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Genetic variability and structure of common carp (*Cyprinus carpio*) populations throughout the distribution range inferred from allozyme, microsatellite and mitochondrial DNA markers

Klaus Kohlmann [a,*], Riho Gross [b], Asiya Murakaeva [a,c], Petra Kersten [a]

[a] *Department of Inland Fisheries, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm 310, P.O. Box 850119, 12561 Berlin, Germany*
[b] *Department of Fish Farming, Institute of Animal Sciences, Estonian Agricultural University, 1 Kreutzwaldi Street, 51015 Tartu, Estonia*
[c] *Institute of Biochemistry, 56, Abdulaev H. Str., Tashkent 700143, Uzbekistan*

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Abstract

Domesticated/captive stocks and wild/feral populations of common carp from Europe, Central Asia and East/South-East Asia were examined for allozyme (23 populations), microsatellite (11 populations) and mitochondrial DNA (21 populations) variation. Allozyme variability (1.06–1.81 alleles per locus, expected heterozygosity 0.006–0.136 at 16 loci) was much lower than microsatellite variability (2.5–14.0 alleles per locus, expected heterozygosity 0.426–0.887 at four loci). Differences in variability between domesticated/captive stocks and wild-caught ones were more pronounced at microsatellite loci than at allozyme loci, suggesting that microsatellites are better suited to detect population bottlenecks and loss of variation due to inbreeding. All but one European population were fixed for a single composite mtDNA haplotype, which also dominated in Central Asia but was completely missing in East/South-East Asia, indicating a single origin of European carp in Central Asia. All three classes of genetic markers clustered populations into two highly divergent groups: Europe/Central Asia and East/South-East Asia. Hierarchical partition of genetic diversity showed that for microsatellite loci most of variation was due to the within-population component while the highest proportion of mtDNA variation and substantial proportion of allozyme variation was accounted for by differences between geographical regions. Genetic data support the subspecies status of *C. c. carpio* assigned to the European carp and *C. c. haematopterus* assigned to the East/South-East Asian carp but do not justify a separate subspecies status (*C. c. aralensis*) for the Central Asian carp. As demonstrated for a wild/feral carp population from R. Danube, Germany, the genetic markers used in our study may be effectively applied to detect mixing and introgression of intra-species units in the presence of sufficient genetic differentiation.

Résumé

Variabilité et structuration génétiques des populations de carpe (*Cyprinus carpio*) d'après la gamme de distribution des marqueurs : allozymes, microsatellites et ADN mitochondrial. Les stocks de carpes domestiquées et les populations de carpes sauvages de l'Europe, de l'Asie centrale, de l'Asie du Sud-Est et d'Extrême-Orient ont été examinés pour étudier les variations en allozymes (23 populations), en microsatellites (11 populations) et en ADN mitochondrial (21 populations). La variabilité en allozymes (1,06 à 1,81 allèles par locus, hétérozygotie attendue 0,006 à 0,136 pour 16 loci) est largement inférieure à la variabilité des microsatellites (2,5 à 14 allèles par locus, hétérozygotie attendue 0,426 à 0,887 à 4 loci). Les différences en variabilité entre stocks domestiqués et carpes sauvages sont plus prononcées aux loci de microsatellites qu'à ceux des allozymes, laissant penser que les microsatellites sont mieux adaptés pour détecter des goulots d'étranglement et la perte de variation due à la consanguinité. Toutes les populations à l'exception de celle de l'Europe ont été fixées pour un seul haplotype mitochondrial mtDNA qui domine également en Asie centrale mais qui est complètement absent en Asie du Sud-Est et Extrême-Orient, indiquant une origine unique pour la carpe européenne en Asie centrale. Les trois classes de marqueurs génétiques sont groupées en deux groupes hautement divergents : Europe–Asie centrale et Sud-Est asiatique–Extrême-Orient. La partition hiérarchique de la diversité génétique montre que la plupart de la variation chez les loci de microsatellites est due à la composante intra-populations, tandis que la plus grande proportion de variation de mtADN et une partie substantielle de la variation des allozymes proviennent des différences entre régions géographiques. Les données génétiques confortent le statut de sous-espèces à *C. c. carpio* assignée à la carpe européenne et *C. c.*

* Corresponding author.
E-mail address: kohlmann@igb-berlin.de (K. Kohlmann).

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haematopterus assignée à la carpe du Sud-Est asiatique et d'Extrême-Orient mais ne justifient pas un statut de sous-espèce pour la carpe d'Asie centrale (*C. c. aralensis*). Comme pour les carpes sauvages du Danube, Allemagne, les marqueurs génétiques utilisés dans notre étude pour une population sauvage peuvent effectivement être appliqués pour détecter des introgressions d'unités intraspécifiques et des mélanges en présence de différentiation génétique suffisante.

Keywords: Population genetics; Allozymes; mtDNA; Microsatellites; *Cyprinus carpio*

1. Introduction

Common carp has a long history of domestication and numerous strains and breeds have been developed from its ancestor, the wild common carp, *Cyprinus carpio*, both in Europe and Asia. The natural distribution range of wild carp in Eurasia is nowadays divided into disjunct western (Caspian, Aral and Black Sea basins) and eastern (East and South-East Asia) areas, which were supposedly isolated during multiple Pleistocene glaciations (Kirpitchnikov, 1999). Based on differences in morphological and ecophysiological traits, numerous subspecies, races and varieties of wild carp have been distinguished but their taxonomic status and phylogenetic relationships remain uncertain (Balon, 1995; Kottelat, 1997) that warrants further studies.

The genetics of common carp populations has mainly been studied on regional levels using traditional allozyme markers (Macaranas et al., 1986; Sumantadinata and Taniguchi, 1990; Paaver and Gross, 1991; Anjum, 1995; Desvignes et al., 2001; Šlechtova et al., 2002) while mitochondrial DNA (Davis et al., 1999; Froufe et al., 2002; Gross et al., 2002) and microsatellite loci (Tanck et al., 2000; Desvignes et al., 2001; David et al., 2001; Bártfai et al., 2003) have been introduced only recently. In the few cases in which populations from different geographical regions were examined at least two genetically distinct groups—European and East Asian—could be detected (Brody et al., 1979; Paaver, 1983; Kohlmann and Kersten, 1999; Gross et al., 2002) supporting the existence of at least two subspecies, *C. c. carpio* and *C. c. haematopterus*, formerly distinguished on the basis of morphological differences (Kirpitchnikov, 1967, 1999; Balon, 1995).

The present study was aimed at providing more detailed information on the genetic variability and differentiation among populations from the major parts of the natural distribution area of the species. This was achieved by extending our previous studies (Kohlmann and Kersten, 1999; Gross et al., 2002; Murakaeva et al., 2003) in both the number and geographical origins of samples and by applying different classes of genetic markers (allozyme, microsatellite and mtDNA loci) simultaneously. Also, we assessed the utility of polymorphism at allozyme and microsatellite loci, combined with variation in mtDNA, to test genetic purity of the carp populations. Phylogeographic aspects will only briefly be discussed since they are the primary topic of an ongoing study on mtDNA sequence variation.

2. Materials and methods

A total of 31 domesticated, feral and wild common carp populations spanning a geographical area as wide as from Europe (Spain, Germany, Czech Republic, Poland, Hungary, Russia) through Central Asia (Uzbekistan) to East and South-East Asia (China, Japan, Vietnam) were sampled (Table 1). Fig. 1 shows the origins of populations. However, these locations are not always identical to the places where samples were collected: Dor-70, Ropsha, Tata, Pohorelice and Amur wild carp were taken from the live gene bank maintained at the Research Institute of Fish Culture and Hydrobiology, Vodňany, Czech Republic, R. Tisza carp were progenies of wild caught spawners, and ornamental Koi carp were obtained from a German fish breeder. The attribute "wild" does not necessarily mean "native", instead it stands for self-reproducing populations in natural waters (rivers and lakes). Out of these, 23 populations could be examined for allozyme polymorphisms, 11 representative populations were selected for a preliminary study of the microsatellite variability and 21 populations were included into the mtDNA analyses. The subset of populations for analysis of each marker type was somewhat different mainly due to logistic reasons. The allozyme studies (eight enzymatic systems representing 22 loci) were performed as previously described in Kohlmann and Kersten (1999). However, already published data had to be re-examined due to later findings that GPI-B is controlled by one locus only instead of two (Kohlmann and Luczynski, 2000). Data analysis was restricted to the 16 diploid loci. The microsatellite variation was examined at four loci (*MFW1, MFW6, MFW7, MFW28*) with PCR conditions as described in Crooijmans et al. (1997). One primer of each pair was fluorochrome labelled to enable the determination of allele sizes using an ABI 310 Genetic Analyzer and the ABI GeneScan software. The PCR-RFLP analysis of the mitochondrial *ND-3/4* and *ND-5/6* gene polymorphisms was carried out according to Gross et al. (2002).

GENEPOP v. 3.1d (Raymond and Rousset, 1995) was used for allozyme and microsatellite loci to calculate allele frequencies, observed (H_O) and expected (H_E) heterozygosities and to test for deviations from Hardy–Weinberg equilibrium. Adjusted estimates of the mean number of alleles per locus (A_r, allelic richness) were calculated by rarefaction approach using the FSTAT ver. 2.9.3.2 software (Goudet, 2002). FSTAT was also used for testing the significance of differences in average values of A_r, H_E and H_O among the

Table 1
List of common carp populations used for different genetic analyses. Status of populations: D, domesticated; W, wild; W/F, wild/feral (exact status not known)

Population code	Geographic region/population name	Country of origin	Status	Analyzed genetic markers		
				Allozymes	Microsatellites	mtDNA
	Europe					
EU1wf	R. Danube (Maier)	Germany	W/F [a]	x	x	x
EU2wf	R. Danube (Straubing)	Germany	W/F	x	x	x
EU3wf	R. Tisza	Hungary	W/F		x	x
EU4wf	R. Rhine	Germany	W/F	x	x	x
EU5d	Scaly pond carp	Germany	D	x	x	x
EU6d	Fiedler	Germany	D	x	x	x
EU7d	Kauppa	Germany	D	x		
EU8d	Seckendorff	Germany	D	x		
EU9d	Wiesinger	Germany	D	x		
EU10d	Scheuermann	Germany	D	x		
EU11d	Hertlein	Germany	D	x		
EU12d	Glinzig	Germany	D	x		
EU13d	Kreba	Germany	D	x		
EU14d	Petkampsberg	Germany	D	x		
EU15d	Petershain	Germany	D			x
EU16d	Dor-70	Israel	D	x		
EU17d	Ropsha	Russia/Europe	D	x		
EU18d	Badajoz	Spain	D			x
EU19d	Tata	Hungary	D			x
EU20d	Pohorelice	Czech Republic	D			x
EU21d	Zator	Poland	D			x
	Central Asia					
CA1w	L. Tuzkan	Uzbekistan	W	x	x	x
CA2w	R. Syr-Darya	Uzbekistan	W	x	x	x
CA3w	Syr-Darya channels	Uzbekistan	W	x		x
CA4w	L. Arnasaiskie	Uzbekistan	W	x		x
CA5w	L. Aidar	Uzbekistan	W	x		x
CA6w	R. Kli	Uzbekistan	W			x
	East and South-East Asia					
EA1w	R. Amur	Russia/Asia	W [a]	x	x	x
EA2w	R. Red	Vietnam	W	x	x	x
EA3d	Wuhan	China	D			x
EA4d	Koi	Japan	D	x	x	x

[a] Captive stock.

groups of populations (1000 permutations, one-sided test of the null hypothesis of no difference). Population differentiation was evaluated by calculating pairwise estimates of F_{ST} values and testing their significance by bootstrapping analysis (1000 replicates) using the Arlequin v.1.1 package (Schneider et al., 1997). For allozyme loci, the relationships among populations were illustrated by an UPGMA dendrogram based on the standard genetic distances, D_S (Nei, 1972) and the significance of population grouping was tested by bootstrapping analysis (1000 replicates) running the appropriate routines of BIOSYS-2 (Black, 1997) together with PHYLIP ver. 3.573c (Felsenstein, 1995). The genetic relationships among samples were also examined by multidimensional scaling analysis (MA) of the matrix of pairwise D_S distances using the STATISTICA v. 6.0. software package. MA plots allow representation of the genetic relationship among samples with minimum loss of information and in multi-dimensional space. For microsatellite loci, the D_A distances (Nei et al., 1983) among pairs of populations were calculated, a NJ dendrogram was constructed and population grouping was tested by bootstrapping analysis (1000 replicates) using the DISPAN program (Ota, 1993). The D_A distance measure was used because it is independent of the mutation models (Nei, 1987) and superior to other distance measures in correct tree topology construction using microsatellites (Takezaki and Nei, 1996). The genetic relationships among mtDNA haplotypes were analysed using the programs FITCH and RESTML from PHYLIP ver. 3.573c. Populations were clustered in an UPGMA dendrogram based on the estimated number of net nucleotide substitutions between all pairs of populations using the software package REAP ver. 4.0 (McElroy et al., 1992) and the NEIGHBOR program from PHYLIP ver. 3.573c. Hierarchical partition of genetic diversity based on allozyme, microsatellite and mtDNA data was evaluated by analysis of molecular variance (AMOVA; Excoffier et al., 1992) using Arlequin ver. 2.0.

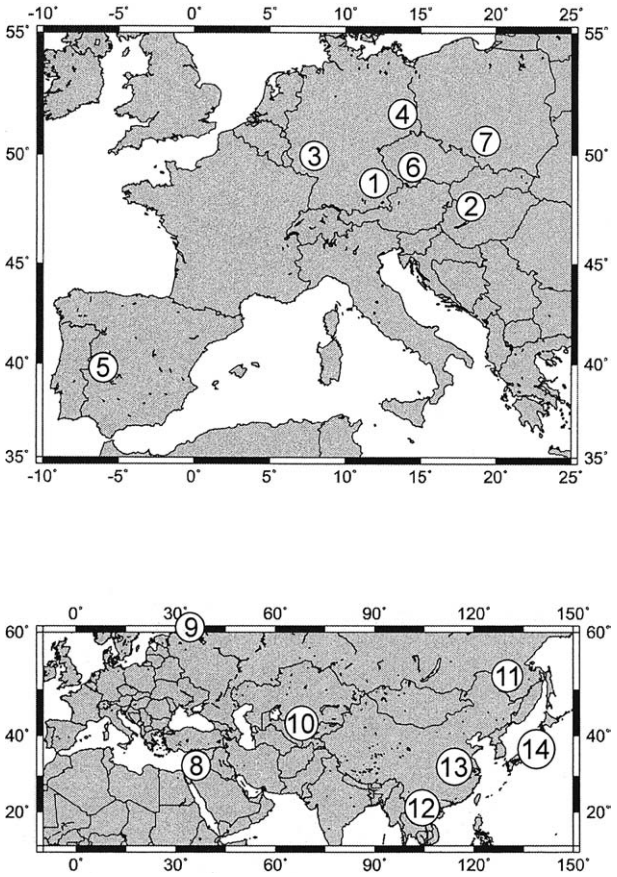

Fig. 1. Map showing the origin of studied common carp populations.1, EU1wf, EU2wf, EU5d, EU6d, EU8d, EU9d, EU10d, EU11d; 2, EU3wf, EU19d; 3, EU4wf; 4, EU7d, EU12d, EU13d, EU14d, EU15d; 5, EU18d; 6, EU20d; 7, EU21d; 8, EU16d; 9, EU17d; 10, CA1w-CA6w; 11, EA1w; 12, EA2w; 13, EA3d; 14, EA4d.

3. Results

3.1. Genetic variability

At allozyme loci, a mean number of 1.06–1.81 alleles per locus, 6.2–43.8% of polymorphic loci and expected heterozygosities ranging from 0.006 to 0.136 were observed (Table 2). The East and South-East Asian populations showed on an average significantly higher levels of variability (measured as allelic richness and heterozygosity) than the European and Central Asian populations (Table 2). Among European populations, the domesticated stocks displayed significantly lower level of variation than the wild populations: mean A_r = 1.19 and 1.39, respectively ($P < 0.05$) and mean H_E = 0.050 and 0.115, respectively ($P < 0.01$). A tendency towards reduced variability in captive (EA1w, A_r = 1.25) or domesticated (EA4d, A_r = 1.31) stocks compared to a wild population (EA2w, A_r = 1.62) was evident also among East and South-East Asian carp populations.

The microsatellite loci displayed a much higher level of variability than the allozyme loci. A total of 125 alleles were observed over four loci, ranging from 22 at *MFW28* to 42 at *MFW7*. However, the mean number of alleles per locus was substantially smaller: from 2.5 in the captive stock EA1w to 14.0 in Central Asian wild populations (Table 2). Average expected heterozygosities were also larger for microsatellite loci ranging from 0.426 in EA4d to 0.887 in CA1w. The differences in variability between domesticated/captive populations (average A_r = 3.87 and H_E = 0.605) and wild-caught populations (average A_r = 7.33 and H_E = 0.851) were more pronounced than at allozyme loci ($P < 0.01$). In contrast to the allozyme data, the average levels of variability were significantly higher in European and Central Asian populations than in East and South-East Asian populations (Table 2). These estimates, however, are probably biased due to unequal representation of domesticated/captive stocks and wild-caught populations in different regions as the allelic richness and heterozygosity estimates among wild-caught populations (e.g. EU2wf, EU4wf, CA1w, CA2w and EA2w) were rather similar.

The digestion of the two mtDNA genes by 10 restriction enzymes resulted in a total of 10 composite haplotypes detectable on agarose gels (Table 3). The rare haplotypes H2 and H4 described in Gross et al. (2002) could not be confirmed by a later sequencing analysis and had to be rejected. The highest variation (number of haplotypes, haplotype and nucleotide diversities) was observed in Central Asian, Chinese and Vietnamese carp populations (Table 4). In contrast, there was no variation within 10 out of the 11 wild/feral and domesticated European populations studied. All of them were fixed for composite haplotype H1, which also dominated in the six wild populations from Uzbekistan (one of them was even fixed for this haplotype). Surprisingly, among the 27 wild/feral carp from the German part of R. Danube (EU2wf) two individuals had a composite haplotype, which differed by only two substitutions from the typical R. Amur wild carp haplotype. The latter population and Koi carp were other populations fixed for distinct haplotypes (H3 and H7, respectively).

At allozyme loci, most of the populations were found to be in Hardy–Weinberg equilibrium. Only four domesticated stocks (EU5d, EU13d, EU16d and EA4d) and the R. Amur wild carp captive stock displayed significant to highly significant deviations (Table 2). In contrast, at microsatellite loci significant to highly significant deviations from Hardy–Weinberg expectations were observed in six out of the 11 populations studied (Table 2).

3.2. Genetic differentiation and relationships between populations

Significant population differentiation based on allozyme loci was mainly found between geographical regions whereas several of the population pairs within regions (especially domesticated stocks in Germany and wild populations in Central Asia) displayed nonsignificant F_{ST} values (Table 5). But there were also some inter-regional (Europe vs. Central Asia) nonsignificant differences among population pairs: between EU16d and CA3w, and between EU9d and three of the Uzbek wild populations (Table 5). The average level of differentiation within geographical regions

Table 2
Genetic variability of common carp populations at 16 allozyme and four microsatellite loci (*n*, mean sample size; *p*, percentage of polymorphic loci (99% criterion); *A*, mean number of alleles per locus; A_r, allelic richness; H_E, expected heterozygosity; H_O, observed heterozygosity; P_{HW}, significance of Hardy–Weinberg probability test: * $P < 0.05$, ** $P < 0.001$, *** $P < 0.01$, n.s. = nonsignificant)

Population	Allozyme loci							Microsatellite loci					
	n	*P*	*A*	A_r	H_E	H_O	P_{HW}	*n*	*A*	A_r	H_E	H_O	P_{HW}
Europe													
EU1wf	49.8	0.312	1.38	1.37	0.129	0.131	n.s.	28.0	5.25	4.37	0.641	0.722	*
EU2wf	27.3	0.312	1.38	1.36	0.102	0.118	n.s.	27.5	13.00	7.38	0.826	0.669	**
EU3wf	–	–	–	–	–	–	–	8.8	4.75	4.74	0.771	0.795	n.s.
EU4wf	29.8	0.375	1.50	1.44	0.098	0.103	n.s.	29.3	13.50	8.16	0.872	0.861	n.s.
EU5d	47.0	0.188	1.19	1.16	0.042	0.061	**	29.3	4.75	4.24	0.720	0.905	**
EU6d	49.5	0.250	1.31	1.29	0.076	0.073	n.s.	30.0	7.75	5.63	0.752	0.775	n.s.
EU7d	48.4	0.250	1.25	1.22	0.053	0.054	n.s.	–	–	–	–	–	–
EU8d	49.8	0.188	1.19	1.18	0.052	0.045	n.s.	–	–	–	–	–	–
EU9d	29.9	0.250	1.25	1.17	0.014	0.015	n.s.	–	–	–	–	–	–
EU10d	24.8	0.188	1.19	1.18	0.057	0.047	n.s.	–	–	–	–	–	–
EU11d	49.8	0.188	1.19	1.18	0.054	0.054	n.s.	–	–	–	–	–	–
EU12d	24.7	0.250	1.25	1.25	0.078	0.087	n.s.	–	–	–	–	–	–
EU13d	24.9	0.312	1.31	1.30	0.087	0.117	***	–	–	–	–	–	–
EU14d	49.3	0.188	1.19	1.18	0.048	0.053	n.s.	–	–	–	–	–	–
EU16d	19.9	0.125	1.13	1.13	0.037	0.025	*	–	–	–	–	–	–
EU17d	38.7	0.062	1.06	1.05	0.006	0.006	n.s.	–	–	–	–	–	–
Average		0.229	1.25	1.232 [b]	0.062 [b]	0.066 [b]			8.17	5.75 [a,b]	0.764 [a]	0.788 [a]	
Central Asia													
CA1w	49.6	0.312	1.44	1.29	0.047	0.044	n.s.	28.5	14.00	8.66	0.887	0.805	***
CA2w	18.9	0.125	1.19	1.19	0.042	0.034	n.s.	27.0	14.00	8.33	0.854	0.835	n.s.
CA3w	24.8	0.188	1.25	1.22	0.042	0.050	n.s.	–	–	–	–	–	–
CA4w	24.8	0.188	1.25	1.21	0.047	0.038	n.s.	–	–	–	–	–	–
CA5w	49.6	0.188	1.31	1.23	0.047	0.045	n.s.	–	–	–	–	–	–
Average		0.200	1.29	1.226 [b]	0.045 [b]	0.042 [b]			14.00	8.50 [a]	0.871 [a]	0.820 [a]	
East and South-East Asia													
EA1w	48.6	0.250	1.25	1.25	0.099	0.149	**	27.8	2.50	2.49	0.468	0.633	**
EA2w	45.6	0.438	1.81	1.62	0.125	0.112	n.s.	26.5	9.25	6.69	0.797	0.771	**
EA4d	45.4	0.312	1.31	1.31	0.136	0.181	**	28.8	3.00	2.60	0.426	0.492	n.s.
Average		0.333	1.46	1.395 [a]	0.120 [a]	0.147 [a]			4.92	3.93 [b]	0.564 [b]	0.632 [b]	

[a,b] Different letters in superscript designate significant differences ($P < 0.05$) between the averages of population groups.

Table 3
Composite mtDNA haplotypes of common carp resulting from the digestion of *ND-3/4* and *ND-5/6* coding regions by restriction enzymes. Variant restriction patterns are designated by capital letters

Composite haplotype	ND-3/4										*ND-5/6*									
	Eco 47I	*Bsu* RI	*Hin* fI	*Rsa* I	*Alu* I	*Mbo* I	*Hpa* II	*Hin* 6I	*Taq* I	*Xba* I	*Eco* 47I	*Bsu* RI	*Hin* fI	*Rsa* I	*Alu* I	*Mbo* I	*Hpa* II	*Hin* 6I	*Taq* I	*Xba* I
H1	A	A	A	A	A	A	A	A	A	B	A	A	*A*	A	A	A	A	A	A	A
H1b	A	A	A	A	A	A	A	A	A	B	**A**	A	A	A	**C**	A	A	A	A	A
H3	A	B	B	A	B	C	B	A	B	B	B	B	A	A	A	B	A	A	B	A
H5	A	A	B	A	B	A	B	A	B	B	B	B	A	A	**C**	A	A	A	A	A
H6	A	A	B	A	B	B	B	A	B	A	B	B	A	A	A	A	A	A	A	A
H7	B	A	B	A	B	A	B	A	B	B	B	C	B	B	A	A	A	A	A	A
H9	A	A	A	A	A	A	B	A	A	B	B	A	A	A	A	A	A	A	A	A
H11	A	C	A	A	A	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A
H12	A	B	B	A	B	A	B	A	B	B	B	B	A	A	B	**A**	A	A	**A**	A
H13	A	B	B	A	A	A	B	A	B	B	B	D	A	A	**D**	A	A	A	A	A

was highest among East/South-East Asian populations (average $F_{ST} = 0.29$), followed by European (average $F_{ST} = 0.10$) and Central Asian carp populations (average $F_{ST} = 0.008$). Pairwise population comparisons from different geographical regions revealed substantially lower differentiation between European and Central Asian populations (average $F_{ST} = 0.18$) than between these two regions and the East/South-East Asia (average $F_{ST} = 0.45$ and 0.52, respec-

Table 4
Distribution of the composite mtDNA haplotypes described in Table 3 and haplotype and nucleotide diversities at *ND-3/4* and *ND-5/6* coding regions of common carp populations

Population code	*n*	Composite haplotype										Haplotype diversity	Nucleotide diversity
		H1	H1b	H3	H5	H6	H7	H9	H11	H12	H13		
EU1wf	8	8										0	0
EU2wf	27	25		2 [a]								0	0
EU3wf	9	9										0	0
EU4wf	29	29										0	0
EU5d	29	29										0	0
EU6d	23	23										0	0
EU15d	30	30										0	0
EU18d	18	18										0	0
EU19d	19	19										0	0
EU20d	18	18										0	0
EU21d	17	17										0	0
CA1w	29	16	6					6	1			0.631	0.002
CA2w	28	15	7					6				0.627	0.002
CA3w	28	28										0	0
CA4w	27	12	4					9	2			0.690	0.003
CA5w	29	15	6					8				0.636	0.002
CA6w	28	11	8					9				0.686	0.003
EA1w	22			22								0	0
EA2w	26				15	11						0.508	0.003
EA3d	20			7						12	1	0.542	0.003
EA4d	30						30					0	0

[a] Based on two nucleotide substitutions designated as H3a later on.

Table 5
Matrix of F_{ST} values between pairs of common carp populations based on microsatellite (above diagonal) and allozyme (below diagonal) loci. Nonsignificant values ($P > 0.05$) in bold

Pop	EU 1wf	EU 2wf	EU 3wf	EU 4wf	EU 5d	EU 6d	EU 7d	EU 8d	EU 9d	EU 10d	EU 11d	EU 12d	EU 13d	EU 14d	EU 16d	EU 17d	CA 1w	CA 2w	CA 3w	CA 4w	CA 5w	EA 1w	EA 2w	EA 4d
EU1wf		0.185	0.249	0.137	0.235	0.227											0.193	0.215				0.354	0.214	0.442
EU2wf	0.139		0.119	0.056	0.106	0.065											0.073	0.102				0.208	0.119	0.333
EU3wf				0.046	0.145	0.138											0.097	0.104				0.299	0.154	0.402
EU4wf	0.060	0.188			0.103	0.073											0.056	0.067				0.233	0.114	0.307
EU5d	0.139	0.328		0.012		0.051											0.086	0.090				0.314	0.185	0.395
EU6d	0.087	0.181		0.067	0.127												0.094	0.107				0.303	0.170	0.381
EU7d	0.123	0.237		0.040	0.085	0.017																		
EU8d	0.118	0.296		**0.008**	**0.000**	0.076	0.044																	
EU9d	0.276	0.369		0.169	0.199	0.160	0.096	0.165																
EU10d	0.152	0.305		0.019	**0.000**	0.109	0.058	**0.001**	0.155															
EU11d	0.125	0.254		0.107	0.161	**0.000**	0.029	0.100	0.187	0.143														
EU12d	0.035	0.258		0.023	0.064	0.098	0.098	0.049	0.326	0.101	0.132													
EU13d	0.038	0.241		0.010	0.052	0.098	0.086	0.042	0.289	0.082	0.134	**0.000**												
EU14d	0.129	0.253		0.041	0.072	0.018	**0.000**	0.032	0.093	0.045	0.028	0.100	0.089											
EU16d	0.219	0.189		0.157	0.245	0.118	0.125	0.203	0.151	0.216	0.170	0.304	0.276	0.125										
EU17d	0.427	0.262		0.608	0.714	0.540	0.647	0.684	0.873	0.789	0.622	0.686	0.671	0.656	0.746									
CA1w	0.292	0.365		0.182	0.217	0.155	0.099	0.180	**0.000**	0.165	0.182	0.346	0.312	0.097	0.079	0.801		**0.003**				0.227	0.099	0.332
CA2w	0.239	0.292		0.152	0.208	0.114	0.079	0.165	**0.009**	0.169	0.139	0.294	0.262	0.077	0.064	0.853	**0.000**					0.254	0.133	0.364
CA3w	0.255	0.271		0.167	0.227	0.143	0.117	0.191	0.050	0.189	0.185	0.325	0.293	0.114	**0.015**	0.814	**0.011**	**0.004**						
CA4w	0.254	0.338		0.159	0.199	0.125	0.076	0.157	**0.000**	0.159	0.143	0.302	0.270	0.072	0.123	0.862	**0.000**	**0.000**	0.042					
CA5w	0.286	0.362		0.194	0.235	0.128	0.089	0.186	0.023	0.192	0.148	0.350	0.320	0.085	0.079	0.811	**0.002**	**0.000**	**0.023**	**0.000**				
EA1w	0.299	0.040		0.342	0.454	0.303	0.370	0.424	0.443	0.428	0.360	0.419	0.411	0.380	0.263	0.204	0.431	0.370	0.344	0.411	0.427		0.230	0.453
EA2w	0.372	0.147		0.438	0.543	0.427	0.487	0.521	0.545	0.515	0.474	0.483	0.478	0.497	0.406	0.222	0.551	0.483	0.471	0.517	0.550	0.094		0.293
EA4d	0.413	0.321		0.511	0.619	0.548	0.594	0.608	0.657	0.605	0.593	0.536	0.520	0.601	0.569	0.518	0.667	0.615	0.613	0.641	0.674	0.425	0.352	

tively). The Ropsha carp (EU17d) which displayed very high level of differentiation from both European and Central Asian populations was excluded from these calculations because of its hybrid origin (see below).

At microsatellite loci, the F_{ST} values for all but one pairwise comparison of the 11 populations were highly significant (Table 5). The only exception was the Uzbek population pair CA1w and CA2w. General pattern of differentiation within and among geographical regions was consistent with the allozyme results: the average F_{ST} was highest among East/South-East Asian populations (0.33), followed by European (0.12) and Central Asian stocks (0.003), while the average level of differentiation between East/South-East Asian and European or Central Asian carp (0.27 and 0.24, respectively) was more than twice as high as between the latter two regions (0.11).

In a first version of an UPGMA dendrogram (not shown) allozyme data clustered populations generally according to their geographical origins (Europe, Central Asia and East Asia) with only two exceptions: the domesticated Ropsha carp (EU17d) and wild/feral population EU2wf from the Danube (despite exclusion of two individuals possessing the typical Amur mtDNA composite haplotype) formed one group (51% bootstrap support) together with East Asian wild carp populations EA1w and EA2w. On the MA three-dimensional plot (Fig. 2) these two populations occupied an intermediate position between the European/Central Asian and East/South-East Asian populations. In case of Ropsha carp this observation could be explained by its hybrid origin: this breed was developed in the former Soviet Union by initial crossing of R. Amur wild carp with domesticated European-Galician mirror carp and selection of the hybrids for increased viability and resistance to low temperatures (Kirpitchnikov, 1999). Intermediate position of the EU2wf population between the European/Central Asian and East/South-East Asian populations (together with the occurrence of R. Amur mtDNA haplotypes) suggests that the particular Danube population has been hybridized (or may have been already introgressed) with the introduced East Asian carp. This is clearly supported by allele frequencies at two allozyme loci: the *GPI-A2*85* allele was recorded at identical frequencies in Amur wild carp (0.240) and in EU2wf (0.232) but could not be found in any other European population and the typical for East Asian carp *PGM*85* allele displayed similar frequencies in both populations (0.730 in Amur wild carp; 0.607 in EU2wf) but was rare (less than 0.220) or missing in all other European carp populations. Based on these findings the two hybrid/introgressed populations were excluded from the further analysis, which improved the resolution of the UPGMA dendrogram (Fig. 3). All remaining 21 populations clustered into two major groups, Europe/Central Asia and East/South-East Asia, supported by a bootstrap value of 73%. European and Central Asian populations clustered also separately but with a lower bootstrap support. The NJ dendrogram constructed on the basis of microsatellite data revealed the same two major groups (after excluding the introgressed EU2wf population which initially occupied an intermediate position): the European and Central Asian populations formed one cluster and the East and South-East Asian populations the other one with 66% bootstrap support (Fig. 4).

The 10 composite mtDNA haplotypes clustered into two distinct groups: Europe/Central Asia (four haplotypes) and East/South-East Asia (six haplotypes), separated from each other with 100% bootstrap support (Fig. 5). The distribution of haplotypic groups strictly followed the geographical origin of populations (Table 4). The UPGMA clustering of populations again revealed the two highly distinct groups:

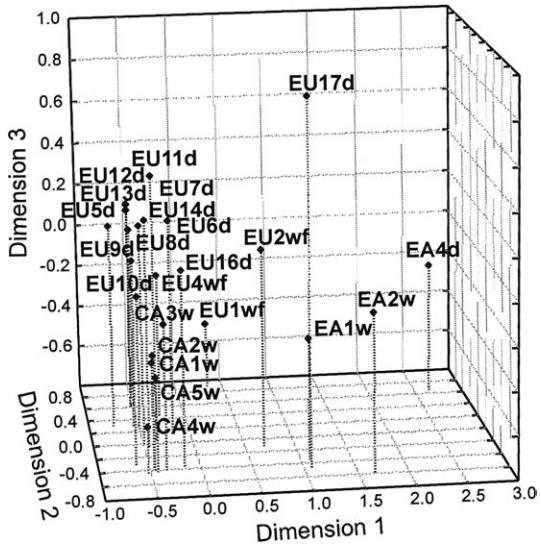

Fig. 2. Three-dimensional plot of common carp populations based on the matrix of allozyme derived Nei's standard genetic distances.

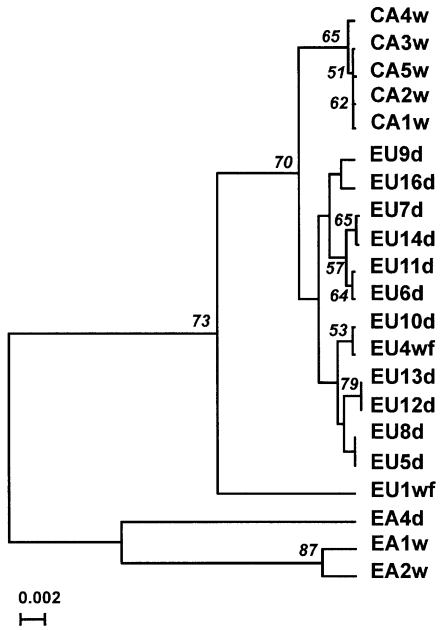

Fig. 3. UPGMA clustering of common carp populations based on allozyme polymorphisms at 16 diploid loci and Nei's standard genetic distances. Bootstrap values were taken from the most consensus tree generated by PHYLIP ver. 3.573c.

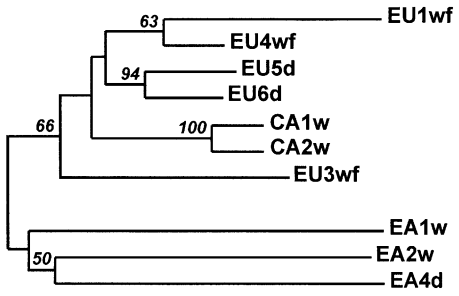

Fig. 4. NJ clustering of common carp populations based on microsatellite variability at four loci and D_A distances (Nei et al., 1983) among pairs of populations. The bootstrap values were generated by DISPAN software.

Europe/Central Asia and East/South-East Asia (Fig. 6) confirming the results of the allozyme and preliminary microsatellite study.

Considering the results of population clustering, hierarchical gene diversity analysis by AMOVA was performed on only two geographical regions, Europe/Central Asia and East/South-East Asia, excluding the two hybrid/introgressed populations. Most of the total variation of microsatellite loci (76.0%) was due to variation within the populations, while the highest proportion of mtDNA variation (49.5%) and substantial part of allozyme variation (44.5%) was accounted for by the differences between the two geographical regions (Table 6). Within geographical regions, the proportion of variation among populations was higher for microsatellites (16.7%) than for allozymes (9.7%), suggesting that the microsatellite data were more effective for differentiating between the populations within regions than the allozyme markers.

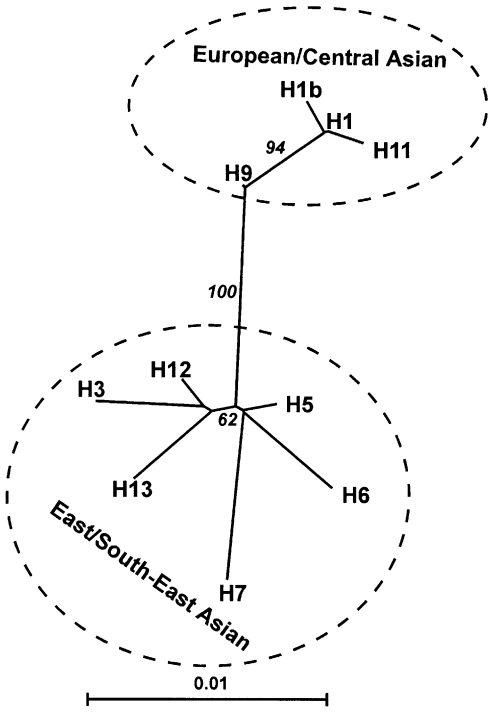

Fig. 5. Unrooted network of common carp mtDNA haplotypes at the *ND-3/4* and *ND-5/6* genes estimated by the Fitch–Margoliash method using the average number of nucleotide substitutions per site (d_{ij}) as an estimate of evolutionary distance between the haplotypes. The bootstrap values were obtained from the maximum likelihood tree. The composite haplotypes are designated according to Table 3.

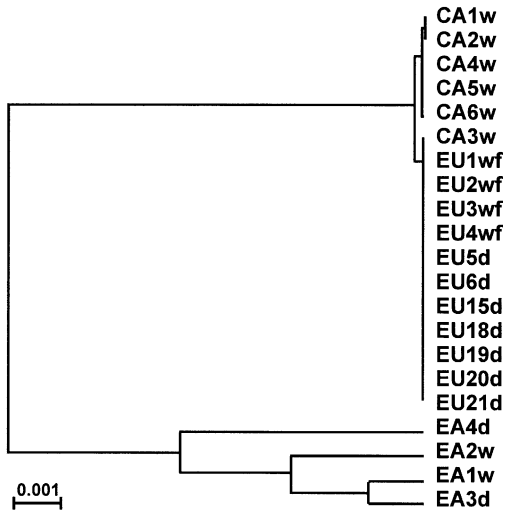

Fig. 6. UPGMA clustering of common carp populations based on the estimated number of net nucleotide substitutions between all pairs of populations (d_A) at the mitochondrial *ND-3/4* and *ND-5/6* genes.

4. Discussion

4.1. Genetic variability in domesticated/captive vs. wild/feral common carp

Small founder numbers, low effective population size and selection in domesticated and captive stocks may lead to loss of genetic diversity and result in inbreeding and reduced reproductive fitness (Frankham et al., 2002). The reduction of genetic variability in domesticated/captive carp stocks compared to wild-caught populations was evident in our study with both types of nuclear genetic markers, though it was less pronounced at allozyme loci than at microsatellite loci. One possible explanation for this observation could be that allozyme loci are not strictly neutral and that some natural balancing selection may act at these loci to maintain a certain level of polymorphism as already proposed by Macaranas et al. (1986) to explain the observed excess of heterozygotes in the cultured carp populations they studied. We also found significant excess of heterozygotes at allozyme loci in several domesticated (EU5d, EU13d, EA4d) and captive

Table 6
Hierarchical partition of genetic diversity by AMOVA among common carp populations based on two geographical regions (Europe/Central Asia and East/South-East Asia); EU2wf and EU17d populations excluded from analysis

Source of variation	Percentage of variation		
	Allozymes	Microsatellites	mtDNA
Among geographical regions	44.5	7.3	49.5
Among populations within geographical regions	9.7	16.7	21.9
Within populations	45.8	76.0	28.6

stocks (EA1w). On the other hand, since the microsatellite loci showed much higher numbers of alleles than the allozyme loci, they might loose genetic variation more rapidly just due to genetic drift and/or founder effects. However, in both cases, microsatellite loci would be better suited than allozyme loci to detect population bottlenecks and losses of variation due to inbreeding with allelic richness being a more sensitive variability measure than mean heterozygosity (Nei et al., 1975; Leberg, 1992; Spencer et al., 2000). Lack of mtDNA polymorphism in both domesticated and wild/feral European carp populations does not allow to estimate the effect of domestication on the variability of mtDNA in this geographical region. However, in the captive stock of River Amur wild carp, loss of variability due to a severe population size bottleneck was evident: we could detect only a single mtDNA haplotype among 22 fish while Froufe et al. (2002) found unique haplotypes in all five wild-caught individuals they analysed. The loss of variability in this captive stock was also indicated by low microsatellite and allozyme variability levels compared to Vietnamese wild carp and literature data (Paaver, 1983). A direct comparison of the present results with literature data, however, is difficult because different sets of allozyme and microsatellite loci have been studied and the number and nature of loci could strongly influence the variability estimates. Moreover, at allozyme loci, a unified locus and allele nomenclature is still lacking in common carp and differing electrophoretic methodologies applied at different laboratories may affect allele designation through altered electrophoretic mobilities. Even at microsatellite loci where alleles are designated according to their size, adjustment is needed due to different instrument types used to measure these sizes.

4.2. Genetic evidence for subspecies status and the origin of European carp

All three genetic marker systems revealed at least two distinct groups of populations: Europe/Central Asia and East/South-East Asia. Genetic divergence between the two regions was substantial as illustrated by high F_{ST} values, high bootstrap support for the genetic distance based population clusters and reciprocal monophyly of mtDNA haplotypes. Thus, the taxonomic status of subspecies *C. c. carpio* and *C. c. haematopterus*, assigned formerly mainly on the basis of morphological characteristics (Kirpitchnikov, 1967, 1999; Balon, 1995) is also justified by our genetic data. The high level of differentiation based on nuclear genes and the presence of endemic mtDNA haplotypes among the East/South-East Asian carp populations suggest that further evolutionary significant units may be worth of delineation in this region. This, however, warrants further detailed studies.

All three genetic marker systems also indicate a close relationship of Central Asian and European carp and do not support the separate subspecies status of *C. c. aralensis* assigned formerly to the Central Asian carp (Kirpitchnikov, 1967). Lack of polymorphism in all European carp populations (except the potentially introgressed EU2wf population) at the mitochondrial *ND-3/4* and *ND-5/6* genes is consistent with the results of Froufe et al. (2002) who sequenced a 565 bp segment of the control region and could not detect any variation among 21 samples of European carp. This suggests a relatively recent bottleneck event in the history of European carp, which most probably is the result of postglacial colonization of the Danube drainage from the Caspian basin refugia. This is supported by our observation that the fixed in Europe mtDNA haplotype is predominant also in Central Asian wild carp populations (which showed also the highest haplotypic diversity) but is missing in all East and South-East Asian populations. An alternative explanation for the mtDNA monomorphism in European carp could be that the wild-caught carp in the Danube and the Rhine drainages represented in fact feral, i.e. offspring of escaped or stocked domesticated carp which have lost their mtDNA variation due to breeding practices in fish farms where very low numbers of spawners are commonly used for reproduction. This is, however, less likely because there should be no significant differences in variability of nuclear genes between the wild-caught and domesticated carp under this scenario.

Estimates of divergence times from a common ancestor do not support dispersal of common carp populations from Central Asia eastward in the last postglacial period as suggested by Balon (1995). The net mean distance of mtDNA sequences (4705 bp, encompassing the *ND-3, 4, 5* and *6* gene regions) between the European/Central Asian and East Asian haplotype groups (0.376%, our unpublished data) corresponds with a divergence time of about 500,000 years BP using the mutation calibration rate of 0.76% divergence per million years for cyprinid mtDNA (Zardoya and Doadrio, 1999), suggesting that splitting between these two groups of mitochondrial lineages took place already before the Weichselian glaciation period.

4.3. Identification of introgression between European and Asian carp

Of more practical relevance is the successful application of the diagnostic restriction enzymes described in Gross et al. (2002) to detect mixing of European and Asian populations. Doing so, two individuals with a composite mtDNA haplotype very similar to the Amur haplotype H3 could be identified among the wild-caught carp from the Danube river near the city of Straubing, Germany (population EU2wf). In combination with allozyme markers, it could also be shown that carp of these two origins were not only mixed but did already hybridize. Hybrid origin of the Ropsha carp breed, as known from the breeding history (Kirpitchnikov, 1999), was also detectable with allozyme markers.

The Japanese Koi carp in our study were fixed for a unique mtDNA haplotype which belonged to the East/South-East Asian haplotypic group, whereas Froufe et al. (2002) found the four Koi individuals in their study to be identical with European carp. The only explanation for this observation is that crossing with the European carp females (leading to the presence of the European mitochondrial haplotype) may

have occurred in their material since in our study not only the mtDNA markers but also allozyme and microsatellite markers revealed strong differentiation between the European and Koi carp.

These results indicate that genetic markers may be effectively applied to detect not only inter-species hybridization (as described in abundant literature) but also mixing and introgression of intra-species units in the presence of sufficient genetic differentiation.

5. Conclusion

To characterize and distinguish common carp populations microsatellite loci should be preferred because of their generally higher variability and better performance, in particular if populations within geographical regions are concerned. Microsatellite loci are also more sensitive to detect population bottlenecks and loss of variation due to inbreeding. The genetic purity of European strains can successfully be checked by combination of nuclear and mtDNA markers. All three classes of genetic markers indicate an ancient separation of East/South-East Asian carp from other geographical areas. The degree of differentiation supports the subspecies status of *C. c. carpio* assigned to European carp and *C. c. haematopterus* assigned to Asian carp but does not justify a separate subspecies status (*C. c. aralensis*) for the Central Asian carp. European and Central Asian carp are closely related and there is evidence for a single origin of present day European domesticated and wild/feral carp from a common ancestor with Central Asian carp.

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