

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/227011487>

Potential impact of genome exclusion by alien species in the hybridogenetic water frogs (*Pelophylax esculentus* complex)

Article in *Biological Invasions* · January 2009

DOI: 10.1007/s10530-009-9427-2

CITATIONS

49

READS

599

2 authors, including:



Robert Jooris

Research Institute for Nature and Forest

28 PUBLICATIONS 161 CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



Analysis of scutellation in populations of *Cerastes vipera* (Linnaeus, 1758): Scale characters co-vary with environmental temperature [View project](#)



INVEXO [View project](#)

Potential impact of genome exclusion by alien species in the hybridogenetic water frogs (*Pelophylax esculentus* complex)

G. Holsbeek · R. Jooris

Received: 23 January 2008 / Accepted: 12 January 2009 / Published online: 30 January 2009
© Springer Science+Business Media B.V. 2009

Abstract Globalization and increasing human impact on natural aquatic systems have facilitated the movement of species and the establishment of nonindigenous species enhancing hybridisation opportunities between naturally allopatric species. In this review, we focus on a special case of natural hybrid speciation and the consequences of recent anthropogenic hybridisation in the water frog complex (*Pelophylax esculentus* complex), which consists of two parental species, *Pelophylax lessonae* and *Pelophylax ridibundus* and a hybrid taxon. The hybrid water frogs reproduce hybridogenetically and eliminate the genome of the syntopic water frog species. Although the actual cause triggering chromosome exclusion remains elusive, it has been proposed that chromosome elimination takes place prior to meiosis and may involve enzymatic degradation of the discarded genome. Translocations of

water frogs in Western Europe have become frequent the last decade leading to rapid expansion of the range of the marsh frog *P. ridibundus*. Subsequent hybridisation of the exotic *P. ridibundus* may dramatically affect the viability and maintenance of hybrid water frog populations throughout Europe. Interestingly, the impact of this introduced species may differ depending on their geographic origin, which defines the ability to induce genome elimination. This may result in fertile or sterile hybrids, making global conservation guidelines challenging. We predict a severe genetic and ecological impact of nonindigenous *P. ridibundus* prompting for strict conservation measures to reduce species translocations and for studies on the geographic origin of exotic frog species.

Keywords European water frogs · Hybrid complex · Hybridisation · *Pelophylax ridibundus* · Translocations

G. Holsbeek (✉)
Laboratory of Aquatic Ecology and Evolutionary Biology,
Katholieke Universiteit Leuven, Ch. Deberiotstraat,
32, 3000 Louvain, Belgium
e-mail: Griet.Holsbeek@bio.kuleuven.be

G. Holsbeek
Laboratory of Animal Diversity and Systematics,
Katholieke Universiteit Leuven, Ch. Deberiotstraat,
32, 3000 Louvain, Belgium

R. Jooris
Natuurpunt, Michiel Coxiestraat 11,
2800 Mechlin, Belgium

Introduction

Translocations of alien species, habitat modifications and fragmentation are the primary factors that are believed to contribute to the current increase of hybridisation rates between naturally allopatric species (Allendorf et al. 2001; Frankham et al. 2002; Rhymer and Simberloff 1996). Global trade has facilitated the movement of species outside their

natural environment and enhanced the establishment of nonindigenous species in new habitats (Mooney and Cleland 2001), increasingly putting pressure on indigenous species already suffering from human-induced habitat loss and fragmentation (Fahrig 2003; Forester and Machlist 1996; Pimm and Raven 2000). The establishment of invading species in new habitats may result in maladaptive hybridisation with autochthonous species and genetic pollution, ultimately leading to the extinction of native species (Rhymer and Simberloff 1996). Translocation of nonindigenous species may cause outbreeding depression in native populations, because reproductive efforts are wasted on producing hybrids with reduced fitness or even sterile offspring (Levin et al. 1996; Rhymer and Simberloff 1996). Alternatively, hybridisation may positively influence species diversification in animals (Dowling and Secor 1997; Grant and Grant 1992; Mooney and Cleland 2001; Seehausen 2004). Inter-specific hybridisation may increase genetic diversity more rapidly than through mutations, and populations with high genetic diversity are expected to be on average better equipped to cope with and adapt to environmental changes and diseases than populations with lower genetic diversity (Dowling and Secor 1997; Ellstrand and Schierenbeck 2006; Frankham et al. 2002; Seehausen 2004; Tsutsui et al. 2000).

Since natural hybridisation is an evolutionary process, natural hybrids are believed to be eligible for protection, while hybrids resulting from human actions are not (Allendorf et al. 2001). It is therefore of primary importance to distinguish between natural and anthropogenic hybridisation. The consequences of natural and anthropogenic hybridisation on native species have been extensively investigated (e.g. Arnold 2004; Grant and Grant 2002; Huxel 1999; Lamont et al. 2003; Mallet 2005; Rieseberg 1997). In the present review, we focus on a special case, being anthropogenic hybridisation taking place in a hybrid complex that originated from a hybridisation event in the past. We discuss the water frog complex and enquire to what extent hybridisation through translocation may have an effect in hybrid populations where natural hybridisation has occurred or is still occurring. Are hybrid species at risk as a result of anthropogenic hybridisation?

The native water frog populations of Western and Central Europe have recently been subjected to multiple and recurrent introductions of other water

frog species (Arano et al. 1995; Grossenbacher 1988; Holsbeek et al. 2008; Pagano et al. 2001a, 2003; Vorburger and Reyer 2003). The water frog complex forms a suitable model system to investigate the population genetics of alien species and translocation effects in a hybrid complex, because the unique way of hybridogenetic reproduction allows for very rapid introgression between native and exotic species (Holsbeek et al. 2008; Pagano et al. 2003). Studies on hybridisation events through translocation in hybridogenetic species are necessary to design more suitable conservation policies for these species. In order to identify and interpret the possible ecological and/or genetic consequences of introductions in the native water frog species, it is crucial to fully understand the hybridogenetic reproduction of the water frog complex and the genetic mechanism underlying hybridogenesis. We provide an overview of the hybridogenetic reproduction of the water frog complex. We then discuss the potential impact of translocated populations of members of this hybrid complex on the native species.

The water frog complex

The *Pelophylax esculentus* complex, formerly known as *Rana esculenta* complex (Dubois 1992; Frost et al. 2006), consists of two parental species, *Pelophylax ridibundus* Pallas 1771 (genotype RR) and *Pelophylax lessonae* Camerano 1882 (genotype LL), and the hybrid species *P. kl. esculentus* Linnaeus 1758 (genotype RL, Berger 1988; Graf and Polls Pelaz 1989). Whereas other hybridogenetic species are characterized by all-female and diploid hybrid populations, the hybridogenetic water frog complex is unique due to the presence of female as well as male hybrids (Dubois and Günther 1982; Graf and Polls Pelaz 1989). Multiple fertilisation experiments performed by Berger (1967) revealed the extraordinary reproductive strategy in *P. kl. esculentus*. The hybridogenetic nature of reproduction in the hybrid frogs of this complex (Berger 1973) was recognized a few years later after the definition of the term 'hybridogenesis' by Schultz (1969).

Natural interspecific hybrid lineages that persist through hybridogenetic reproduction are widespread among the European water frogs (Berger 1988; Graf and Polls Pelaz 1989). Hybrid gametogenesis in the

P. esculentus complex shows peculiar geographical variation. The hybridogenetic system can be categorized into three major breeding strategies (Fig. 1): the *P. lessonae*—*P. kl. esculentus* (L–E) system, the *P. ridibundus*—*P. kl. esculentus* (R–E) system, and all-hybrid (E) populations (Graf and Polls Pelaz 1989). The L–E system (*P. lessonae*—*P. kl. esculentus*; Fig. 1), which inhabits Western Europe, is the most widespread hybridogenetic system, and its breeding strategy has been studied to the greatest detail. In this L–E system, the hybrid, which originated through an ancient hybridisation between *P. ridibundus* and *P. lessonae*, lives syntopically with *P. lessonae* (Berger 1988; Graf and Polls Pelaz 1989). During the hybridogenetic reproduction of *P. kl. esculentus*, the *lessonae* genome is discarded and gametes containing only the *ridibundus* genome are produced. The *lessonae* genome exclusion from the germ line occurs prior to meiosis during the prolonged phase of oogonial proliferation (Tunner and Heppich 1981). The chromosome exclusion itself appears to be a gradual elimination from the cell during successive mitotic divisions instead of one-step elimination during one mitotic division (Ogielska 1994a). The remaining *ridibundus* genome is then replicated either by a premeiotic or by a prediplotene meiotic event. This results in the restoration of a diploid chromosome number, which enables a regular meiosis that produces haploid, non-recombined gametes (Tunner and Heppich-Tunner 1991; Tunner and Heppich 1981). Hybrids are re-established in each generation through backcrosses with the syntopic parental species *P. lessonae*, whose genome is subsequently always excluded from the germ line (Graf and Polls Pelaz 1989; Pagano et al. 1997). The outcome is a hybrid that remains genetically unaltered at the level of the *ridibundus* genome, which is clonally transmitted each generation (Fig. 1).

Matings between two *P. kl. esculentus* almost invariably (97%) lead to RR tadpoles with developmental difficulties resulting in death before they reach sexual maturity (Berger 1967; Graf and Polls Pelaz 1989). This inviability is usually explained by the accumulation of homozygote-recessive deleterious mutations on the non-recombining *ridibundus* genomes of the hybrid lineages due to the operation of Muller's ratchet (Muller 1964) since the ancient origin of the hybridogenetic system. Numerous experimental crosses confirmed lethality of the

offspring of *P. kl. esculentus* inter se matings and the operation of the ratchet (Berger 1967; Binkert et al. 1982; Ogielska 1994b; Uzzell et al. 1980; Vorburger 2001c). Vorburger (2001a, b) and Geux et al. (2002) also revealed that *P. ridibundus* tadpoles from matings between *P. kl. esculentus* individuals with two different *ridibundus* genomes survived. Even though most offspring of matings between two *P. kl. esculentus* with the same *ridibundus* genomes are nonviable, a low percentage (3%) of viable *P. ridibundus* are produced from hybrid matings. Since the primary hybridisation likely occurred between a bigger female *P. ridibundus* (higher fecundity) and a smaller male *P. lessonae* (Berger 1970), the original clonally transmitted *ridibundus* genome is carrying the X-chromosome (the sex-determining mechanism in frogs is XX-XY, males being heterogametic; Berger 1988; Hotz et al. 1992). The viable individuals are therefore all female (Vorburger 2001c). In these *P. ridibundus* females, Mendelian segregation can occur, and recombination may possibly reduce the amount of deleterious mutations on the *ridibundus* genome. Because conspecific males are absent in L–E systems, the *P. ridibundus* females have to breed with either *P. kl. esculentus* males, leading to more *P. ridibundus* females, or with *P. lessonae* males, generating new hybrid lineages with different mutations on the *ridibundus* genome (Som and Reyer 2006). The survival of a small fraction of these RR tadpoles may in this way reintroduce recombination into an otherwise hemiclinal reproductive system. Simulations have shown that only a small percentage of recombination (1–10%) is needed to prevent fixation and accumulation of deleterious mutations. This mechanism may thus beneficially influence the long-term average fitness of individuals in a hybridogenetic population (Som and Reyer 2006).

The strictly maternally inherited mitochondrial DNA (mtDNA) of the two parental species *P. lessonae* and *P. ridibundus* shows a clear distinction. The latter species has two strikingly different types of mtDNA: type A (*P. ridibundus* specific) and type B (*P. lessonae*-like) (Spolsky and Uzzell 1984, 1986). Generally, introgression of mtDNA is connected to interspecific hybridisation mediated by (partially) fertile hybrids (Plötner et al. 2008). As primary hybridisations most likely occurred between female *P. ridibundus* and male *P. lessonae* due to

size-related behavioral reasons (Berger 1970), the hybrid species *P. kl. esculentus* is expected to have *P. ridibundus* mtDNA. Because hybrid lineages of the L–E system are usually maintained through backcrosses of a female *P. kl. esculentus* with a male *P. lessonae*, one would expect to find *P. ridibundus* mtDNA in all *P. kl. esculentus* individuals. However, the majority of these hybrids appear to contain mtDNA of their conspecific parental species *P. lessonae* (Spolsky and Uzzell 1986). There are two plausible explanations for this phenomenon. Either the primary hybridisations did occur in the reversed combination (female *P. lessonae* and male *P. ridibundus*), which would have led to *P. kl. esculentus* containing *P. lessonae* mtDNA, or the expected crosses within the L–E system may be contrary to expectations (female *P. lessonae* and male *P. kl. esculentus*) resulting also in hybrids with *P. lessonae* mtDNA. Spolsky and Uzzell (1986) believe the second explanation to be most likely. The wide distribution of hybrids with *P. lessonae* mtDNA may be accounted for by rare matings between female *P. lessonae* and male *P. ridibundus* in the L–E system. In the absence of the parental species *P. ridibundus*, such a transfer of *P. lessonae* mtDNA into *P. kl. esculentus* is irreversible (Plötner et al. 2008; Spolsky and Uzzell 1986). Through an intermediated stage involving the hybrid *P. kl. esculentus*, interspecific transfer of mtDNA has occurred from *P. lessonae* to *P. ridibundus* (Plötner et al. 2008; Spolsky and Uzzell 1984). This introgression of *P. lessonae* mtDNA in *P. ridibundus* may be the result of two scenarios. Although unlikely due to nonviable offspring of matings between two *P. kl. esculentus*, matings between two hybrids both carrying *P. lessonae* mtDNA may produce *P. ridibundus* offspring containing *P. lessonae* mtDNA. Another more likely way to obtain *P. ridibundus* individuals with *P. lessonae* mtDNA might be crosses between hybrids with *P. lessonae* mtDNA and *P. ridibundus* individuals (Spolsky and Uzzell 1984). Plötner et al. (2008) compared the extent of introgression of *P. lessonae* mtDNA in *P. ridibundus* individuals of different regions. Several geographical patterns were detected, such as the restriction of *P. ridibundus* individuals with *P. lessonae* mtDNA to Central Europe north of 48°N latitude and between 8 and 22°E longitude, which also corresponds to the L–E and R–E system. Plötner et al. (2008) also suggest that individuals of both *P. kl. esculentus* and *P. ridibundus*

with *P. lessonae* mtDNA may have an adaptive advantage. Plénet et al. (2000a, b) stated that *P. ridibundus* larvae under hypoxic conditions develop less well than larvae of *P. lessonae*. If this difference depends on mitochondrially encoded enzymes, *P. ridibundus* larvae with *P. lessonae* mtDNA might be less sensitive to oxygen deficiencies than ‘normal’ *P. ridibundus* larvae. However, the latter statement has yet to be tested. Finally, Plötner et al. (2008) suggest that the extent of transfer of *P. lessonae* mtDNA into *P. ridibundus* (33.7%) does not influence mitochondrial–nuclear interaction, and consequently the fitness of this species, despite large mitochondrial divergences between the two parental species *P. ridibundus* and *P. lessonae*.

The R–E (*P. ridibundus*—*P. kl. esculentus*) system (Fig. 1) is basically the inverse of the L–E system, although not quite as regular. *P. kl. esculentus* discards the *ridibundus* genome in a 3:1 ratio, leading to the production of mainly *lessonae* gametes and a minority of *ridibundus* gametes (Uzzell et al. 1977; Vinogradov et al. 1991). When the hybrids of this system transmit *ridibundus* gametes to the germ line, all offspring are *P. ridibundus* females. When the *lessonae* gametes are passed on, *P. kl. esculentus* males are formed (Fig. 1). This peculiar phenomenon in the R–E system is known as hybrid-amphispermy (Vinogradov et al. 1991).

All-hybrid populations (Fig. 1), also known as the E system, lack both parental species. They consist solely of *P. kl. esculentus* individuals. All-hybrid populations occur frequently in both the L–E and the R–E system. However, we will focus on all-hybrid populations in Western Europe (L–E system), as they are studied in most detail. In addition to LR diploids, these populations harbor large numbers of LLR triploids, which have taken over the role of *P. lessonae* individuals as providers of *lessonae* gametes (Christiansen et al. 2005; Uzzell et al. 1980). The formation of unreduced diploid gametes by diploid *P. kl. esculentus* can be considered a strategy to escape extinction in the absence of *P. lessonae*. Gamete formation in triploids differs from that in diploid hybrids. In LLR-triploids the *ridibundus* genome, of which only one copy is available, is discarded (Fig. 1). This results in diploid cells, in which normal segregation is possible. In contrast to diploid hybridogenesis, no endoreduplication occurs and haploid gametes containing the *lessonae* genome

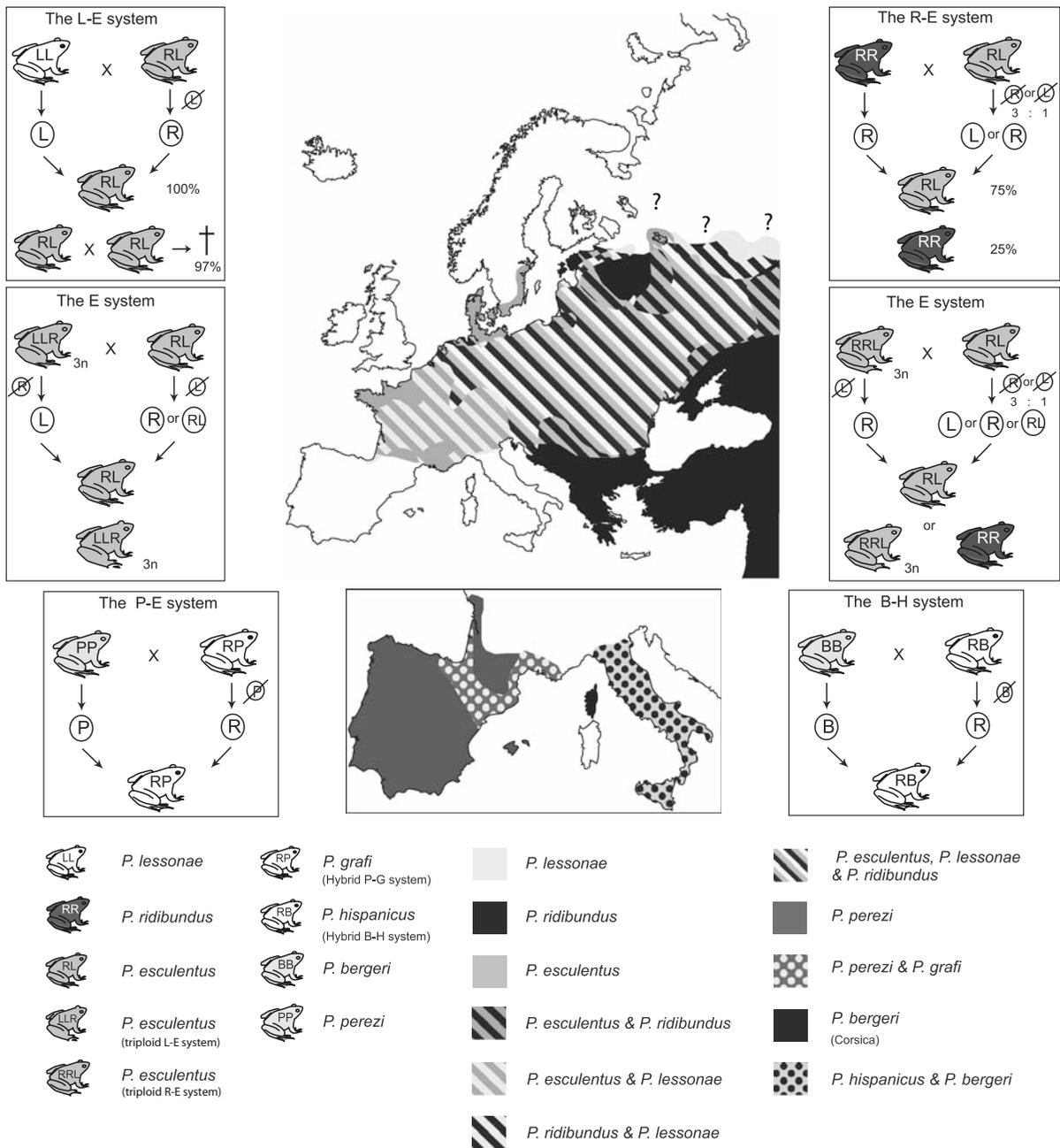


Fig. 1 Overview of the various reproductive systems of the four water frog complexes in Europe and the geographical distribution of the parental and hybridogenetic species belonging to these complexes. In the L–E system (Western Europe), the hybrid *P. kl. esculentus* eliminates the *lessonae* genome from the germline and backcrosses with the syntopic parental species, *P. lessonae*. Hybrid matings produce nonviable tadpoles (97%). In E systems, however, hybrids can mate with each other, as long as there are triploid individuals that provide the *lessonae* gametes. The distribution of these all-hybrid populations is not indicated on the map, since these populations

occur in the whole geographic region of the water frog complex. The R–E system (Eastern Europe) is basically the inverse of the L–E system. The hybrid discards, however, the *ridibundus* genome in a 3:1 ratio, leading to progeny consisting of *P. kl. esculentus* (75%) and *P. ridibundus* (25%). This is also the case for the E-system in Eastern Europe. In the P–G system (Spain), *P. grafi* hybrids are re-established through backcrosses with the syntopic parental species *P. perezi*, whose genome is excluded from the germline. The B–H system (Italy) involves the hybrid *P. hispanicus* discarding the *bergeri* genome, and the syntopic parental species *P. bergeri*

are created (Christiansen et al. 2005; Günther et al. 1979). In most cases, pure hybrid populations consist of diploid females and triploid males (Christiansen et al. 2005; Mikulicek and Kotlik 2001). Although the parental species *P. lessonae* and *P. ridibundus* are absent from these populations, they have been observed among juveniles. These larvae apparently never reach metamorphosis (Berger 1988). The cause of this early death is still unknown. However, recombination between the two homologous genomes of the triploid hybrids occurs, which suggests that accumulation of deleterious mutations is probably not the reason for this mortality (Plötner 2005). Overall, the degree of genetic compatibility of gametes has a pronounced influence on the structure and dynamics of these all-hybrid populations, as is reflected by high embryonic mortality (Berger 1988) and fertilisation difficulties (Ogielska 1994b).

Widespread hybridogenetic systems are present in at least two other water frogs, *P. hispanicus* Bonaparte 1839 (Italy) and *P. grafi* Crochet 1995 (Spain) (Fig. 1). These hybrids also reproduce hybridogenetically and eliminate the genome of the syntopic species. The Italian hybrid originated from an ancestral hybridisation between *P. ridibundus* and *P. bergeri* Günther 1985 (B–H system) and reproduces through backcrosses with *P. bergeri* (Uzzell and Hotz 1979). The origin of the hybridogen *P. grafi* remains elusive, as it is at present unknown whether this hybrid is the result of a primary hybridisation between *P. perezi* Seoane 1885 and *P. ridibundus* or between *P. perezi* and *P. kl. esculentus* transmitting the *ridibundus* genome (P–G system). In this P–G system, the hybrid *P. grafi* mates with *P. perezi* to produce *P. grafi* offspring (Graf et al. 1977).

The mechanism behind hybridogenetic reproduction in the genus *Pelophylax*

The actual cause triggering chromosome exclusion in water frog hybridogenesis and the exact mechanisms governing this elimination remain an issue of debate. Several observations suggest that alleles present on the *ridibundus* genome account for the germ line elimination of the other parental genome: (1) the *lessonae* genome is in most cases excluded and the *ridibundus* genome is passed on to the gametes (Graf and Polls Pelaz 1989; Guerrini et al. 1997;

Vinogradov et al. 1991) (2) the *ridibundus* genome is present in all known hybridogenetic types, and (3) none of the interspecific hybrids that were experimentally created without *P. ridibundus* as a parent have been found to reproduce hybridogenetically. Yet, there are some hybrids that exclude the *ridibundus* genome instead of the *lessonae* genome (Hotz et al. 1985; Hotz and Uzzell 1983; Uzzell et al. 1980; Vinogradov et al. 1991), or even may eliminate any of the two genomes (hybrid-amphispermy) (Vinogradov et al. 1991). Some authors suggest that hybridogenetic gametogenesis is induced by the co-operation of complex and species specific genetic factors (Hotz et al. 1985; Vinogradov et al. 1991). Two hypotheses have been formulated on the mechanisms of the actual genome elimination. First, it has been proposed that chromosome elimination takes place during mitosis, which is supported by observations on meta-, ana- and telophases (Ogielska 1994a). Alternatively, the elimination of the chromosomes may involve the enzymatic degradation of the rejected genome during the interphase. The degraded chromatin is then subsequently removed from the nucleus by enclosing it in nucleus-like bodies (NLB), which are then exported from the cell. The latter hypothesis is supported by the occurrence of NLBs during apoptosis and seems to be a probable mechanism (Ogielska 1994a; Vinogradov and Chubinshvili 1999). The nuclear envelope forms a bud that detaches from the main nucleus when the inner and the outer membrane fuse. As a result, the NLBs are surrounded by a double membrane and contain material similar to nuclear chromatin (Ogielska 1994a). The NLBs have been observed mainly in tadpoles of *P. kl. esculentus*. Comparable structures to the NLBs have sporadically been observed in the parental species, which may indicate that also the parental species are capable to discard chromosomes from the germ line, leading to hybridogenetic reproduction (Ogielska 1994a).

An important feature of the hybridogenetic system of the water frog complexes is that there is geographic variation in the capacity of *P. ridibundus* to induce genome elimination. Many Southern populations of *P. ridibundus* (Hotz et al. 1985; Hotz and Uzzell 1983) fail to induce hybridogenesis in hybrids with *P. lessonae*. *P. bedriagae*, the closest known relative of *P. ridibundus*, is equally incapable of inducing hybridogenesis (Plötner 2005). This suggests that the acquisition of the capacity for genome exclusion may

have been a relatively recent event, and may have arisen only once within *P. ridibundus* (Guerrini et al. 1997). Under the assumption that the *ridibundus* genome carries the alleles responsible for germ line exclusion (Guerrini et al. 1997), one may expect two distinct groups in *P. ridibundus*: a group with the ability to induce hybridogenesis and a nonhybridogenetic group. However, besides *P. kurtmulleri* and *P. ridibundus* being sister species and forming a sister group to *P. bedriagae* and *P. cerigensis* (Lymberakis et al. 2007), the phylogenetic relationships among *P. ridibundus* individuals from Central, Eastern and Southern European populations still remain unresolved (Lymberakis et al. 2007; Plötner 2005).

The impact of translocations in the water frog complex

Of the three European water frog species, *P. ridibundus* displays the largest distribution range across Europe, which contributes to an increased ability to cross species' range borders (Schmeller et al. 2005). Since the overview published by Berger (1988), the distribution area of *P. ridibundus* (Fig. 2) has expanded naturally to the Ural Mountains in the east, to the Rhine and Oder banks in the west and to the Black Sea in the south (Plötner 2005). However, human-mediated translocations of water frogs have become very frequent the last decade, either accidentally (e.g. associated with fish stocking and escapees from garden centers) or on purpose (e.g. stocking of garden ponds and culinary purposes). These translocations have led to an rapid additional expansion of the range of *P. ridibundus* (Pagano et al. 2001a, 2003; Plötner 2005; Vorburger and Reyer 2003). Several countries have experienced introductions of this water frog. In Britain, *P. ridibundus*, which was introduced from Hungary for the first time in 1935 and multiple times over the last decades, is the most successful introduced amphibian. It became abundant by the end of the twentieth century (Zeisset and Beebee 2003). In Switzerland, the first *P. ridibundus* specimens were sighted in 1950 (Vorburger and Reyer 2003). Once established, they have continued to expand their range. Most of the introduced animals originated from Eastern or South-eastern Europe, although some water frogs originated from Anatolia (Turkey) (Pagano et al. 1997). In the middle of the



Fig. 2 The distribution of *P. ridibundus* in Europe (gray) and translocations of lineages (gray circles) known from published data in Switzerland (Vorburger and Reyer 2003), France (Pagano et al. 1997), and South-East England (Zeisset and Beebee 2003). Translocations into Belgium (data from the authors) are also added

twentieth century, a large number of *P. ridibundus* animals escaped or were deliberately released into the wild (Vorburger and Reyer 2003). In France, multiple introductions were observed. In the 1960s, most of the water frog import occurred from Balkan countries. This shifted, however, in the 1980s towards Albany, Turkey and Egypt, the main current sources for frog import (Pagano et al. 1997, 2003; Schmeller et al. 2007). Arano et al. (1995) conducted a two-year follow-up study of several water frog populations in Spain. The native populations of the Iberian Peninsula normally consist of *P. perezi* and *P. grafi*, which is a hybrid between *P. ridibundus* and *P. perezi*. In this country, several introductions of *P. ridibundus*, *P. lessonae* and even *P. kl. esculentus* have occurred. As a consequence, new hybridisation opportunities between *P. perezi* and *P. ridibundus*, and between *P. lessonae* and *P. ridibundus*, have arisen. The hybridogenetic hybrids between *P. grafi* and *P. kl. esculentus* could be harmful for the local water frog populations, because the number of surviving hybrids may exceed the parental species (Rist et al. 1997). Translocations of *P. ridibundus* have also surfaced in

other countries, such as Belgium, Germany, Luxembourg, Latvia, Libia and Russia (Plötner 2005). Taxonomic uncertainties of *P. ridibundus* and the presence of many cryptic species (Plötner 2005) make the documentation of the distribution of introduced *P. ridibundus* difficult. The likelihood of misidentifications of translocated animals is high. Some countries may thus have experienced introductions by other or additional water frog species than those documented in the literature. In France, Pagano et al. (2003) anticipates the presence of other water frog species, such as *P. kurtmuelleri*, *P. bedriagae* and *P. saharicus*, based on the source areas for import, Turkey and Egypt. So far, however, this prediction has not yet been confirmed by data. In Belgium, Holsbeek et al. (2008) recently showed that besides *P. ridibundus* another water frog species, *P. cf. bedriagae*, has been introduced. Phylogenetic analyses could link the invasion of this Anatolian frog *P. cf. bedriagae* to trade (Holsbeek et al. 2008).

The impact of introduced *P. ridibundus* may differ depending on the geographic region they come from. Introductions of Central or Eastern European *P. ridibundus* into a mixed population of *P. lessonae* and *P. kl. esculentus* may give rise to mating opportunities between all three taxa, since their breeding seasons overlap (Pagano et al. 2001c; Vorburger and Reyer 2003). Matings among *P. ridibundus* will produce *P. ridibundus* offspring, as do crossings between *P. ridibundus* and the hybridogen *P. kl. esculentus*, as the latter transmits only gametes containing the *ridibundus* genome. Natural hybridisations of the introduced *P. ridibundus* with *P. lessonae* result in new hybrid lineages (referred to as “polluted hybrids” by Pagano et al. 2003). If the introduced Central European *P. ridibundus* are able to induce hybridogenesis, newly formed hybrids will discard the *lessonae* genome and clonally transmit the *ridibundus* genomes just like the original hybrids in the population do. However, since the introduced *P. ridibundus* display Mendelian reproduction with normal recombination, the inherited *ridibundus* genomes carry less or different deleterious mutations than the original *ridibundus* genomes of the hybridogenetic lineage (Schmeller et al. 2001). Matings of such new hybrids with the original hybridogenetic lineages may lead to the production of viable *P. ridibundus* progeny, because the mutations on the *ridibundus* genome of the hybridogenetic lineage are compensated for by the

second *ridibundus* genome carrying less or other mutations. The survival probability of *P. kl. esculentus* × *P. kl. esculentus* progeny will increase as more primary hybridisations have taken place in the population. These various crosses with newly introduced *P. ridibundus* may create new dynamics and deeply affect the population structure of the water frogs in former L–E systems. First, the number of *P. ridibundus* individuals will increase in the population, because all matings within *P. ridibundus*, all crossings between *P. ridibundus* and *P. kl. esculentus*, and an increasing fraction of matings among *P. kl. esculentus* individuals produce *P. ridibundus* progeny. *P. lessonae* will decrease in numbers, because only matings inter se produce *P. lessonae* offspring, and because of competition with the larger *P. ridibundus*. Because their sexual host becomes less frequent, hybrids will also decline. This may finally lead to a pure *P. ridibundus* population and the loss of the hybridogenetic system. At the same time, however, *P. ridibundus* introductions result in new *P. kl. esculentus* hemiclones, which may contribute to an increase in genetic diversity in hybridogenetic hybrids and may reduce the fixed and accumulated mutation load on the *ridibundus* genome through recombination, which may potentially stabilize the hybridogenetic system (Fig. 3; Plötner 2005; Som and Reyer 2006).

On the other hand, one might think that primary hybridisation between Eastern European *P. ridibundus* (belonging to the R–E system) and *P. lessonae* would lead to hybridogenetic hybrids transmitting the *lessonae* genome instead of the *ridibundus* genome. These hybrids may then function as providers of the *lessonae* genome, and would hybridize with *P. ridibundus*. In this way, they would maintain the number of *P. kl. esculentus* individuals in the invaded populations as the majority of the crossings produce hybrids. In fact, the L–E system would be turned into an R–E system.

From the above, it is clear that it is a priority to determine which genome is transferred in hybridisations between *P. ridibundus* and *P. lessonae*. This can be verified by means of experimental crosses. A first attempt of such crosses between water frogs of the L–E and R–E system has been made by Ragghianti et al. (2007). They concluded that a particular combination of the R–E system *lessonae* genome and the R–E system *ridibundus* genome is necessary to induce a *ridibundus* genome elimination (Ragghianti et al. 2007).

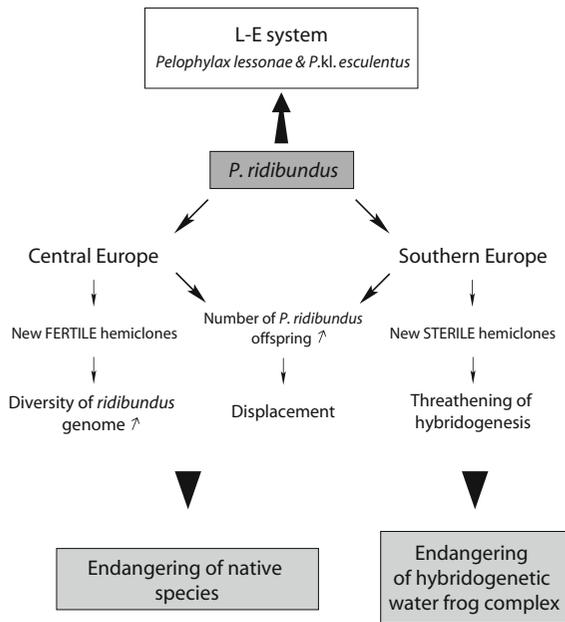


Fig. 3 Scheme indicating the consequences of translocations of *Pelophylax ridibundus* in the Western European water frog complex (L–E system). After introduction, the majority of the crossings in the L–E system will produce *P. ridibundus* offspring. As a result, the number of invading species will increase rapidly, which may lead to a displacement of the native species. The scheme also depicts the different outcomes the system will face depending on the origin of the invading *P. ridibundus* species. In the case of Southern European *P. ridibundus*, the hybridogenetic reproduction may become endangered, because Southern *P. ridibundus* have lost the ability to induce hybridogenesis in hybridisation with *P. lessonae*. In contrast, Central European *P. ridibundus* are capable of producing fertile primary hybrids, but they will be differing from the originals, and may change the structure of the complex

Introducing Southern European *P. ridibundus* in L–E systems may result in a destabilization of the hybridogenetic system. Primary hybridisation between Southern European *P. ridibundus* and *P. lessonae* of the L–E system will produce sterile hybrids without the possibility of hybridogenetic reproduction, as Southern *P. ridibundus* lack the capacity for inducing genome elimination (Hotz et al. 1985; Hotz and Uzzell 1983; Plötner 2005). These Southern introductions may thus have major implications for the preservation of the *P. kl. esculentus* complex. It will ultimately lead to a decline in reproductive hybrids and *P. lessonae*, because of their wasted reproductive efforts. Although the progeny of Southern European *P. ridibundus* and *P. lessonae* is

sterile, they still pose an ecological threat on the fertile hybrids as sexual competitors for mates. Introductions of Southern European *P. ridibundus* in L–E system may therefore potentially lead to the disappearance of hybridogenetic reproduction and eventually to the extinction of *P. kl. esculentus* (Fig. 3).

The possible threat of losing native species upon invasion of *P. ridibundus* is illustrated by several studies in several regions. In 1985, native *P. lessonae*—*P. kl. esculentus* populations in the Valais canton and Rhodan Valley (Switzerland) were observed to be replaced almost completely by introduced *P. ridibundus* individuals (Rey et al. 1985). Later on, other native populations in Western Switzerland were replaced by the rapidly expanding *P. ridibundus* individuals (Vorburger and Reyer 2003). These authors investigated the possible genetic replacement of native populations by *P. ridibundus* in Switzerland. In order for this genetic mechanism to play a role, several predictions have to be met. First, hybrids of primary hybridisations between *P. ridibundus* and *P. lessonae* have to be fertile. Secondly, populations introduced by *P. ridibundus* should contain more and different hemiclones than uninvaded populations. Finally, at least few matings between *P. kl. esculentus* inter se should produce viable *P. ridibundus* offspring (Vorburger and Reyer 2003). Although all these conditions were experimentally verified and were fulfilled, the reduced fertility of male F_1 hybrids raised some doubt on the proposed mechanism, leading researchers to believe that the ecological influence of *P. ridibundus* on the native species is likely to be important (Vorburger and Reyer 2003). In these studies, however, no distinction was made between Southern and Central European *P. ridibundus*. The presence of Southern European *P. ridibundus* producing sterile primary hybrids may account for the obtained decrease in fertility of F_1 hybrids. Pagano et al. (2003) also discovered another possible consequence of translocations of alien species, i.e. genetic pollution. Exotic (Egyptian) alleles were found in individuals of native water frog populations, suggesting introgression of exotic genes. This provides evidence of matings between native and alien species (Pagano et al. 2003). Besides matings between alien species and native species, hybridisations among different alien species are also a possible outcome. Based on nuclear microsatellite data, Holsbeek et al. (2008) showed that hybridisations between two introduced alien species

P. ridibundus and *P. cf. bedriagae* occurred frequently. These authors are convinced that the obtained hybrids are fertile, but are uncertain whether they are able to reproduce hybridogenetically (Holsbeek et al. 2008).

In addition to a genetic impact, translocations may also have an important ecological impact. Niche partitioning has been observed among the three species of the *P. esculentus* complex (Holenweg Peter et al. 2002; Pagano et al. 2001b; Wijnands 1976). *P. lessonae* individuals prefer small breeding habitats with dense submerged vegetation and tolerate ponds with hypoxic water. *P. ridibundus* individuals, on the other hand, inhabit larger less vegetated water bodies that are under strong river influence with low salinity, near-to-neutral pH and well oxygenated water (Holenweg Peter et al. 2002; Pagano et al. 2001a; Plénet et al. 2000b; Schmeller et al. 2007). The habitat difference between both parental species may be accounted for by the lower tolerance of *P. ridibundus* to hypoxic conditions, which may be related to the oxygen affinity of the blood and different modes of hibernation (Pagano et al. 1997, and references herein). Probably as a result of its heterozygotic nature, the hybrid species *P. kl. esculentus* occupies a broader range of habitats (Holenweg Peter et al. 2002; Pagano et al. 2001a) and tends to occupy habitats that are intermediate in nature of those occupied by the parent species (Plénet et al. 2000a). Irrespective of the differences in habitat use between the three species and the high site fidelity in adult water frogs (Holenweg Peter et al. 2001), the water frog species are not true habitat specialists and may show some plasticity in their habitat preferences. *P. ridibundus* individuals may be found in all kinds of habitat types (Pagano et al. 2001a, c). Furthermore, Holenweg Peter (2001) showed that the dispersal rates decreased and dispersal distances increased from *P. lessonae* through *P. kl. esculentus* to *P. ridibundus*. The long dispersal distances and the preference for habitats associated with rivers favors the migration of introduced *P. ridibundus* individuals into new habitats, allowing a rapid invasion. Schmeller et al. (2007) assessed the danger of replacement of the indigenous species *P. perezi* and *P. grafi* by introduced *P. ridibundus* individuals in France. Once established, the replacement by *P. ridibundus* may be rapid due to its greater longevity and faster growth rate compared to *P. perezi* and *P. grafi*, suggesting higher competitive strength.

The larger and older *P. ridibundus* females also tend to produce more eggs, since fecundity is positively related to age and snout-to-vent length (Schmeller et al. 2007). Bearing all these characteristics in mind, Schmeller et al. (2007) concluded that the indigenous water frog taxa are under risk of extinction, especially in the preferred habitats of *P. ridibundus*. Additionally, *P. ridibundus* individuals, which are morphologically larger than the hybrids or *P. lessonae*, may compete with the other water frog taxa for food and mating opportunities (Jooris 2002). Male water frogs try to mate with large females because of their higher fecundity (Berger 1970), and will thus prefer the larger *P. ridibundus* females. This results in a further increase in number of *P. ridibundus* individuals. Moreover, introduced *P. ridibundus* have even been observed to prey on hybrids and *P. lessonae* individuals (Grossenbacher 1988; Hofer-Polit 1998; Pagano et al. 1997). Finally, there is also the threat of introduction of new diseases to which endemic species are not immune (Plötner 2005). All these ecological consequences as well as the genetic impacts seem to all be primarily favoring *P. ridibundus* individuals, and are therefore expected to strongly influence the water frog complex.

Conclusion

The *P. kl. esculentus* system is complex and exceptional in the presence of both male and female hybrids as well as in its peculiar geographic variation. However, many aspects of this intriguing species complex remain unclear. Detailed studies on the phylogeography and the molecular mechanisms underlying the hybridogenetic process are necessary to provide a more accurate account on the unique aspects of the water frog complex and to assess the dangers of translocations within this complex.

Several studies on translocations in the water frog complex point to a strong genetic impact as well as important ecological influences (Arano et al. 1995; Pagano et al. 2003; Schmeller et al. 2007; Vorburger and Reyer 2003). Only an integrated approach combining population genetic data with ecological insights will enable us to predict the outcome of these novel interactions. So far, no studies investigated the impact of geographic origin of nonindigenous water frogs on their impact on native water frog

populations. It is likely, however, that it will be crucial to know the origin of invasive populations, because of the potential sterility primary hybrids may face if confronted with Southern European invaders. The different consequences depending on the geographic origin of invasive *P. ridibundus* have to be taken into account when drawing up an appropriate conservation policy. From the above, however, it is also clear that the impact is likely to be very severe in many or most cases, and strict measures need to be taken to restrict translocation of water frogs. Autochthonous water frog species should receive proper legal protection, and more restrictive laws and effective controls on the import of foreign species should be implemented (Arano et al. 1995). In case such control fails, the unique hybridogenetic water frog species are likely to be severely impacted and even risk extinction.

Acknowledgments The authors thank L. De Meester whose input improved this manuscript to a great extent, and G. Maes, F. Bossuyt and F. Volckaert for providing valuable comments on earlier drafts. We thank J. Mergeay for skilful graphical assistance. GH enjoys a Ph.D. grant of the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen).

References

- Allendorf FW, Leary RF, Spruell P, Wenburg JK (2001) The problems with hybrids: setting conservation guidelines. *Trends Ecol Evol* 16:613. doi:[10.1016/S0169-5347\(01\)02290-X](https://doi.org/10.1016/S0169-5347(01)02290-X)
- Arano B, Llorente G, Garcia-Paris M, Herrero P (1995) Species translocation menaces iberian waterfrogs. *Conserv Biol* 9:196–198. doi:[10.1046/j.1523-1739.1995.09010196.x](https://doi.org/10.1046/j.1523-1739.1995.09010196.x)
- Arnold ML (2004) Natural hybridization and the evolution of domesticated, pest and disease organisms. *Mol Ecol* 13:997–1007. doi:[10.1111/j.1365-294X.2004.02145.x](https://doi.org/10.1111/j.1365-294X.2004.02145.x)
- Berger L (1967) Embryonal and larval development of F1 generation of green frogs of different combinations. *Acta Zool Cracov* 12:123–160
- Berger L (1970) Some characteristics of the crosses within *Rana esculenta* complex in postlarval development. *Ann Zool* 27:373–416
- Berger L (1973) Some characteristics of backcrosses within forms of *Rana esculenta* complex. *Genet Pol* 14:413–430
- Berger L (1988) On the origin of genetic systems in European water frog hybrids. *Zool Pol* 35:5–32
- Binkert J, Borner P, Chen PS (1982) *Rana esculenta* complex: an experimental analysis of lethality and hybridogenesis. *Cell Mol Life Sci* 38:1283
- Christiansen DG, Fog K, Pedersen BV, Boomsma JJ (2005) Reproduction and hybrid load in all-hybrid populations of *Rana esculenta* water frog in Denmark. *Evol Int J Org Evol* 59:1348–1361
- Dowling TE, Secor CL (1997) The role of hybridization and introgression in the diversification of animals. *Annu Rev Ecol Syst* 28:593–619. doi:[10.1146/annurev.ecolsys.28.1.593](https://doi.org/10.1146/annurev.ecolsys.28.1.593)
- Dubois A (1992) Notes sur la classification des Ranidae (Amphibiens, Anoures). *Bull Mens Soc Linn Lyon* 61:305–352
- Dubois A, Günther R (1982) Klepton and synklepton: two new evolutionary systematics categories in zoology. *Zool Jahrb Syst* 109:290–305
- Ellstrand N, Schierenbeck K (2006) Hybridization as a stimulus for the evolution of invasiveness in plants? *Euphytica* 148:35–46. doi:[10.1007/s10681-006-5939-3](https://doi.org/10.1007/s10681-006-5939-3)
- Fahrig L (2003) Effects of habitat fragmentation on biodiversity. *Annu Rev Ecol Evol Syst* 34:487–515. doi:[10.1146/annurev.ecolsys.34.011802.132419](https://doi.org/10.1146/annurev.ecolsys.34.011802.132419)
- Forester DJ, Machlist GE (1996) Modeling human factors that affect the loss of biodiversity. *Conserv Biol* 10:1253–1263. doi:[10.1046/j.1523-1739.1996.10041253.x](https://doi.org/10.1046/j.1523-1739.1996.10041253.x)
- Frankham R, Ballou JD, Briscoe DA (2002) Introduction to conservation genetics. Cambridge University Press, Cambridge, p 640
- Frost DR, Grant T, Faivovich J, Bain RH, Raxworthy CJ, Wheeler W (2006) The amphibian tree of life. *Bull Am Mus Nat Hist* 297:1–370. doi:[10.1206/0003-0090\(2006\)297\[0001:TATOL\]2.0.CO;2](https://doi.org/10.1206/0003-0090(2006)297[0001:TATOL]2.0.CO;2)
- Graf JD, Polls Pelaz M (1989) Evolutionary genetics of *Rana esculenta* complex. In: Dawley RM, Bogart JP (eds) Evolution and ecology of unisexual vertebrates. The New York State Museum Bulletin, Albany, pp 298–302
- Graf JD, Karch J, Moreillon MC (1977) Biochemical variation in the *Rana esculenta* complex: a new hybrid form related to *Rana perezi* and *Rana ridibunda*. *Experientia* 33:1582–1584. doi:[10.1007/BF01934010](https://doi.org/10.1007/BF01934010)
- Grant PR, Grant BR (1992) Demography and the genetically effective sizes of two populations of Darwin's finches. *Evol Int J Org Evol* 73:766–784
- Grant PR, Grant BR (2002) Unpredictable evolution in a 30-year study of Darwin's finches. *Science* 296:707–711. doi:[10.1126/science.1070315](https://doi.org/10.1126/science.1070315)
- Grossenbacher K (1988) Verbreitung der wasserfrösche in der schweiz. In: Günther R, Klewen R (eds) Beiträge zur biologie und bibliographie (1960–1987) der europäischen wasserfrösche. Jahrbuch für Feldherpetologie, Duisburg
- Guerrini F, Bucci S, Ragghianti M, Mancino G, Hotz H, Uzzell T et al (1997) Genomes of two water frog species resist germ line exclusion in interspecies hybrids. *J Exp Zool* 279:163–176. doi:[10.1002/\(SICI\)1097-010X\(19971001\)279:2<163::AID-JEZ7>3.0.CO;2-M](https://doi.org/10.1002/(SICI)1097-010X(19971001)279:2<163::AID-JEZ7>3.0.CO;2-M)
- Guex G-D, Hotz H, Semlitsch RD (2002) Deleterious alleles and differential viability in progeny of natural hemiclinal frogs. *Evol Int J Org Evol* 56:1036–1044
- Günther R, Uzzell T, Berger L (1979) Inheritance patterns in triploid *Rana "esculenta"* (Amphibia, Salientia). *Mitt Zoolog Mus Berl* 55:35–57
- Hofer-Polit D (1998) Aussterben von *Rana lessonae* und *Rana esculenta* durch die Ausbreitung von *Rana ridibunda*. *Elaphe* 6:79–80
- Holenweg Peter A-K (2001) Dispersal rates and distances in adult water frogs, *Rana lessonae*, *R. ridibunda* and their

- hybridogenetic associate *R. esculenta*. *Herpetologica* 57:449–460
- Holenweg Peter A-K, Reyer H-U, Tietje GA (2001) Homing behavior of *Rana lessonae*, *R. ridibunda* and their hybridogenetic associate *R. esculenta* after experimental displacement. *Amphib-reptil* 22:475–480. doi:[10.1163/15685380152770435](https://doi.org/10.1163/15685380152770435)
- Holenweg Peter A-K, Reyer H-U, Abt G (2002) Species and sex ratio differences in mixed populations of hybridogenetic water frogs: the influence of pond features. *Eosience* 9:1–11
- Holsbeek G, Mergeay J, Hotz H, Plötner J, Volckaert FAM, De Meester L (2008) A cryptic invasion within an invasion and widespread introgression in the European water frog complex: the toll of uncontrolled commercial trade and weak international legislation. *Mol Ecol* 17:5023–5035
- Hotz H, Uzzell T (1983) Interspecific hybrids of *Rana ridibunda* without germ line exclusion of a parental genome. *Experientia* 39:538–540. doi:[10.1007/BF01965196](https://doi.org/10.1007/BF01965196)
- Hotz H, Mancino G, Bucci-Innocenti S, Raghianti M, Berger L, Uzzell T (1985) *Rana ridibunda* varies geographically in inducing clonal gametogenesis in interspecies hybrids. *J Exp Zool* 236:199–210. doi:[10.1002/jez.1402360210](https://doi.org/10.1002/jez.1402360210)
- Hotz H, Beerli P, Spolsky C (1992) Mitochondrial DNA reveals formation of nonhybrid frogs by natural matings between hemiclinal hybrids. *Mol Biol Evol* 9:610–620
- Huxel GR (1999) Rapid displacement of native species by invasive species: effects of hybridization. *Biol Conserv* 89:143–152. doi:[10.1016/S0006-3207\(98\)00153-0](https://doi.org/10.1016/S0006-3207(98)00153-0)
- Jooris R (2002) Pelophylax: de groene wachters aan de waterkant. Natuurpunt, Mechelen
- Lamont BB, He T, Enright NJ, Krauss SL, Miller BP (2003) Anthropogenic disturbance promotes hybridization between *Banksia* species by altering their biology. *J Evol Biol* 16:551–557. doi:[10.1046/j.1420-9101.2003.00548.x](https://doi.org/10.1046/j.1420-9101.2003.00548.x)
- Levin DA, Francisco-Ortega J, Jansen RK (1996) Hybridization and the extinction of rare plant species. *Conserv Biol* 10:10–16. doi:[10.1046/j.1523-1739.1996.10010010.x](https://doi.org/10.1046/j.1523-1739.1996.10010010.x)
- Lymberakis P, Poulakakis N, Manthou G, Tsigenopoulos CS, Magoulas A, Mylonas M (2007) Mitochondrial phylogeography of *Rana (Pelophylax)* populations in the Eastern Mediterranean region. *Mol Phylogenet Evol* 44:115–125. doi:[10.1016/j.ympev.2007.03.009](https://doi.org/10.1016/j.ympev.2007.03.009)
- Mallet J (2005) Hybridization as an invasion of the genome. *Trends Ecol Evol* 20:229–237. doi:[10.1016/j.tree.2005.02.010](https://doi.org/10.1016/j.tree.2005.02.010)
- Mikulicek P, Kotlik P (2001) Two water frog populations from western Slovakia consisting of diploid females and diploid and triploid males of the hybridogenetic hybrid *Rana esculenta* (Anura, Ranidae). *Mitt Mus Naturkunde Berl. Zoolog Reihe* 77:59–64
- Mooney HA, Cleland EE (2001) The evolutionary impact of invasive species. *Proc Natl Acad Sci USA* 98:5446–5451. doi:[10.1073/pnas.091093398](https://doi.org/10.1073/pnas.091093398)
- Muller HJ (1964) The relation of recombination to mutational advance. *Mutat Res* 1:2–9. doi:[10.1016/0027-5107\(64\)90047-8](https://doi.org/10.1016/0027-5107(64)90047-8)
- Ogielska M (1994a) Nucleus-like bodies in gonial cells of *Rana esculenta* (Amphibia, Anura) tadpoles—a putative way of chromosome elimination. *Zool Pol* 39:461–474
- Ogielska M (1994b) *Rana esculenta* developmental syndrome: fates of abnormal embryos from the first cleavage until spontaneous death. *Zool Pol* 39:447–459
- Pagano A, Joly P, Hotz H (1997) Taxon composition and genetic variation of water frogs in the Mid-Rhone floodplain. *Life Sci* 320:759–766
- Pagano A, Crochet PA, Graf JD, Joly P, Lodé T (2001a) Distribution and habitat use of water frog hybrid complexes in France. *Glob Ecol Biogeogr* 10:433–441. doi:[10.1046/j.1466-822X.2001.00246.x](https://doi.org/10.1046/j.1466-822X.2001.00246.x)
- Pagano A, Joly P, Plénet S, Lehman A, Grolet O (2001b) Breeding habitat partitioning in the *Rana esculenta* complex: the intermediate niche hypothesis supported. *Ecoscience* 8:294–300
- Pagano A, Lodé T, Crochet PA (2001c) New contact zone and assemblages among water frogs of Southern France. *J Zool Syst Evol Res* 39:63–67. doi:[10.1046/j.1439-0469.2001.00156.x](https://doi.org/10.1046/j.1439-0469.2001.00156.x)
- Pagano A, Dubois A, Lesbarreres D, Lodé T (2003) Frog alien species: a way for genetic invasion? *C R Biol* 326:S85–S92. doi:[10.1016/S1631-0691\(03\)00043-X](https://doi.org/10.1016/S1631-0691(03)00043-X)
- Pimm SL, Raven P (2000) Biodiversity: extinction by numbers. *Nature* 403:843–845. doi:[10.1038/35002708](https://doi.org/10.1038/35002708)
- Plénet S, Hervant F, Joly P (2000a) Ecology of the hybridogenetic *Rana esculenta* complex: differential oxygen requirements of tadpoles. *Evol Ecol* 14:13–23. doi:[10.1023/A:1011056703016](https://doi.org/10.1023/A:1011056703016)
- Plénet S, Pagano A, Joly P, Fouillet P (2000b) Variation of plastic responses to oxygen availability within the hybridogenetic *Rana esculenta* complex. *J Evol Biol* 13:20–28. doi:[10.1046/j.1420-9101.2000.00141.x](https://doi.org/10.1046/j.1420-9101.2000.00141.x)
- Plötner J (2005) Die westpaläarktischen wasserfrösche von märtyrern der wissenschaft zur biologischen sensation. Laurenti-Verlag, Bielefeld, p 160
- Plötner J, Uzzell T, Beerli P, Spolsky C, Ohst T, Litvinchuk SN et al (2008) Widespread unidirectional transfer of mitochondrial DNA: a case in western Palaearctic water frogs. *J Evol Biol* 21:668–681. doi:[10.1111/j.1420-9101.2008.01527.x](https://doi.org/10.1111/j.1420-9101.2008.01527.x)
- Raghianti M, Bucci S, Marracci S, Casola C, Mancino G, Hotz H et al (2007) Gametogenesis of intergroup hybrids of hemiclinal frogs. *Genet Res* 89:39–45. doi:[10.1017/S0016672307008610](https://doi.org/10.1017/S0016672307008610)
- Rey A, Michellod B, Grossenbacher K (1985) Inventaire des batraciens du Valais: situation en 1985. *Bull Maurithienne* 103:3–38
- Rhymer JM, Simberloff D (1996) Extinction by hybridization and introgression. *Annu Rev Ecol Syst* 27:83–109. doi:[10.1146/annurev.ecolsys.27.1.83](https://doi.org/10.1146/annurev.ecolsys.27.1.83)
- Rieseberg LH (1997) Hybrid origins of plant species. *Annu Rev Ecol Syst* 28:359–389. doi:[10.1146/annurev.ecolsys.28.1.359](https://doi.org/10.1146/annurev.ecolsys.28.1.359)
- Rist L, Semlitsch RD, Hotz H, Reyer H-U (1997) Feeding behaviour, food consumption, and growth efficiency of hemiclinal and parental tadpoles of the *Rana esculenta* complex. *Funct Ecol* 11:735–742. doi:[10.1046/j.1365-2435.1997.00147.x](https://doi.org/10.1046/j.1365-2435.1997.00147.x)
- Schmeller D, Seitz A, Crivelli A, Pagano A, Veith M (2001) Inheritance in the water frog *Rana ridibunda* Pallas, 1771—is it Mendelian or hemiclinal? *Mitt Mus Naturkunde Berl. Zoolog Reihe* 77:39–42

- Schmeller D, Seitz A, Crivelli A, Veith M (2005) Crossing species' range borders: interspecies gene exchange mediated by hybridogenesis. *Proc R Soc Lond B Biol Sci* 272:1625–1631. doi:[10.1098/rspb.2005.3129](https://doi.org/10.1098/rspb.2005.3129)
- Schmeller DS, Pagano A, Plenet S, Veith M (2007) Introducing water frogs—Is there a risk for indigenous species in France? *C R Biol* 330:684–690. doi:[10.1016/j.crvi.2007.04.005](https://doi.org/10.1016/j.crvi.2007.04.005)
- Schultz R (1969) Hybridization, unisexuality, and polyploidy in the teleost *Poeciliopsis* (Poeciliidae) and other vertebrates. *Am Nat* 103:605–609
- Seehausen O (2004) Hybridization and adaptive radiation. *Trends Ecol Evol* 19:198–207. doi:[10.1016/j.tree.2004.01.003](https://doi.org/10.1016/j.tree.2004.01.003)
- Som C, Reyer HU (2006) Hemiclonal reproduction slows down the speed of Muller's ratchet in hybridogenetic frog *Rana esculenta*. *J Evol Biol* 20:650–660. doi:[10.1111/j.1420-9101.2006.01243.x](https://doi.org/10.1111/j.1420-9101.2006.01243.x)
- Spolsky C, Uzzell T (1984) Natural interspecies transfer of mitochondrial DNA in amphibians. *Proc Natl Acad Sci USA* 81:5802–5805. doi:[10.1073/pnas.81.18.5802](https://doi.org/10.1073/pnas.81.18.5802)
- Spolsky C, Uzzell T (1986) Evolutionary history of the hybridogenetic hybrid frog *Rana esculenta* as deduced from mtDNA analyses. *Mol Biol Evol* 3:44–56
- Tsutsui ND, Suarez AV, Holway DA, Case TJ (2000) Reduced genetic variation and the success of an invasive species. *Proc Natl Acad Sci USA* 97:5948–5953. doi:[10.1073/pnas.100110397](https://doi.org/10.1073/pnas.100110397)
- Tunmer HG, Heppich S (1981) Premeiotic genome exclusion during oogenesis in the common edible frog, *Rana esculenta*. *Naturwissenschaften* 68:207–208. doi:[10.1007/BF01047207](https://doi.org/10.1007/BF01047207)
- Tunmer HG, Heppich-Tunmer S (1991) Genome exclusion and two strategies of chromosome duplication in oogenesis of a hybrid frog. *Naturwissenschaften* 78:32–34. doi:[10.1007/BF01134041](https://doi.org/10.1007/BF01134041)
- Uzzell T, Hotz H (1979) Electrophoretic and morphological evidence for two forms of green frogs (*Rana esculenta* complex) in peninsular Italy (Amphibia, Salientia). *Mitt Zoolog Mus Berl* 55:13–27
- Uzzell T, Günther R, Berger L (1977) *Rana ridibunda* and *Rana esculenta*: a leaky hybridogenetic system (Amphibia Salientia). *Proc Acad Nat Sci Phila* 128:147–171
- Uzzell T, Hotz H, Berger L (1980) Genome exclusion in gametogenesis by an interspecific *Rana* hybrid: evidence from electrophoresis of individual oocytes. *J Exp Zool* 214:251–259. doi:[10.1002/jez.1402140303](https://doi.org/10.1002/jez.1402140303)
- Vinogradov AE, Chubinshvili AT (1999) genome reduction in a hemiclonal frog *Rana esculenta* from radioactively contaminated areas. *Genetics* 151:1123–1125
- Vinogradov AE, Borkin LJ, Günther R, Rosanov JM (1991) Two germ cell lineages with genomes of different species in one and the same animal. *Hereditas* 114:245–251. doi:[10.1111/j.1601-5223.1991.tb00331.x](https://doi.org/10.1111/j.1601-5223.1991.tb00331.x)
- Vorburger C (2001a) Fixation of deleterious mutations in clonal lineages: evidence from hybridogenetic frogs. *Evol Int J Org Evol* 55:2319–2332
- Vorburger C (2001b) Heterozygous fitness effects of clonally transmitted genomes in waterfrogs. *J Evol Biol* 14:602–610. doi:[10.1046/j.1420-9101.2001.00307.x](https://doi.org/10.1046/j.1420-9101.2001.00307.x)
- Vorburger C (2001c) Non-hybrid offspring from matings between hemiclonal hybrid waterfrogs suggest occasional recombination between clonal genomes. *Ecol Lett* 4:628–636. doi:[10.1046/j.1461-0248.2001.00272.x](https://doi.org/10.1046/j.1461-0248.2001.00272.x)
- Vorburger C, Reyer H-U (2003) A genetic mechanism of species replacement in European waterfrogs? *Conserv Genet* 4:141–155. doi:[10.1023/A:1023346824722](https://doi.org/10.1023/A:1023346824722)
- Wijnands HEJ (1976) Distribution and habitat of *Rana esculenta* complex in the Netherlands. *Neth J Zool* 27:277–286. doi:[10.1163/002829677X00135](https://doi.org/10.1163/002829677X00135)
- Zeisset I, Beebee TJC (2003) Population genetics of a successful invader: the marsh frog *Rana ridibunda* in Britain. *Mol Ecol* 12:639–646. doi:[10.1046/j.1365-294X.2003.01775.x](https://doi.org/10.1046/j.1365-294X.2003.01775.x)