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Morphology of the F_1 Generation of Various Crosses within *Rana esculenta*-Complex

[Pp. 301—324, pl. X and 8 text-figs.]

Morfologia pokolenia F_1 różnych kombinacji żab zielonych (*Rana esculenta*-Complex)

Морфология поколения F_1 различных комбинаций зеленых лягушек (*Rana esculenta*-complex)

Abstract. Metamorphosed F_1 specimens produced by crosses of three forms of green frogs, viz. *Rana lessonae* Camerano, *R. esculenta* Linnaeus and *R. ridibunda* Pallas, fall into three morphological groups corresponding with them. The indices analysed suggest that only the progeny of *lessonae* and *ridibunda* inherit characters of their parents, whereas the offspring of *esculenta* exhibit, almost exclusively, characters typical of *ridibunda*. The group which reveals esculenta characters consist of hybrids obtained by crossing the form *lessonae* with *esculenta* or *ridibunda*. The author concludes that out of the three forms of green frogs, those of *lessonae* and *ridibunda* are species and the form *esculenta* is a hybrid produced, above all, by crossing the two others.

INTRODUCTION

A morphological analysis of adult specimens taken at the time of mating shows (Berger, 1966) that the green frogs in the Poznań region fall into three morphological groups, *Rana lessonae* Cam., *R. esculenta* L. and *R. ridibunda* Pall., there being no specimens with intermediate characters. Further observations on the development of various crosses between these forms show (Berger, 1967) that adult specimens of all the forms are highly fertile, and yet their progeny display distinctly varying degrees of viability. These data together with the results of a morphological analysis of metamorphosed specimens of this progeny will allow a fairly accurate determination of the degree of relationship between the three forms of green frogs.

In the literature devoted to green frogs I failed to find any data concerning the morphology of the F_1 generation except a mention on its colour (MANDEVILE and SPURWAY, 1949).

I should like to take the opportunity of expressing my gratitude to Prof. P. V. TERENTIEV, Leningrad, with whom I have discussed some problems of this paper, and Prof. H. SZARSKI, Cracow, for helpful advice and criticism in the course of this work.

I. MATERIAL AND METHOD

In the years 1963—1965 I obtained over 1700 metamorphosed specimens by various types of mating between three forms of green frogs, namely, *lessonae, esculenta* and *ridibunda* (BERGER, 1967). In this work I shall discuss only 1216 specimens (Table I), leaving out 482 specimens from 1963, intended for further breeding (I confined myself to measuring their body length), and about 30 from 1964, with markedly enlarged lymph sacs (BERGER, 1967), which made measuring impossible.

Specimens subjected to close morphological analysis were either fully metamorphosed or in the final stage of metamorphosis with their fore limbs already present. I shall deal with all the specimens together, as the morphological analyses have not detected any essential differences between them. The material was fixed in 3·5% formalin.

Measurement was confined to only those organs which in the case of adult green frogs enabled their division into three well-differentiated morphological forms (BERGER, 1966). The measurements, denoted by the abbreviations adopted in a previous work (BERGER, 1966), include:

L. — body length. In the presence of numerous specimens with tails the body length was measured from the snout tip to the base of the hind limbs and not to the end of the urostyle as in adult specimens. This length is slightly greater than the snout-vent length.

T. — length of the tibia.

D. p. — length of the first toe (digitus primus) of the hind limb.

C. int. — length of the internal metatarsal tubercle (callus internus).

Measurements were taken on the organs of the right side of body to an accuracy of 0·1 mm in the case of L. and T. and 0·02 mm for D. p. and C. int. A vernier caliper was used to measure the body lenght and in the other cases a binocular microscope furnished with eyepiece micrometer. In 1964, 263 specimens were intended for further breeding; these were measured to an accuracy of 0·1 mm.

The types of mating between the three forms of green frogs used to obtain F_1 animals are given in Table I. Complete series of 9 types of mating (the same specimens were used for crossing) are marked with the letter S. Each series and its particular crosses are marked with serial numbers and symbols of temperature (e. g., L. — 15°C, M. — 20°C) at which the eggs developed (Berger, 1967). Other details will be discussed, if need be, in the later sections.

Table I

Metamorphosed specimens of the F_1 generation from various crosses of green frogs. Classification according to morphological phenotypes. Description in the text

P generation			F_1 generation			
Combination			Phenotype			Total
female	male	Symbol	*lessonae*	*esculenta*	*ridibunda*	
les. × *les.*		LL	136	—	—	136
les. × *esc.*		LE	—	128	—	128
les. × *rid.*		LR	—	158	—	158
esc. × *les.*		EL	—	139	—	139
esc. × *esc.*		EE	—	6	166	172
esc. × *rid.*		ER	—	2	111	113
rid. × *les.*		RL	—	101	1	102
rid. × *esc.*		RE	—	—	99	99
rid. × *rid.*		RR	—	—	169	169
Total			136	534	546	1216

II. RESULTS

a. Morphology and coloration

A close morphological analysis shows that the division of metamorphosed specimens into morphological groups can be performed, as in adult individuals (Berger, 1966), on the basis of the size and shape of the internal metatarsal tubercle as well as the length of the first toe. Both these characters, easy to distinguish and correlated with other morphological features, and the colouration of specimens will be dealt with first.

The length of the metatarsal tubercle and that of the first toe have various mean values for different crosses (Table II). A comparison of these values shows clearly that the specimens form groups whose members are characterized by similar values. The progenies of the forms *esculenta* and *ridibunda* (EE, RR) and hybrids produced by their crosses (ER, RE) have the lowest mean values of the length of metatarsal tubercle (C. int.), i. e., 0·63—0·77 mm, whereas the highest value, 0·99 mm, clearly detached from the rest, is pertinent to the progeny of *lessonae* (LL). Hybrids obtained by crossing *lessonae* with *esculenta* and *ridibunda* (LE, LR, EL, and RL) make up a very compact group with intermediate values (0.88—0·91 mm).

The mean lengths of the first toe (D. p.) are distributed in reverse order. They are the highest, 2·13—2·41 mm, in EE, RE and RR specimens. The smallest body size of all (Table II) characterizes the hybrids ER, belonging to the same group. Their first toe is rather often distinctly shortened (Fig. 8), its length averaging 1·83 mm. Specimens LL attain the lowest mean value, 1·81 mm, and the hybrids LE, LR, EL and RL are, as previously, intermediate, their values being between 1·98 and 2·08 mm.

This pattern of both parameters accounts for the fact that the values obtained for the D. p./C. int. index allow the division of all the metamorphosed F_1 specimens into three distinct groups (Figs. 1 and 2), which in respect of morphological phenotype correspond to the adult individuals of the three forms of green frogs, *lessonae*, *esculenta* and *ridibunda* (Berger, 1966). For clarity, the same names are therefore applied for these morphological groups of the F_1 generation.

Table II

Lengths of different parts of body measured on metamorphosed F_1 specimens of green frogs (in mm.). Mean (M) and extreme (min., max.) values are given

Combination	N	L. min. M max.	T. min. M max.	D.p. min. M max.	C.int. min. M max
LL	136	14·0—18·32—22·5	5·1—7·00— 9·3	1·24—1·81—2·42	0·64—0·99—1.30
LE	128	14·0—17·68—21·8	5·0—7·25— 9·4	1·40—1·98—2·70	0·70—0·89—1·20
LR	158	15·0—17·82—24·5	5·3—7·20—10·0	1·40—1·98—2·80	0·62—0·88—1·20
EL	139	14·3—17·48—22·0	5·8—7·36— 9·8	1·50—2·08—2·74	0·68—0·89—1·32
EE	172	14·2—18·28—25·2	5·5—7·93—12·3	1·30—2·21—3·50	0·30—0·69—1·20
ER	113	14·0—16·17—20·0	5·2—6·86— 9·5	0·90—1·83—2·70	0·40—0·63—0·90
RL	102	14·0—17·87—26·5	4·7—7·61—10·8	1·30—2·08—2·82	0·56—0·91—1·30
RE	99	14·2—17·79—22·2	5·9—7·87—10·3	1·24—2·13—2·70	0·54—0·71—1·00
RR	169	14·5—18·98—23·5	5·9—8·67—11·8	1·70—2·41—3·50	0·50—0·77—1·05

The phenotype *lessonae* comprises only the progeny from one type of mating, LL. The metatarsal tubercles of its members are very poorly differentiated in morphological respect (Fig. 1). In most specimens they are very high and strongly compressed laterally; they are usually distinctly semicircular in shape, with their culmination situated at about half length, and only in a few cases they reveal some asymmetry.

The remaining two phenotypes include specimens from four crosses each, in which the metatarsal tubercles are always asymmetrical but of different

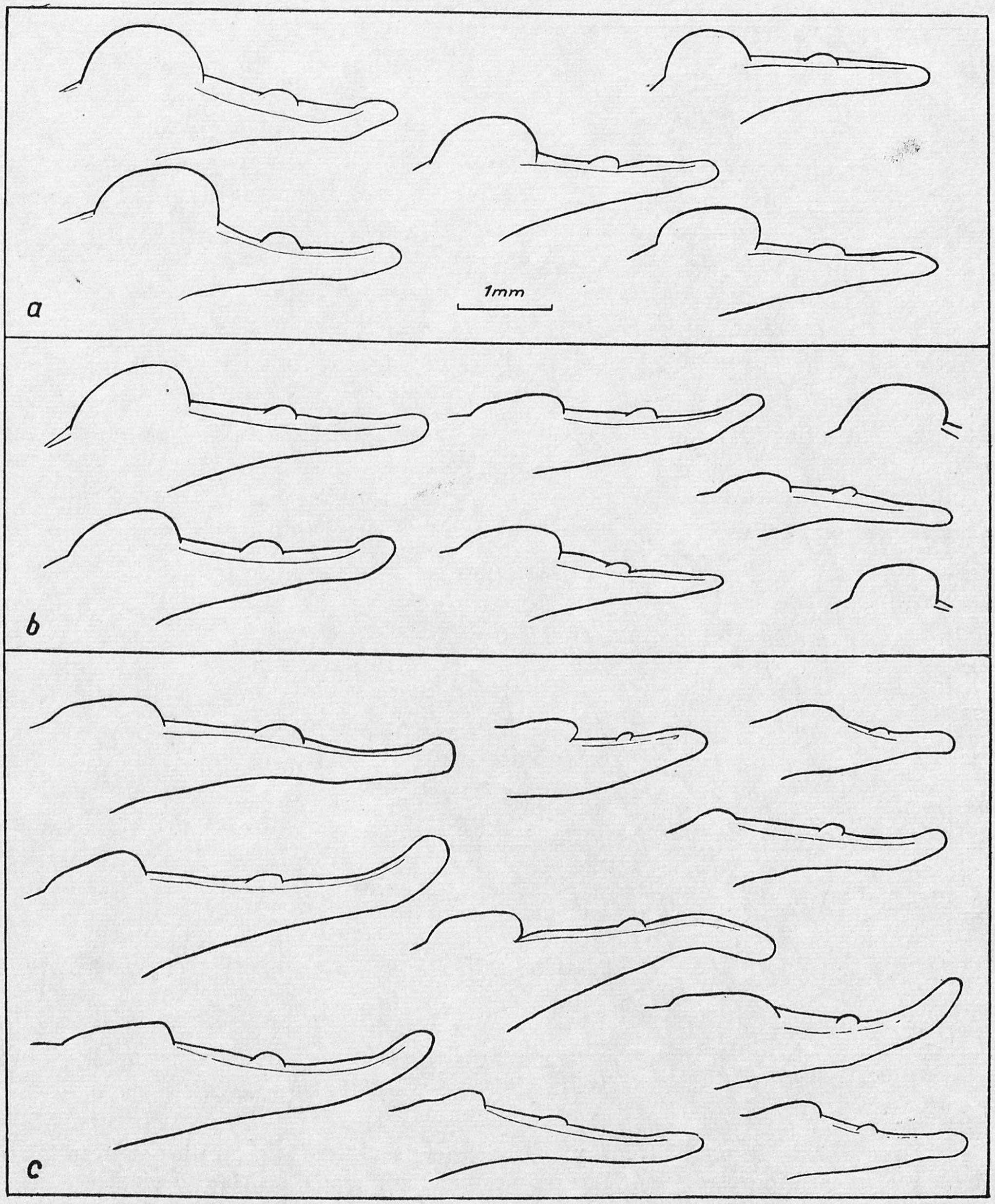

Fig. 1. Examples of differentiation in the internal metatarsal tubercle (C. int.) and the first toe (D. p.) of the F_1 generation of green frogs. Phenotypes: a. *lessonae*, b. *esculenta*, c. *ridibunda*. On the left-hand side are the drawings of typical specimens.

structure in each group. In the specimens showing characters of *esculenta* they are relatively large, rather high, markedly asymmetrical and never semicircular in shape. The highest point of the tubercle is shifted considerably towards the foot. Their structure is hardly differentiated within each of the four crosses

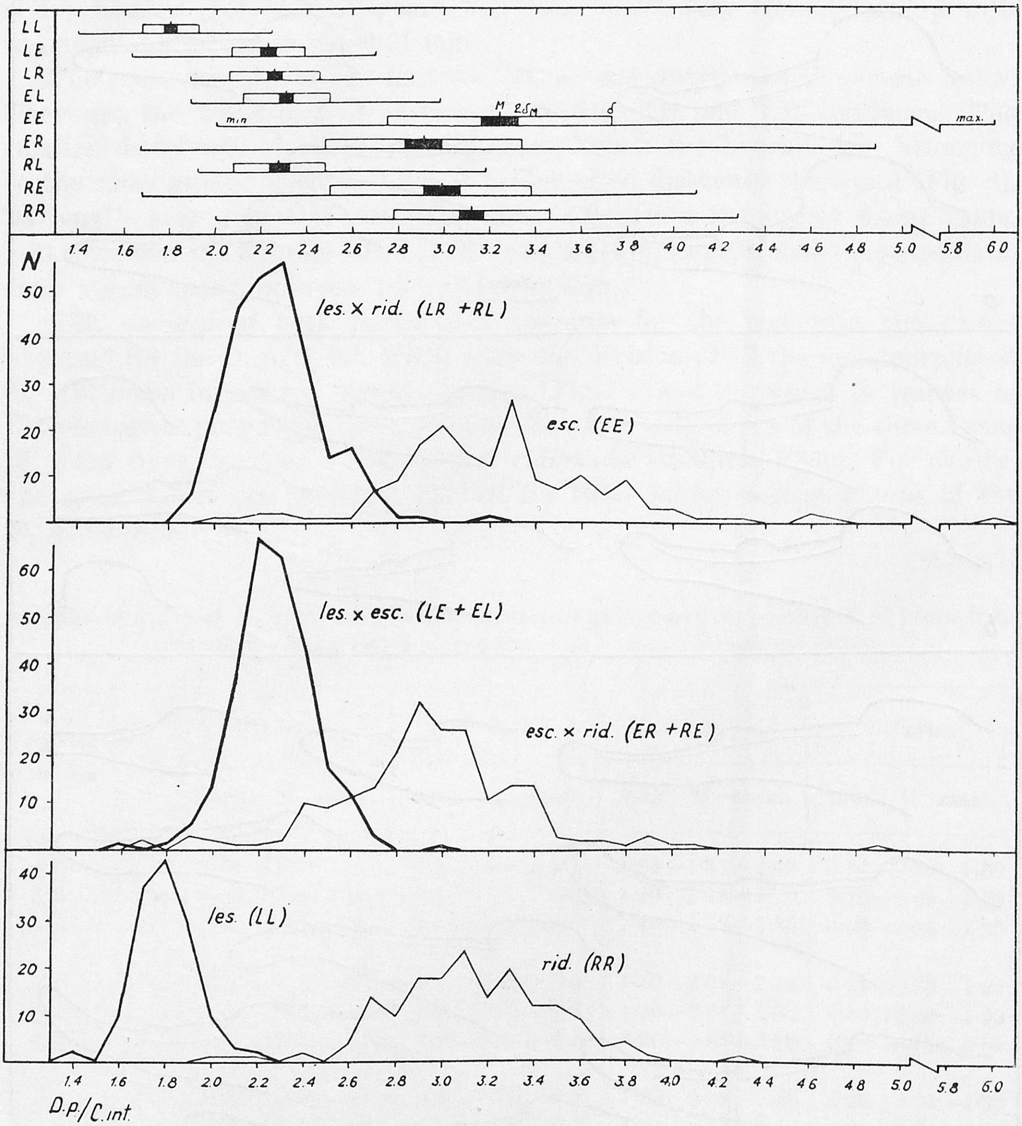

Fig. 2. Graphs of the D. p./C. int. index for various crosses of the F_1 generation of green frogs The linear graphs show the mean (M), extremes (min. and max.), standard deviation (δ) and two standard errors of the mean ($2\delta_M$). The axes of coordinates indicate the values of the D. p./C. int. index and the number of specimens (N).

representing the phenotype *esculenta*. Only one specimen in the RL group (Table I, Fig. 8) whose metatarsal tubercle resembles in structure that found in the specimens with characters of the form *ridibunda* differs apparently from this general description. It will be described in detail in a later section.

In the individuals of the phenotype *ridibunda* the metatarsal tubercles are very small, low and mostly with no distinctly marked culminations. However, there are frequent specimens whose tubercles are similar in shape to those in the phenotype *esculenta* but differ greatly from them in size (Fig. 1). In several specimens, which will be described more thoroughly a little further, the metatarsal tubercle has even a structure typical of *esculenta* and distinguishing them decidedly from the other specimens produced by the same parents (Figs. 7 and 8). On the other hand, some specimens of the phenotype *ridibunda* exhibit tubercles which in spite of their considerable length are quite low, poorly visible or only marked on the side of the toe and vanishing gradually without any conspicuous boundary towards the tarsus.

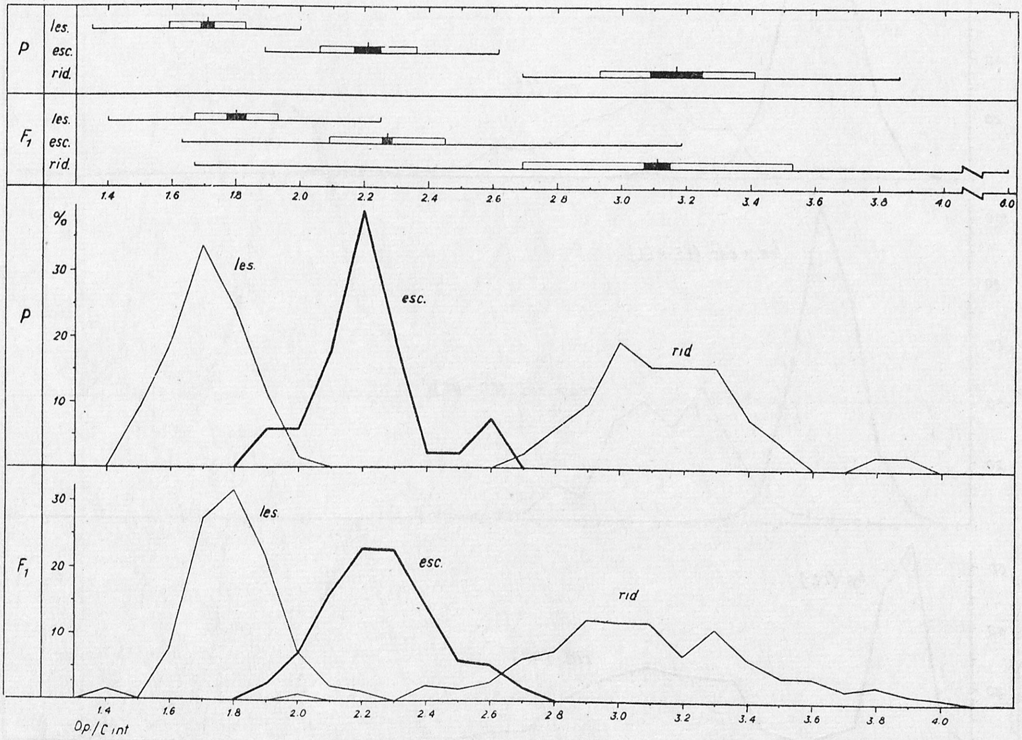

Fig. 3. Collective graphs of the D. p./C. int. index for the phenotypes *lessonae*, *esculenta* and *ridibunda* in the F_1 generation. The graphs for the P generation after BERGER (1966). Groups of less than 0·5% of the specimens have been excluded in the case of the F_1. The axes of coordinates indicate the values of the index and the percentage of specimens. For explanations see Fig. 2.

There is generally no difference in morphological structure of the first toe (D. p.) between the specimens of particular phenotypes. The hybrids ER, in which this toe is rather frequently obviously shortened and sometimes even underdeveloped (Fig. 8), make an exception. However, as mentioned above, there is in general a clearcut difference in length of the first toe between the individuals of particular phenotypes, and this is why a comparison of its length with the size and structure of the metatarsal tubercle makes it possible to

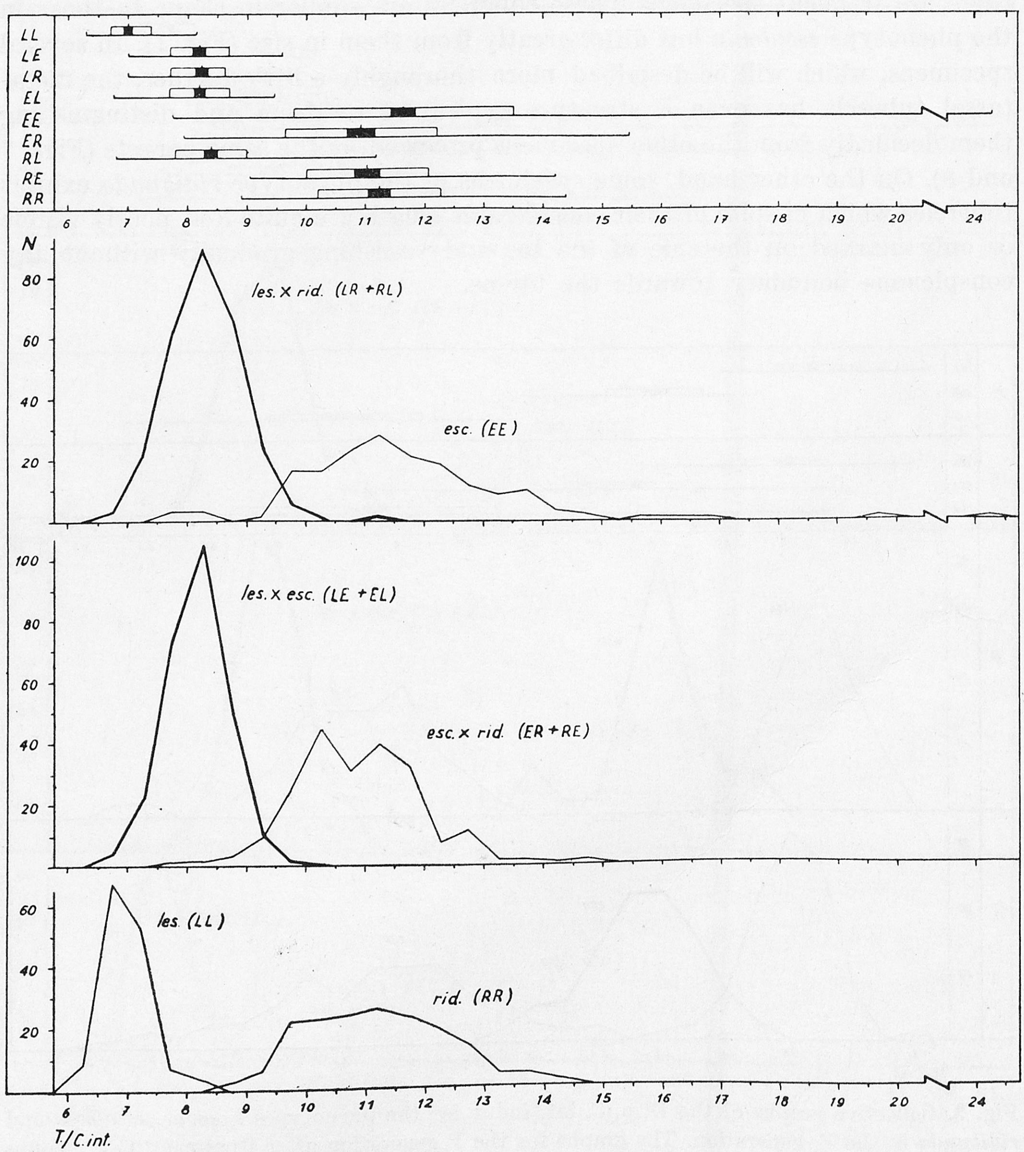

Fig. 4. Graphs of the T./C. int. index for various crosses of the F_1 generation of green frogs. For explanations see Fig. 2.

divide all the individuals of various crosses into three distinct morphological groups, even on cursory visual inspection. The other measurements correlated with the D. p./C. int. index do not allow this division without statistical analysis.

The morphological differentiation of F_1 individuals can be most readily demonstrated by the help of the D. p./C. int. and T./C. int. indices, which not only render it possible to divide all the specimens obtained by 9 different types of mating into the above-mentioned three frog forms (Figs. 2 and 4) but also facilitate a comparison between the P and F_1 generations (Figs. 3

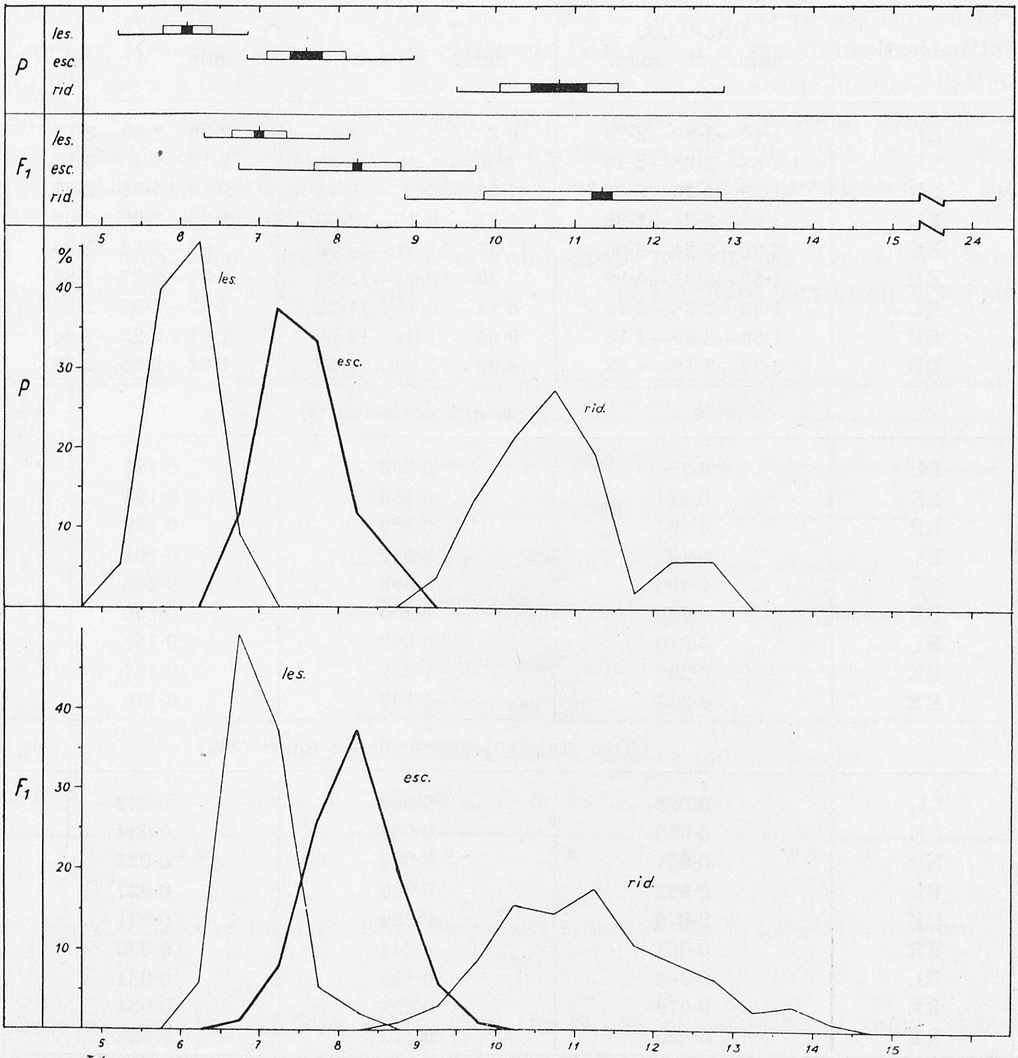

Fig. 5. Collective graphs of the T./C. int. index for the phenotypes *lessonae*, *esculenta* and *ridibunda* in the F_1 generation. The graphs for the P generation after Berger (1966). Groups of less than 0·4% of the specimens have been axcluded in the case of the F_1. For explanations see Fig. 3.

and 5). The indices are marked by the fact that their mean values for the morphological phenotypes of the F_1 generation approximate the corresponding values for adult individuals of *lessonae*, *esculenta* and *ridibunda*.

The differences between the phenotypes, as can be seen from the diagrams

Table III

Statistical parameters calculated for metamorphosed F_1 specimens of green frogs. Mean (M) and extreme (min., max.) values are given for indices

Combination	D.p./C.int. min. M max.	T./C.int. min. M max.	L./T. min. M max.
LL	1·40—1·80—2·25	6·30— 7·03— 8·15	2·29—2·63—3·12
LE	1·63—2·23—2·70	7·00— 8·20— 9·56	2·18—2·46—3·12
LR	1·89—2·26—2·86	7·00— 8·26— 9·75	2·19—2·48—2·97
EL	1·89—2·31—2·98	6·77— 8·21— 9·39	2·05—2·37—2·92
EE	2·00—3·24—6·00	7·67—11·67—24·30	1·90—2·32—2·96
ER	1·67—2·91—4·88	7·93—10·94—15·50	2·01—2·37—2·89
RL	1·92—2·28—3·18	6·82— 8·40—11·21	2·10—2·35—2·98
RE	1·68—2·99—4·10	9·05—11·04—14·20	1·97—2·25—2·86
RR	2·00—3·12—4·28	8·90—11·25—14·40	1·62—2·20—2·73
		Standard deviation (δ)	
LL	0·126	0·353	0·187
LE	0·164	0·496	0·194
LR	0·197	0·526	0·158
EL	0·190	0·510	0·161
EE	0·496	1·899	0·205
ER	0·429	1·295	0·190
RL	0·210	0·602	0·158
RE	0·392	1·031	0·167
RR	0·344	1·133	0·170
		Two standard errors of the mean ($2\delta_M$)	
LL	0·022	0·060	0·032
LE	0·029	0·088	0·034
LR	0·031	0·084	0·025
EL	0·032	0·086	0·027
EE	0·076	0·289	0·031
ER	0·081	0·244	0·036
RL	0·042	0·119	0·031
RE	0·079	0·205	0·034
RR	0·053	0·173	0·026
		Coefficient of variation (υ)	
LL	7·00	5·02	7·11
LE	7·35	6·05	7·89
LR	8·72	6·37	6·37
EL	8·22	6·12	6·79
EE	15·32	16·15	8·84
ER	14·74	11·82	8·02
RL	9·22	7·17	6·73
RE	13·11	9·04	7·42
RR	11·02	10·08	7·73

in Figures 2 and 4, are very great. They are particularly well reflected in standard deviation values, which are a measure of disperion of measurements in relation to their means, calculated for the specimens of all the 9 groups. The diagram presenting the D. p./C. int. index (Fig. 2) shows that the values of standard deviation overlap slightly for only three types of mating producing the phenotypes of *esculenta* (EL, RL) and *ridibunda* (ER). In the remaining cases they mostly show a distinct disjunction, thus bringing out the morphological individuality of phenotypes. And though all the ranges of variation, even those for crosses with extreme mean values, overlap considerably, yet this is true of a relatively small group of individuals, as is clearly demonstrated by the distribution curves (Fig. 2).

The differences between the morphological phenotypes are still better expressed by the T./C. int. index. The values of standard deviation are here

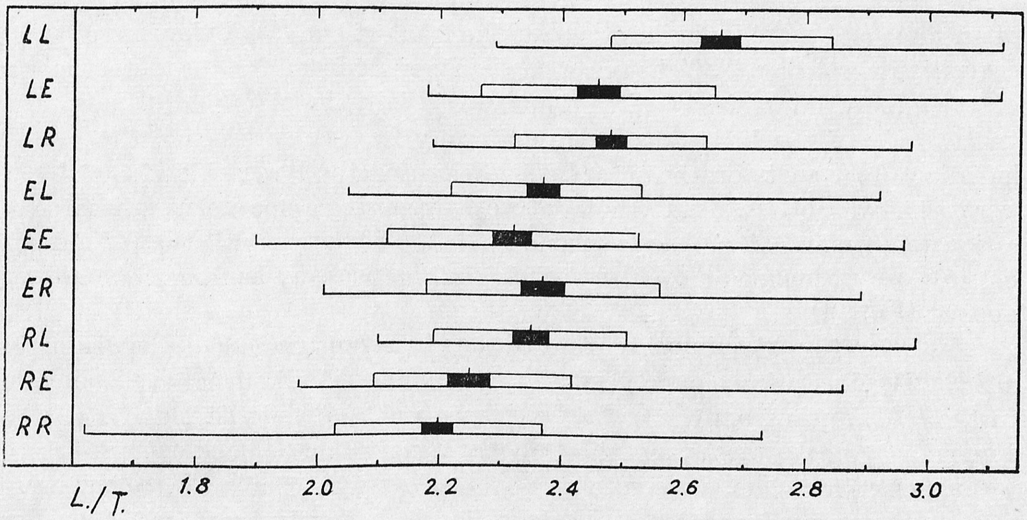

Fig. 6. Graphs of the L./T. index for various crosses of the F_1 generation of green frogs.

more distant (Fig. 4) than before, and the ranges of variation of some crosses representing the phenotypes with extreme values, i. e., *lessonae* and *ridibunda*, even show a disjunction.

The L./T. index indicates a distinct gradient of variation for the progeny obtained from crosses of all types, ranging from the lowest value for the offspring of *ridibunda* (RR) to the highest one for that of *lessonae* (LL, Fig. 6).

A clear correlation has been found between the morphological characters on the basis of which the frog forms were distinguished and the superficial structure of the skin as well as the coloration of body (Pl. X). Since these characters are meristic and qualitative, it is difficult to define them precisely and, therefore, I shall confine myself to their description.

A characteristic structural feature of the skin is the presence or absence of warts on its surface. In most cases the skin of individuals of the phenotype *lessonae* is smooth and glossy, whereas the individuals of the phenotype *ridibunda* have warts all over the body, but grouped most profusely on the upper side of the tibia and in the rear part of the dorsum. Specimens of the phenotype *esculenta* show intermediate characters; their skin is generally covered with warts only in the rear of the dorsum and belly (Pl. X) as well as in the upper part of the hind limbs, the rest of the body being usually smooth. However, among the specimens of all the phenotypes there are numerous divergencies from this general pattern.

Almost all the crosses belonging under the morphological phenotypes *lessonae* (LL) and *esculenta* (LE, LR, EL and RL) were characterized by a very similar colour of body when alive (Pl. X). Their dorsal side was mostly grass-green, more rarely dark-green and exceptionally grey-brown. Black spots with well-defined outlines were scattered on such a background. The spots were often more and more indistinct towards the front of body, but they were rarely completelly wanting. A light-green median streak from the head to the tip of the urostyle was fairly often found, especially in older individuals. The ventral side of the body was light, uniformly flesh-coloured, sometimes with indistinct dark spots on the throat. The belly and the lower side of the thighs were usually spotless. There, however, occurred single specimens whose coloration was typical of *ridibunda* (Table I, RL). They resembled the individuals of this last phenotype also in morphological respect, as has benn mentioned above (Fig. 8).

The coloration of living specimens of the phenotype *ridibunda* was distinctly differentiated. Dark colours generally predominated all over their bodies. In the individuals from the crosses *esculenta* and *ridibunda* (ER and RE) the dorsum was for the most part uniformly dark grey, almost black or dark-brown with a steel-brey hue, without any spots or streaks. Similar in colour were the specimens produced by *esculenta* (EE) which developed from the smaller eggs (Berger, 1967) whereas the specimens obtained from the larger eggs had usually their dorsum lighter. They very often appeared green, with distinct irregular black spots and, generally, a brighter streak along the dorsal median line. The dorsum of specimens (RR) produced by *ridibunda*, commonly dark-brown with a stell-grey hue had always black spots with blurred outlines. A brighter streak was seen along the dorsum, whose forepart as well as the head region were rather often green, but only in larger specimens.

In the specimens of *ridibunda* the ventral side was mostly grey with numerous dark spots. In smaller specimens, chiefly ER and RE, the spots were indistinct and usually distributed on the thighs and throat, whereas in the larger specimens they occured all over the body. A characteristic dark spot was fairly often seen above the sternum in the breast region in RE specimens.

Several individuals derived from *esculenta* parents (EE) and the ER hybrids differed evidently from this type of coloration, their dorsum being grass-green and belly spotless. These specimens resembled those of the phenotype *esculenta* (Fig. 8) also in respect of their morphological characters.

b. Heredity

The heredity of some morphological characters in green frogs presents itself as follows: virtually, the specimens of each group reveal distinct characters of one of the three frog forms (Figs. 2 and 4), but in some types of crosses there occur single individuals showing characters of other forms (Table I, Fig. 8). Omitting the phenotype *lessonae*, which includes the specimens of only one type of mating, LL, I shall discuss in detail the phenotypes *esculenta* and *ridibunda*, which are better differentiated.

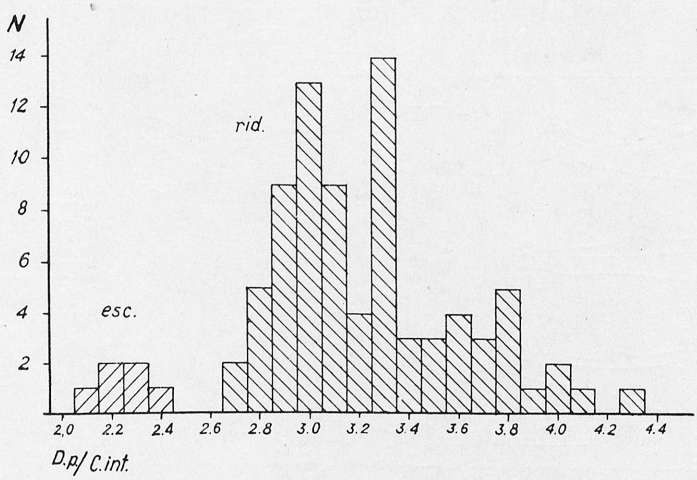

Fig. 7. Histogram of the D. p./C. int. index for the four series of the EE (*esculenta*) groups. containing metamorphosed specimens with characters of two forms (cf. Table IV). The axes of coordinates indicate the index values (D. p./C. int.) and the number of specimens (N)

Table IV

Series of the EE type of mating (*esculenta*) in which metamorphosed specimens with *esculenta* and *ridibunda* characters were found

Serie	Initial number of tadpoles	Dead tadpoles	Number of metamorphosed specimens		
			Phenotype		Total
			esculenta	*ridibunda*	
S4X	249	188	3	58	61
S4M	28	18	1	9	10
S3L	29	16	1	12	13
7L	84	83	1	—	1
	390	305	6	79	85

The phenotype *esculenta* contains hybrids from the crosses *lessonae* x *esculenta* (LE and EL) and *lessonae* x *ridibunda* (LR and RL), of which only the RL group comprises individuals with characters typical of the other two forms (Table I). Out of the total number of 102 metamorphosed specimens of this cross one individual of obscure origin had characters of *ridibunda*. This specimen belonged to the series RL-2M of 21 tadpoles, which developed together with 21 tadpoles of the series EE-S4X (this series will be described later) in a common aquarium, seperated from each other by a net partition (BERGER, 1967). Exchange of specimens between the two series through the net was hardly probable but not excluded. The individual in question was in no respect similar to the specimens of its own form (Fig. 8) it was extremely vigorous and in coloration and morphology closely resembled the metarmorphosed EE-S4X specimens, the members of the only series within the EE type of mating that provided

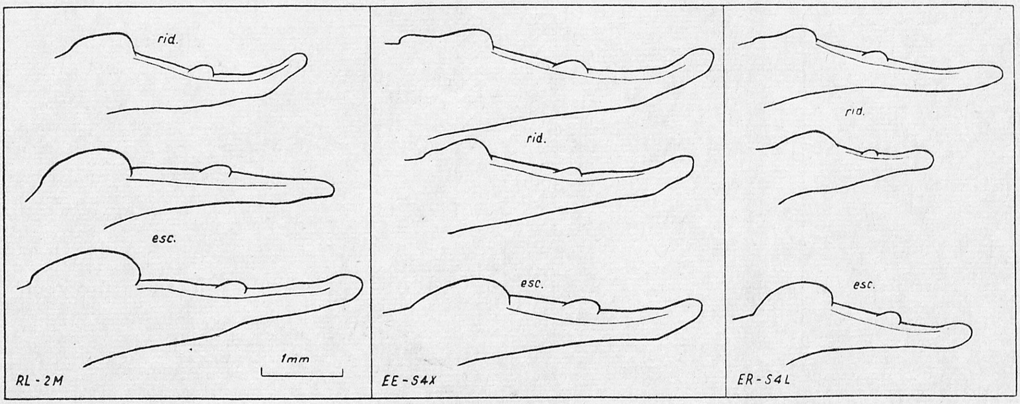

Fig. 8. Drawings of the internal metatarsal tubercle and the first toe of the members of three series (RL-2M, EE-S4X and ER-S4L) in which specimens with characters of the forms *esculenta* and *ridibunda* occurred. If the specimens of one of the forms were in the majority, two of them with extreme characters were selected for drawing. Description in the text.

numeras viable specimens, developing into adults. The remaining 19 metamorphosed specimens of the series RL-2M (1 tadpole was fixed) with their D. p./C. int. index ranging from 2·28 to 2·70 (on the average 2·47) revealed characters of the form *esculenta*, the value of this index for an individual with the characters of *ridibunda* being 3·18 (see Fig. 2).

The phenotype *ridibunda* comprises specimens descending from *esculenta* (EE) and *ridibunda* (RR) parents and their reciprocal combinations (ER and RE). Among the EE and ER specimens there were some single ones with distinct characters of *esculenta* (Table I, Fig. 8).

Most of the progeny of the form *esculenta* had morphological features characteristic of *ridibunda* (166 specimens) and only a few (6 specimens) had distinct parental characters. On closer inspection these last specimens appear to have derived from three or four different series, as shown in Table IV, the values of the D. p./C. int. index being presented for all the individuals of these series in a diagram (Fig. 7).

The tadpoles of the series S4M hatched from eggs squeezed out of three *esculenta* females into a suspenion of sperm from four *esculenta* males contained in a common vessel. In the same way was obtained the series S4X only that the eggs used were taken from only one of the females. Thus, it may well be that in these series all the F_1 individuals with morphological characters of the form *esculenta* came from the eggs of the same female. Two of the specimens died at the final stage of metamorphosis, and two others metamorphosed and were very viable. In the remaining two series there were only single individuals with the characters of *esculenta* and they were the last within this group to being metamorphosis, but died without attaining adult form as soon as their fore limbs had appeared.

It will be seen from Figures 7 and 8 that the individuals of these series fall into two morphological groups, these being no intermediate specimens. In collective surveys the differences between them are blurred (Fig. 2) owing to the presence of individuals with intermediate characters in the other series.

Out of the total number of 113 metamorphosed individuals of the cross *esculenta* × *ridibunda* (ER), two specimens showed the characters of *esculenta*, and the clear-cut characters of *ridibunda* were found in the others (Table I). The specimens with *esculenta* characters occurred in the series ER-S4L. Out of the 100 tadpoles of this series, 37 attained the final stage of metamorphosis, and 2 of them had the characters of *esculenta*. These two individuals metamorphosed on August 10, 1964, whereas the first metamorphosed specimens with *ridibunda* characters appeared in this series 12 days later, i.e., on August 22. Among the tadpoles descending from the same parents such a long interval between the first metamorphosed specimens and those succeeding them happened only once during my rearing of frogs, but I did not mention it in my previous papers (Berger, 1967).

c. Morphological Variation

The average body length (L.) of individuals of various types (Table II) may make the best example of morphological differentiation. The crosses representing the phenotype *ridibunda* (EE, ER, RE, RR) attain extreme values, which are 16·17 mm and 18·98 mm for ER and RR specimens, respectively. The specimens of the phenotype *esculenta* (LE, LR, EL, RL) form a very compact group with an average body length ranging from 17·48 to 17·82 mm. The phenotype *lessonae* holds a separate position (LL), its specimens attaining one of the greatest average body length, 18·32 mm.

A similar pattern is presented by the mean values of indices, as will be seen clearly from Table III and, above all, from the graphs in Figs. 2 and 4. These values are always more differentiated for the specimens with characters of the form *ridibunda* than *esculenta*.

The D. p./C. int. and T./C. int. indices have very similar mean values for the forms of both generations (Figs. 3 and 5), but each of them defines relations between them in a different way. The mean values of the T./C. int. index are always higher for the F_1 than for the P (Fig. 5). The mean values of the D. p./C. int. index are arranged in a similar way only for the phenotypes *lessonae* and *esculenta* (Fig. 3), whereas within the phenotype *ridibunda* its value is lower for the F_1 than for the P.

The differentiation of the F_1 individuals produced by different crosses can be evaluated best on the basis of the coefficient of variation, V, which for the D. p./C. int. and T./C. int. indices calculated for the specimens of all types of crossing, gives three well-marked groups corresponding to the three morphological phenotypes (Table III). The values of V, which are reflected by the course of the curves in Figures 2 and 4, accentuate the morphological distinctness of the particular phenotypes to a great extent. The phenotype *lessonae* (LL) has the lowest value of V, 7.00 for the D. p./C. int. index and 5.02 for the T./C. int. ratio and, at the same time, the most regular curves. The EE, ER, RE and RR individuals of the phenotype *ridibunda* show the highest values of V for both indices, 11·02—15·32 and 9·04—16·15, respectively, and simultaneously they have the most irregular curves. The phenotype-*esculenta* specimens (LE, LR, EL and RL) hold and intermediate position for both indices in respect of their coefficients of variation (7.35—9·22 and 6·05—7·14) as well as the course of the curves. The standard deviation and two standard arrors display a similar pattern (Table III). The foregoing three parameters show no connections with the phenotypic groups in the case of the L./T. index.

A comparison of the curves presenting both the indices worked out for the specimens of all the types of crossing together (Figs. 3 and 5) shows a situation similar to that described above. The values of the coefficient of variation for the D. p./C. int. index are 7.00 for *lessonae*, 7.75 for *esculenta* and 13·60 for *ridibunda*, and in the case of the T./C. int. index 5·02, 6·68 and 12·97, respectively. It should be emphasized that the specimens with characters of a definite form generally resemble each other very much also in respect of other morphological characters and body coloration. A close analysis of the specimens with characters of the form *esculenta*, which characters appeared in specimens obtained from as many as 6 different types of crossing (Table I) did not bring about the detection of characters in which they differed from each other. The same is true of the specimens with *ridibunda* characters, representing 5 different crosses.

d. Abnormalities

In general, individuals with abnormally developed organs were not frequent. None the less, some abnormalities, especially the absence of certain organs, influenced the number of obserwations and are, therefore, well worth mentioning.

One of the LR individuals had an abnormal left fore-limb. Its proximal part (humerus) was single, but the distal portion was duoble and ended in two normally developed palms, either of them with four fingers.

One specimen of the EL progeny had a stiff right hind limb and another lacked the first toe in both hind limbs.

In the EE progeny there were individuals with characters of the form *ridibunda* having various defects. One specimen of the series S4M lacked the left hind leg at all and in the series 5M the mandible was wanting in one individual. Out of the 4 specimens of the series 3M, one was normal, two had a stiff right hind-limb and in one both of them were stiff. In two specimens of the series 12M both hind-limbs were lacking in the first toe.

Lack of the first toe in the left hind-limb was found in two individuals of the ER progeny. In some dozen specimens of the RE progeny the skin in the region of the sternum on the abdominal side was fissured so that there was an opening into the abdominal cavity.

III. DISCUSSION

To begin with, it should be stated that the numbers of specimens in particular cross groups used for the elaboration of statistical parameters (Table III, Figs. 2—6) „indicate at a glance that statistical adequacy... is ordinarily fully attained when the white bar is more than twice as long as the dark" (Hubbs and Hubbs, 1953). This condition is fulfilled for all the indices studied, which guaranties that the conclusion made on the morphological differentiation of the F_1 generation of green frogs are adequately certain.

The characters on the basis of which all F_1 specimens may be readily and infallibly classified in definite forms of frogs are, as in adults (Berger, 1957, 1966), the structure and size of the metatarsal tubercle (C. int.) and the length of the first toe (D. p.).

The mean values of the D. p./C. int. and T./C. int. indices make it possible to divide the F_1 specimens derived from all the 9 types of mating into three distinct groups (Figs. 2 and 4), which, in respect of morphological phenotypes, correspond to the three forms of adult green frogs, viz., *lessonae*, *esculenta* and *ridibunda* (Figs. 3 and 5), well-known in Central Europe (Boulenger, 1898; Berger, 1966). The differences between the means of the D. p./C. int. index and those of the T./C. int. index (lower for the P generation and higher for the F_1) are obvious, as their values decrease with the growth of the specimens (Kauri, 1959; Terentiev, 1962).

On the other hand, the L./T. index, which divides the P specimens into three sepaerate groups (Berger, 1966), is not so important to F_1 specimens, as it shows a well-marked gradient of variation and has the lowest value for RR specimens and the highest for LL (Fig. 6).

Numerous reports concerning hybridization in amphibians (VOLPE, 1959; MECHAM, 1960a, 1960b; KAWAMURA and KOBAYASHI, 1960) and other groups of animals show that the „first-generation (F_1) hybrids are generally intermediate between the parental species and tend to be uniform in most characters“ (MAYR, 1963: 112). In relation to green frogs this general rule is confirmed only for F_1 specimens obtained from some of the hybrid combinations

It will be seen from an analysis of some morphological characters that only hybrids from the crossing of the forms *lessonae* and *ridibunda* (LR and RL, Figs. 2 and 4) show intermediate parental characters (Figs. 3 and 5). On the other hand, the hybrids obtained by crossing these forms with *esculenta* — *lessonae* × *esculenta* and *esculenta* × *ridibunda* — reveal the characters of the parent mentioned above in the second place. It might be inferred from this fact that, as regards the inheritance of these characters, the forms *lessonae* and *ridibunda* appear to be distinctly genetically differentiated toward the form *esculenta*. The characters of the form *lessonae* are recessive and those of *ridibunda* dominant.

Owing to this pattern of recessiveness and dominance of the characters of green frog forms, the particular phenotypes of the F_1 generation show a well-marked gradient of differentiation, in respect of origin of the progeny, in the direction from *lessons* through *esculenta* to *ridibunda*. The phenotype *lessonae* comprises individuals from only one type of mating, *lessonae* × *lessonae* (LL), the phenotype *esculenta* is represented by the specimens obtained by two types of crosses, *lessonae* × *esculenta* (LE and EL) and *lessonae* × *ridibunda* (LE and RL) and the phenotype *ridibunda* includes three groups of individuals: the progeny of the forms *esculenta* (EE) and *ridibunda* (RR) and their hybrid combinations (ER and RE).

A similar gradient of differentiation is also observed in the statistical parameters (δ, $2\delta_M$ and V; Table III) calculated for the D. p./C. int. and T./C. int. indices, which made the basis for the division of all the F_1 specimens into three phenotypes. These data would indicate that the morphological differentiation in F_1 specimens of green frogs reflects their genetic differentiation.

Special attention should be given to the specimens with characters of the forms *esculenta* and *ridibunda*, most of which were produced by hybridization. Before dealing with them I should like to recall the facts that have been found so far.

The experiments on hybridization of green frogs suggest (BERGER, 1967) that all the three forms of these frogs are highly fertile and the progeny obtained from 9 types of their crossing are viable and metamorphose. They form 3 distinct groups (Figs. 2 and 4), which agree in morphological phenotype with adults of the 3 forms of green frogs (Figs. 3 and 5) taken in amplexus at breeding sites (BERGER, 1966).

On the basis of the foregoing facts it may be supposed that these phenomena have, in all probability, a similar course in the wild. This conclusion is confirmed by the observations made by LÁC (1959) and BERGER (1964, 1966), who found specimens of various forms of green frogs in amplexus in the field, which would point to a possible origin of the hybrids. BERGER (1964), besides, found specimens belonging to different forms among newly metamorphosed green frogs. The D. p./C. int. index calculated for these specimens, caught in a pond, ranged from 1·41—2·04 (averaging 1·72) for 166 specimens of *lessonae* and from 1·90 to 2·69 (averaging 2·24) for 45 esculenta specimens, which is fully consistent with the values characterizing the F_1 specimens of the corresponding forms obtained experimentally (Table III).

In the light of these data special importance is, above all, ascribed to the specimens with characters of the form *esculenta*, for Table I and the graphs in Figures 2 and 4 show that most of these specimens (98·86%) are hybrids produced by the crosses *lessonae* × *esculenta* (LE and EL) and *lessonae* × *ridibunda* (LR and RL), whereas the offspring of the form *esculenta*, which should make the stock of the F_1 generation with *esculenta* characters, for the most part reveal the characters of the form *ridibunda* (Table IV, Fig. 7). VOLPE'S statement (1959) that the „convincing data requires the demonstration that experimental hybrids between the two species resemble individuals collected in mixed breeding aggregations" is confirmed here to the full extent, being in accordance with the findings presented in this report in reference to individuals of both generations of the phenotype *esculenta*.

If it is therefore assumed that under natural conditions, as in a laboratory, individuals axhibiting characters of the form *esculenta* are produced by hybridization, it must be accepted, according to the foregoing reasoning, that adult individuals caught in the field are also hybrids and their progeny obtained experimentally would constitute the F_2 generation.

Whether this is true is not known. It is, however, known that the progeny of the form *esculenta* have characters unencountered in a progeny of normal populations of a definite species. The most important of these charaters will be discussed below.

Hybrids produced by crossing different species of amphibians are usually marked by numerous negative characters if they reach adult age at all. The most important of them are: the adaptive inferiority of hybrids, their sterility or limited reproductive power (MECHAM, 1961; MAYR, 1963; MICHAŁOWSKI, 1964). This is not the case with green frogs, since all the three forms of these frogs are very fertile and in all their possible crosses they show a very high proportion of fertilized eggs (BERGER, 1967), in which they would resemble the hybrids and parental species of some American anurans (VOLPE, 1959; MECHAM, 1960a, 1960b). However, the progeny of green frogs are characterized by peculiar features. In all the series of each cross they nearly alwyas went through complete metamorphosis and the metamorphosed specimens were viable. An axception was the offspring from different series of the form *esculenta*; these exhibited clear-cut differences in degree of viability (BERGER, 1967). Out of the 35 females fertilized by males of the same form, only one produced offspring which, in large numbers, attained complete metamorphosis (Table IV,

Fig. 7), hardly a few offspring (5 specimens all together) from 4 other females metamorphosed: it should be emphasized that these specimens had developed from exceptionally large eggs. The progeny of the remaining 30 females died at various stages of development (Berger, 1967).

The morphology of individuals obtained from the form *esculenta* (EE), however, appears to be the most enigmatic. In some characters most of them are not similar to their own parents but resemble the offspring of *ridibunda* parents (RR) (Figs. 2 and 4).

These facts seem to indicate that the form *esculenta* is really made up of F_1 specimens produced by hybridization. To the best of my knowledge, in literature there are no examples (Mayr, 1963) of individuals belonging to an indisputable species — the form *esculenta* is regarded as such — and producing offspring which either die in the course of development or, in fact the greater part of them, are similar to the offspring of the related species as is in the present case, according to many authors, the form *ridibunda*.

The conclusion that the progeny of the form *esculenta* is an F_2 generation would also be supported by the fact that the EE specimens have the highest value of standard deviation (dispersion; Table III), the widest ranges of variation of morphological characters (Figs. 2 and 4) and the most strongly differentiated coloration of body. These findings resemble the data obtained by Volpe (1959) and concerning the inheritance of characters in toads. Toad specimens caught in the field and similar to the hybrids obtained by crossing *Bufo terrestris* with *B. fowleri* gave offspring (F_2) marked by a considerably wider range of variation of characters than they were themselves.

Observations made by Lác (1959) and Berger (1964, 1966) show that mixed pairs of various forms of green frogs are met with under natural conditions. Thus, it may be assumed that, as in a laboratory, hybrids of various origin and with similar variation of characters appear in the wild. A comparison of variation in morphological characters between the specimens of both generations, P and F_1 (Figs. 3 and 5), however, reveals that under natural conditions there are some rather obscure mechanisms, which effectively counteract the disappearance of morphological differences between specimens of particular forms. This opinion is corroborated by observations made by Lác (1959) in Slovakia and by Berger (1964, 1966) in the Poznań region. Among the 679 adults of the three forms of green frogs collected in different habitats, Lác found only 2 individuals with characters intermediate between the forms *esculenta* and *ridibunda*, whereas Berger (1966) found no such specimens among the 276 individuals caught at breeding sites and only two intermediate specimens among the 796 newly metamorphosed members of the forms *lessonae* and *esculenta* (Berger, 1964).

These mechanisms, about whose action we know very little, probably play part of isolating mechanisms contributing to the speciation of green frogs. Further investigations on adults and F_1 specimens are needed for their detection.

IV. SUMMARY AND CONCLUSIONS

It will be seen from the material presented that the relationship between the forms of green frogs is a complex matter. The occurrence of three distinct morphological forms, *lessonae*, *esculenta* and *ridibunda*, whose taxonomic rank is undoubtedly various, side by side under natural conditions, being an obvious fact, should be amployed as a starting point for their discussion.

The metamorphosed F_1 specimens obtained from 9 different crosses of these forms also fall into three groups (Figs. 2—5), which agree in phenotype with the groups of adults of green frogs caught in amplexus.

General analysis of the inheritance of characters reveals that

1. only the forms *lessonae* and *ridibunda* produce offspring which resemble their parents, whereas the hybrids from their crosses have intermediate characters and constitute the form *esculenta* (Figs. 2 and 4),

2. the *esculenta* form produces offspring which have, for the most part (96·51% of the specimens), characters typical of the progeny of the form *ridibunda*, and

3. specimens of the F_1 generation with characters of the form *esculenta* were almost all (98·86%) produced by hybridization (Table I).

These facts seem to confirm the conclusion made in the previous paper (BERGER, 1967) that the forms *lessonae* and *ridibunda* are species and the form *esculenta* is a hybrid produced by crossing them. This would, in turn, lead to the conclusion that the offspring of the form *esculenta* is the F_2 generation.

Such individuals with *esculenta* characters are also produced by the cross *lessonae* × *esculenta* and they appear occasionally among the offspring from other types of mating as well (Table I). In spite od such a great genetic differentiation, all the specimens with *esculenta* characters much resemble each other in phenotype.

It is very difficult to explain these phenomena and the interrelation between the groups of these individuals, which may be associated with the differences in viability of the F_1 generation obtained from *esculenta* parents (BERGER, 1967).

The form *esculanta* can be crossed with the forms *lessonae* and *ridibunda* and produces viable offspring (introgressive hybridization?), which, however, have *esculenta* characters in the case of the cross *lessonae* × *esculenta* and *ridibunda* characters in the case of the cross *esculenta* × *ridibunda*. Hence it follows that the characters of the form *esculenta* are dominant in relation to those of *lessonae* but recessive in relation to the characters of *ridibunda*. The dominance of *ridibunda* characters is also manifested in the progeny of the form *esculenta* (Figs. 2 and 4).

Owing to this dominance of characters, the phenotypes of the F_1 generation show a distinct gradient in their genetic differentiation from *lessonae* (one type of offspring) through *esculenta* (two types) to *ridibunda* (three types), which is best seen from the arrangement of the curves in Figures 2 and 4. A similar gradient in differentiation is exhibited by the statistical parametres of the D. p./C. int. and T./C. int. indices (Table III). It might be inferred that there is an unknown correlation between these two phenomena.

The progeny obtained by mating specimens belonging to definite forms have, as a whole, the characters of a definite form of green frogs (Table I, Figs. 2 and 4). This fact allows a very likelyanticipation of the occurrence of some characters in the F_1 generation.

The F_1 offspring obtained experimentally display a very great dispersion of characters and, therefore, there are some intermediate specimens between the particular phenotypes (Figs. 2 and 4); on the other hand, there are no such specimens or only very few among both young (Berger, 1964) and adult (Lác, 1959; Berger, 1966) animals caught in the field. These observations suggest that there are some unknown isolating mechanisms acting in nature, which probably contribute to the speciation of green frogs by preventing the obliteration of morphological differences.

So far, the investigations of relashionships between the forms *lessonae*, *esculenta* and *ridibunda* have been confined exclusively to the frog population inhabiting the Poznań region. They show that the forms *lessonae* and *ridibunda* have characters of species, whereas the form *esculenta* is a hybrid. However, it is not known whether relations similar to those described above exist between the populations of these forms living together in other parts of Europe. In order to solve these problems, connected directly with those of speciation, further studies are needed.

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STRESZCZENIE

Autor omawia morfologię 1216 przeobrażonych osobników pokolenia F_1 różnych kombinacji żab zielonych (Tab. I). W oparciu o dwa wybrane wskaźniki i opracowane parametry statystyczne wykazano (Tab. III), że osobniki F_1 rozpadają się na trzy grupy morfologiczne (Fig. 2, 4), które odpowiadają pod względem fenotypu trzem formom żab zielonych *Rana lessonae* CAM., *R. esculenta* L. i *R. ridibunda* PALL. (Fig. 3, 5).

Do fenotypu *esculenta* należą w przeważającej większości hybrydy (98,86% okazów) powstałe głównie ze skrzyżowania form *lessonae* × *esculenta* i *lessonae* × *ridibunda*, natomiast fenotyp *ridibunda* obejmuje potomstwo formy *esculenta* i *ridibunda*, oraz hybrydy powstałe z ich skrzyżowania.

Wśród wszystkich kombinacji tylko dwie kombinacje homospecyficzne, *lessonae* i *ridibunda*, wydają potomstwo, które dziedziczy cechy rodziców. W potomstwie formy *esculenta* w przeważającej większości (96,51% okazów) pojawiły się osobniki o cechach potomstwa formy *ridibunda*, a tylko nieznaczna ich część (3,49%) ma wyraźne cechy rodziców.

W konkluzji autor dochodzi do wniosku, że forma *Rana esculenta* LINNAEUS, którą uważano dotąd za dobry gatunek, jest hebrydem, natomiast formy *Rana lessonae* CAMERANO i *R. ridibunda* PALLAS wykazują cechy gatunków.

РЕЗЮМЕ

Автор обсуждает 1216 экземпляров после метаморфоза поколения F_1 различных комбинаций зеленых лягушек (Табл. I). Опираясь на двух показателях и отработанных статистических параметрах обнаружено (Табл. III), что особи F_1 распадаются на три морфологические группы (Фиг. 2, 4), которые по фенотипу соответствуют трём формам зеленых лягушек *Rana lessonae* Cam., *R. esculenta* L. и *R. ridibunda* Pall. (Фиг. 3, 5).

К фенотипу *esculenta* принадлежат в большинстве случаев гибриды (98,86% экземпляров) полученные, главным образом, путём скрещивания форм *lessonae* × *esculenta* и *lessonae* × *ridibunda*, зато фенотип *ridibunda* охватывает потомство формы *esculenta* и *ridibunda*, а также гибриды полученные из скрещивания их.

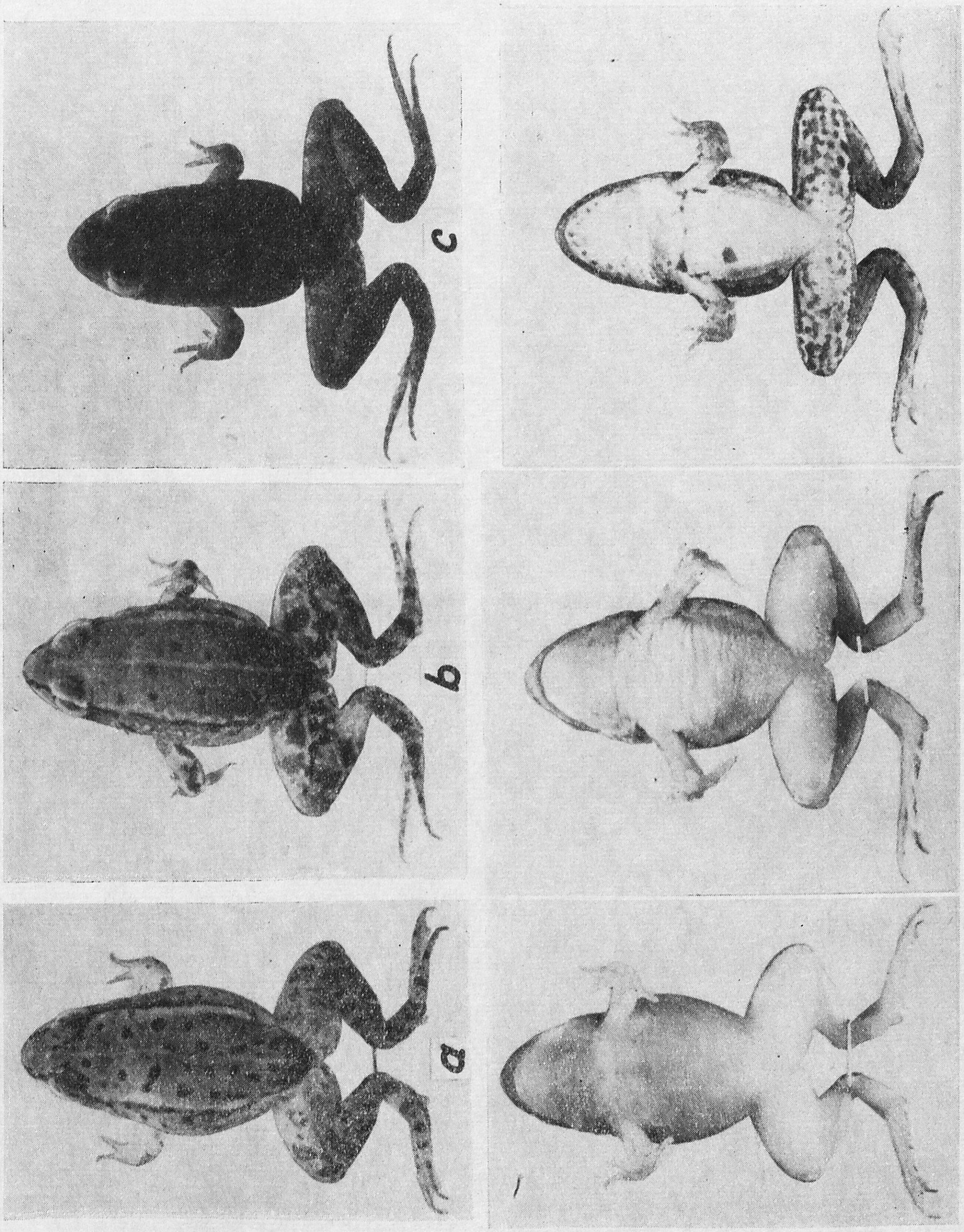
Среди всех комбинаций только две гомоспецифические, *lessonae* и *ridibunda*, дают потомство, которое наследует признаки родителей. У потомства формы *esculenta* в большинстве случаев (96,51% экземпляров) появились особи по признакам похожим на потомство *ridibunda*. Лишь незначительная их часть (3,49%) имеет чёткие признаки родителей.

В заключении автор приходит к выводу, что форма *Rana esculenta* L., которую до сих пор считали за вид, является гибридом, а формы *Rana lessonae* Cam. и *R. ridibunda* Pall. обладают признаками видов.

PLATES

Plate X

Metamorphosed specimens representing the phenotypes *lessonae* (a), *esculenta* (b) and *ridibunda* (c)



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