Conference Paper

Testicular Anomalies in the Hybridogenetic Frog *Pelophylax esculentus* (Amphibia: Anura: Ranidae)

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Abstract

Testicular anomalies in the hybridogenetic frog *Pelophylax esculentus* (586 adults) and two parental species (193) were analyzed. The hybrids were characterized by an increased number of males with testicular anomalies (61%) compared to the parental species (17–20%). Diploid hybrids had more males with abnormal testes when compared to triploid hybrids. Among the hybrids with asymmetrical testes, males with a larger left testis were prevalent.

Keywords: hybridization, polyploidy, green frogs, asymmetry, helminths, Eastern Europe.

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1. Introduction

Gonadal anomalies in amphibians have attracted attention for a long time. Prior to the beginning of the 20th century, about a hundred such cases were registered [13]. It has been assumed that several factors affect the appearance of gonadal anomalies in amphibians: chemicals, helminth invasions, hybridization and polyploidy [4, 5, 9, 10].

The edible frog *Pelophylax esculentus* is widespread in temperate Europe and consists of numerous genetic lineages of hemiclonal or meroclonal di- and polyploid hybrids [6]. The species originates from crosses of *P. lessonae* and *P. ridibundus* and is characterized by a special mechanism of clonal inheritance named hybridogenesis [12]. The aim of the present study is to study the effect of natural hybridisation and poliploidy on the appearance of testicular anomalies in *P. esculentus*.

2. Material and methods

For the study, 539 diploid and 47 triploid adult males of *P. esculentus* from Russia, Belarus', Ukraine and Moldova were analyzed. For comparison, males of two parental species (127 *P. ridibundus*and66 *P. lessonae*) were used as well. Species and ploidy level were determined via the use of flow DNA cytometry [3]. The percentage of males with the following testicular anomalies was calculated: *1)* the absence of at least one testis; *2)* reduced testes (both testes less than 3 mm in length); *3)* extremely pronounced asymmetry; and *4)* abnormal shape (elongated, flattened, segmented, lobed, lumpy, etc.). Finally, the percentage of males with any testicular anomaly was counted. The correlation between the number of males with testicular anomalies in the sample, the geographical coordinates and the significance of differences (T-test for independent samples) in the number of testicular anomalies were calculated via Statistica 6.0. Males with black areas and/or cysts (metacercariae of the trematode *Codonocephalus urnigerus*) were counted as well.

3. Results and discussion

The number of hybrid males (diploids plus triploids: 61%) with testicular anomalies was more than three times higher than the parental species (17–20%; Table 1). These differences were significant (Table 2). Among the hybrids, the number of males with anomalies was significantly larger in diploids (63%) than in triploids (34%). In *P. esculentus*, the right testis was larger than the left in most cases (60%). The absence of both testes was noted in 4% of diploid and 2% of triploid hybrid males. The absence of both testes was found in one specimen (1%) of *P. ridibundus* from Valya Adynke (Moldova; 48.00°N 28.83°E). Previously, only one such case has been mentioned in a sample of *P. ridibundus* from the Rostov province of Russia [8].

There was no correlation between the number of males with testicular anomalies and the geographical coordinates. Nevertheless, several samples of *P. esculentus* from the southern part of the range had an increased number of anomalies. For example, hybrids from Beryozovsky forest (Odessa province, Ukraine; 47.17°N 30.92°E; n=9) were characterized by a large number of males with absent (33%), asymmetrical (55%) and abnormally shaped testes (67%). Hybrids from the villages of Valya Adynke (n=7), Rashkovo (47.95°N 28.83°E; n=3) and Kolbasnoe (47.77°N 29.20°E; n=3) in Moldova had a large number of males with abnormally shaped testes (89, 67 and 67%, respectively). All seven hybrids from Lisovschina village (Zhitomir province, Ukraine; 50.79°N 28.57°E) had strongly reduced testes with large black areas.

Helminth invasions may have caused an increased number of testicular anomalies in these localities. The metacercariae of *C. urnigerus* can change the form and color of frog testes, or even castrate them [4, 7]. However, the obtained data did not support this

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assumption. Males of *P. esculentus* with *C. urnigerus* cysts on the testes were found only in two localities, namely Gaydary village (Kharkov province, Ukraine; 49.63°N 36.33°E; n=183, 2.7% of males) and Tiraspol' city (Moldova; 46.85°N 29.63°E; n=5, 20.0%). Additionally, several males of *P. ridibundus* with such cysts were found in Gaydary village (Kharkov province, Ukraine; 49.63°N 36.33°E; n=14, 7.1% of males), Doybany village (Moldova; 47.41°N 29.20°E; n=1, 100%) and the settlement of Nikita (Crimea; 44.52°N 34.24°E; n=2, 50.0%).

Previously, Reminnyi (2005) analyzed the effect of natural hybridization on the number of gonadal anomalies in green frogs in Ukraine. This author revealed that specimens with anomalies were more frequent in hybrids (44%) than the parental species (1–4%). Several other authors [1, 2, 11] have mentioned that natural hybrids are characterized by the absence of testes or their reduced size.

Pisanets (1992) studied the effect of natural polyploidy on the appearance of gonadal anomalies in green toads of the genus *Bufotes*. He did not reveal any such anomalies in diploid toads from Russia and Ukraine, but found several cases in polyploids, including four cases of hermaphroditism (18%) in triploid *B. baturae* ("*B. danatensis*") from Ishkashim village (Pamirs, Tajikistan).

Table 1: Percentage of males with various testicular anomalies, black areas and cysts in species of the *Pelophylax esculentus* complex. *2n* is diploids and *3n* is triploids.

Type of anomaly	Esculentus (2n)	Esculentus (3n)	Lessonae	Ridibundus
Absence of testis	7.2	2.1	0	2.4
Reduced testes	50.1	29.8	5.1	10.8
Asymmetry	33.0	4.3	3.1	7.4
Abnormal shape	22.0	2.1	10.6	6.2
Anomaly (generally)	63.3	34.0	16.7	20.0
Black areas	1.7	2.1	0	0
Cysts	5.7	0	0	2.3

Table 2: Significance of differences (p) in the number of testicular anomalies between species of the *Pelophylax esculentus* complex. *e2* is diploid *P. esculentus*, *e3* is triploid *P. esculentus*, *l* is *P. lessonae* and *r* is *P. ridibundus*. Significant differences ($p \leq 0.05$) are indicated by underlining.

Type of anomaly	e2/e3	e2/l	e2/r	e3/l	e3/r	l/r
Absence of testis	0.13	$\underline{0.02}$	$\underline{0.04}$	0.28	0.84	0.22
Reduced testes	0.58	$\underline{0}$	$\underline{0}$	$\underline{0}$	$\underline{0}$	0.25
Asymmetry	$\underline{0}$	$\underline{0}$	$\underline{0}$	0.73	0.52	0.27
Abnormal shape	$\underline{0}$	$\underline{0.03}$	$\underline{0}$	0.08	0.28	0.27
Anomaly (generally)	$\underline{0}$	$\underline{0}$	$\underline{0}$	$\underline{0.03}$	0.05	0.58

4. Conclusions

1. In green frogs, hybridization is strongly influenced by the number of testicular anomalies.
2. The number of testicular anomalies in diploid hybrids is higher than in triploid hybrids.
3. Among the specimens of *P. esculentus* with asymmetric testes, males with a larger left testis were prevalent.

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