Full Papers

Rana esculenta complex: An experimental analysis of lethality and hybridogenesis[1]

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Summary. Experiments designed to analyze the lethality and hybridogenesis in the European green frog complex have yielded the following results: 1. As a rule the inter-se cross of *Rana esculenta* is lethal, but several crosses have produced fully viable progeny. The frequency of such 'break-through' crosses appears to be related to parental population structure. 2. Parabiotic joining of lethal to viable embryos indicates that manifestation of the lethal effect is autonomous. There is, however, a 16–18% increase in the life span of the lethal partner. 3. Studies of LDH isozyme patterns revealed that the *lessonae*-specific alleles coding for the B^a and B^c subunits can be passed to the F_1 progeny from a parental female or male of the *esculenta* phenotype. This demonstrates that there is no total elimination of the *lessonae* genome in the *esculenta* germ cells. 4. Immunologically, offspring from the inter-se cross of *R. esculenta* show a closer relationship to the *ridibunda* than to the *esculenta* phenotype. Variations of antigenic protein patterns suggest the possibility of chromosomal recombination between *lessonae* and *ridibunda* in the *esculenta* hybrid. These results are confirmed by two-dimensional electrophoretic analysis of proteins in the oocytes of the three frog phenotypes.

Introduction

Recent morphological and biochemical investigations of the 3 types of European green frogs indicate clearly that *Rana lessonae* (les) and *Rana ridibunda* (rid) are 2 distinct species, while *Rana esculenta* (esc) represents their hybrid (see references in a review by Berger[2]). Our previous studies on the genetic control of lactate dehydrogenase (LDH) isozymes are in agreement with such a conclusion[3,4]. However, the problem is by far not so simple, since *R. esculenta* inter-se crosses show abnormal meiosis[5,6], reduced fertility[5] and high lethality[7,8]. Yet the presence of *R. lessonae* is sufficient for the reproduction of *R. esculenta*, as crosses between these 2 phenotypes yield always viable *esculenta* progeny. In order to gain more insight into this complex phenomenon further hybridization experiments have been carried out by us and special emphasis has been laid on the analysis of the lethal cross *esculenta* × *esculenta* by developmental, biochemical and immunological approaches.

Materials and methods

Adult frogs of *R. lessonae* and *R. esculenta* were collected from 4 different populations in the vicinity of Zürich which differ distinctly in the frequency of the 2 phenotypes as well as in the sex ratio within each phenotype (table 1). Frogs of *R. ridibunda* were obtained from the Balkans. Individuals of the F_1 generation were produced by either natural pairing or artificial insemination. In the latter case each female frog was injected with extract of 2 hypophyses on the previous day to induce egg maturation. The parental phenotypes of each cross were identified, in addition to morphological criteria, by electrophoretic examination of their serum albumin patterns. *R. lessonae* and *R. ridibunda* have each a single albumin band of different mobilities, whereas *R. esculenta* exhibits a double albumin band with the corresponding mobilities of both[9–11]. Embryos and larvae were cultured in water under conditions described previously[8,12].

Parabiotic twins were produced by the standard technique[13,14]. Two embryos at the tailbud stage were joined by cutting out a piece of ectoderm in the trunk region and then pressed in an agar dish with the wound surfaces contacting each other. After complete healing the joined pairs were first cultured in Holtfreter's solution[15] and then in water at 18 °C. Their development was followed as long as the lethal partner remained alive. In some cases the larvae were fixed and sectioned for histological examination.

The growth of larvae from different crosses was analyzed by determination of their body weight. For this purpose 10 randomly selected tadpoles were dried on a piece of filter paper and weighed.

The metamorphic capacity of the lethal offspring was tested by treatment with thyroxine (Fluka, Buchs). Three different concentrations ($1:50\times10^6$, $1:100\times10^6$, $1:200\times10^6$) were used. Both viable and lethal larvae raised in water without hormone served as controls.

In order to clarify whether triploid individuals were present among the adult frogs and their progeny we

Table 1. Summary of adult frogs collected from 4 different biotopes (A–D) during the spawning seasons 1978–1980

Phenotype	Biotope A		B		C		D		
	Female	Male	Female	Male	Female	Male	Female	Male	
R. esculenta	23	22	5	8	72	26	30	17	
R. lessonae	2	1	6	7	9	12	38	53	

Table 2. Mean life span of parabiotic and singly cultured lethal larvae from the inter-se cross *R. esculenta* × *R. esculenta* (E × E). Additional partners are from the crosses *R. esculenta* × *R. lessonae* (E × L) and *R. lessonae* × *R. lessonae* (L × L), *indicates break-through crosses

Class	Series	Parabiotic combination	n	Life span of lethal larvae (days) Parabiosis	Single
I	A	$E \times E_C + E \times E_B$	3	23 ± 11	42
	B	$E \times E_C + E \times E_B$	5	31 ± 14	42
	H	$E \times E_C + E \times E_{A1}$	5	45 ± 4	36
Ia	J	$E \times E_{A2}^* + E \times E_{A3}$	3	19 ± 0	28
	K	$E \times E_{A2}^* + E \times E_{A3}$	4	22 ± 6	-
II	D	$E \times E_C + E \times L_{D1}$	6	33 ± 9	40
	E	$E \times E_{A1} + E \times L_{D1}$	4	44 ± 3	32
	N	$E \times E_D + E \times L_{D2}$	3	37 ± 6	24
	P	$E \times E_{A3} + E \times L_{D2}$	4	37 ± 15	28
IIa	L	$E \times E_{A2}^* + E \times L_{D2}$	6	42 ± 6	-
III	F	$E \times E_C + L \times L_{D1}$	4	42 ± 7	40
	G	$E \times E_{A1} + L \times L_{D1}$	7	38 ± 3	32
	O	$E \times E_D + L + L_{D2}$	2	38 ± 8	24

have determined the long axes of the erythrocytes[16]. According to Uzzell et al.[17] erythrocytes with a diameter of 29.38 µm are considered to be triploid.

LDH isozymes were separated by both polyacrylamide gel (7.5%) electrophoresis according to Davis[18] and isoelectric focusing (pharmalyte pH 3–10) according to Vesterberg[19]. Following incubation tracing of the stained bands was conducted with a Beckman densitometer (Model CDS-2000).

For preparation of antisera, serum was collected from adult frogs of *R. ridibunda* and *R. lessonae* and injected into rabbits. The antiserum titer was estimated by the double diffusion test of Ouchterlony[20]. Two-dimensional immunoelectrophoresis was performed on 1% agarose gel according to procedures given by Crowle[21].

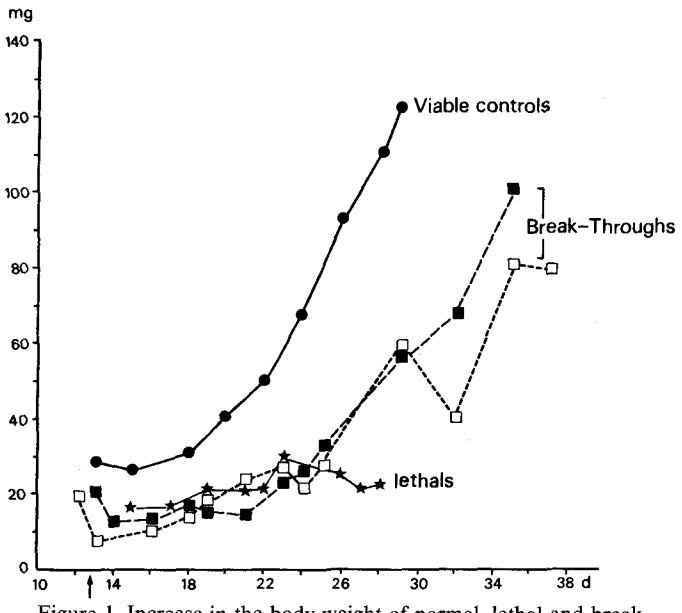

Figure 1. Increase in the body weight of normal, lethal and break-through *R. esculenta* tadpoles. After the initiation of feeding (indicated by arrow) the viable controls (●) from the cross les × esc show a rapid linear increase in body weight, whereas the growth of the break-through (■, □) and lethal (*) larvae from the inter-se cross esc × esc is retarded at the very beginning of development.

For comparison of protein synthesis in the oocytes of the 3 frog phenotypes 10 oocytes were incubated in 50 µl Barth's solution containing 42 µCi ^{35}S-methionine (sp. act. 1090 Ci/mM, Radiochemical Centre, Amersham) for 24 h at 18 °C. Extraction of the labeled soluble proteins was carried out according to procedures given by Bienz[22]. The proteins were separated by isoelectric focusing (pH 4–6.5) in the 1st dimension, followed by SDS electrophoresis (10% polyacrylamide gel) in the 2nd dimension according to O'Farrell[23]. The individual proteins were visualized by autoradiography.

Results

1. *Development.* Development of the progeny from the *R. esculenta* inter-se cross is retarded at the very beginning of embryogenesis, compared to viable individuals of the cross *R. esculenta* × *R. lessonae*. Under identical culturing conditions a distinct delay in both gastrulation and neurulation can be observed. As illustrated in figure 1, subsequent to feeding at Shumway stage 25 the normal controls show a rapid, linear increase in body weight. The 'break-through' tadpoles exhibit a similar increase, but at a much slower rate. The corresponding weight values of the lethals remain virtually constant throughout the culturing period. It should be emphasized that all three types of larvae are of the *esculenta* phenotype, yet they differ clearly in their growth pattern which is obviously under genetic control.

Parallel results have been obtained from experiments of thyroxine treatment. Increase in the body weight of normal esc tadpoles from the cross esc × les and that of the break-through larvae from the cross esc × esc in the presence of thyroxine are summarized in figure 2. The latter, though viable, exhibit again a distinct delay in growth. Their reduced metamorphic potential is further manifested in the shorter posterior legs and the delayed appearance of the anterior legs (see Binkert[12]).

Triploid frogs have been reported for *R. esculenta* by previous authors[17], suggesting that lethality of the *esculenta* inter-se cross could be caused by a certain meiotic defect. In amphibians the size of erythrocytes can be used to estimate polyploidy[16]. As shown by the diagram in figure 3, the mean value of the erythrocyte long axis is 23.9 µm (range 22.7–25.0 µm) in *ridibunda*, 28.8 µm (range 27.1–30.5 µm) in *lessonae* and 26.1 µm (range 23.5–28.8 µm) in *esculenta*. According to Uzzell et al[17] triploid *esculenta* frogs have an average long axis of 29.38 µm, and diploid individuals 23.5 µm. Thus triploidy appears to be excluded in the frogs studied by us. Values of the F_1 individuals from 3 crosses indicated at the lower left region in figure 3 are within the expected ranges, since both crosses esc × esc yielded rid offspring, and the cross esc × les yielded esc offspring.

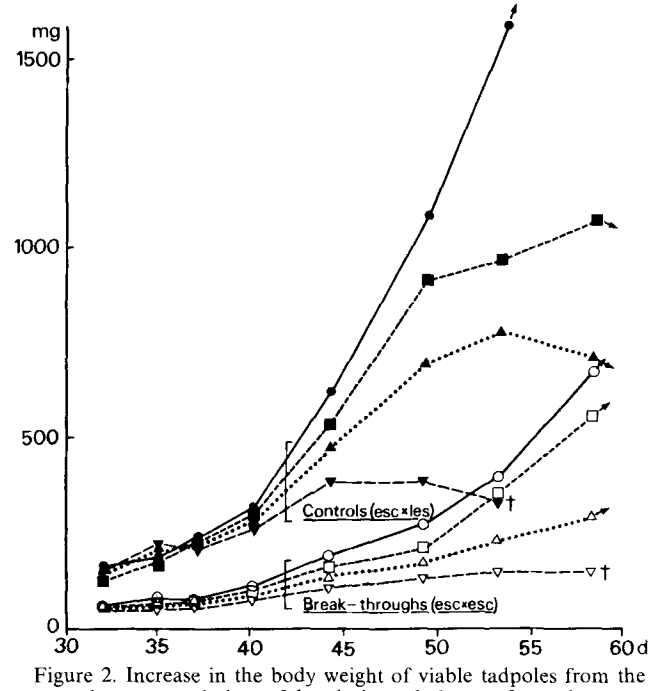

Figure 2. Increase in the body weight of viable tadpoles from the cross les × esc and that of break-through larvae from the cross esc × esc in the presence of thyroxine. The hormone concentrations were 1:30 × 10^6 (▽, ▼), 1:100 × 10^6 (△, ▲), 1:200 × 10^6 (□, ■) and 1:zero (○, ●).

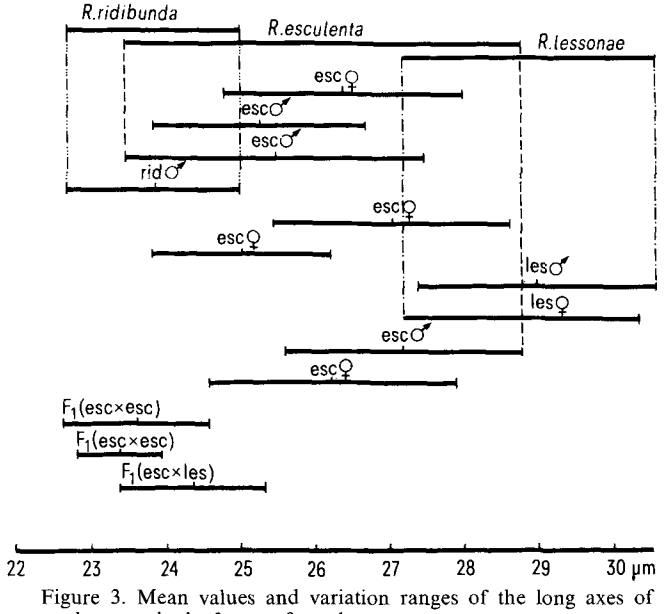

Figure 3. Mean values and variation ranges of the long axes of erythrocytes in the 3 green frog phenotypes.

For experiments of parabiosis, progeny from a total of 13 crosses in various combinations were used (table 2). In principle embryos of the lethal cross esc × esc (E × E) were joined to an individual of another esc lethal cross (class I), or to a viable partner of either the cross esc × les (E × L, class II), or the inter-se cross les × les (L × L, class III). Each class was again divided into different series according to the biotopes of the parental phenotypes. As indicated in table 2, in 2 series of class I and in 1 series of class II larvae of the lethal cross E × E_{A2} turned out to be break-throughs. These are designated as class Ia and class IIa respectively.

Altogether 148 parabiotic twins were prepared and their development has been followed in detail as long as the lethal partner remained alive. A typical example for the external morphology is illustrated in figure 4. This is a parabiotic pair from class III at the age of 14 days following operation. The reduced body length and other morphological symptoms of the lower lethal larva (E × E_{A3}) are evident, compared to normal development of the upper control larva (L × L_{D2}). Figure 5 shows the cross section of a parabiotic pair (class Ia, series J) fixed 19 days after operation. Degeneration of muscle (M) and notochord (N) in the right lethal larva is clearly visible, whereas in the left break-through partner both tissues are of normal appearance. The occurrence of creatine in the free amino acid pool of the lethals (fig. 6, peak indicated by arrow) but not in that of the viable

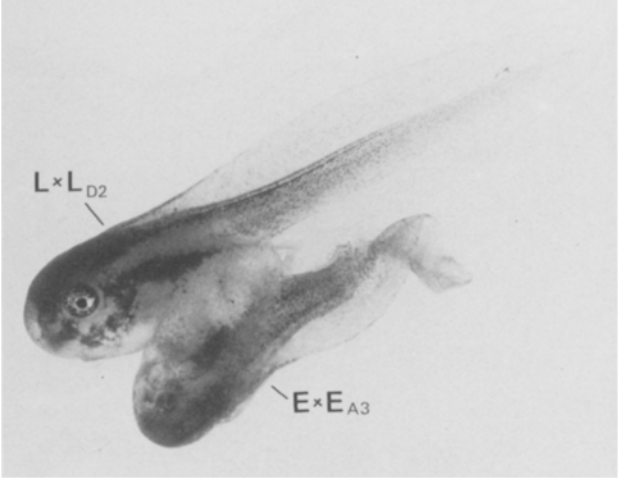

Figure 4. A typical parabiotic pair of tadpoles at the age of 14 days after operation. The lower lethal esc × esc (E × E_{A3}) larva shows abnormal morphology and retarded growth compared with the upper viable les × les (L × L_{D2}) partner.

Table 3. Inheritance of B subunit of LDH isozymes in 12 crosses of the 3 green frog phenotypes, * break-through cross

No. of crosses	Female Phenotype	Genotype	Male Phenotype	Genotype	F_1 Genotype
1	esc	B^a/B^b	esc	B^b/B^c	B^b/B^b
2	esc	B^a/B^d	esc	B^b/B^c	B^d/B^b B^d/B^c
3	esc	B^a/B^d	esc	B^c/B^d	B^d/B^c
4	esc	B^b/B^c	esc	B^a/B^b	B^b/B^b
5	esc	B^a/B^b	esc	B^b/B^c	B^b/B^b
6*	esc	B^a/B^b	esc	B^c/B^d	B^b/B^d
7	les	B^a/B^c	les	B^a/B^a	B^a/B^a B^c/B^a
8	les	B^a/B^c	les	B^a/B^a	B^a/B^a B^c/B^a
9	esc	B^b/B^c	les	B^a/B^a	B^b/B^a
10	esc	B^a/B^b	les	B^a/B^c	B^b/B^a B^b/B^c
11	esc	B^a/B^b	rid	B^d/B^d	B^a/B^d B^b/B^d
12	esc	B^a/B^b	les	B^c/B^c	B^b/B^c B^a/B^c

tadpoles may possibly be related to the muscular degeneration. The only positive effect of the viable larva in parabiosis is a 16–18% increase in the life span of the lethal partner (table 2). Otherwise the over-all results indicate that manifestation of lethality in the *R. esculenta* inter-se cross is autonomous.

2. *Inheritance of LDH isozymes.* Our previous studies[3,4] demonstrated that the A subunit of LDH tetramers is the same in all 3 green frog phenotypes, whereas there are 4 different B subunits, designated as B^a, B^b, B^c and B^d in the order of their increasing anodal moblility. B^a and B^c are synthesized only in *R. lessonae,* B^b and B^d only in *R. ridibunda,* but all 4 have been found in *R. esculenta.*

One difficulty in the analysis of the LDH isozymes is that B^c and B^d subunits are not distinguishable by polyacrylamide gel electrophoresis (PAGE) at pH 8.3, though they can be separated at pH 7 (see Vogel and Chen[4]). In a further effort to solve this problem we succeeded to achieve a clear-cut identification of the 4 genotypes B^a/B^a, B^b/B^b, B^c/B^c and B^d/B^d by isoelectric focusing (IEF) at pH 3–10 (fig. 7). When the isozymes are separated by PAGE in the 1st dimension and subjected to a further separation by IEF in the 2nd dimension, the LDH bands from both systems can be easily correlated. As demonstrated in figure 8, isoelectric focusing exhibits a higher resolution power, and a larger number of bands can be visualized on the two-dimensional gels.

By means of the above electrophoretic procedures we carried out a careful study of the inheritance of the B subunits in the 3 green frog phenotypes. Table 3 summarizes the phenotypes and genotypes of the parental animals and the genotypes of the F_1 generation from a total of 12 crosses. It can be noted that the allele coding for B^c in the F_1 heterozygote B^d/B^c in crosses 2 and 3 must be derived from the esc male frog. By analogy the allele coding for B^a in the F_1 heterozygote B^a/B^d in cross 11 and in the F_1 heterozygote B^a/B^c in cross 12 must have its origin from the esc female frog. As already mentioned, both subunits B^a and B^c are synthesized only in *R. lessonae.* Therefore, we conclude that there is not always a total elimination of the *lessonae* genome during gametogenesis of the *esculenta* hybrid.

3. *Patterns of immunologically active proteins.* The titer of the antisera prepared against either *ridibunda* or *lessonae* serum was determined by the Ouchterlony double diffusion test (fig. 9). For application in two-dimensional immunoelectrophoresis the optimal dilution ratio of antigen to antibody was found to be 1/32:1/2. This means that the antisera were immuno-

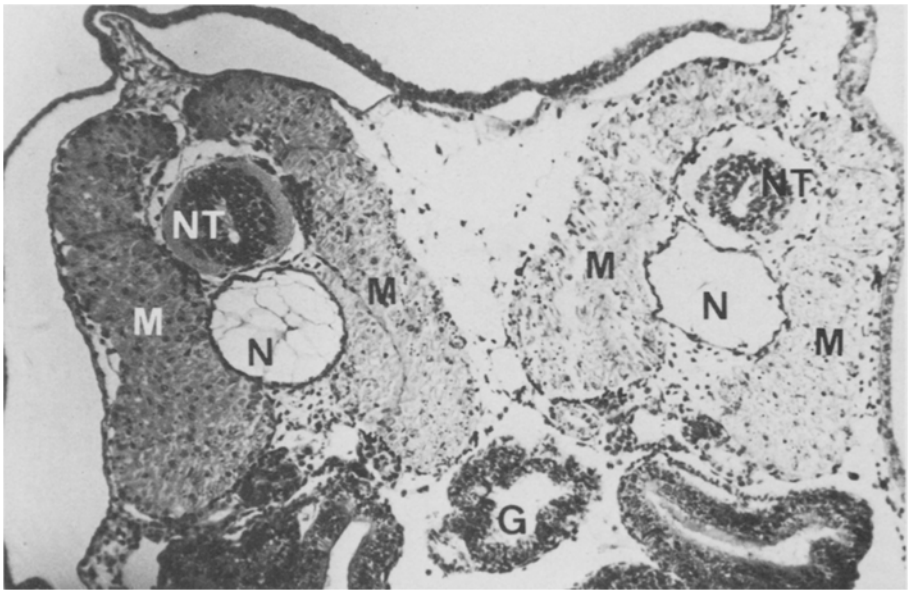

Figure 5. Cross section of a parabiotic pair between a breakthrough (left) and a lethal larva (right) from a *R. esculenta* × *R. esculenta* cross. The tadpoles were fixed on the 19th day of development. NT, neural tube; M, myotome; N, notochord; G, gut. × 120.

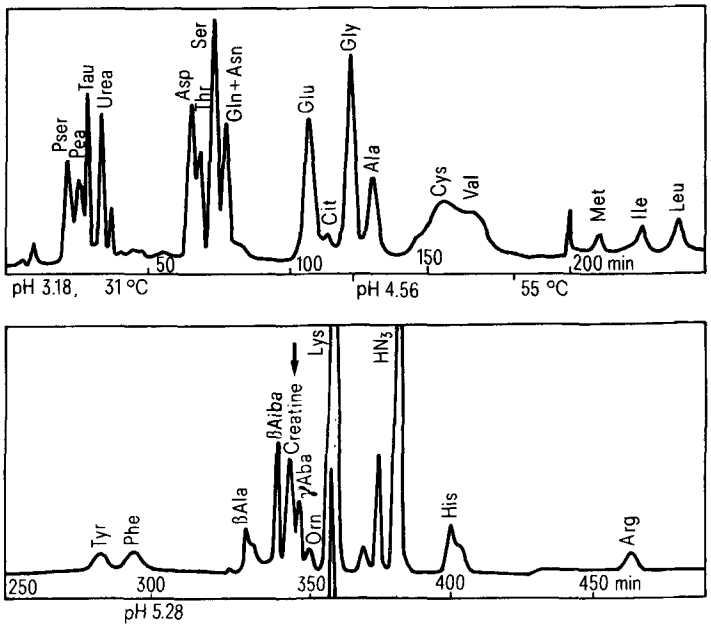

Figure 6. Elution profile of free amino acids and related compounds in the lethal larvae of the inter-se cross esc × esc on amino acid analyzer. Arrow indicates the occurrence of creatine.

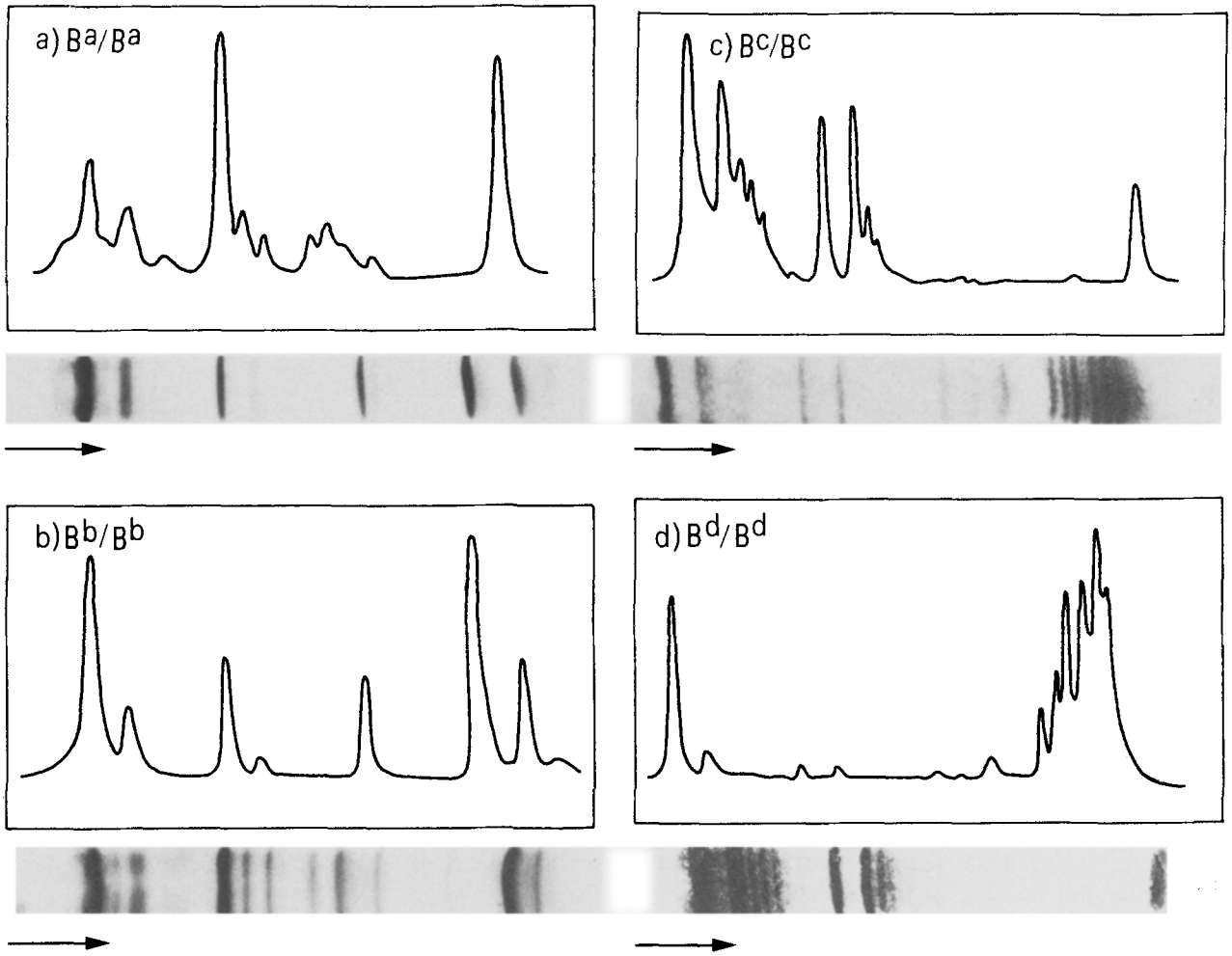

Figure 7. Isoelectric focusing of LDH isozymes in green frogs with genotypes B^a/B^a (a), B^b/B^b (b), B^c/B^c (c) and B^d/B^d (d). The densitometric tracing curve is depicted above each zymogram.

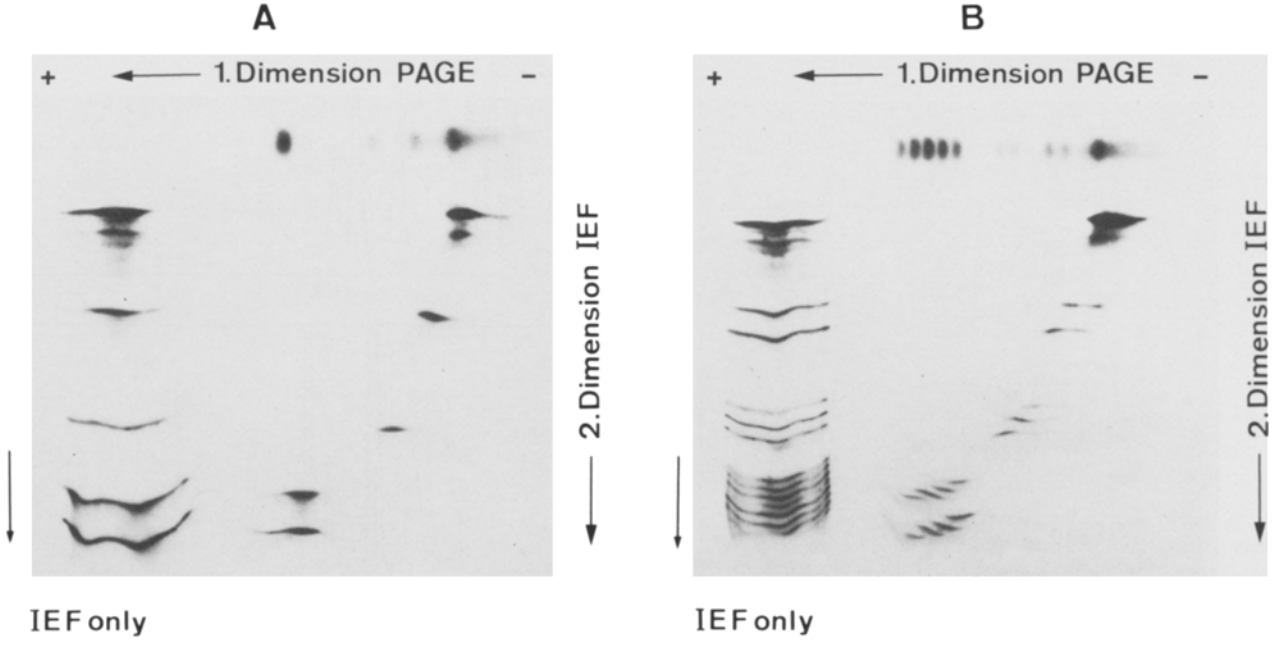

Figure 8. Separation of LDH isozymes by two-dimensional gel electrophoresis. As can be seen, the LDH bands separated by PAGE in the 1st dimension can be correlated to those separated by IEF in the 2nd dimension. *A* esc, genotype B^b/B^b. *B* esc, genotype B^d/B^b. In both zymograms the pattern of one-dimensional IEF is given at the left as reference.

logically 16-fold less concentrated than the serum of the adult frogs.

For analysis of the antigenic patterns progeny of crosses 5 (esc × esc), 6 (esc × esc), 11 (esc × rid) and 12 (esc × les) were used (see table 3). Cross 6 yielded viable break-through larvae and thus differs from cross 5 which, as expected, was lethal. Based on the elimination of *lessonae* genome cross 6 and cross 11 would produce F_1 offspring of the *ridibunda* phenotype and cross 12 those of the *esculenta* phenotype.

The lethal larvae from cross 5 were too small to yield sufficient serum. Thus in our first series of experiments total homogenates were subjected to two-dimensional immunoelectrophoresis. Anti-*ridibunda*-antiserum was used. As can be seen in figure 10, dilution of serum proteins in total homogenate and the small larval size resulted in the detection of only 2 precipitating fractions in cross 5 (E × E). Cross 6 (E × E) yielded break-through larvae and four precipitating fractions could be detected. Their antigenic pattern show a great similarity to that of cross 11 (E × R) which, owing to the elimination of *lessonae* genome, produced also progeny of the *ridibunda* phenotype. Cross 12 (E × L) produced viable *esculenta* larvae in the F_1 generation. Compared to cross 11 fraction 2 occurs in a much higher concentration, and the electrophoretic mobilities of all 3 fractions are reduced. Therefore, the antigenic patterns between these 2 crosses differ both quantitatively and qualitatively.

When serum proteins were used for two-dimensional immunoelectrophoretic separation the antigenic patterns appear much more complicated. With anti-*ridibunda*-antiserum a minimum of 7 to 10 precipitat-

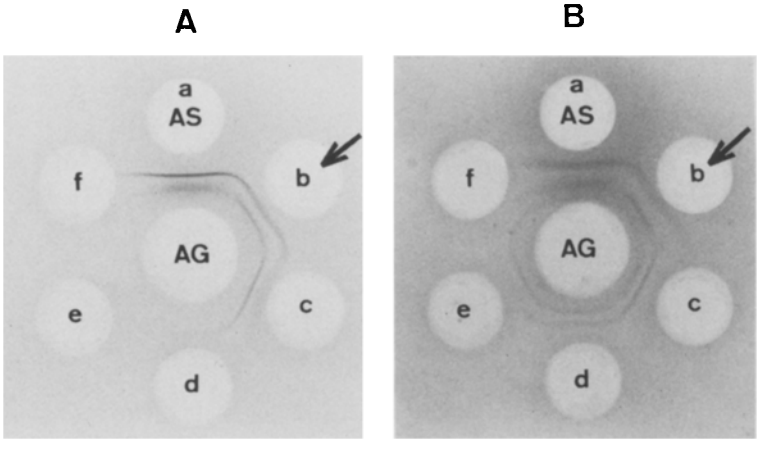

Figure 9. Ouchterlony double diffusion test. *A* AG, antigen from *ridibunda;* AS, anti-*ridibunda* antiserum. *B* AG, antigen from *lessonae;* AS, anti-*lessonae* antiserum. Antigen concentration in both cases: 1/32. Antiserum concentrations: a, 1/1; b, 1/2; c, 1/4; d, 1/8; e, 1/16; f, 1/32. The optimal concentration ratio is indicated by arrow.

ing fractions can be visualized (fig. 11). A detailed analysis of individual homolog fractions based on their mobilities confirmed our previous conclusion that the break-through cross E × E shows a greater similarity to cross E × R than to cross E × L.
Serum proteins from the progeny of all 3 crosses were also examined with anti-*lessonae*-antiserum. In general, the number of detectable precipitating fractions is strikingly small, and in each cross the antigenic pattern differs totally from that obtained with anti-*ridibunda*-antiserum (data not shown).

4. *Protein synthesis in oocytes.* Incubation of the frog oocytes with ^{35}S-methionine followed by two-dimensional electrophoretic separation of the labeled proteins revealed more than 300 spots, and their patterns appear highly complex (fig. 12). In general, esc shows again a greater similarity to rid than to les in the synthesis of oocyte proteins. For example, proteins numbered 1–14 are all synthesized in both rid and esc (indicated by arrow in fig. 12), but there is apparently no synthesis of proteins 7–14 in les (indicated by + in fig. 12). On the other hand, the two-dimensional gels of rid and esc are by no means identical. These observations confirm again that, at least in the frog populations studied by us, hybridogenetic reproduction of *R. esculenta* is accomplished by retaining the *ridibunda* genome which, however, does not exclude recombinations between *ridibunda* and *lessonae* chromosomes.

Discussion

1. *Lethality and structure of the frog population.* Our data suggest that lethality of the inter-se cross in *R. esculenta* is related to the structure of the parental frog population. All 4 break-through crosses observed in the present study were limited to populations A and C. Adult frogs collected from A yielded one and those from C 3 such crosses. As can be seen in table 1, the ratio of *R. esculenta* to *R. lessonae* is 45:3 in A, 98:21 in C. The corresponding values are 13:13 in B and 47:91 in D. Thus it seems that in a population, in which the phenotype ratio is in favour of *esculenta*, the inter-se cross has a higher probability to yield viable progeny in the F_1 generation. Another significant point of the inter-se crosses studied by us is that all larvae within a single cross were either fully viable or died before Shumway stage 25. Berger[25] reported previously a large variation in the lethality of the esc × esc cross. The different results could be due to regional variations of the parental animals used for the breeding experiments.

Based on our detailed developmental studies of the parabiotic twins lethality of the *R. esculenta* inter-se cross is doubtless under genetic control. Although the break-through tadpoles are viable, both their growth and reacting capacity towards thyroxine are reduced (figs 1 and 2). It should be mentioned that in the biotopes where adult frogs for the present study were collected only *lessonae* and *esculenta* exist (L-E system). Obviously the *ridibunda* genome has been carried by the *esculenta* gametes since the initiation of hybridogenesis. Such an isolation would lead to the accumulation of lethal factors the effects of which are expressed in the homozygotic offspring of the inter-se cross.

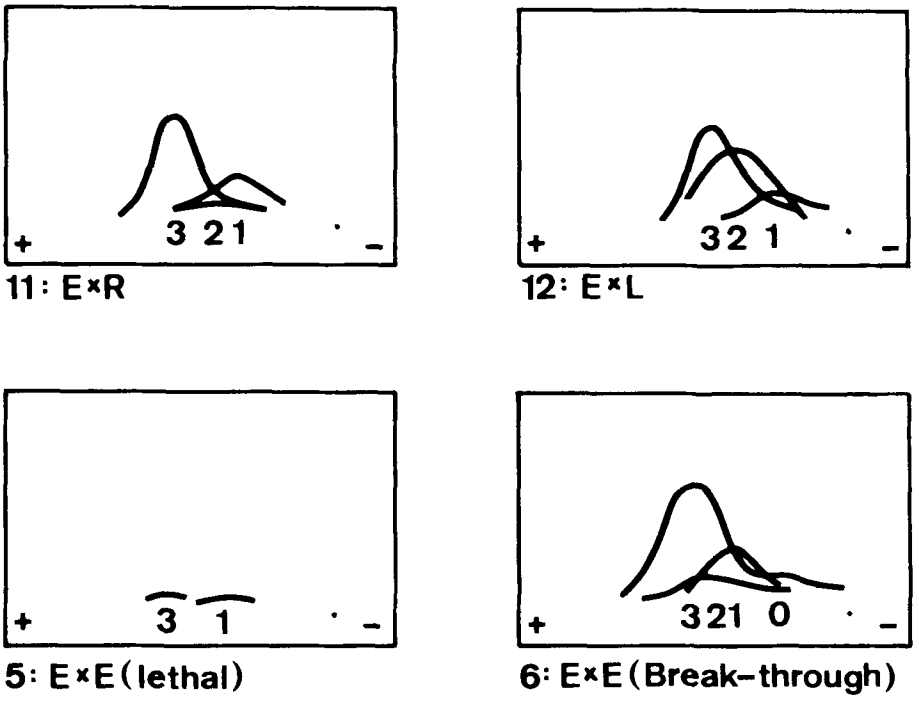

Figure 10. Two-dimensional immunoelectrophoresis of total homogenates from F_1 progeny of 4 different crosses, as indicated under each electropherogram. Anti-*ridibunda* antisera were used. Further explanation is given in text.

2. *Inheritance of the B subunit of LDH isozymes.* Previous hybridization experiments indicated that allelic genes coding for the *lessonae*-specific LDH subunits B^a and B^c are not transmitted from *R. esculenta* to the F_1 generation[8]. In an extensive study for 4 enzyme systems by using single primary oocytes from *R. esculenta* Uzzell et al.[26] detected no expression of *lessonae*-specific alleles, including the B locus of LDH. These results are understandable if there is a premeiotic exclusion of the total *lessonae* genome during formation of the hybrid gametes[27].

As described in 'results', in 4 out of 12 crosses in which *R. esculenta* was involved convincing evidence for the transmission of both B^a and B^c alleles from parental *esculenta* frogs to the F_1 progeny has been obtained by us (see crosses 2, 3, 11 and 12 in table 3). This demonstrates that *lessonae* chromosomes may not be totally discarded prior to meiosis, and recombination between les and rid genomes does occur in the hybrid frog. We shall discuss this point in more detail in the following section.

3. *Significance of protein patterns in relation to hybridogenesis and genomic recombination.* Immunologically offspring of the cross esc × esc resemble more the *ridibunda* than the *esculenta* phenotype (compare 6, 11 and 12 in figs 10 and 11). This is to be expected in view of the elimination of the *lessonae* genome in the *esculenta* gametes. On the other hand, the antigenic patterns between cross 6 (E × E) and cross 11 (E × R), in spite of their similarity, are not identical. The same is true in the synthesis of oocyte proteins. Compared to *lessonae,* the pattern of *esculenta* is much more similar to that of *ridibunda,* but the latter 2 show no identity (fig. 12). Our present working hypothesis is that modification of both mobility and concentration of individual protein components may result from the recombination of *lessonae* and *ridibunda* genomes, by either crossing over or chromosomal assortment. In a recent study of the premeiotic exclusion of *lessonae* chromosomes during hybridogenesis of *R. esculenta* Tunner and Heppich[28] mentioned the occasional retaining of *lessonae* chromosomes, resulting in the

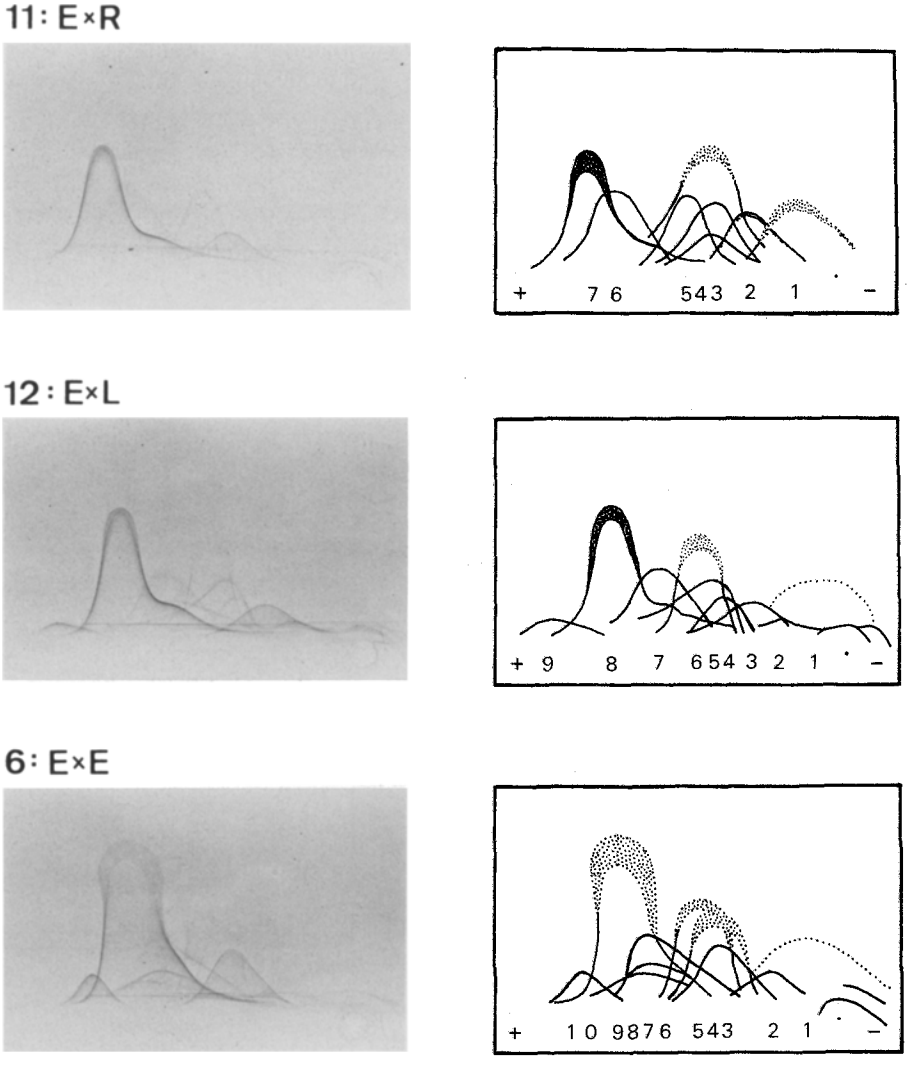

Figure 11. Two-dimensional immunoelectrophoresis of serum proteins from F_1 progeny of crosses 6 (E × E), 11 (E × R and 12 (E × L). Antisera against *ridibunda* were used. As indicated by the diagram at right of each electropherogram 7–10 precipitating fractions are visible.

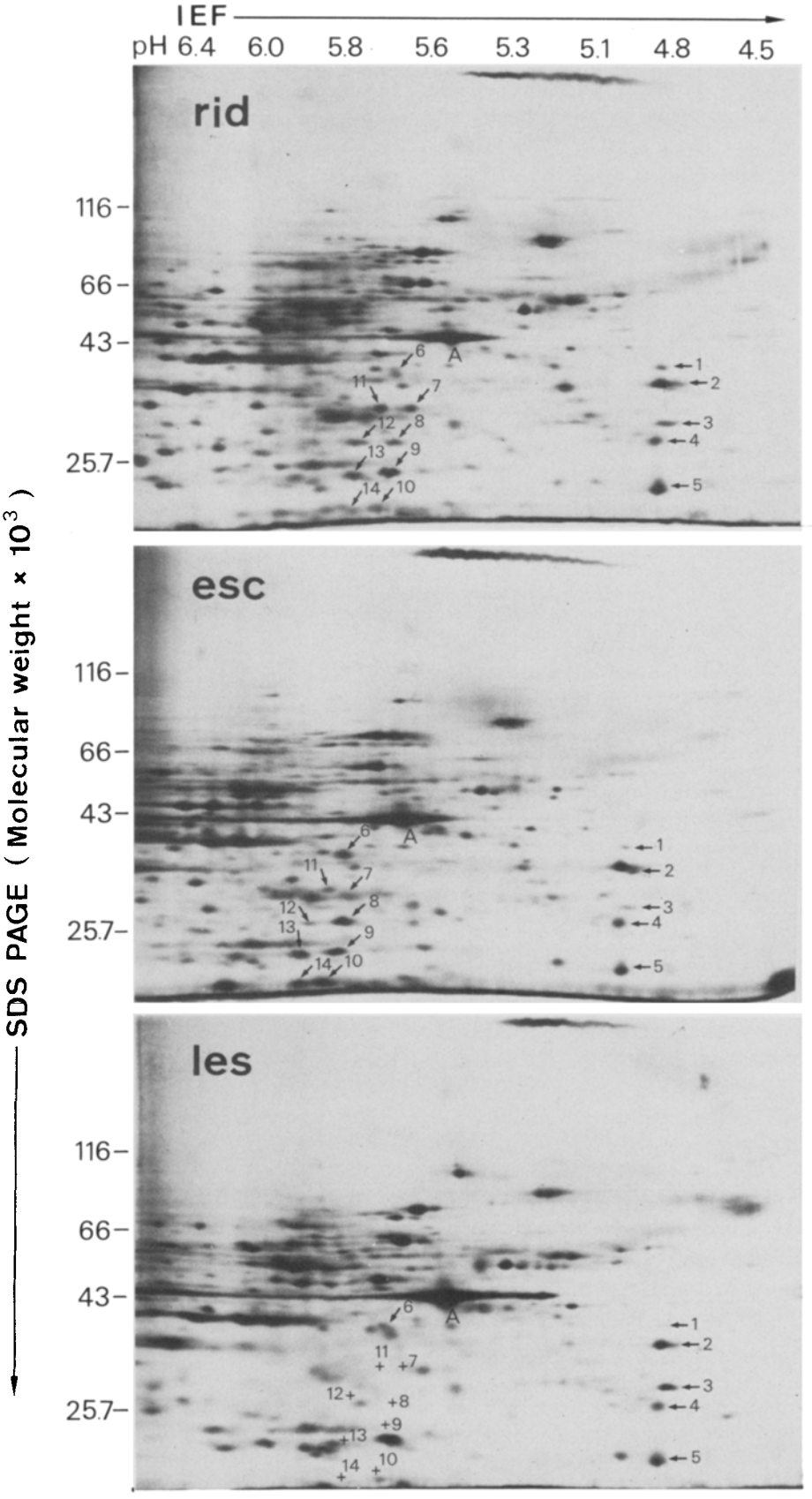

Figure 12. Autoradiographs of two-dimensional polyacrylamide gel electrophoresis of ^{35}S-labeled oocyte proteins in the three phenotypes *R. ridibunda* (rid), *R. esculenta* (esc) and *R. lessonae* (les). Positions of molecular weight markers are indicated at left (beta-galactosidase 116,000, bovine serum albumin 66,000, ovalbumin 43,000, alpha-chymotrypsinogen 25,700). Arrows indicate protein spots which are present in all 3 phenotypes, and crosses (+) indicate those which are not synthesized in *lessonae*. A refers to actin position. Exposure time of the autoradiograph was 4 days.

recombination of the two parental chromosome sets. Recombination of electrophoretic phenotypes in *R. esculenta* inter-se crosses has been previously reported by these authors[29,30]. A recombination rate of 2–3% for the R-E system has been estimated by Uzzell et al.[31] from a similar study of 13 crosses involving *R. esculenta* males. Further cytological and biochemical experiments by using specific chromosomal markers and more specific antisera are needed to clarify the situation.

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Reversibility of the transformed and neoplastic phenotype. III. Long-term treatment with electrophoretically pure mouse interferon leads to the progressive reversion of the phenotype of X-ray transformed C3H/10T1/2 cells

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Summary. Electrophoretically pure mouse interferon induced the progressive reversion of the transformed phenotype of a clone of X-ray transformed C3H/10T1/2 cells. Cells of this clone did not harbor C-type particles and reverse transcriptase activity was not detected. Interferon-treated transformed cells were aligned without cellular overlapping and attained low cell densities. Morphologic changes were associated with the