# IS RECOMBINATION LESS NEGLIGIBLE THAN PREVIOUSLY DESCRIBED IN HYBRIDOGENETIC WATER FROGS ?

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Abstract: Hybridogenesis have been described as a hemiclonal reproductive mode excluding recombination events. However, recombination have been evidenced by several authors. Our data suggests that the recombination rate may be upper than previously thought and propose some hypothesis to explain this paradox.

KEY WORDS: hybridogenesis, clonal, recombination, introgression.

## INTRODUCTION

Water frogs have been described as a hybridization complex maintained by hybridogenesis (Berger, 1968; Tunner, 1974). Such a reproductive mode is characterized by a peculiar hemiclonal reproduction (Schultz, 1969; Tunner, 1974). Several experimental studies corroborated this model revealing that the hybridogenetic hybrid excludes one parental genome from its germinal cells (mostly *lessonae* genome in the L-E system; Uzzell and Berger, 1975; *perezi* genome in the P-G system; Graf et al., 1977; Graf and Polls-Pelaz, 1989) while the remaining genome (mostly from R. *ridibunda*) is endoreduplicated prior to meiosis. Thus, in hybridogenetic water frog systems, the hybridogen is considered to produce clonal *ridibunda* gametes (e.g. Tunner and Heppich-Tunner, 1991; Berger, 1988).

Although hybridogenesis theoretically implies an absence of recombination between the two parental genomes of the hybrid, several studies evidenced recombination (e.g. Uzzell *et al.*, 1975; Günther and Hähnel, 1976; Tunner, 1979; Pagano *et al.* 1997, Plötner and Klinkhardt, 1992; Schröer, 1997). As several authors assumed an absence of recombination or minimized the importance of such a process (e.g. Uzzell *et al.*, 1980; Berger *et al.*, 1988; Graf and Polls-Pelaz, 1989), a specification is necessary: is hemiclonality the main characteristics of the water frog complexes or are these recombination events less rare and negligible than previously described?

An answer to such alternative hypotheses implies, both numerous descriptive genetic studies on the complexes throughout Europe, and analyses of the cellular mechanisms of genome exclusion. In this respect the presented study can only provide a preliminary contribution in reporting new cases of recombination within several clutches from a pond located in France.

## Material and Methods

Three groups of eggs were collected in the field in May 1998 in one pond (Fondation Verots, 25 km North East Lyon). Groups 1B and 2B belonged to "big clutches" while 1S belonged to a "small clutch". Embryos are suitable for protein electrophoresis investigations (Pagano and Plénet, in prep.). Thus, thirteen embryos were haphazardly sampled in each "clutch" for taxonomic identification. Such an identification is performed by specific allozymic markers (e.g.: Hotz, 1983; Beerli, 1994; Pagano *et al.*, 1997) such as those used in this study: lactate dehydrogenase (ldh-b; E.C. 1.1.1.27), mannose-6-phosphate isomerase (mpi; E.C. 5.3.1.8), and phosphoglucomutase (pgm-2; E.C. 5.4.2.2, formerly 2.7.5.1). Because introgression may affect a diagnostic marker, we used several markers in order to avoid identification errors.

## Results and Discussion

As revealed by the specific markers each "clutch" was composed of either one or two taxa. Because embryos from 1B and 2B corresponded to two distinct taxa and 1S to one taxon, 1B and 2B were assumed to be in fact the mixture of several clutches (at least two) originating from distinct parental pairs. Because offspring belonged to *R.* kl. *esculenta* and R.*lessonae*, we believe that they originated from a L-E population system (Uzzell and Berger, 1975).

As observed in genotypic frequencies (table 1) introgression affected both pgm-2 and ldh-b loci and both taxa. Introgressions were revealed either from R. *ridibunda* to *R.* kl. *esculenta* or vice-versa. The mean amount of individuals affected by introgression was 23%; 12.5% of R. *lessonae* and 30% of *R.* kl. *esculenta* did show recombined genotypes. Such an amount can be considered to be much higher than those previously detected (1.9% to 4.5% in R. *lessonae* and 3.7% to 6.5% in *R.* kl. *esculenta*; Günther and Hähnel, 1976; Günther, 1983; Heppich and Tunner, 1979; Tunner, 1979; 1980; Uzzell *et al.*, 1975; 1977; 1980; Plötner, 1990). This holds also true for introgression of *R.* kl. *esculenta*, found to be 5.8% in our study and at most 3.3% in the above mentioned studies. Introgression of R. *lessonae* shows about the same degree as previously reported (2.1% our data, 0.9% - 2.2% in literature).

As most genetic investigations on water frogs focused on a small number of loci, it is possible that the low recombination rates recorded in the literature represent a bias. Our data on several loci (this study, but also Pagano, unpubl. data; Schmeller *et al.*, in prep.) evidenced higher recombination rates than previously described. However, a study on water frogs from Western Germany (Schröer, 1997), in which a large number of individuals (765) and loci (7) were used, revealed introgression greater than 12% and an average amount of recombined individuals greater than 80%.

Introgression and recombination events in hybridogenetic water frogs are still controversially discussed. On the one hand, hybridogenesis is a clonal reproduction characterized by genome exclusion before meiosis (Tunner and Heppich- Tunner, 1991) and is not coherent with recombination and introgression. On the other hand, recombination events have been evidenced independently in several studies by several authors (e.g. Uzzell *et al.*, 1975; Günther and Hähnel, 1976; Tunner, 1979; Plötner and Klinkhardt, 1992; Pagano *et al.* 1997, Schröer, 1997, Schmeller *et al.* in prep.).

Some hypotheses have been addressed to explain such a paradox. Heppich (1978) assumed that in several populations "imperfect" hybridogenesis would allow introgression while others would get a "perfect" hybridogenesis. Uzzell *et al.* (1980) argued

for occasional recombination events and spoke of a "leaky hybridogenesis". Graf and Polls-Pelaz (1989) suggested that recombination is limited to the scarce R-E systems.

However, several facts may be noted in favour of recombination events. Different authors detected besides a high proportion of R. *ridibunda* gametes also R. *lessonae* and diploid gametes containing both parental genomes in the hybrid R. *esculenta* (e.g. Kawamura and Nishioka, 1986, Vinogradov *et al*, 1991). Also, diverse meiotic abnormalities in the hybridogens were described and some led to the production of recombined gametes (Tunner and Heppich-Tunner, 1991; Berger and Ogielska, 1994). As such irregularities in gametogenesis of the hybrids occur recombination events seem to be probable and introgression might vary in dependence to unknown factors enforcing such irregularities. Thus, Schröer (1997) suggested that recombination events could be influenced by abiotic and biotic factors (such as presence or absence of diverse taxa). In addition, Ogielska (comm. pers.) hypothesizes that the exclusion of the R. *lessonae* genome is gradual rather than achieved in one step. If so, it provides another possibility for recombination events. The validation of such a hypothesis would contribute to explain both meiosis abnormalities and introgression events. As mechanisms of hybridogenesis remained unclear, these questions need urgent investigation.

Because recombination have often been evidenced from allozyme data by inference (i.e. by comparison of an expected genotype at a specific marker locus with the observed genotype) it cannot be considered as a direct proof of recombination. A proof will be established by comparison of parental genotypes with offspring genotypes, which seems to be only possible in controlled crossing experiments. Such experiences bear disadvantages, as they are (i) not frequent enough to detect rather seldom

| clutch | Taxon | N | *ldh-b* | | | | *mpi* | | | *pgm-2* | | | N recomb. (%) | I (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | a/e | c/e | b/e | e/e | a/h | c/h | h/h | d/d | c/d | c/c | | |
| 1B | *R.* kl. *esculenta* | 7 | 7 | - | | | 7 | | | | 6 | 1# | 1<br>14.3% | 2.1% |
| | *R. lessonae* | 6 | | | *4* | *2* | | | *6* | | 1* | *5* | 1<br>16.6% | 2.8% |
| 2B | *R.* kl. *esculenta* | 3 | 3 | - | | | 3 | | | | 3 | | 0 | 0.0% |
| | *R. lessonae* | 10 | 1* | | *1* | *8* | | | *10* | | | *10* | 1<br>10.0 % | 1.7% |
| 1S | *R.* kl. *esculenta* | 13 | 2 | 11 | | | 3 | *10* | | 6* | 7 | | 6<br>46.2% | 7.7% |
| Total | *R.* kl. *esculenta* | 23 | | | | | | | | | | | 7<br>30.4% | 5.1% |
| | *R. lessonae* | 16 | | | | | | | | | | | 2<br>12.5% | 2.1% |

**Tab. 1:** Electrophoretically detected genotype frequencies at three variable enzyme loci. Genotypes in italic characters are markers of *R. lessonae,* those in bold characters markers of *R. ridibunda* and those underlined markers of *R.* kl. *esculenta*. The genotype marked by * highlights an introgression of a *R. ridibunda* locus in a *R.lessonae* genome and those by # an introgression of a *R. lessonae* locus in a *R. ridibunda* genome.
$N_{recomb.}$ = total number (above) and proportion (below in %) of recombined individuals, I = Introgression, as proportion of foreign alleles to all alleles.

recombination events and (ii) might be performed with individuals, reproducing in imperfect hybridogenesis.

Some authors suggest that electrophoretic studies could lead to pitfalls in interpretation such as in the case of null alleles (Hoare and Beaumont, 1995). Especially, interpretation of monomeric enzyme patterns lead to wrong results, as phenotypic homozygotes are genetically heterozygotes. A detection of null alleles is only possible via "blank" enzyme pattern in homozygotes. As extensive studies were carried out on the LE-system in the study area (Pagano, unpubl.) and other areas such as Germany (Schröer 1997) and Southern France (Schmeller *et al.*, in prep.) and blanks in either taxon of water frogs were not found, we consider that no null alleles occur whether in R. *lessonae* nor in R. *ridibunda*. Hence, we doubt that null alleles in the LE-complex remained undetected, while many studies were carried out on this hybridogenetic complex in many different areas (e.g. in Uzzell *et al.*; 1975; Günther and Hähnel, 1976; Tunner, 1979; Plötner and Klinkhardt, 1992; Pagano *et al.* 1997; Schröer, 1997).

However, in our study, only one individual (R. *esculenta* from clutch 1B) can be considered to potentially carry a null allele, as the shown genotype resembles R. *lessonae* alleles. The null allele than would be located on the R. *ridibunda* genome and might be accumulated due to the hemiclonal inheritance due to hybridogenesis. As null alleles occur only in very low frequency (Leary *et al.*, 1993) it seems to be unlikely, but cannot be fully refused.

Because parental genotypes are unknown, it is not possible to specify if recombination occurred in one generation or several. In case of the progeny from clutch 1S, it has to be pointed out that it most likely originated from one mating. The high amount of recombined individuals than would be due to recombination events in earlier generations and would be biased by sampling of siblings. Our goal is not to propose a quantitative estimation of recombination and introgression at the level of a generation. It is rather to underline that recombination and introgression occur and may have importance on an evolutionary point of view as introgressed alleles lead to the permanent incorporation of foreign alleles into a species gene pool and introgression might steadily increase over time.

Indeed, considering the literature on hybrid zones, introgressive hybridization had been assumed to generate additive genetic diversity, thus allowing speciation processes if populations within the hybrid zone are genetically isolated from other populations outside of the hybrid zone (e.g. Harrisson, 1990). As a consequence, the introgression and the gene flow among populations are key parameters to predict the evolution of the complex.

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## REFERENCES


- BEERLI, P. (1994): Genetic isolation and calibration of an average protein clock in Western Paleartic water frogs of Aegean region. Ph.D. Dissertation, Universität Zürich.
- BERGER, L. (1968): Morphology of the F1-generation of various crosses within *Rana esculenta*-complex. Acta. Zool. Cracov. 13: 301 - 321.
- BERGER, L. (1988): On the origin of genetic systems in European water frogs hybrids. Zool. Pol. 35: 5-32.

- Berger, L., Uzzell, T., Hotz, H. (1988): Sex determination and sex-ratio in Western Palearctic water frogs: XX and XY female hybrids in the Pannonian Basin ? Proc. Acad. Nat. sci. Philadelphia 140: 220-239.
- Berger, L., Ogielska, M. (1994): Spontaneous haploid-triploid mosaicism in the progeny of a *Rana* kl. *esculenta* female and *Rana* lessonae males. Amphibia-Reptilia 15: 143-152.
- Graf, J.D., Karch, F., Moreillon, M.C., (1977): Biochemical variation in the *Rana esculenta* complex: A new hybrid form related to *Rana perezi* and *Rana ridibunda*. Experientia 33: 1582-1584.
- Graf, J.-D., Polls Pelaz, M. (1989): Evolutionary genetics of the *Rana esculenta* complex. In: R.M. Dawley and Bogart, J.P. (eds): Evolution and Ecology of Unisexual Vertebrates, New York State Mus. Bull. 446, Albany, USA.
- Günther, R. (1983): Zur Populationsgenetik der mitteleuropäischen Wasserfrösche des *Rana esculenta*-Synkleptons (*Anura*, Ranidae). Zool. Anz. Jena 211: 43 - 54.
- Günther, R., Hähnel, S. (1976): Untersuchungen über den Genflufl zwischen *Rana ridibunda* und *Rana* lessonae sowie die Rekombinationsrate bei der Bastardform R. *esculenta* (*Anura*, Ranidae). Zool. Anz 197: 23 - 38.
- Harrisson, R.G. (1990): Hybrid zones: windows on evolutionary process. Oxford Surveys in Evolutionary Biology 7: 69-128.
- Heppich, S.(1978): Hybridogenesis in *Rana esculenta*: C-band karyotypes of *Rana ridibunda*, *Rana* lessonae and *Rana esculenta*. Z. zool. Syst. Evolut. -forsch 16: 27-39.
- Heppich, S., Tunner, H. G. (1979): Chromosomal constitution and C-banding in homotypic *Rana esculenta* crosses. Mitteilungen aus dem zoologischen Museum in Berlin 55: 111-114.
- Hoare, K., Beaumont, A.R. (1995): Effects of an ODH null allele and a GPI low-activity allozyme on shell length in laboratory-reared Mytilus edulis. Marine Biology (Berlin) 123 (4): 775 - 780
- Hotz, H. (1983): Genetic diversity among water frog genomes inherited with and without recombination. Ph.D Dissertation, Universität Zürich.
- Kawamura, T. and Nishioka, M. (1986): Hybridization experiments among *Rana* lessonae, *Rana ridibunda* and *Rana esculenta*, with special reference to hybridogenesis. Sci. Rep. Lab. Amphibian Biol., Hiroshima University 8: 117 -271.
- Leary, R.F., Allendorf, F.W., Knudsen, K.L. (1993): Null alleles at two lactate dehydrogenase loci in rainbow trout are associated with decreased developmental stability. Genetica 89 (1-3): 3 - 13.
- Pagano, A., Joly, P., Hotz, H. (1997): Taxon composition and genetic variation of water frogs in the Mid-Rhône floodplain. C. R. Acad. Sci. Paris, Sciences de la vie / Life science 320: 759-766.
- Plötner, J. (1990): Populationsgenetische Untersuchungen an europäischen Wasserfröschen (*Anura*, Ranidae), Ph. D. thesis, Humbodlt-Universität, Berlin.
- Plötner, J., Klinkhardt, M. (1992): Investigations on the genetic structure and the morphometry of a pure hybrid population of *Rana kl. esculenta* (*Anura*, Ranidae) in North Germany. Zool. Anz. 229: 163-184.
- Schröer, T. (1997): Untersuchungen zur Populationsgenetik und ÷kologie westfälischer Wasserfrösche (*Anura*: Ranidae). Ph.D. Dissertation, Universität Düsseldorf.
- Schultz, R.J. (1969): Hybridization, unisexuality and polyploidy in the teleost Poeciliopsis (Poecilidae) and other vertebrates. Amer. Nat. 103: 605 - 619.
- Tunner, H.G. (1974): Die klonale Struktur eine Wasserfroschpopulation. Z. zool. Syst. Evol. Forsch. 12: 309-314.
- Tunner, H. G (1979): The inheritance of morphology and electrophoretic markers from homotypic crosses of the hybridogenetic *Rana esculenta*. Mitteilungen aus dem zoologischen Museum in Berlin 55: 89-109.
- Tunner, H.G (1980): Kreuzungsexperimente mit Wasserfröschen aus österreichischen und polnischen Mischpopulationen (*Rana lessonae* + *Rana esculenta*). Eine Analyse biochemischer und morphologischer Merkmale. Z. Zool. Syst. Evolut. Forsch. 18: 257 - 297.
- Tunner, H.G., Heppich-Tunner, S. (1991): Genome exclusion and two strategies of chromosome duplication in oogenesis of a hybrid frog. Naturwissenschaften 78: 32 - 43.

- 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.
- UZZELL, T., BERGER, L., GÜNTHER, R. (1975): Diploid and triploid progeny from a diploid female of *Rana esculenta* (Amphibia Salientia. Proc. Acad. Nat. Sci. Philadelphia 127: 81-91.
- UZZELL, T., GÜNTHER, R., BERGER, L. (1977): *Rana ridibunda* and *Rana esculenta*: a leaky hybridogenetic system (Amphibia Salientia). Proc. Acad. Nat. Sci. Philadelphia 128: 147 - 171.
- UZZELL, T., HOTZ, H., BERGER, L. (1980): Genome exclusion in gametogenesis by an interpsecific *Rana* hybrid: evidence from electrophoresis of individual oocytes. J. Exp. Zool. 214: 251 - 259.
- VINOGRADOV, A.E., BORKIN, L.J., GÜNTHER, R. AND ROSANOV, J.M. (1991): Two germ cell lineages with genomes of different species in one and the same individual. Hereditas 114: 245-251.