Reintroduction of the Poljana and Nasice Common carp strains to their farms of origin

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Vukovar. 2010.04.14-15

Introduction

- Haki ex-situ genebank (15 Hungarian strains, 15 strains from all over the world)
- War in ex-Yugoslavia, strains possibly mixed, lost broodfish, lost genetic variability
- Cooperation between Zagreb Univesity and HAKI, main goal of project: reintroduction of Nasice and Poljana Common carp strains

Objectives

- Propagation, transport and reintroduction (Nasice, Poljana) of Croatian carp strains (100.000 larvae/strain)
- Description of the genetic variability of the local and genebank strains (number of alleles, allelic richness, number of individual alleles) using microsatellite DNA markers
- Description of genetic distance between the strains
- Searching for signs of inbreeding

Material and methods 1.

- samples from Poljana and Nasice strains kept at the fish farms (thanks to Tea!), samples from HAKI genebank strains (all preserved in 96% ethanol)

Material and Methods 2.

- extraction of total genomic DNA:

- digestion of proteins with Proteine-Kinase enzyme
- using high salt concentration, phenol, chlorophorm, isopropanol (**Miller et al., 1988**)

- checking DNA quality and quantity using agarose gel-electrophoresis and spectophotometry

- 80-150 ng/ml genomic DNA, dilution or repeated extraction if necessary

Material and methods 3.

- 6 microsatellite DNA markers: MFW1, MFW4, MFW6, MFW7, MFW16, MFW28 (**Crooijmans et al. 1997.**)
- PCR:

  first cycle: 2 minutes on 94°C (denatuing step)

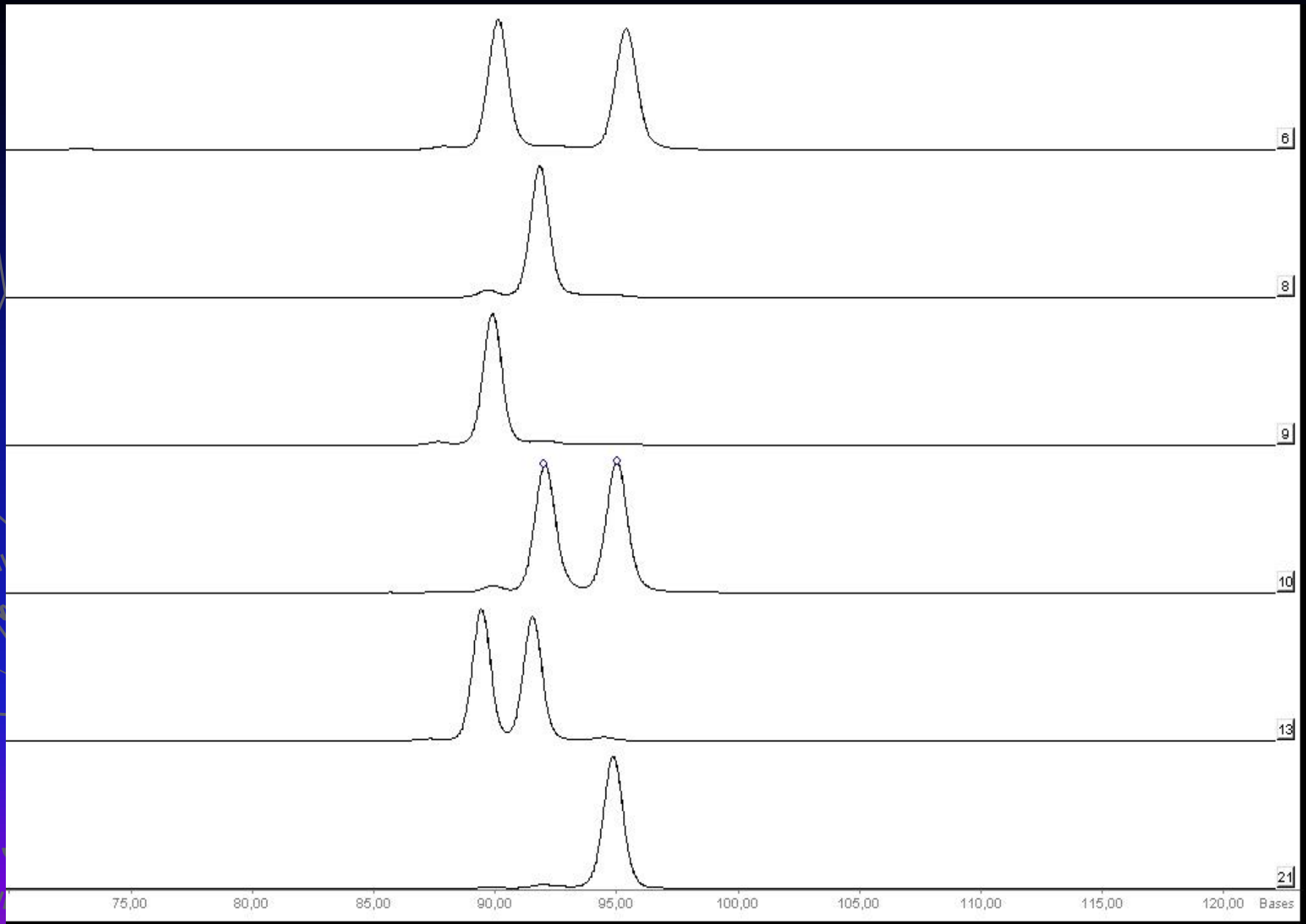
  next 30 cycles: 40 seconds 94°C-on (denaturing), 50 seconds on 55°C (primer-annealing) and 90 seconds on 72°C (elongation)

  last cycle: 10 minutes on 72°C (for T*aq* polymerase terminal transpherase activity)

Material and methods 4.

- separation of PCR products using 7% polyacrylamid gel (32% formamide, 5% urea), ALF Express II (Amersham Biosciences) fragment analyser

- sizing of PCR products : size standards
  - 50,100,150,200,250,300,350,400,450,500 bp standards
  - standard samples with known lenght
  - Fragment Analyser 1.03 (Amersham-Biosciences) software


6
8
9
10
13
21
75,00
80,00
85,00
90,00
95,00
100,00
105,00
110,00
115,00
120,00
Bases

Material and methods 5.

data analysis:

- Genepop (**Raymond and Rousset, 1995**): allele-frequences mean number of alleles, H_e, H_o, deviation from Hardy-Weinberg equilibrium (p values)
- Convert 1.31 (**Glaubitz, 2004**): private alleles
- F-Stat (**Goudet, 1995**): pairwise fixation index (F_{st} -value), allelic richness
- Populations (**Langella, 1999**):Nei's genetic distance between populations (**Nei, 1972**; **Nei, 1978**; **Nei et al., 1983**)

Material and methods 6.

- Populations (**Langella, 1999**): dendrogram (Neighbour Joining method- NJ)
- Treeview (**Page, 1996**): drawing dendrogram
- GeneClass (**Piry et al., 1999**):
  assignment test, self-classification, Bayesian method **Rannala and Mountain, 1997)** checking individual genotypes in order to assign them to a population


Results

Results

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Genetic variabiliy within the strains

Allele numbers by strain and locus, (number of privete alleles)

- 6 loci: 90 alleles
- allele number/locus: from 13 (MFW28) to 23 (MFW1)

	strain			
locus	Nasice	Genbank Nasice	Poljana	Genbank Poljana
MFW1	10	11 (3)	10 (4)	14 (4)
MFW4	10 (2)	7	8 (1)	13 (2)
MFW6	6	7	10 (3)	10 (3)
MFW7	10 (2)	9 (1)	11 (1)	13 (3)
MFW16	6 (1)	11 (5)	6 (1)	14 (4)
MFW28	9	11 (1)	13 (1)	10 (1)
∑	51 (5)	56 (10)	58 (11)	74 (17)
mean	8,5	9,33	9,6	12,33

Results

- the observed heterozygosity (Ho) values are between 6,66 (Poljana) and 93.3 % (genebank Poljana)

- in most cases the strains are significantly deviate from Hardy-Weinberg equilibrium

- the mean observed heterozygosity is lower than the mean expected heterozygosity in case of all strains (sign of inbreeding!!!)

- 97.4% of individuals was assigned correctly by test (only 3 individual to other strain)

Results

Expected (He) and observed (Ho) heterozygosity

Nasice	He	Ho
MFW1	85,96	24,13
MFW4	79,11	74,19
MFW6	61,23	41,19
MFW7	78,53	92
MFW16	61,18	19,35
MFW28	85,06	75
mean	75,18	54,31

Genebank Nasice	He	Ho
MFW1	90,57	86,66
MFW4	73,71	76
MFW6	66,12	16
MFW7	80,32	64
MFW16	83,67	84
MFW28	86,77	84
mean	80,19	68,44

Results

Expected (He) and observed (Ho) heterozygosity

Poljana	He	Ho
MFW1	84,02	25
MFW4	86,40	54,83
MFW6	87,17	76,66
MFW7	79,67	75,86
MFW16	63,84	6,66
MFW28	89,09	40
mean	81,69	46,50

Genebank Poljana	He	Ho
MFW1	88,64	63,33
MFW4	88,58	93,33
MFW6	80,15	20,68
MFW7	88,53	86,66
MFW16	86,27	26,66
MFW28	82,66	72,72
mean	85,80	60,56

Allelic richness

The number of alleles in a sample (allelic richness) is a fundamental measure of genetic diversity. However, this diversity measure has been difficult to use because large samples are expected to contain more alleles than small samples. The statistical technique of rarefaction compensates for this sampling disparity.

Results

Allelic richness

	strain			
locus	Nasice	Genebank Nasice	Poljana	Genebank Poljana
MFW1	8,7	11,0	8,5	11,1
MFW4	7,3	6.1	7,4	10,7
MFW6	5,6	5,4	8,6	7,5
MFW7	7,8	7,5	9,1	10,6
MFW16	4,6	9,4	4,4	10,6
MFW28	7,8	9,5	10,4	8,5
mean	7,0	8,1	8,1	9,8

Fixation index

Fixation index (Fst) is a measure of population differentiation based on genetic polymorphism data, such as single nucleotide polymorphisms (SNPs) or microsatellites. It is a special case of F-statistics, the concept developed in the 1920s by Sewall Wright.

This statistic compares the genetic variability within and between populations and is frequently used in the field of population genetics.

Results

Pairwise Fst values

	Nasicei	Poljanai	Genebank Poljana	Genebank Nasice
Nasice	0	*	**	**
Poljana	0,1066	0	*	*
Genebank Poljanai	0,1475	0,0786	0	*
Genebank Nasicei	0,1614	0,1097	0,0680	0

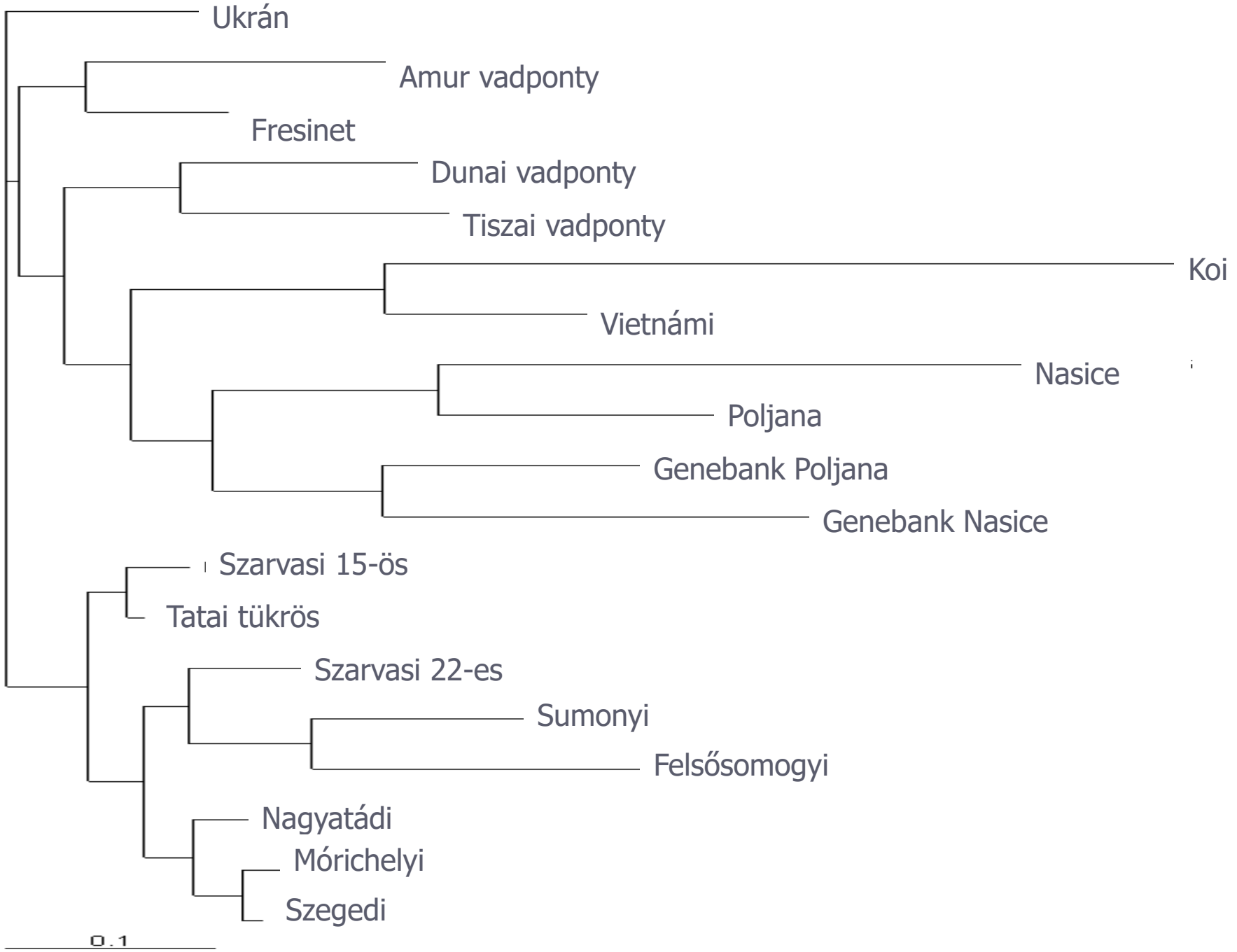
* Significant at 5% level

** Significant at 1% level

Results

Genetic Distance between the strains (Da distance by Nei)

	Nasice	Poljana	Genbank Poljana	Genbank Nasice
Nasice	0			
Poljana	0.44	0		
Genbank Poljana	0.69	0.464898	0	
Genbank Nasice	0.67	0.573435	0.38222	0


Ukrán
Amur vadponty
Fresinet
Dunai vadponty
Tiszai vadponty
Koi
Vietnámi
Nasice
Poljana
Genebank Poljana
Genebank Nasice
Szarvasi 15-ös
Tatai tükrös
Szarvasi 22-es
Sumonyi
Felsősomogyi
Nagyatádi
Mórichelyi
Szegedi
0.1

Conclusions

- all strains are inbred
- strains from the genebank are more variable
- strains are genetically not too close to each other
- genebank strains are good source of repatriating projects
- genetic markers are useful tools in conservation
- international cooperation!!!

Thank you for your attention!