Appendix S1. Details on erythrocyte length of Kreminna and Brusivka water frogs.

The mean erythrocyte length was obtained for 178 individuals collected in the wild populations (125 adults and 53 juveniles), and for six individuals from Kreminna progeny (five froglets and one metamorphosing tadpole at the stage 45) (Table S1). Distributions of and summary on the erythrocyte sizes by systems, ages, and ploidies are provided in Table S4 and Fig. S3. Triploids in both systems and of both ages contain only female LLR-*P. esculentus*, and diploids contain only *P. ridibundus* (see main sections of present paper).

Normality of numeric data (erythrocyte length, snout-vent length) was assessed by the Shapiro-Wilk test. Respectively, the samples of numeric data were compared using either Student's t-test or Mann-Whitney tests, and correlations were tested using either Pearson ϱ or Spearman r coefficients. Outliers were checked out using Grubbs' test.

Table S4. Erythrocyte size statistics for different groups of water frogs collected in the wild in Kreminna and Brusivka population systems.

Locality	Age	Ploidy	Erythrocyte size statistics, μm					
			Minimum	1st Q	Median	Mean	3rd Q	Maximum
Brusivka	adults	2	18.86	19.71	20.91	20.71	21.81	23.48
		3	24.69	27.41	28.34	28.29	29.60	30.74
	juveniles	2	18.12	19.00	19.33	19.93	20.41	25.48
Kreminna	adults	2	19.48	20.76	21.62	21.82	22.88	25.19
		3	26.59	28.58	30.39	30.47	32.32	34.28
	juveniles	2	19.48	19.96	21.22	21.70	21.84	28.98
		3	27.25	27.53	28.64	28.71	29.90	30.27

Frogs from both systems exhibited a gap in erythrocyte lengths between ploidies. Diploid frogs from Kreminna differed significantly from diploids in Brusivka by their mean erythrocyte length, comparing either two ages together (Mann-Whitney U = 979.5, $P < 0.001$) or separately adults (Student's t = –3.802, $P < 0.001$) and juveniles (U = 67, $P = 0.002$). Adult hybrids (triploids) also significantly differed between localities (t = –3.045, $P = 0.005$). Removal of outliers, i.e. triploids with small erythrocytes or diploids with large erythrocytes (see Results and Fig. S3), did not affect the significance of these differences.

Erythrocyte length of adult triploids significantly correlated with their SVL both in Kreminna (ϱ = 0.628, $P = 0.0007$) and in Brusivka (r = 0.637, $P = 0.035$). Adult diploids demonstrated such a correlation only in Kreminna (r = 0.368, $P = 0.01$). No similar correlations were found among any groups of juveniles.

All five froglets and one tadpole from Kreminna progeny with measured erythrocyte lengths were LLR-females. The mean erythrocyte length was 26.26 μm in a tadpole, and 25.14-27.29 μm in froglets. Tadpole's erythrocyte size lies quite close to a lower margin of triploid erythrocyte size in the Kreminna system (26.59 μm). In Kreminna froglets, erythrocyte sizes differed insignificantly from Kreminna triploid juveniles (U = 0, $P = 0.095$) but differed much stronger from adult LLR-hybrids

from Kreminna (U = 0, $P < 0.001$). In Brusivka, froglets did not differ from adult LLR-hybrids by erythrocyte length (U = 6, $P = 0.073$).

Erythrocyte data demonstrated certain differences between two studied systems. The size of erythrocytes in diploids and triploids generally lay within typical ranges known for *P. esculentus* complex in Eastern Ukraine (Bondarieva et al. 2012, Meleshko et al. 2014, Drohvalenko et al. 2021). Diploid juveniles demonstrated a wider range of erythrocyte lengths than diploid adults, while triploid juveniles had a narrower range than triploid adults – such a pattern has been observed for other population systems in the Siverskyi Donets Center of Diversity of water frogs (Drohvalenko et al. 2021). To clarify the differences in age changes of erythrocyte size between both systems, Brusivka triploid juveniles and froglets need to be studied. Moreover, the erythrocyte length in tadpoles and its discriminative capacity as a ploidy marker may also possess a substantial interest.

Literature

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