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Premeiotic Chromosome Doubling After Genome Elimination During Spermatogenesis of the Species Hybrid *Rana esculenta*

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Summary. Gamete production in the hybridogenetic species hybrid *Rana esculenta* (*Rana ridibunda* × *Rana lessonae*) is preceded by a premeiotic elimination of the *R. lessonae* genome and subsequent duplication of the remaining *R. ridibunda* genome, so that only *ridibunda* chromosomes enter a quasi normal meiosis, and only *ridibunda* gametes are formed. This is demonstrated by differences in genome specific centromere fluorescence and electrophoretic patterns between somatic and gonadal tissue.

Key words: *Rana* hybrid – Hybridogenesis – Genome elimination – Fluorescence – Electrophoresis

Introduction

Two problems have been settled within the *Rana esculenta* complex: 1. The common European water frog *R. esculenta* is a hybrid between the species *R. ridibunda* and *R. lessonae* (Berger 1968). 2. The mode of its reproduction is hybridogenesis (Tunner 1974). Hybridogenesis appears to function in such a way that hybrid *R. esculenta* males and females propagating with *R. lessonae* produce only non recombined haploid *ridibunda* gametes (Tunner 1973, 1979; Uzzell and Berger 1975; Heppich and Tunner 1979). Earlier investigations of spermatogenesis in *R. esculenta* have not produced any evidence for a particular chromosomal mechanism underlying the hybridogenetic pattern of inheritance (Wickbom 1945; Galgano and Morescalchi 1966; Morescalchi and Galgano 1973; Günther 1975). In the case of oogenesis, however, a demanded premeiotic elimination of the complete set of *lessonae* chromosomes followed by a duplication of the remaining *ridibunda* chromonstrated (Tunner and Heppich 1981). In the presmonstrated (Tunner and Heppich in press). In this present paper, we report that by means of a fluorescence double staining technique it is possible to distinguish the *ridibunda* and *lessonae* chromosomes by centromere fluorescence. It can be demonstrated, therefore, that *R. esculenta* is a somatic hybrid with only the *ridibunda* chromosomes entering a quasi normal meiosis. Consequently, a premeiotic haploidization by genome elimination and a following premeiotic rediploidization must exist not only in female but also in male *R. esculenta.* This cytological evidence is supported by results from the electrophoresis of somatic and gonadal tissue.

Materials and Methods

The chromosomes were fixed and prepared as described by Heppich (1978). The double staining technique was carried out according to Schweizer (1976). Actinomycin D treatment was performed in a 0.25 mg/ml (McIlvaine buffer: citric acid 0.1 M, di-natriumhydrogenphosphat 0.2 M; pH 7) solution for 20 min. The concentration of the 33 258 Hoechst solution was 0.5 µg/ml distilled water.

Electrophoresis was carried out on acrylamide block gels by the disc method. Two pore sizes were used: a large pore spacer gel (2.5% acrylamide concentration, pH 7.8) and a small pore separation gel (7.5% acrylamide concentration, pH 8.4).

Results and Discussion

Of several tested single and double staining fluorescence techniques, the most satisfactory results were obtained with actinomycin D in combination with DAPI or 33 258 Hoechst (specific for AT-rich DNA) (Latt and Wohlleb 1975; Comings 1975; Schweizer 1976). Metaphase plates of squashed intestine tissue of colchicinized frogs treated with this technique reveal a marked difference between the genomes of the two parental species. Whereas all 26 chromosomes of *R. ridibunda* show intensive fluorescence in the centromere region (Fig. 1 a), the centromeres of *R. lessonae* chromosomes exhibit

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almost no differential fluorescence (Fig. 1 b). In both species the nucleolus-organizing-region shows diminished fluorescence indicating GC-richness (Schweizer 1976; Schmid 1978). The hybrid constitution of male and female *R. esculenta* is reflected by the presence of this genome specific difference in centromere fluorescence within homologous pairs (Fig. 1 c). This can best be demonstrated in the easily identifiable second chromosome pair and the NOR-chromosomes (Fig. 1 e). Regrettably, exact homologization of water frog chromosomes is somewhat disputed due to the absence of a typical Q-type banding pattern (Greilhuber 1977; Heppich 1978).

Provided with these genome specific chromosome markers we examined testicular tissue of adult *R. esculenta.* During late summer and autumn months, chromosomes are seen mostly in the stage of spermatogonial multiplication with 26 metaphase chromosomes or in diakinesis of the first meiotic division with 13 bivalents. In contrast to the soma, all these chromosomes show the intensive centromere fluorescence typical for *R. ridibunda* (Fig. 1 d, f). This observation implies that only *ridibunda* chromosomes are participating even as early as proliferation of spermatogonia A (Fawcett 1972; Phillips 1974). We can therefore assume that elimination of the *lessonae* genome from the germline and subsequent duplication of the remaining haploid *ridibunda* genome occurs during an early stage of the spermatogenetic cycle (probably during the division of those stem cell spermatogonia characterized by complete cytokinesis (Phillips 1974)). We observed haploid metaphases in phase contrast preparations of squashed testes of a young *R. esculenta* male. Although previously reported (Günther 1975), such stages have been misinterpreted.

The pattern of differential fluorescence proved to be identical in all populations checked. The analysed material comprises *R. ridibunda* from Austria, Yugoslavia and Greece, *R. lessonae* and *R. esculenta* from Austria and France, and offspring of the three genotypes from experimental crosses. We examined a total of 11 *R. ridibunda,* 8 *R. lessonae,* 16 *R. esculenta* females and 4. *R. esculenta* males. It must be mentioned that these males originate from a population (Neusiedlersee, Austria) where the sex ratio is extremely biased in favour of females (frequency of males 3%) (Tunner and Dobrowsky 1976). Crossing experiments with frogs of these populations also yielded very few males (Tunner 1980). Despite the low number of *R. esculenta* males examined, this analysis gains further strong support from 1. evidence from oogenesis (Tunner and Heppich 1981), from 2. a C-banded marker chromosome derived from *R. lessonae* and heterozygously present in the soma yet absent during the meiosis in *R. esculenta* males (Heppich 1980), and from 3. the vast amount of morphological, electrophoretic and chromosomal data on these populations and crossing experiments (Tunner 1979, 1980; Tunner and Dobrowsky 1976; Heppich and Tunner 1979).

Parallel to the cytological investigation, we examined the genetic constitution of male soma and testes by electrophoresis. When comparing the electrophoretic phenotypes of polymorphic enzymes (i.e. lactate dehydrogenase – 1 or glucosephosphate isomerase) from muscle homogenates with that of macerated testes, a

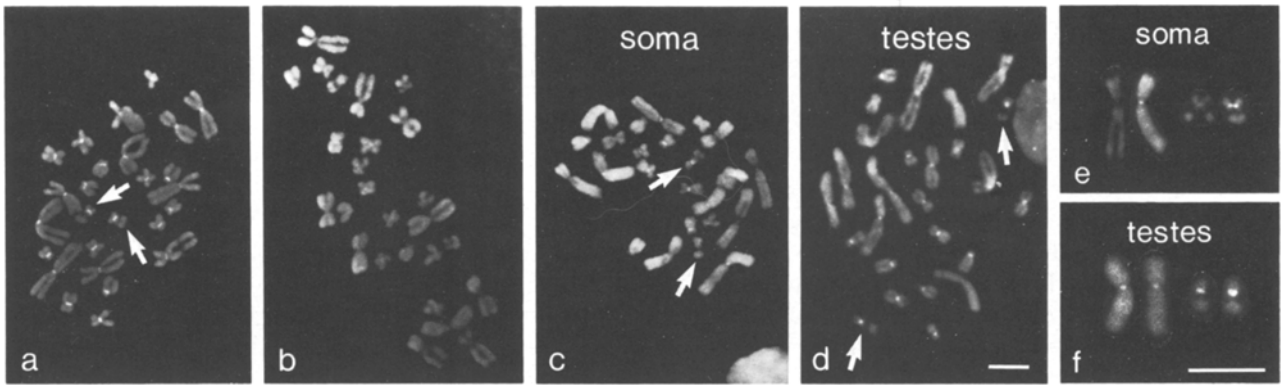

Fig. 1 a–f. Actinomycin D – 33 258 Hoechst fluorescence staining of metaphase chromosomes from the parental species *Rana ridibunda* and *R. lessonae* and their hybrid *R. esculenta.* **a–d** Metaphase plates (2n = 26). **a** *R. ridibunda,* soma: all chromosomes with intensive centromere fluorescence; **b** *R. lessonae,* soma: almost no intensive centromere fluorescence; **c** *R. esculenta,* soma: genome specific difference in centromere fluorescence; **d** *R. esculenta,* testes of the same individual: all chromosomes with intensive centromere fluorescence corresponding to a diploid *ridibunda* genome. Arrows point to the nucleolus-organizing chromosomes recognizable by their diminished NOR-fluorescence. **e,f** Chromosome pairs (the second large and the NOR-chromosome) from another *R. esculenta* male. **e** Difference in centromere fluorescence in the soma according to the hybrid constitution (chromosomes of 1 cell); **f** No difference in centromere fluorescence in the testes, indicating the presence of *ridibunda* chromosomes only (chromosomes of 1 cell). Bars represent 10 μm

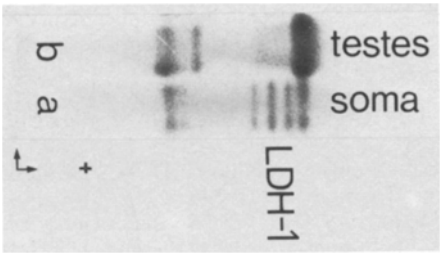

Fig. 2 a and b. Electrophoretic phenotypes of lactate dehydrogenase from the species hybrid *Rana esculenta.* In somatic tissue (muscle homogenates) a heterozygous *R. ridibunda* + *R. lessonae* allozyme pattern is seen **(a).** In gonadal tissue (macerated testes), however, only the translational products of the *R. ridibunda* alleles are visible **(b)**

distinct difference is apparent: muscle tissue always shows the typical heterozygous *ridibunda* + *lessonae* allozyme pattern (Fig. 2 a) observed in numerous investigations (Uzzell and Berger 1975; Graf et al. 1977; Tunner 1979). Testicular tissue, on the other hand, only shows the translation products of the *ridibunda* alleles (homozygous *ridibunda* allozyme pattern) (Fig. 2 b).

These concordant chromosomal and electrophoretic data clearly show that, as in females, meiotic pairing in *R. esculenta* males takes place between sister chromosomes whose recombination is without consequence. Using conventional techniques the bivalents cannot be distinguished from normal pairing of maternal and paternal chromosomes.

A genome elimination followed by a shortened haploid meiosis was described in the fish genus *Poeciliopsis* (Cimino 1972 a) – the only other known hybridogenetic system. A premeiotic elevation of the chromosome number from triploid to hexaploid is known from two all-female vertebrate taxa of hybrid origin (the Urodele *Ambystoma* and a second Poeciliid fish) which reproduce by gynogenesis (Macgregor and Uzzell 1964; Cimino 1972 b; Cuellar 1976). In *R. esculenta* the hybridogenetic pattern of inheritance is achieved through both elimination and subsequent elevation. This leads to the exclusive production of only one gamete type, carrying a haploid *ridibunda* chromosome set.

It seems of general relevance that this unusual and highly controlled mechanism of genome elimination is probably caused by a genome specific difference in the centromere structure, most likely due to particular repetitive sequences. The hybrid taxon *R. esculenta* thus provides hitherto unique evidence for the often presumed, yet frequently disputed involvement of repetitive – or satellite-DNA in centromere function (Walker 1971; Yamamoto and Miklos 1978; John and Miklos 1979).

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