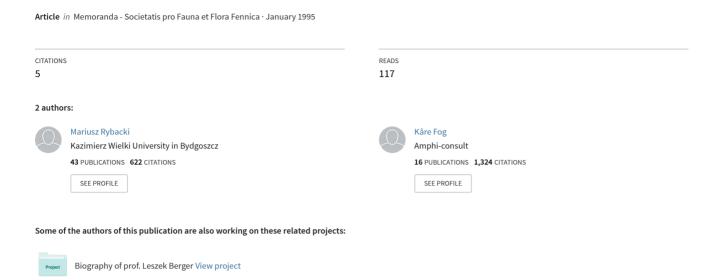
Populations of Green Frogs (Rana ridibunda and Rana esculenta) on Bornholm, Denmark



Populations of Green Frogs (*Rana ridibunda* and *Rana* kl. esculenta) on Bornholm, Denmark

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By sampling of water frogs on Bornholm in 1992, *Rana ridibunda* was found to have declined since 1949, but was still widely distributed. *Rana ridibunda* from Bornholm tends to deviate from central European *R. ridibunda* in certain morphological traits. The green frogs of Bornholm show many different, interesting mating systems, especially a system with *R. ridibunda* females and *R. esculenta* males. Such a system is also known from the Polish island Wolin at the south coast of the Baltic. The data suggest that *R. ridibunda* colonized Bornholm via a land bridge from Wolin at least c. 9000 years ago.

Introduction

At the middle of this century, many European herpetologists considered that there were just two species of green frogs in Europe, viz. Rana esculenta L. and Rana ridibunda Pallas (Mertens & Wermuth 1960, Terentjev 1962). Some herpetologists considered also a third species, R. lessonae Cam. (Karaman 1948, Berger & Michalowski 1963). In Denmark, only esculenta was known (Pfaff 1943).

In 1949, lecturer Arne Larsen was mapping the occurrence of amphibians on Bornholm. Near Vestermarie and Nyker, he found some very large green frogs, up to 12 cm in the females, which he considered to be *Rana ridibunda*, a species hitherto unknown in Denmark. The determination was confirmed at the Zoological Museum in Copenhagen (Larsen 1950).

Up to 1955, Larsen recorded *R. ridibunda* from 10 localities on Bornholm; his determinations, based on morphology, were probably correct, as they were confirmed by voice recordings, and as specimens from several localities preserved at the Zoological Museum of Copenhagen are indeed *R. ridibunda*.

In 1950, Larsen cooperated with Hans Kauri in Sweden, who was studying European green frogs. Larsen measured c. 400 frogs and concluded that two types could indeed by discerned, but that the *R. ridibunda* type was somewhat variable. Specimens from Praestemose near Klemensker were the most pronounced *R. ridibunda* types (Larsen 1954). Kauri, however, making more detailed biometry on 139 of Larsen's frogs, concluded that all transitions from typical *lessonae* via *esculenta* to typical *ridibunda* were represented, and concluded from the Bornholm material that all European green frogs were one species (Kauri 1954).

This wiev was challenged by L. Berger (1966), who concluded that in Poland, *R. lessonae*, *R. esculenta* and *R. ridibunda* are well separated forms, and later demonstrated that *R. esculenta* is a cross product (RL) between *R. lessonae* (LL) and *R. ridibunda* (RR) (Berger 1968). Later research has demonstrated that in some parts of Europe, *esculenta* may also be triploid, either LLR or LRR (Günther 1975).

Based on this new knowledge, K. Knudsen and J. Scheel (1975) again investigated green frogs on

Bornholm, in cooperation with Larsen. In Praestemose, they did find true *R. ridibunda*, as shown by karyotype and electrophoresis, whereas frogs at all other localities were only *R. esculenta*. Again, in 1982 J. Vahl collected frogs at Praestemose and confirmed by electrophoresis that both *R. esculenta* and *R. ridibunda* was present there (Vahl 1985).

Thus, it was believed that true *R. ridibunda* occurred on Bornholm, but probably only in one single locality. In 1985–86, K. Fog mapped calling amphibians all over Bornholm and noted *ridibunda*-like calling on many localities (Fog 1988). So the question was: Does *R. ridibunda* occur only in Praestemose, or is it widely distributed over the island?

Present investigations

In 1992, M. Rybacki, working at Berger's laboratory in Poznan (Poland), engaged in a cooperation with K. Fog and F. Hansen on Bornholm. During one week in August, green frogs were sought on many localities. Six of Larsen's *R. ridibunda* localities were investigated, as well as many localities where K. Fog had heard *ridibunda*-like calling, and some localities with probably only *esculenta*.

Determination of the frogs was carried out by M. Rybacki, based on a combination of biometry and investigation of blood samples. Erythrocyte size in the blood samples allows determination of whether the frog is diploid or triploid (Günther 1977). The biometrical indices (tibia length)/(callus internus length) and (digitus primus length)/(callus Internus length) usually allow determination of diploid frogs (either LL, RL or RR) and of triploid frogs (either RLL or RLL) (e.g. Günther 1990). Some newly metamorphosed froglets were determined as either *R. esculenta* or *R. ridibunda*.

A number of adults and newly metamorphosed froglets were brought to Poznan for closer inspection, e.g. sex determination of froglets, and for breeding experiments.

Results

Green frogs were sought in six of Larsen's localities, including Praestemose. In none of these, any frogs were found. This could be partially due to the very dry weather conditions during the investigation period, but in any case it was clear that a severe decline had occurred since 1949. However, *R. ridibunda* frogs were caught in nine other localities, scattered over the whole Island.

Surprisingly, in several instances *R. ridibunda* was found — with breeding success — also in quite small ponds, created recently by the Bornholm county to improve amphibian populations.

In an abandoned kaolin pit north of Rönne, now a large lake with fishes, newly metamorphosed *ridibundas* were found, but no *R. esculenta*. All other *R. ridibunda* populations occurred together with *esculenta*. Two populations had c. 70% *R. ridibunda* and 30% *R. esculenta*. The remaining populations had maximally 30% *R. ridibunda* among the adults (among the new froglets, the proportion of *R. ridibunda* could be higher than that). Frogs were also sampled from five pure *R. esculenta* populations.

We found *R. esculenta* in the forms RLL, RL and RRL. Compared to the rest of Denmark (cf. Fog, 1994), RLL was relatively rare (on average 8% of a population, and maximally 21%), and RRL relatively numerous (on average 39%, maximally 88%). RL frogs made out on average 35% of a population, and maximally 86%. Population structures varied greatly from locality to locality. Details are given by Rybacki (1994a).

A closer inspection shows some structural differences between populations on the western and the eastern half of Bornholm. This is evident from Table 1. The only genotype with equal sex ratio all over the island is RRL. This again is a difference from the rest of Denmark, where RRL frogs are predominantly female (Fog, 1994).

Table 1. Sex and genotypes of frogs caught on west Bornholm (Ankermyr, Bromme, Krakken, Korsmyr, Rö Plantage, Sorthat) and east Bornholm (Ypnasted, Vandtappergård, Smålyngsvaerket, Snogebaek, Dueodde). The figures give the number of adult specimens caught.

	RR		RRL		RL		RLL	
	Q	ð	Q	♂	Q	ď	Q	oʻ
West	5	9	19	17	15	1	1	3
East	18	0	21	23	37	14	0	12

On west Bornholm, RL frogs are nearly all female. On east Bornholm, the *R. ridibunda* frogs caught were all female. The likelihood that no male should be caught out of 18 frogs is less than 5% if the true sex ratio female: male is less than 5:1. Thus it is concluded that the sex ratio is more skew than 5:1.

If the female *R. ridibunda* frogs on East Bornholm have no male *ridibundas* to mate with, they must mate with male *R. esculenta*.

The most interesting population found was in a garden pond at Ypnastedgård, where we caught 8 RR females and 3 RL males. Some *R. ridibunda* tadpoles before metamorphosis and some newly metamorphosed froglets caught here were all females, according to dissection. One RL male from this population gave interesting results in breeding experiments (see below).

Among a few newly metamorphosed *R. ridibunda* froglets from other ponds near Ypnastedgård, there were also some males, proving that *R. ridibunda* males do occur in low number on East Bornholm, at least in young stages. The total sex ratio of all *R. ridibunda* froglets and tadpoles from east Bornholm was 43:9.

Concerning the biometrical indices, some populations on Bornholm showed unusual values. Thus, in 2 localities, RL frogs had unusually low values of both indices (for details, see Rybacki, in 1994a).

The biometrical indices of R. ridibunda were not quite typical for central European R. ridibunda, e.g. from Wolin in NW Poland, as shown in Table 2. When all data from Bornholm in 1992 are combined and compared with those from Wolin, the differences in both indices shown are highly significant (t-test, p < 0.01). As animals have been measured by M. Rybacki on both islands, the differences are not artifacts due to different measuring techniques of different investigators.

Within Bornholm, average values of the index ti/c.i. varied considerably from population to population, but due to low numbers of animals, these differences were mostly non-significant. Larsen (1954) found that frogs with a tibia length typical of central European *R. ridibunda* were common in Praestemose, but rare in 3 other *R. ridibunda* localities. The *ridibunda*s sampled from 1949 to 1982 are mainly from Praestemose; their average tibia length is significantly larger than in ridibunda populations sampled in 1992 mainly on mid and east Bornholm

(t-test, p < 0.05). It must be remembered, however, that the two data sets originate from measurements made by different investigators.

Strangely, in some places where Fog had heard *R. ridibunda* calling (Fog 1988), either no *ridibundas* were found, or only *R. ridibunda* females. This discrepancy must be investigated further, but as *R. esculenta* males calling like *R. ridibunda* are known from Christiansö north of Bornholm, it is probable that also some *R. esculenta* males on Bornholm call like *R. ridibunda*.

Results of mating experiments

The mating experiments with frogs from Bornholm will be only very briefly referred to.

Preliminary data (Rybacki, unpublished) indicate that *R. esculenta* x *R. esculenta* matings where both parents are from east Bornholm, may produce *R. ridibunda* offspring, with more females than males. However, the relevance of these results cannot yet be evaluated, before the viability of this offspring is known.

Results with an RL male from the interesting population at Ypnastedgård showed that if such a male mates with RR females, the offspring will again be RR females and RL males, i.e. this could be a self-reproducing population type.

Conclusions

Although one could want much more extensive investigations of the interesting green frog populations on Bornholm, just one week of sampling frogs enable us to draw a number of conclusions:

- 1) R. ridibunda has declined markedly since 1949.
- 2) *R. ridibunda* is still widely distributed on the island.
- Many different population systems of green frogs are represented on Bornholm, and ridibundas are involved in different population systems on east and west Bornholm.

Discussion

There seems little doubt that the occurrence of R.

ridibunda on Bornholm is of natural origin. This is indicated, e.g. by the wide distribution on the island, already in 1949.

It is also indicated by the geographical variation in morphology, as shown in Table 2. The average length of tibia and digitus primus is shorter than in central Europe. Already Larsen (1950, 1954) found that the difference between *R. esculenta* and *R. ridibunda* in the index d.p./c.i. was smaller on Bornholm than elsewhere.

It can be added that an unusual colour form has been observed in a few animals on Bornholm: grass green with dark green spots; this colour form is unknown in Central Europe.

Also, there seems to be geographical variation within Bornholm. On the basis of a large number of frogs, Larsen (1954) found that frogs in Praestemose had longer tibias than frogs from three other *R. ridibunda* localities on Bornholm. The data in Table 2 seem to corroborate this trend.

The geographical variation in morphology between Bornholm and central Europe, and within Bornholm, suggests a long history on the island.

But the strongest indication that *R. ridibunda* on Bornholm has a natural origin is that a peculiar population system with a very skew sex ratio (no or very few males) is present, but only on a part of the island. A similar population system is known from only one other place in Europe, viz. the island Wolin in NW Poland, where populations with only female *R. ridibunda* are living (Rybacki, 1994b). Such a population system is only possible if we have *R. esculenta* males that produce R sperm with female factor and L sperm with male factor. The mating experiments performed by M. Rybacki—although only preliminary — have demonstrated

Table 2. Average values of the indices (digitus primus)/ (callus internus) and (tibia)/(callus internus) in samples of *Rana ridibunda*. Data from Wolin, NW Poland, are from Rybacki (1994b). Data from Bornholm 1949–82 are from preserved museum specimens and from Vahl (1985). n = number of specimens.

	d.p./c.i	ti/c.i.	n
Wolin	3.13	10.93	50
Bornholm: West, 1949-82	2.76	10.78	19
West, 1992	2.77	10.28	14
East, 1992	2.71	10.13	18

that indeed some *R. esculenta* males from Wolin and from Bornholm are able to do this.

Thus, we have a peculiar similarity between Wolin and Bornholm, which is interesting because until about 8900 years ago, Bornholm was connected via a land bridge to some place at the south coast of the Baltic near Wolin (Kolp 1990; Björck 1995). The results strongly suggest that *R. ridibunda* colonized Bornholm long time ago via this land bridge. That animals from Wolin should have been moved by man to Bornholm in relatively recent time is unlikely because of the morphological differentiations.

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