Types and frequencies of multivalent associations and chiasma frequency at diakinesis in the parent, S_1 and S_2 plants

Plant	Association of 5 chromosomes				Association of 4 chromosomes		Trivalents	Bivalents Ring	Bivalents Rod	Univalents	Chiasma frequency Mean	Chiasma frequency Variance	Total No. of cells
	*8	11	13	18	6	17	4						
Parent interchange trisomic	34	2	12	6	10	8	28	408	120	18	12.68	0.7375	100
s_1	126	–	–	3	6	9	6	534	213	33	12.40	0.6845	150
s_2	80	–	–	–	6	24	40	612	128	130	12.32	0.6577	150

* Numbers refer to the types of configurations given by Sybenga[5].

chromosomes besides 5 bivalents; 18% showed a ring of 4 or chain of 4 and a univalent (figure a). In addition to the different types of configurations observed, a few others occurred which were the consequence of the presence of interstitial chiasmata and pairing initiation. From the data of the configurations at diakinesis, it is possible to assess preferential pairing, if present. 1 type of association resulting from preferential pairing is a trivalent with a ring bivalent (figure b). The frequency of this configuration is very low (3.7%) indicating that preferential pairing is practically absent in this material. The orientation of the multiple associations at metaphase I was studied. The association of 5 was alternate in 18% of the cases, adjacent in 24% and linear in 10%. Association of 4 chromosomes was alternate in 4%, adjacent in 14% of the cells. Among the trivalents, adjacent orientation was observed in 18%, linear in 12% and alternate in 2% of the cells. At anaphase I the distribution of chromosomes was 7–8. In 2% of the cells, 1 lagging chromosome was observed. The second division was normal. The 2 interchange trisomics were selfed and their s_1 and s_2 progenies were raised. A comparative study of the cytology of the s_1 and s_2 and the parental interchange trisomics was attempted with a view to assess the effect of inbreeding on the types of multiple associations and the mean chiasma frequency. The following features are observed (table): in the parents, the bivalent frequency was low when compared to the s_1 and s_2 generations. This might be due to the higher chiasma frequencies in the parent. This difference is significant at the 5% level.

In the parent, multivalent associations showing higher chiasma numbers were observed, whereas in the s_1 and s_2 generations the associations of 5 or 4 chromosomes were predominantly of chain types. In the s_1 and s_2 generations, the orientation of the multiple associations at metaphase I was adjacent or linear, alternate orientation being observed in 4% of the cells only in s_1. The pollen fertility in the parents, s_1 and s_2 was 58, 42 and 38% respectively. Inbreeding leads to reduced chiasma frequency and occurrence of meiotic abnormalities. This effect has been previously reported for *Pennisetum typhoides*[6,8]. In the present study, inbreeding for 1 and 2 generations has produced a significant difference in the mean chiasma frequencies, and a change in the types and frequencies of multiple associations.

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Biochemical variation in the *Rana esculenta* complex: A new hybrid form related to *Rana perezi* and *Rana ridibunda*

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Summary. Investigations of the green frogs from western Europe for electrophoretic variations at 4 enzyme loci demonstrated a new form which must be considered as a hybrid between *Rana ridibunda* and *R. perezi*. Biochemical evidence supports the hypothesis that its reproduction is hybridogenetic, as it is for *R. esculenta*.

The common European green frog, *Rana esculenta* Linnaeus 1758, has been shown to result from natural hybridization between *Rana ridibunda* Pallas 1771 and *Rana lessonae* Camerano 1882[1–8]. Breeding experiments, as well as morphological and genetic evidence, indicate that little or no introgression occurs between the 2 species[2,5–7]. Furthermore, they suggest that R. esculenta, reproductively speaking, is virtually a R. ridibunda. As a matter of fact, the cross ridibunda × esculenta produces exclusively ridibunda offsprings, while the cross escu-

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lenta × lessonae produces only esculenta offsprings. Results of the cross esculenta × esculenta, when not lethal, are essentially of the ridibunda type[2,5,8]. On this basis, Tunner[6] formulated the hypothesis that the reproduction of R. esculenta was hybridogenetic, which means that during the gametogenesis of the hybrid a set of parental chromosomes (namely the lessonae chromosomes) is eliminated. This hypothesis was supported by the electrophoretic analyses of LDH isozymes in male and female germ cells of R. esculenta[8]. These germ cells exhibited only the allozyme inherited from ridibunda, suggesting that the lassonae genome had been discarded from them.

Our electophoretic studies on green frogs were initiated in order to clarify the systematic status of the species or subspecies *Rana ridibunda perezi* Seoane 1885, occurring in Spain and southwestern France. During these studies, we discovered in southern France a population of green frogs, which, like R. esculenta, have all the features of hybrids. However, while R. esculenta is a hybrid between R. ridibunda and R. lessonae, this 'new' type must have resulted from hybridization between R. ridibunda and R. perezi, as will be shown in the present paper. (For convenience, we have adopted the nomenclature used by Hotz[9], who admits the specific status of Rana perezi Seoane 1885.)

Materials and methods. Frogs were collected from the following localities: Rana perezi: Banyuls-sur-mer (France, Dép. Pyrénées-orientales). Rana ridibunda × perezi: Goudargues (France, Dép. Gard). Rana ridibunda, Rana esculenta and Rana lessonae: Vicinity of Lausanne (Switzerland) (figure 1).

Skeletal muscle and heart were removed from freshly killed frogs and homogenized in 5 volumes of 0.1 M Tris-HCl (pH 8.0), then centrifuged at 18,000 × g. Supernatant fractions were subjected to vertical starch gel electrophoresis, using a discontinuous buffer system (Poulik)[10]. Electrophoresis was conducted at 12 V/cm for 5 h. All preparations were done at 4 °C.

Gel slices were stained for lactate dehydrogenase (LDH)[10], aspartate aminotransferase (AAT)[10] and creatine kinase (CK)[11]. Masses of oocytes (about 100 mg) from ovaries of adult females were prepared in the same way and were analyzed electrophoretically for the isoenzymes of LDH and AAT. These zymograms were then compared with those obtained from somatic tissues of the same individuals.

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Table 1. Enzyme phenotypes of the green frogs which were analyzed in this study. The alleles detected at each locus were named according to the relative mobility of their allozymes

Species	N	LDH-B					CK			AAT-1			AAT-2	
		100	91	86	75	71	100	97	85	147	100	26	100	–21
R. perezi	2					II	II				II			II
	1			I		I	II					II		II
	3					II	II					II		II
R. ridibunda × perezi	6	I				I	I		I		I	I		II
R. ridibunda	2	II							II		II			II
	2	I		I					II		II			II
	1	I		I					II	1	I			II
	1	II							II	I	I			II
	1	I	I						II		II			II
R. esculenta	3	I			I			I	I		I	I	I	I
	2	I			I				II		I	I	I	I
	8			I	I			I	I		I	I	I	I
	2			I	I				II		I	I	I	I
R. lessonae	11				II			I	I			II	II	
	1		I		I			I	I			II	II	
	4				II			II				II	II	
	2				II				II			II	II	

Homozygosity for an allele is indicated by the presence of a double bar in the corresponding column. *N* Number of individuals with identical phenotypes.

Table 2. Comparison of enzyme phenotypes in somatic tissues and oocytes I of heterozygous females

Species	N		LDH-B					AAT-1			AAT-2	
			100	91	86	75	71	147	100	26	100	–21
R. ridibunda	2	Soma	I		I				II			II
		Oocytes	I		I				II			II
	1	Soma	I		I			I	I			II
		Oocytes	I		I			I	I			II
R. ridibunda × perezi	5	Soma	I				I		I	I		II
		Oocytes	II						II			II
R. esculenta	2	Soma			I	I			I	I	I	I
		Oocytes			II				II			II
	1	Soma	I			I			I	I	I	I
		Oocytes	II						II			II

For further details see above (table 1).

Results. 1. Enzyme phenotypes in somatic tissues (table 1). LDH (figure 2). The isoenzymes resolved are encoded in 2 loci, designated A and B[12]. The A-locus did not show any variation detectable by the electrophoresis. The B-locus is polymorphic. R. perezi, R. ridibunda and R. lessonae clearly differ from one another by their major alleles; most individuals were homozygous. On the contrary, all individuals of R. esculenta and R. ridibunda × perezi were heterozygous, the former possessing a typical lessonae-allele and a ridibunda-allele, whereas the latter has a typical perezi and a ridibunda-allele.

CK. Creatine kinase is a mutilocus enzyme system like LDH[13]. However, we worked with the 'muscle enzyme' only, which is encoded in a single locus. Homozygous individuals exhibit a single band on zymograms, while heterozygous individuals have a 3-banded phenotype due to the dimeric structure of the enzyme[14]. R. perezi and R. ridibunda are both monomorphic at this locus, but differ from one another. All individuals of R. ridibunda × perezi were heterozygous for the ridibunda and the perezi-allele. R. lessonae is polymorphic, and shares one of its alleles (CK^{85}) with R. ridibunda. Therefore the situation is not as clearcut in the hybrid R. esculenta, where heterozygous and homozygous individuals are observed.

AAT. 2 loci were found to be active in heart. At the AAT-1 locus 3 alleles have been detected. All individuals of R. esculenta and R. ridibunda × perezi were heterozygous, exhibiting a 3-banded phenotype. At the AAT-2-locus 2 alleles have been detected. One of them ($AAT\text{-}2^{+100}$) was found in both R. ridibunda and R. perezi, the other being specific to R. lessonae. All individuals of R. esculenta were heterozygous (3-banded phenotype).

2. Enzyme phenotypes in oocytes (table 2). The patterns of LDH-B, AAT-1 and AAT-2 found in oocytes I from females of R. ridibunda were identical to those obtained from their somatic tissues. In contrast, the phenotypes found in oocytes I from hybrid females (R. esculenta, as well as R. ridibunda × perezi) exhibited only the ridibunda-type allozymes. We may thus assume that only the ridibunda alleles were active in the oocytes of the 2 hybrid forms.

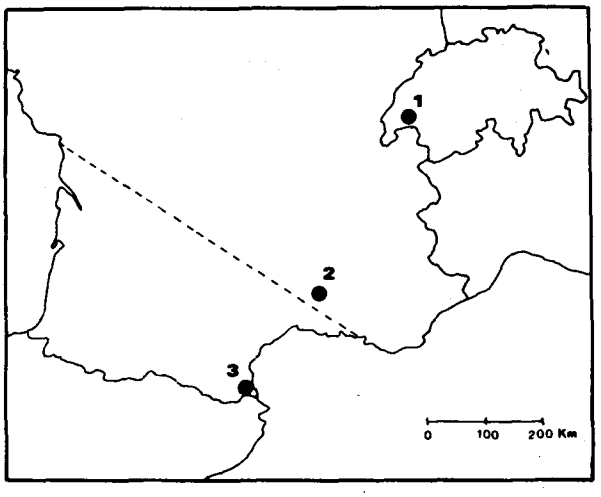

Fig. 1. Geographical situation of the populations of green frogs which were examined in this study. *1.* Switzerland: Vicinity of Lausanne. *2.* France: Goudargues. *3.* France: Banyuls-sur-mer. The dashed line indicates the northern limit of the range of Rana perezi.

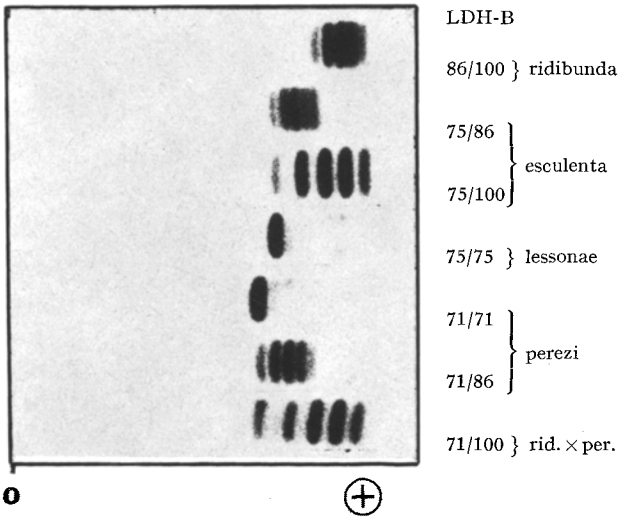

Fig. 2. Starch gel electrophoresis of supernatant fractions of heart homogenates. Specific staining for LDH activity. *O* Origin.

Discussion. 1. Concerning the Rana esculenta complex sensu stricto, our results are in agreement with those reported by Uzzel and Berger[7]. All individuals of R. esculenta were heterozygous for LDH-B, AAT-1 and AAT-2. At each of these loci, we could detect both a typical ridibunda and a typical lessonae-allele. At the CK-locus, our results differ from Uzzel and Berger's data. East European populations of R. lessonae and R. esculenta studied by these investigators were monomorphic and electrophoretically identical at the CK-locus. In our material, however, polymorphism is recorded. The CK-polymorphism of R. lessonae apparently involves the same polymorphism in R. esculenta. This is of interest as it is consistent with the hypothesis that the latter needs the former for its reproduction.

Tunner's hypothesis[6] of a hybridogenetic mode of reproduction of R. esculenta is well-supported by our observations on enzyme phenotypes in oocytes I as opposed to somatic tissues. Furthermore, the enzyme phenotypes observed in oocytes I suggest a premeiotic exclusion of the lessonae genome, as oocytes I still possess a double set of chromosomes. Such a phenomenon has been reported in all female hybrid fishes of the genus Poeciliopsis by Cimino[15].

2. Enzyme phenotypes of Rana ridibunda × perezi are strikingly similar to those of R. esculenta both with respect to the high incidence of heterozygosity and to the difference of enzyme phenotypes in oocytes I as opposed to somatic tissues. It thus appears very likely that R. ridibunda × perezi is a hybrid with a similar mode of reproduction as R. esculenta.

3. The occurrence of R. ridibunda × perezi has some bearing on the systematic status of R. perezi. If our assumption on the origin of R. ridibunda × perezi and its mode of reproduction is correct, Rana ridibunda Pallas 1771 and Rana perezi Seoane 1885 must be considered as 2 distinct species. In fact, electrophoretic analyses of albumin[16] and other proteins (Graf, unpublished) also show that these 2 'forms' are genetically very different.

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