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STUDY OF THE GENETIC STRUCTURE OF TWO ECOMORPHOLOGICAL GROUPS OF ROACH (*RUTILUS RUTILUS*) OF THE KANIV RESERVOIR

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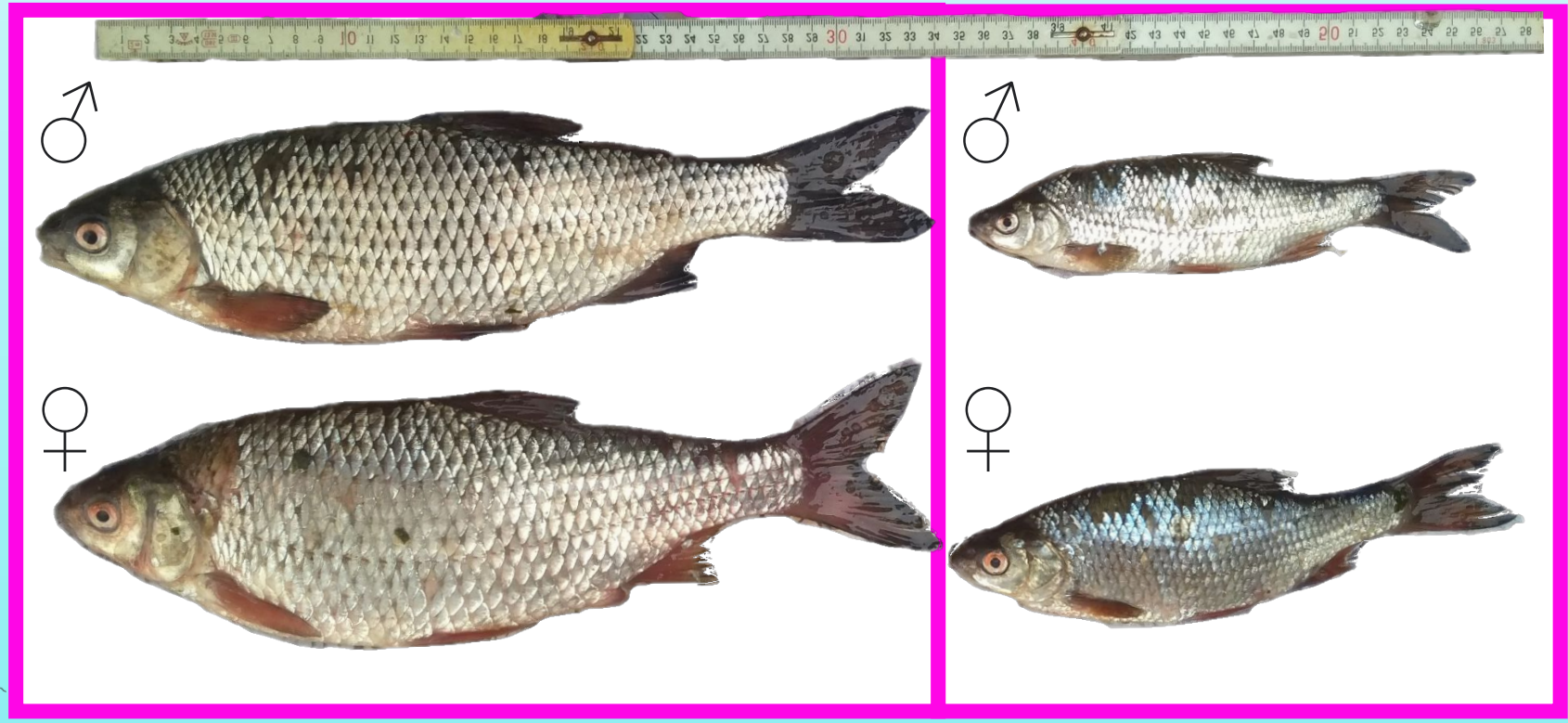
Materials & Methods: aim, object, sampling

The aim of the study was to investigate the genetic structure of two forms of roach (large fast-growing forms – group I and small slow-growing – group II) of the Kaniv reservoir using microsatellite loci (Rru: 3, 4 and 7; Ca: 3, 5 and 12).

Forms of roach (*Rutilus rutilus*)

large fast-growing forms group I	small slow-growing group II



Materials & Methods: Microsatellite Analysis

Locus	Genbank accession number	Marker sequence (5'→3')	PCR conditions				Reference
			D	AT	E	C	
Rru 3	AB112739	F: GGGGAGTCTGGCTTCAGG R: CGGCACACAGGGAGGTTA	90° C 30 s	55°C 30 s	72 °C 30 s	33	[Barinova, 2004]
Rru 4	AB112740	F: TAAGCAGTGACCAGAATCCA R: CAAAGCCTCAAAAGCACAA		55°C* 30 s			
Rru 7	AB112741	F: GTCTCCACAAAAATAGCGAACC R: CGATTGATGCCGTCAGAA		55°C 30 s			
Ca 3	AF277575	F:GGACAGTGAGGGACGCAGAC R:TCTAGCCCCCAAATTTTACGG	92 °C 1 min	57°C* 1 min	72 °C 1.5 min	27	[Dimsoski, 2000]
Ca 5	AF277577	F:TTGAGTGGATGGTGCTTGTA R:GCATTGCCAAAAGTTACCTAA		57°C* 1 min			
Ca 7	AF277579	F:ACACGGGCTCAGAGCTAGTC R:CAAATGTCAGGAGTTCTCCGA		59°C 1 min			
Ca 12	AF277584	F:GTGAAGCATGGCATAGCACA R:CAGGAAAGTGCCAGCATACAC		57°C* 1 min			
MFW 6	-	F*: ACCTGATCAATCCCTGGCTC R**:TTGGGACTTTTAAATCACGTTG	95°C 30 s	55 °C 45 s	72 °C 1 min	35	[Crooijmans, 1997]
MFW 15	-	F: CTCCTGTTTTGTTTTGTGAAA R: GTTCACAAGGTCATTTCCAGC					
MFW 23	-	F:GTATAATTGGGAGTTTTAGGG R:CAGGTTTATCTCCCTTCTAG					
MFW 31	-	F:CCTTCCTCTGGCCATTCTCAC R:TACATCGCAGAGAATTCGTAAG					
Hmo 02	AM086449	F: CATCTGTTCTGAGGGGCTGAG R: CCCCACTTTACCACCAATTATTAT	94° C 50 s	60°C 50 s	72 °C 50 s	28	[Gheyas, 2006]
Hmo 27	AM086456	F: CTGTAATTCCGTTTTATCTGTGT R: ATTGCTGTAAACCATAAAATGTAA		60°C 50 s			
Hmo 33	AM086458	F: GTGCAGCAGTATGTGAATCAGGACAC R: GTGCTTCGGGATACCACACTCTTG		59°C 50 s			

Notes:

* - Temperature was optimized

F - forward primer's sequence;

R - reverse primer's sequence;

D - denaturation;

AT - annealing temperature;

E - extension.

C - Cycles

Separation in 8% PAGE

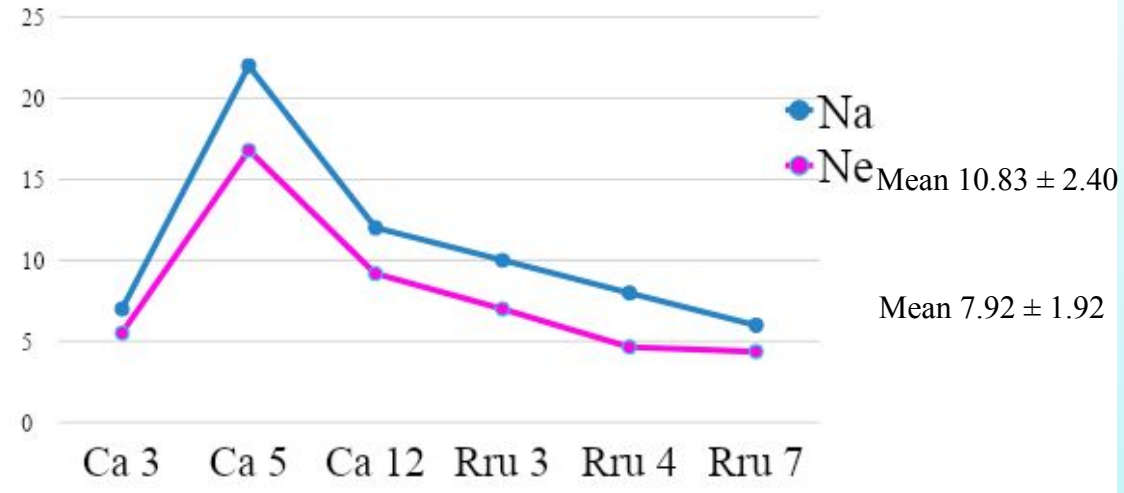
Gel analysis in Totallab v.2.01

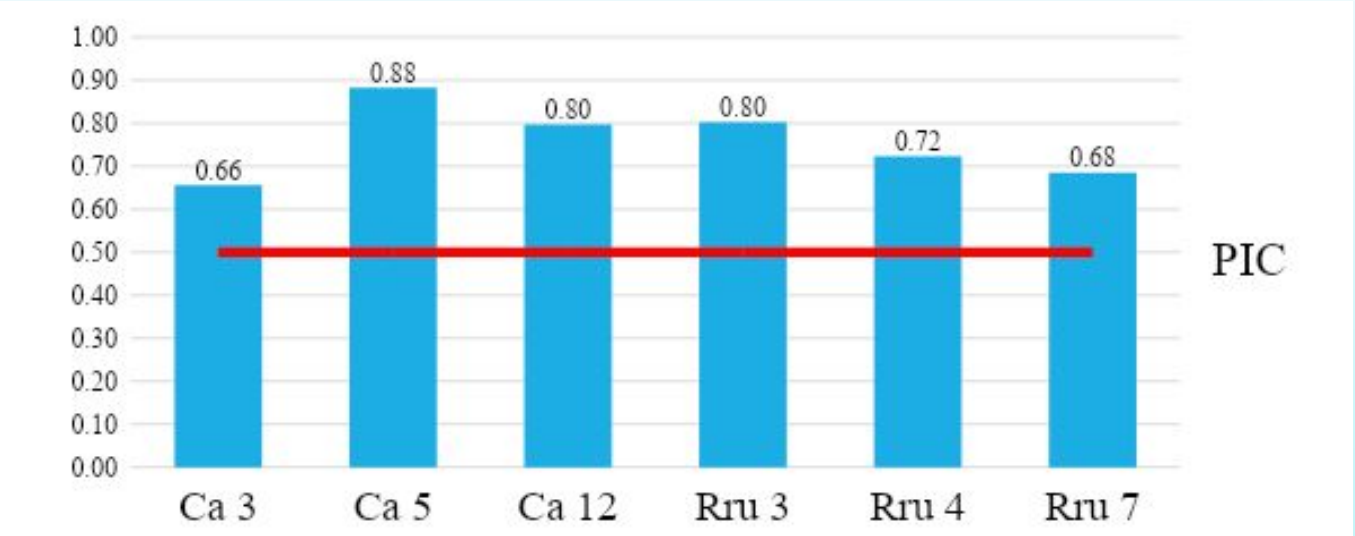
Statistical Analysis in Genalex 6.5 (Peakall & Smouse, 2006, 2012).

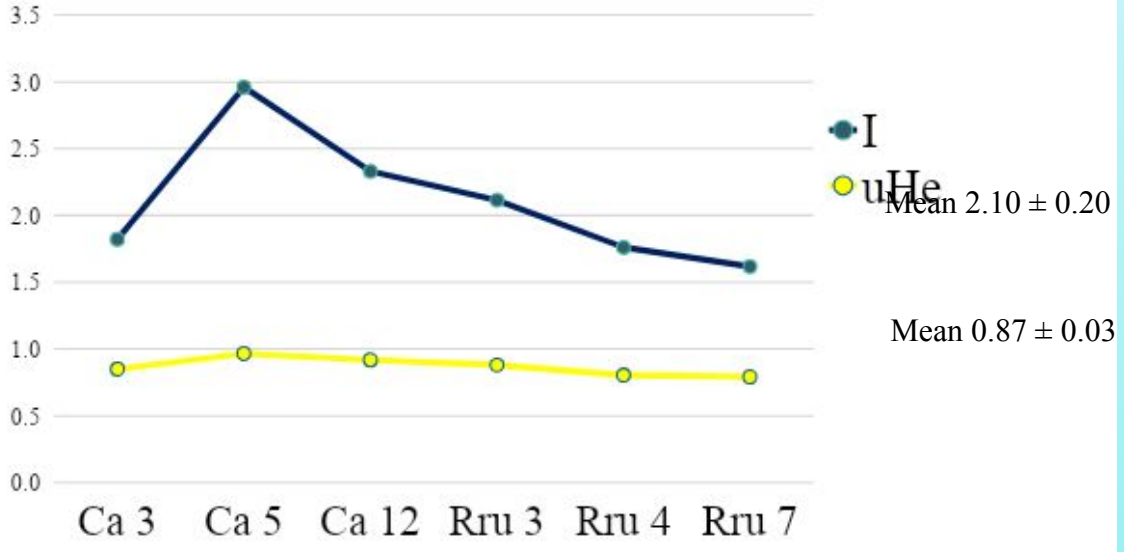
PIC (The polymorphic information content) was calculated using the formulas generally accepted for codominant markers (Nagy et al., 2012).

1. Barinova,A., Yadrenkina,E., Nakajima,M. and Taniguchi,N. (2004). Identification and characterization of microsatellite DNA markers developed in ide Leuciscus idus and Siberian roach *Rutilus rutilus*. *Mol. Ecol. Notes* 4, 86-88
2. Dimsoski P. , Toth G. P. , Bagley M. J. (2000) Microsatellite characterization in central stoneroller *Campostoma anomalum* (Pisces: Cyprinidae). Blackwell Science Ltd, *Molecular Ecology*, 9, 2187–2189
3. Crooijmans R., Bierbooms V., Komen J. et al. Microsatellite markers in common carp (*Cyprinus carpio L.)*. Animal Genetics. 1997. V. 28. P. 129 – 134.
4. Gheyas A. A., Cairney M., Gilmour A. E., Sattar M. A., Das T. K., Mcandrew B. J., Penman D. J. and Taggart J. B. (2006) Characterization of microsatellite loci in silver carp (*Hypophthalmichthys molitrix*), and cross-amplification in other cyprinid species. Molecular Ecology Notes 6, 656–659. doi: 10.1111/j.1471-8286.2006.01288.x © 2006

Results: evaluation of the effectiveness of markers







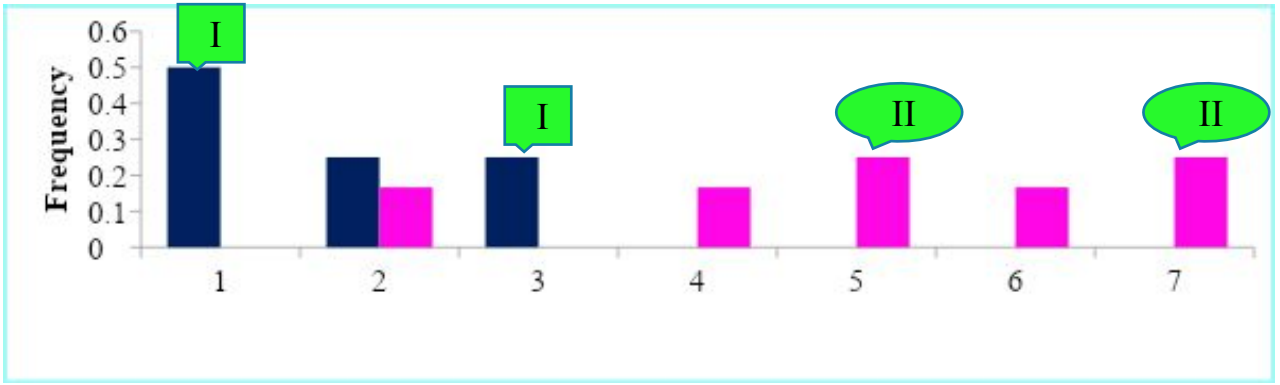
Results: differences between groups

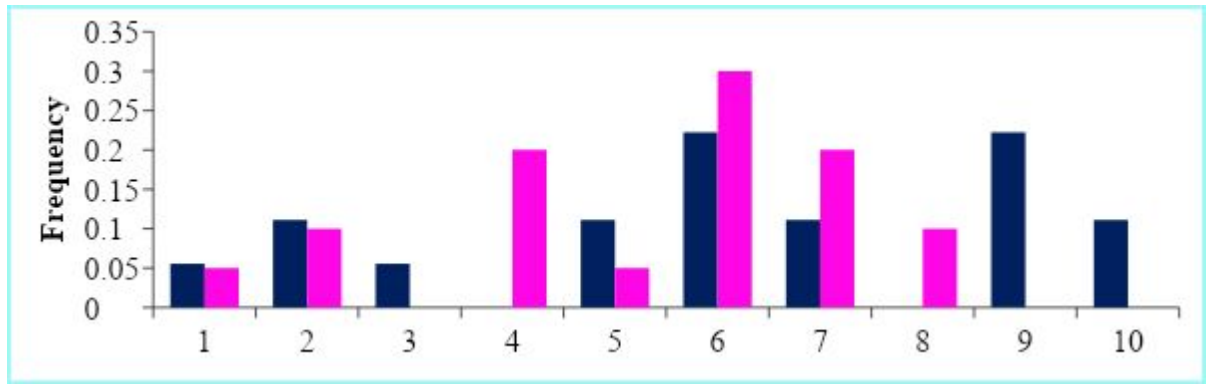
Locus	N		Na		Ne		I		uHe	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
Ca 3	8	6	3.000	5.000	2.667	4.800	1.040	1.589	0.667	0.864
Ca 5	10	9	12.000	11.000	10.000	8.526	2.389	2.274	0.947	0.935
Ca 12	9	8	9.000	7.000	7.364	4.414	2.091	1.689	0.915	0.825
Rru 3	9	10	8.000	7.000	6.480	5.128	1.966	1.765	0.895	0.847
Rru 4	10	10	6.000	7.000	4.545	3.704	1.614	1.591	0.821	0.768
Rru 7	10	10	5.000	5.000	3.846	3.509	1.471	1.392	0.779	0.753
Mean	9.333	8.833	7.167	7.000	5.817	5.013	1.762	1.717	0.837	0.832
SE	0.333	0.654	1.302	0.894	1.092	0.747	0.198	0.123	0.043	0.027

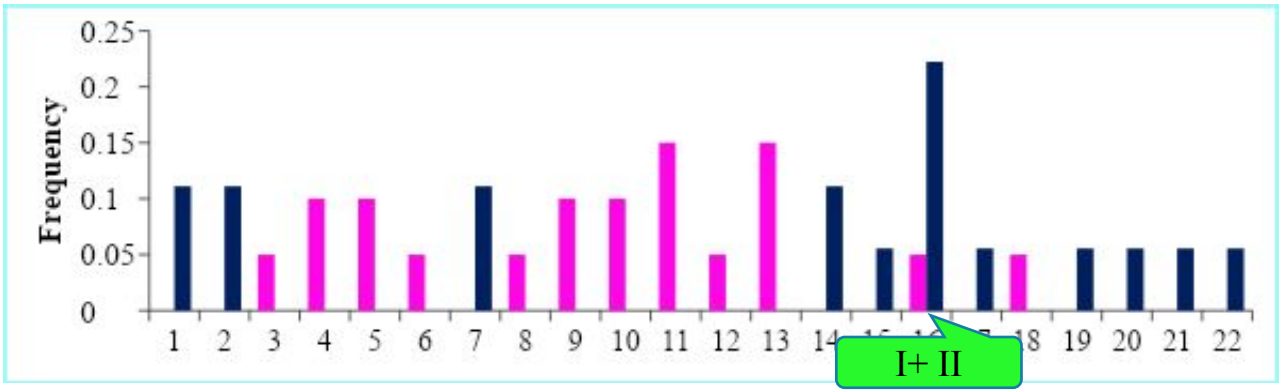
Notes:

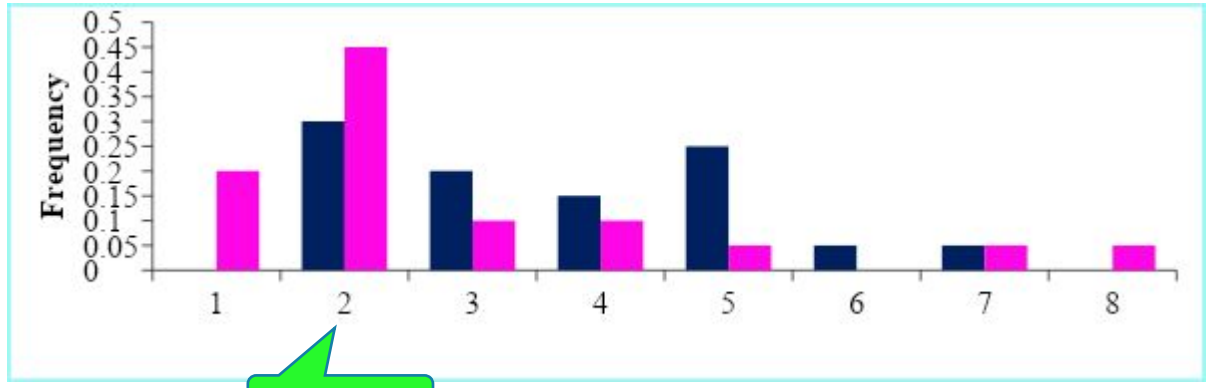
Na = No. of Different Alleles
Ne = No. of Effective Alleles
I = Shannon's Information Index
uHe = Unbiased Expected Heterozygosity

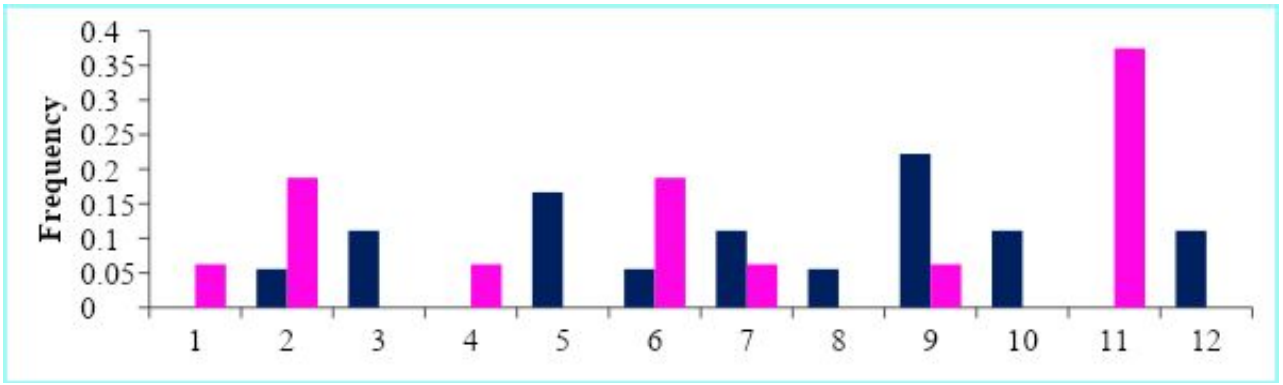
Results: differences in Allele Frequency between groups

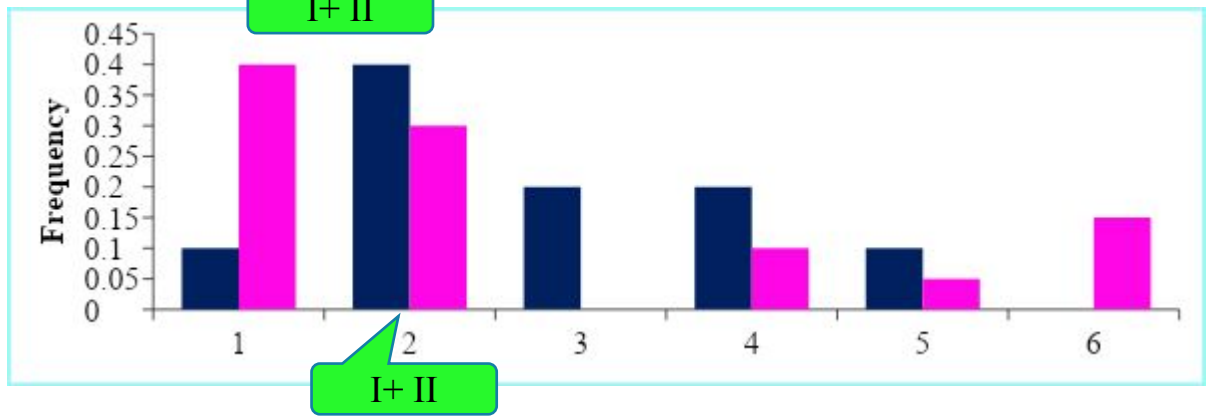












Notes: group I group II

Conclusion

- The studied loci were found to be polymorphic (100%) (average Ne = 7.9±1.9).
- The average polymorphic information content increased in the following sequence
  Ca 3 (0.657) < Rru 7 (0.685) < Rru 4 (0.723) < Ca 12 (0.797) < Rru 3 (0.803) < Ca 5 (0.883).
- Since the PIC value was above 0.5, it was concluded that all SSR primers used were characterized by high polymorphism.

Key Features of studied groups

General:

- There are no significant differences in the value of Shannon index I in the groups (fast-growing 1.762 ± 0.198 and slow-growing 1.717 ± 0.123)
- For Ca5 locus - allelic variant of 287 bp occurred in both groups (0.050 in group I and 0.222 in group II).
- For Rru7 locus - 82 bp, which occurred in both groups with a high frequency: 40% and 30%, respectively.
- For Rru3 locus, the presence of a specific allele of 187 bp was found, which was typical for both groups and occurred with a frequency of 22% in group I and 30% in group II.
- For Rru4 - variant of 140 bp occurring with a high frequency in both groups (30% and 45%, respectively)

Differences:

- For Ca3 locus:
  - in group I allelic variants of 224 bp. (50%) and 237 bp (25%) were private alleles
  - in group II - 275 bp and 297 bp variants were the most frequent (25% each), which were specific for this group.

The obtained results allowed making assumptions about the different genetic origin of the studied roach groups. Confirmation of this hypothesis requires additional comparison of the genetic structure of fast-growing roach and *R. heckelii* inhabiting the lower Dnieper.

THANK YOU
FOR YOUR ATTENTION!