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On the occurrence and identity of triploids of *Rana* kl. *esculenta* Linnaeus and *R. lessonae* Camerano in The Netherlands (Anura: Ranidae)

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Abstract

According to electrophoresis and erythrocyte size the genotypes of 756 waterfrogs, collected during 1986–1988 in 54 localities in The Netherlands, were classified as belonging to 5 different genotypes: 331 diploid *R. lessonae* (LL), 5 triploid *R. lessonae* (LLL), 250 diploid *R.* kl. *esculenta* (LR), 133 triploid *R.* kl. *esculenta* (LLR), and 37 diploid *R. ridibunda* (RR).

The occurrence of triploid *R.* kl. *esculenta* in The Netherlands is reported for the first time and triploid *R. lessonae* has not yet been reported previously. There are indications that LL gametes could be produced by LLR triploids and LL diploids. *R.* kl. *esculenta* in *R.* kl. *esculenta* and *R. ridibunda* – *R.* kl. *esculenta* populations of the western regions seems to be exclusively triploid, whereas the percentage of triploid *R.* kl. *esculenta* in *R. lessonae* – *R.* kl. *esculenta* populations of the eastern regions is about 1%.

Biometrical differences were neither found between *R.* kl. *esculenta* triploid and diploid, nor between *R. lessonae* triploid and diploid.

Résumé

En se basant sur l'électrophorèse et sur les dimensions des érythrocytes, les génotypes de 756 grenouilles vertes, collectées de 1986 à 1988 dans 54 localités des Pays-Bas, sont rangés dans 5 génotypes différents: 331 exemplaires de *R. lessonae* (LL) diploïde, 5 de *R. lessonae* (LLL) triploïde, 250 de *R.* kl. *esculenta* (LR) diploïde, 133 de *R.* kl. *esculenta* (LLR) triploïde et 37 de *R. ridibunda* (RR) diploïde.

L'existence de *R.* kl. *esculenta* triploïde est démontrée pour la première fois dans les Pays-Bas, et des *R. lessonae* triploïdes n'ont pas encore été décrites. Il y a des indications que des gamètes LL peuvent être produits par des LLR triploïdes et des LL diploïdes. Il paraît que tout *R.* kl. *esculenta* est triploïde dans des populations de *R.* kl. *esculenta* ou de *R. ridibunda* – *R.* kl. *esculenta* des zones occidentales du pays, tandis que le pourcentage de *R.* kl. *esculenta* triploïde est d'environ 1% dans des populations de *R. lessonae* – *R.* kl. *esculenta* des zones orientales.

On n'a pas trouvé des différences biométriques entre *R.* kl. *esculenta* triploïde et diploïde, ou bien entre *R. lessonae* triploïde et diploïde.

Introduction

The edible frog *Rana* klepton (thief in Greek) *esculenta* Linnaeus, 1758 is hybridogenetic, its genome containing parental chromosome sets of both the little waterfrog *Rana lessonae* Camerano, 1882 and the lake frog *Rana ridibunda* Pallas, 1771 (cf. Günther, 1987). Both parental species and the hybrid form occur in The Netherlands (Wijnands & Van Gelder, 1976; Wijnands, 1977).

In most cases *R.* kl. *esculenta* coexists with one of the parental species and is maintained by backcrossing with the syntopic parental species, clonally transmitting the genome of the allotopic parental species (Günther & Plötner, 1988).

Mixed diploid *R. lessonae* – *R.* kl. *esculenta* populations are the most widespread within the geographical range of *R.* kl. *esculenta* (western and central Europe). In these populations *R.* kl. *esculenta* generates *ridibunda* (R) gametes and is maintained by backcrossing with *R. lessonae*, which produces *lessonae* (L) gametes. Crosses of *R.* kl. *esculenta* × *R.* kl. *esculenta* are usually lethal and *R. ridibunda* is extremely rare in such populations.

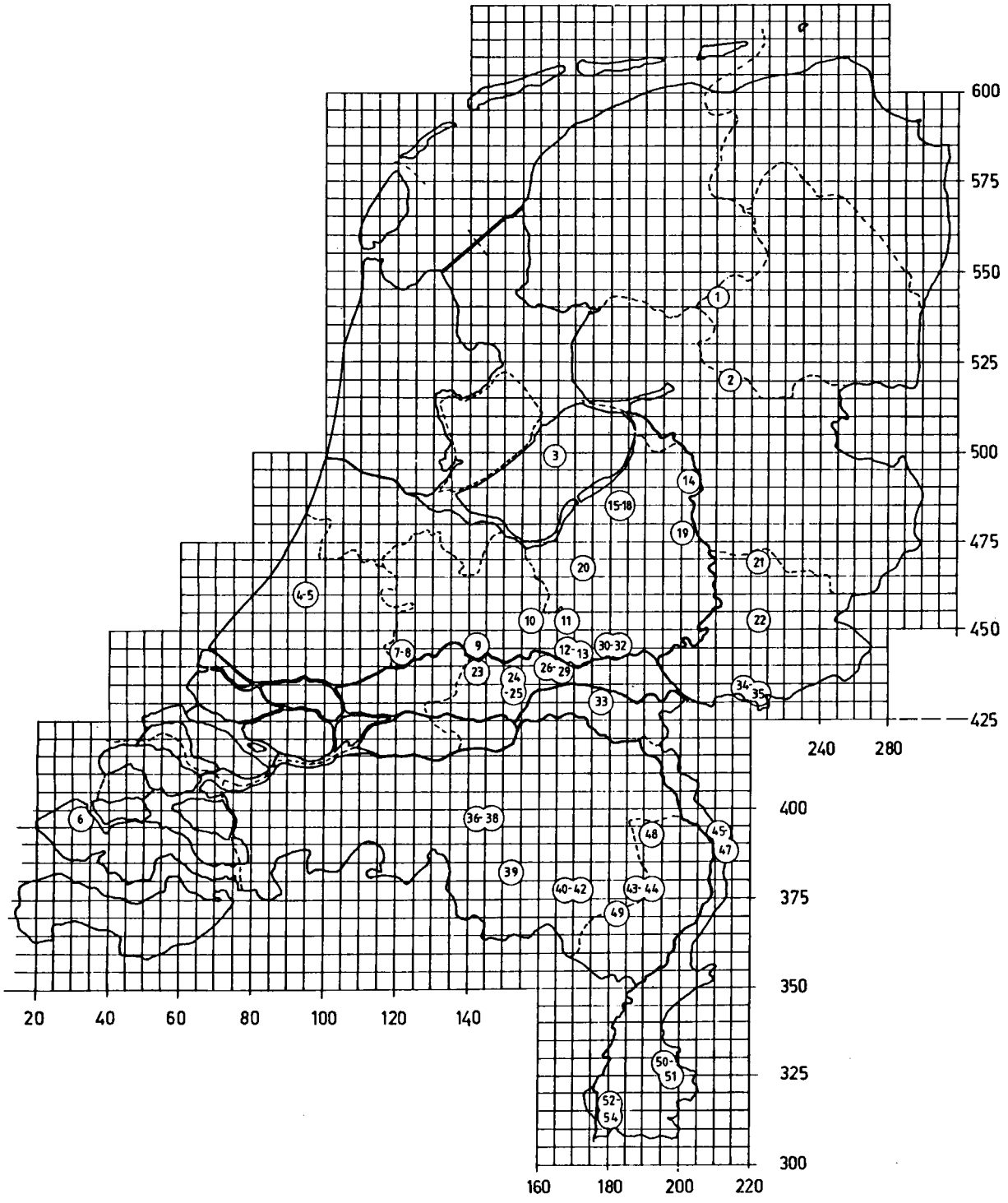

Fig. 1. Distribution of collecting sites. The encircled numbers refer to localities in Table I. Coordinates according to the Amersfoort grid (quadrats are 25 km²).

Both mixed *R. ridibunda* – *R.* kl. *esculenta* populations and *R. lessonae* – *R.* kl. *esculenta* populations are known only from The Netherlands, East and West Germany and Poland (Günther, 1975; Berger, 1977; Wijnands, 1977; Rahmel, 1988).

Populations, containing only *R.* kl. *esculenta* individuals, comprise always a large fraction of triploids and have been found in the northern parts of East and West Germany and Poland and in the southern part of Sweden (Günther, 1975; Berger, 1977; Ebendal & Uzzell, 1982; Eikhorst, 1984). In (2n and 3n) *R.* kl. *esculenta* populations LLR (two *lessonae* and one *ridibunda* genomes) triploids largely outnumber the LRR (one *lessonae* and two *ridibunda* genomes) ones. Studies on these pure *R.* kl. *esculenta* populations indicate that LLR triploids generate mainly haploid fertile L gametes, formed after normal genetic recombination processes, thus replacing the parental *R. lessonae*, while diploid LR males produce R gametes and LR females produce LR or R ova. Reproduction seems to depend essentially on the mating of diploid LR females with triploid LLR males, which results in both LLR and LR progeny. Other crosses seem to be much less successful. The pure *R.* kl. *esculenta* population is thus a modified *lessonae/esculenta* system (Günther et al., 1979; Günther, 1983; Berger & Günther, 1988; Eikhorst, 1988b; Borkin et al., 1989).

The present paper reports on the genotypes of waterfrogs and occurrence of triploid *R.* kl. *esculenta* in The Netherlands. Data were collected during a study on the distribution, ecology, and need for more adequate protection of *R. lessonae*. As the LLR triploid *R.* kl. *esculenta* can take the place of *R. lessonae* functionally in the maintenance of *R.* kl. *esculenta*, it is potentially threatening the survival of *R. lessonae*. Ecological and conservational aspects will be treated elsewhere.

Material and methods

According to the distribution map provided by Bergmans & Zuiderwijk (1986), in which waterfrog identifications are based mostly on biometry, *R. lessonae* occurs mainly in the eastern part (above sea level) of The Netherlands. Hence, the present study focussed on this part of the country. Sample localities are shown in Fig. 1 and listed in Table I.

The ratios tibia length/callus internus length (index 1) and digitus primus length/callus internus length (index 2) are considered by most authors the most useful biometrical characteristics to distinguish different genotypes of waterfrogs from each other (cf. Günther, 1975; Berger, 1977). For all captured (sub)adults the following measurements were taken using a vernier caliper: body length, tibia length, length of digitus primus, length of callus internus, and height of callus internus. Biometrical comparisons are restricted to animals larger than 36 mm.

Blood was taken by severing a blood vessel between the fourth and fifth toe. After measuring, bleeding, and sex determination the frogs were released.

As tadpoles from homotypic *R.* kl. *esculenta* crosses mostly die before metamorphosis or produce clumsy froglets (Berger, 1970; Blankenhorn, 1977) tadpoles were reared until they hatched as vital froglets. Just after metamorphosis, the animals were anaesthetized in a solution of MS 222 (Sandoz, Basel) and blood was taken by heart puncture.

The blood was collected in glass capillaries, a small amount of a 4% Na-citrate or heparine frog saline solution being added to prevent clotting. A blood smear was prepared in most cases and the plasma of the remaining blood, after centrifugation with a hand centrifuge, was mixed with an equal volume of a 40% sucrose solution and stored at −20°C.

The plasma protein pattern was studied by means of vertical polyacrylamide slab-gel electrophoresis (Maurer, 1971). After staining with Coomassie Blue up to three bands of different anodical mobilities were expected to show up on the gels. A fast moving band "Type A" is typical of *R. lessonae* while a slower moving band "Type B" and/or a slightly slower moving band (than "Type B") "Type C" are typical of *R. ridibunda*. *R.* kl. *esculenta* is heterozygous, having both the *lessonae* and a *ridibunda* band (Tunner, 1973; Wijnands, 1977).

Triploid *R.* kl. *esculenta* can be distinguished from diploid animals in two ways: (1) from the gene dosage effect the albumin band, representing the double genome, is darker and broader than the albumin band, representing the single genome (Eikhorst, 1984) and (2) on account of their (about 20%) longer erythrocytes (Günther, 1977). Therefore, the length of at least 10 erythrocytes was measured from the dried smear and scored blind.

For both the biometrical data and erythrocyte sizes Student's *t*-test was used to test the differences found, after application of the normality test.

Results

With electrophoresis the following albumin patterns were found in 756 specimens: A (*lessonae*), B and BC (*ridibunda*), and AB and AC (*esculenta*). In some of the *esculenta* patterns, a darker and slightly broader A band, combined with a lighter and slightly narrower B or C band was observed (Fig. 2). This was interpreted as a gene dosage effect, indicating

Table I. Localities and genotypes of waterfrogs, grouped by province. Coordinates according to the Amersfoort grid. Genotypes: L = *lessonae* genome; R = *ridibunda* genome; LL = *R. lessonae* 2n; LLL = *R. lessonae* 3n; LR = *R.* kl. *esculenta* 2n; LLR = *R.* kl. *esculenta* 3n; RR = *R. ridibunda* 2n. N = number of specimens. * Presence observed. ** Leg. P. Bellink, University of Nijmegen.

Localities	Coordinates	Year	Water bodies	Genotypes (N)				
				LL	LLL	LR	LLR	RR
Province Drenthe								
1. Vledder "Vleddéresch"	211.4–543.0	87	Fen in moorland	5		2	1	
2. De Wijk "Havixhorst"	213.8–521	87	Pool in deciduous forest	4		8		
Province Flevoland								
3. Lelystad "Natuurpark"	164 –499	87	Small lakes					15
Province Zuid-Holland								
4. Leiden "Knotterpolder"	93.5–461.2	88	Ditch in meadow				8	1
5. Zoeterwoude "Weipoort"	96.5–458.4	88	Ditch in meadow				1	*
Province Zeeland								
6. Veere "Oranjezon"	30 –401.4	88	Water-win area in dunes		1		72	
Province Utrecht								
7. Schoonhoven "Willige Langerak"	120.3–439.1	88	Ditch in water-meadow				1	
8. Lopik "Polder Wiel"	124.5–442.1	88	Ditch in water-meadow				4	9
9. Honswijk "Steenwaard"	141.2–442.1	88	Pool in water-meadow	*		3		*
10. Leersum "Leersumsche veld"	158.5–450.4	87	Fen in marsh and reed	8		3		
11. Veenendaal "Fort Buurtsteeg"	166.3–451	87	Moat	24		3		
12. Rhenen	166.8–441.2	86	Pool in water-meadow	7		29		
13. Rhenen "Blauwe Kamer"	170.4–440.1	87	Pool in water-meadow	3		6		
Province Gelderland								
14. Wapenveld "Wapenveldsche Broek"	203 –492.7	87	Ditch in meadow	4		7		
15. Nunspeet "Mythstee"	183.2–485.1	87	Fen in mixed forest	5	2			
16. Nunspeet "Zandenbosch"	183.5–485.7	87	Fen in mixed forest	15				
17. Nunspeet "Huize de Vennen"	183.1–484.5	87	Fen in mixed forest	17	1	1		
18. Nunspeet "Mosterdveen"	184.5–484.6	88	Fen in heath bog	13		1		
19. Terwolde "De Mijntjes"	202.3–478.1	87	Pond in deciduous forest		1			
20. Voorthuizen "Wilbrinksbosch"	172.3–466.9	87	Pool in meadow	11		5		
21. Lochem "IJsbaan"	224 –472	88	Canal in mixed forest	1		21		
22. Vorden "De Wildenborch"	223.1–459.8	87	Ponds in deciduous forest	2		12		
23. Culemborg "Redichemse Waard"	144 –442	88	Ditch and pool in meadow	4		5		
24. Rijswijk "Rijswijksche Veld"	152.8–439.4	87	Ditch in meadow	15		1		
25. Tweesluizen "Het Nieuwland"	152 –433.3	87	Ditch in meadow			3		
26. Maurik "Mauriksche Waarden"	158.4–443.1	87	Ditch and pool in meadow	28		12		
27. Ommeren "Ommerensche Veld"	160.7–439.5	87	Ditch in meadow	31				
28. Lienden	167.7–440.2	86	Pool in water-meadow			1		
29. Kesteren "Schuilenburg"	165.1–439.3	86	Ditch in meadow	15		7		
30. Wageningen "Bovenste Polder"	175.6–441.5	86	Pool in water-meadow	1		19		
31. Renkum	179 –442	87	Pool in water-meadow	6		20		
32. Doorwerth "Doorwerthse Waarden"	182.8–442.2	87	Ditch in water-meadow			3		
33. Winssen "Winssensche Waarden"	177.3–433	88	Ditch in water-meadow	1		11		
34. Varsselder	220 –433.3	88	Sand-pits				3	
35. Gendringen "Landfort"	225.2–429.8	87	Pond in meadow			12		
Province Noord-Brabant								
36. Udenhout "Leemkuilen"	140 –401	88	Loam-pits	3		3		
37. Oisterwijk "Kampinasche Heide"	145 –398.8	88	Fen in moorland and firwood	16		2		
38. Oisterwijk "Kl. Oisterwijksche Heide"	144.9–393.4	88	Pool in meadow	*		*		3
39. Vessem "Grootmeer"	150 –382	88	Fen				10	
40. Heeze	168.9–378.5	88	Pool in meadow	1		10		
41. Maarheeze "Lieropsche Heide, Witven"	171.8–376.8	88	Fen in heath	18		6	1	
42. Maarheeze "Lieropsche Heide, Grafven"	172 –377.6	88	Fen in heather	8		1		
43. Griendtsveen	190.1–382.5	88	Canals in peat bog	13		12		

Table I. (Continuation)

Localities	Coordinates	Year	Water bodies	Genotypes (N)				
				LL	LLL	LR	LLR	RR
44. Griendtsveen	189.9–382	88	Fen in peat bog	5		1		
Province Limburg								
45. Wellerlooi "Hamert, Heerenven"**	208.7–393.7	88	Fen in moorland and firwood	*		*		1
46. Arcen	212 –390	88	Gravel-pits			5		8
47. Lomm "Ravenvennen"	211 –384	88	Fen in moorland and firwood	4				
48. IJsselsteyn "Rouwkuilen"	191.4–390.2	88	Fen in moorland and firwood	35				
49. Meijel "Groote Peel"	183.9–371.1	88	Fen in peat bog	6		1		
50. Brunssum	198.7–327.6	88	Pond on golf-course			1		
51. Brunssum "Brunsummerheide"	197.8–327	88	Fen in moorland	2		*		
52. Eysden "Oost-Maarland"	177.1–311.9	87	Gravel-pits			13	23	
53. Rijckholt "Kasteel"	179 –312.2	87	Moat				6	
54. Rijckholt	179.2–311.7	87	Cattle-pond in meadow				3	
			Total	331	5	250	133	37

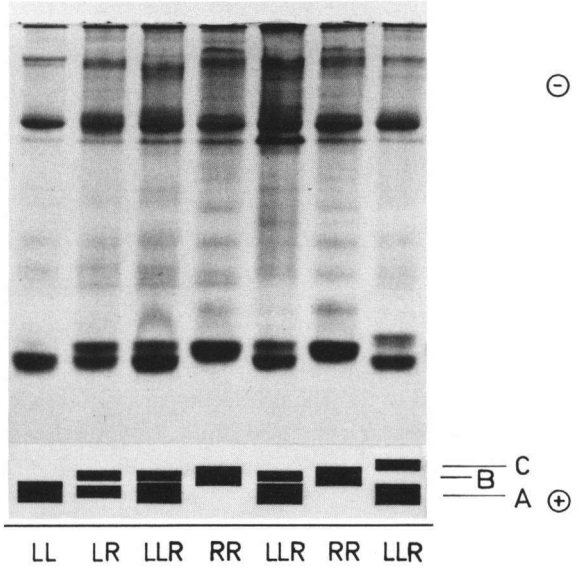

Fig. 2. Albumin pattern as revealed by electrophoresis. A, B, and C refer to albumin bands, L and R to the corresponding genotypes.

a triploid specimen. Since the A band was more intense than the B or C band with no exception, all *R.* kl. *esculenta* triploids apparently possessed the LLR and not the LRR genotype. The C band was only found twice: combined with B in one diploid *R. ridibunda* and together with a more intense A band in one triploid *R.* kl. *esculenta*; both specimens were collected at locality 8 (Table I).

The erythrocyte lengths were measured from blood of 534 specimens. It appears that the lengths of the ovoid erythrocytes in froglets just after metamorphosis are 6–7% smaller than in subadult and adult specimens in all forms, which represents a significant difference ($p < 0.005$) (Table II). Therefore the data on juveniles and (sub)adults are treated separately. Within the latter group, no significant differences in erythrocyte size were found between the sexes, or between animals of different size classes.

Histograms of the average erythrocyte lengths of (sub)adults of *R.* kl. *esculenta* and *R. lessonae* are shown in Fig. 3 and of those in juveniles just after metamorphosis in Fig. 4.

In *R.* kl. *esculenta* diploidy and triploidy is confirmed by electrophoresis and the erythrocyte sizes are grouped according to these findings. The erythrocyte size in triploid *R.* kl. *esculenta* is significantly larger (about 20%) than in diploid specimens, both in (sub)adults and juveniles ($p < 0.001$) and slightly smaller in *R. ridibunda* than in diploid *R.* kl. *esculenta* ($p < 0.01$). The distribution of the average erythrocyte lengths both in *R.* kl. *esculenta* diploid and triploid and in *R. ridibunda* appears normal. All findings agree with Günther (1977), besides he found also a normal distribution of the erythrocyte lengths of *R. lessonae*, in which the

Table II. Average length, standard deviation (SD) and range of erythrocytes in μm. N = number of specimens. All values calculated over the averages per specimen in (sub)adults, and juveniles, just after metamorphosis.

Form	Ploidy level	(Sub)adults					Juveniles				
		N	average	SD	min.	max.	N	average	SD	min.	max.
R. kl. *esculenta*	2n	128	25.3	0.99	22.9	28.2	44	23.6	0.98	21.8	25.5
R. lessonae	2n	118	25.3	1.09	22.7	28.3	88	23.2	1.09	20.9	25.7
R. kl. *esculenta*	3n	55	30.7	1.27	27.4	33.7	65	28.7	1.50	25.0	31.9
R. lessonae	3n	4	29.8	0.54	29.1	30.5	1	27.2			
R. ridibunda	2n	28	24.6	1.08	22.2	26.3	3	22.7	0.62	22.1	23.3

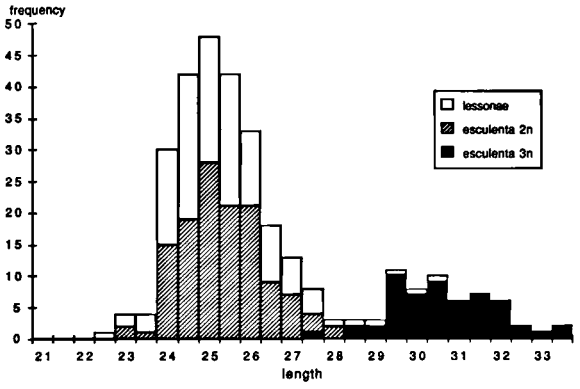

Fig. 3. Histogram of average erythrocyte lengths (μm) of *R. lessonae* and *R.* kl. *esculenta* in (sub)adults.

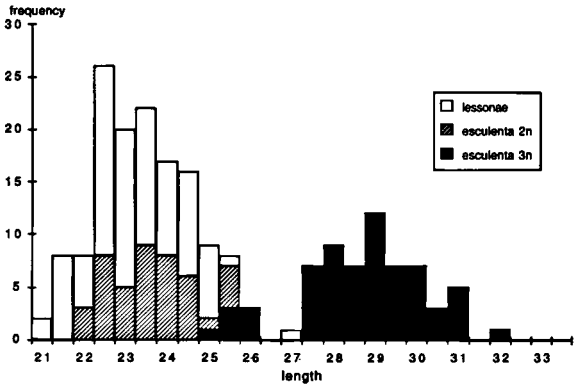

Fig. 4. Histogram of average erythrocyte lengths (μm) of *R. lessonae* and *R.* kl. *esculenta* in juveniles.

erythrocyte sizes were similar to *R.* kl. *esculenta* diploid.

Supposing that the distribution of the erythrocyte sizes of *R. lessonae* is normal and not skewed and the erythrocyte lengths are equal to those of *R.* kl. *esculenta* diploid, one finds at least four large erythrocytes (29.1 μm–30.5 μm) in (sub)adults (Table II, Fig. 3) and one large erythrocyte (27.2 μm) in a juvenile (Table II, Fig. 4) of *R. lessonae*, which are outlyers. Erythrocyte size in (sub)adults is similar in *R. lessonae* (four largest excluded) and diploid *R.* kl. *esculenta* ($p = 0.4$) and in juveniles it is probably slightly smaller in *R. lessonae* (largest excluded) than in diploid *R.* kl. *esculenta* ($p = 0.05$).

In *R. lessonae* diploidy and triploidy cannot be distinguished by electrophoresis (only A band), but the 5 outlying large erythrocytes are not different from *R.* kl. *esculenta* triploid and larger than the maximum average erythrocyte length of *R.* kl. *esculenta* diploid (Table II), suggesting these specimens are triploid.

Since erythrocyte size appears similar in *R. lessonae* and *R.* kl. *esculenta* diploid and is very variable within a specimen, individual erythrocyte lengths were also used to test the difference in size between the 4 *R. lessonae* and the 10 diploid *R.* kl. *esculenta* (sub)adults with the largest average erythrocyte size (≥ 27 μm), in which diploidy is confirmed by electrophoresis. The 68 erythrocytes (average length 29.8 μm, SD 1.58) measured in the 4 *R. lessonae* are also significantly larger than the 140 erythrocytes (average length 27.3 μm, SD 1.41) measured in the 10 diploid *R.* kl. *esculenta* ($p < 0.001$) and the SD of the erythrocyte lengths measured in *R. lessonae* is not smaller than the SD of those measured in *R.* kl. *esculenta*, indicating that the measurements of erythrocytes in *R. lessonae* were not restricted to the largest erythrocytes.

In animals sharing the same genotype and larger than 36 mm, no statistically significant differences were found in the biometrical indexes 1 or 2, nei-

Table III. Biometrical data of waterfrogs with body size over 36 mm. N = number of specimens. Index 1 = tibia length/callus internus length; index 2 = digitus primus length/callus internus length.

Form	Ploidy level	N	Index 1				Index 2			
			average	SD	min.	max.	average	SD	min.	max.
R. lessonae	2n	186	6.5	0.6	5.2	8.1	1.58	0.2	1	2.3
R. lessonae	3n	4	6.8	0.8	5.7	7.3	1.5	0.3	1.3	1.9
R. kl. *esculenta*	2n	101	8	0.8	6.3	10.4	1.92	0.3	1.3	2.5
R. kl. *esculenta*	3n	44	7.9	1.1	5.7	11.7	1.99	0.3	1.3	3.2
R. ridibunda	2n	10	10.6	0.8	9.5	12.6	2.67	0.2	2.4	3.2

ther between male and female, nor between animals of different size. Table III presents the indexes for each genotype.

Both indexes are significantly ($p < 0.001$) different between electrophoretically identified *R. lessonae*, *R.* kl. *esculenta*, and *R. ridibunda*, although there is a clear overlap of *R.* kl. *esculenta* with both *R. lessonae* and *R. ridibunda* and even more so in *R.* kl. *esculenta* 3n than 2n. No statistically significant difference is found in any index, between *R.* kl. *esculenta* 2n and 3n ($p = 0.3$) or between *R. lessonae* 2n and 3n ($p = 0.4$).

According to electrophoresis and erythrocyte size, the genotypes of 756 waterfrogs have been classified (Table I) as belonging to 5 different genotypes: 331 diploid *R. lessonae* (LL); 250 diploid *R.* kl. *esculenta* (LR); 133 triploid *R.* kl. *esculenta* (LLR) and 37 diploid *R. ridibunda* (RR), and 5 probably triploid *R. lessonae* (LLL).

The mixed *R. lessonae* - *R.* kl. *esculenta* population appears widespread in the eastern part of the country. The percentage of *R.* kl. *esculenta* triploids in these populations is extremely low; only two of them were found out of 211. Pure *R. lessonae* and pure *R.* kl. *esculenta* (including a high percentage of triploids) populations seem to be rare in this region, while *R. ridibunda* has been found occasionally.

Although the sample in the western part of The Netherlands is small, it is remarkable that all *R.* kl. *esculenta* specimens from localities 4–8 are triploids and occur either in pure *R.* kl. *esculenta* or mixed *R. ridibunda* - *R.* kl. *esculenta* populations.

The few triploid *R. lessonae* have been found either in *R. lessonae*, *R. lessonae* - *R.* kl. *esculenta* or triploid *R.* kl. *esculenta* populations.

Discussion and conclusions

The occurrence of triploid *R.* kl. *esculenta* in The Netherlands is reported here for the first time. Triploid *R.* kl. *esculenta* is already known from Sweden, Denmark, West and East Germany, and Poland (R. Günther, pers. comm.). All our specimens are of the LLR genotype. The biometrical data show that their phenotypes resemble diploid *R.* kl. *esculenta* much more than *R. lessonae*.

It is striking that no LRR triploids were found. Such triploids occur frequently in *R. ridibunda* - *R.* kl. *esculenta* populations in East Germany and Poland (Günther & Hähnel, 1976; Günther, 1983; Berger, 1987) and sporadically in *R. lessonae* - *R.* kl. *esculenta* or pure *R.* kl. *esculenta* populations of East and West Germany (Günther & Hähnel, 1976; Günther, 1983; Eikhorst, 1988a).

The percentage of triploid *R.* kl. *esculenta* in all mixed *R. lessonae* - *R.* kl. *esculenta* samples from the eastern Netherlands is about 1%, indicating that, in these populations, *R.* kl. *esculenta* is mainly maintained by mating with *R. lessonae*. This percentage is much higher (14–43%) in East Germany, where triploid *R.* kl. *esculenta* has been detected in almost every *R. lessonae* - *R.* kl. *esculenta* population that was studied for genotype (Günther, 1975; Günther & Hähnel, 1976). The percentage of *R.* kl. *esculenta* triploid in a pure *R.* kl. *esculenta* population from the south (locality 52) is about 64%, which is within the normal range for pure *R.* kl. *esculenta* populations (Eikhorst, 1984).

In contrast, *R.* kl. *esculenta* in both the mixed *R. ridibunda* - *R.* kl. *esculenta* and the (almost) pure *R.* kl. *esculenta* populations of the western regions seem to be exclusively triploid. The exclusive occur-

rence of this LLR triploid has not been reported elsewhere, and investigations over a larger area are needed to verify it.

There are indications that *R. lessonae* triploids may also occur. Triploid *R. lessonae* has not yet been reported. Evidently, the LLL genotype cannot be distinguished from type LL by electrophoresis, since only a single fast moving A band is present in both forms. Circumstantial evidence is that the peaks of the average erythrocyte size distribution in diploid *R. lessonae* and diploid *R.* kl. *esculenta* are subequal, with the erythrocyte size in the supposedly *R. lessonae* triploids similar to those of triploid *R.* kl. *esculenta*. Verification is needed, for example by karyotyping.

The albumin type C band has been observed only in one specimen of *R. ridibunda* and in a triploid *R.* kl. *esculenta* (LLR) from the same locality (8). Apparently, these two forms mate and the latter produces (also) LL gametes. The type C band is apparently rare in this country, as Wijnands (1977) observed it only once, in a few specimens from a *R. ridibunda* population. The occurrence of one LLL triploid in the almost exclusively LLR population at locality 6 would also indicate that LL gametes are formed in LLR triploids. The production of LL gametes in LLR triploids appears to be rare (see Introduction). This has been reported only by Berger & Günther (1988) for few ova of some LLR females and by Graf & Polls Pelaz (1989) in LLR males (in an otherwise diploid population). The presence of LLL triploids in almost pure *R. lessonae* populations (localities 15 and 17) would mean that LL gametes are sometimes also produced by diploid *R. lessonae*. The biometrical indexes 1 and 2 appeared only suitable for the classification of 3 types only. Both triploid *R.* kl. *esculenta* and triploid *R. lessonae* show values similar to those of the corresponding diploid form. The additional L in the LLR or LLL genotype is apparently not expressed in these morphometric indexes. In a more extensive study of individual populations, Günther (1975) often found the same similarities, but in some of his populations most *R.* kl. *esculenta* (LLR genotype) resembled more *R. lessonae* than diploid *R.* kl. *esculenta*. This may also be true on a more local level in The Netherlands, but as yet the separate samples were too small.

Whereas this paper deals mainly with other than morphological characters, it should be stressed that some experience generally suffices to identify correctly almost every adult waterfrog in the field. However, to distinguish between diploids and triploids proved impossible (Blommers-Schlösser, in prep.).

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