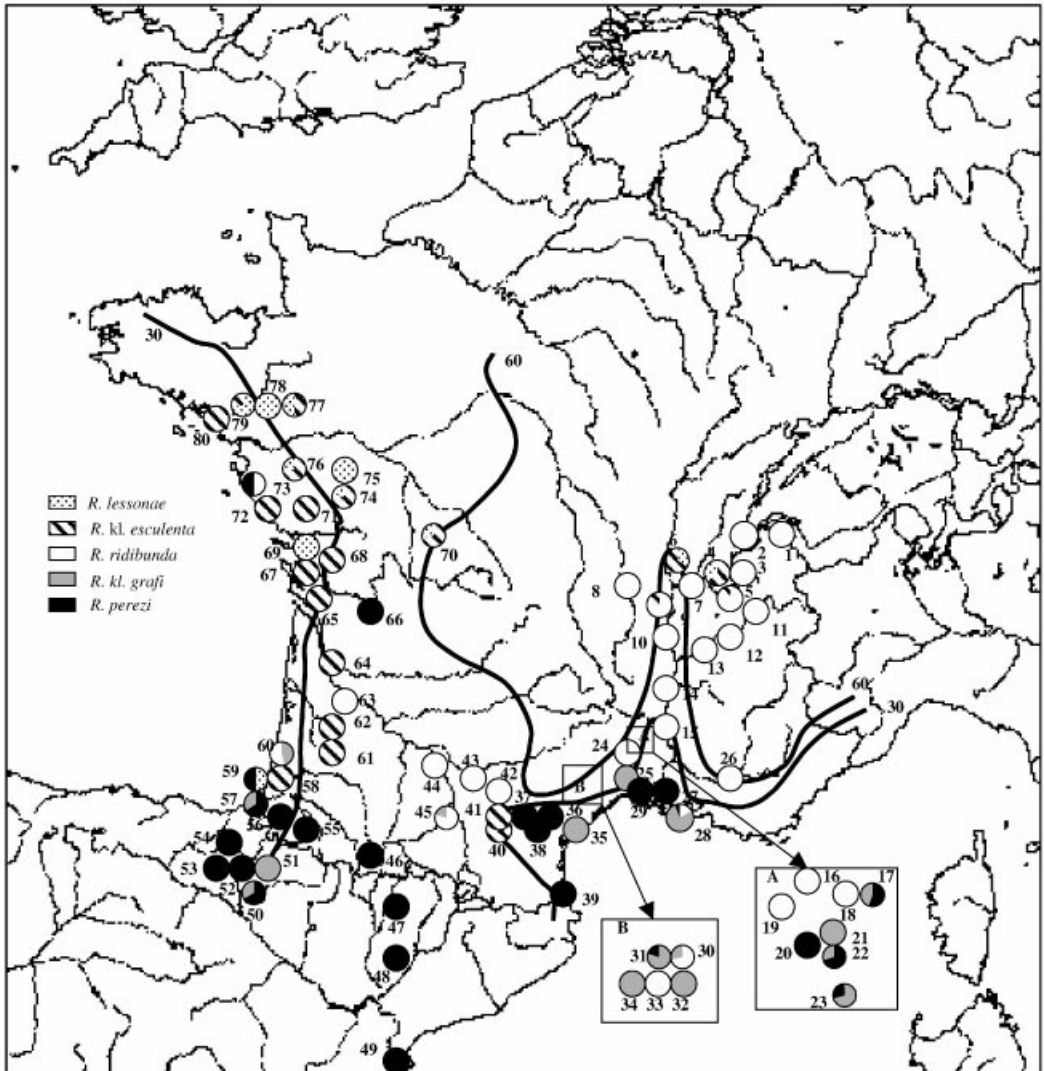
Fig. I Taxonomic composition of populations. Within each circle, *R. ridibunda* is represented in white; *R. lessonae* by dotted line; *R.* kl. *esculenta* by hatching; *R. perezi* in black; and *R.* kl. *grafi* in grey. The thick isoclines show the number of freezing days per year (30-day and 60-day isocline; Kessler & Chambraud, 1986). Names and location of sites are reported in Table 1.

northward expansion because they do not tolerate cold winters. With regard to climatic clines, we propose that the distribution of the *grafi* complex is rather a result of their sensitivity to cold winters.

In fact, the northern range limit of *R. perezi* and *R.* kl. *grafi* follows the limits of the Mediterranean and Oceanic climates (Southern France and Western France, respectively; Fig. 1). In France,

the common characteristic of these climates is mild winters (Fig. 1; Kessler & Chambraud, 1986). Thus, we suggest that the distribution of these taxa may be predicted by global indicators of climatic conditions, e.g. they may be restricted to areas having < 60 days with frost per year (see climatic clines on Fig. 1). The presence of *R. perezi* in sites 66 and 73 is in agreement with this prediction.
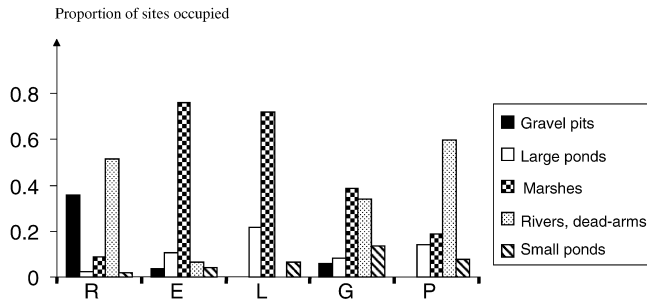
Fig. 2 Occurrence (in proportion) of each species within the five habitat types. R = *R. ridibunda*, E = *R.* kl. *esculenta*, L = *R. lessonae*, G = *R.* kl. *grafi* and P = *R. perezi*.

This hypothesis could be validated by investigating water frog populations along the Atlantic coast of France further north than these sites, or by experimental studies. However, we cannot discard completely the possibility that competition between taxa of the P–G system and those of the *esculenta* complex may be an explanation for the northern range limit of the P–G system.

R. kl. *grafi* may have originated either from a *R. perezi*–*R. ridibunda* hybridization or from a *R. perezi*–*R.* kl. *esculenta* hybridization (Graf *et al.*, 1977; Arano *et al.*, 1995a). *R. ridibunda* exhibits a high amount of heterozygosity (Hotz *et al.*, 1985; Beerli *et al.*, 1996; Pagano *et al.*, 1997 for French populations). In contrast, the *ridibunda* genome of *R.* kl. *esculenta* from France shows lower genetic variation (Pagano, 1999). In the *R.* kl. *grafi* of the present study, the *ridibunda* genome exhibited a very low genetic variation, as also observed in *R.* kl. *esculenta*. Thus, our study suggests rather that these *R.* kl. *grafi* frogs might have received their *ridibunda* genome from a *R. perezi*–*R.* kl. *esculenta* primary hybridization.

We found that *R. ridibunda* was more widespread in France than indicated by Graf & Polls-Pelaz (1989), Neveu (1989) or Gasc *et al.* (1997), i.e. that it is present in Eastern and Southern France (Fig. 1). This widespread distribution may result: (i) from native populations following an expansion from a Balkan refugium or (ii) from various introductions. These two possible origins are not necessarily contradictory but, rather, are complementary. The comparison of range distribution of *R. ridibunda* in France according to two recent maps (Neveu, 1989; Gasc *et al.*, 1997) suggests a contemporary expansion. Nevertheless,

this cannot completely explain the distribution of *R. ridibunda*. Isolated populations suggest introductions. Moreover, the hypothesis of allochthonous origins has been supported for several populations. The rare allele MPI-j (of allochthonous origin; Pagano *et al.*, 1997) was detected in several *R. ridibunda* individuals from populations located near scientific laboratories (sites 33 and 45), but also at other sites (e.g. site 9). It proves that introduction events were — and still are — multiple (Pagano *et al.*, 1997). Thus, the origin(s) of *R. ridibunda* in France has still to be specified.

The southern range limit of *R. lessonae* and *R.* kl. *esculenta* has been previously considered to be the central part of France (Graf & Polls-Pelaz, 1989). We identified these two taxa in several populations in Southern France, indicating that their southern range limit presently lies further south than Central France. A contact zone between *R. lessonae* and *R. perezi*, leading to natural hybridization, has even been discovered (site 50 nine, Pagano *et al.*, 2001). Further investigations should allow to make sure whether these *R. lessonae* individuals are isolated from the main range of the L–E system or not. Moreover, it should be possible to elucidate whether they represent a natural occurrence previously overlooked or whether they result from introduction, as described in Spain for another water frog taxon (Arano *et al.*, 1995b).

With the present data, it is not possible to confirm or to reject the hypothesis of a contemporary northward expansion of *R. perezi* and *R.* kl. *grafi*. If populations 66 and 73 are persistent, this will prove such a recent expansion. The comparison of distribution maps (Graf & Polls-Pelaz, 1989; Gasc *et al.*, 1997) suggests a recent expansion of

both *R. ridibunda* and *R.* kl. *esculenta* into South-ern France either because of introductions or because of evolutionary and/or ecological reasons.

Habitat use

This study demonstrates significant differences in habitat use between the various water frog taxa (Fig. 2). *R. ridibunda* exhibits a clear preference for habitats characterized by river influence (rivers, dead arms and gravel-pits). In contrast *R. lessonae* is very rare in or absent from these habitat types. Interestingly, the habitat preferences of *R.* kl. *esculenta* mainly correspond to those of its sexual host *R. lessonae*, i.e. both co-occur preferentially in marshes, suggesting a kind of vicariance. In the other host–hybridogen system, habitat uses are not as concordant. *R.* kl. *grafi* and *R. perezi* were often found in rivers and dead arms. This is par-ticularly true for *R. perezi* (60% of our sampled individuals were found in such habitats) while *R.* kl. *grafi* mainly inhabits two habitat types (rivers and dead arms 34%, but also marshes 39%).

While none of the species exhibited a ubiquitous distribution, it should be noted that none appeared to be strictly limited to one habitat type. Thus, each of the taxa demonstrated a certain amount of plasticity in habitat use, i.e. they were able to occupy different habitat types either frequently or more occasionally. The broader habitat range of the two hybridogens — which were present in all five habitat types — suggests 'a heterozygositic advantage', i.e. the 'co-occurrence' of two differ-ent genomes confers an ability to occupy habitats from which the parental species are absent or in which they are only marginally present. It is to be underlined that hybrid lineages are expected to exploit a broader ecological niche in the general purpose genotype or in the frozen niche variation models (see, e.g. Vrijenhoek, 1994).

Hybrid zone models

The tension zone model (Barton & Hewitt, 1985) is not supported by the data reported herein because distributions showed neither clinal struc-tures nor independence of habitat use in water frogs. Moreover, whereas one basis of this model is to assume selection against hybrids, hybrid water frogs are fit according to several life his-tory traits (Hotz *et al.*, 1999; Plénet *et al.*, 2000).

The environment-dependent mosaic zone model (Harrisson, 1986) appears to be the more appropri-ate to describe water frog distributions. As pre-dicted by this model: (i) 'pure' parental populations can be found within the hybrid zone and (ii) the distribution of taxa in a mosaic of patches reflects differences in habitat use. Our field data support both these predictions. Thus, pure parental popula-tions were recorded for both *R. perezi, R. ridibunda* (e.g. sites 3, 7 and 20) and *R. lessonae* (e.g. Rybacki & Berger, 1995). Mosaic distribution is also evid-enced by the multiple events of co-occurrence between different taxa (18 sites). In site 28, three taxa co-occurred (*R. perezi, R. ridibunda* and *R.* kl. *grafi*). In numerous sites, two taxa co-occurred either as assemblages (e.g. sites 5 and 9) or as L–E and P–G systems (e.g. sites 4, 6, 17, 22 and 23).

The existence of a new contact zone — between *R. lessonae* and *R. perezi* — described recently from Southern France (Pagano *et al.*, in press), can also be explained by the mosaic zone model. Indeed, this model predicts a high rate of inde-pendent hybridization events.

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