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Deeper insight into the origin and spread of European common carp (*Cyprinus carpio carpio*) based on mitochondrial D-loop sequence polymorphisms

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ABSTRACT

The common carp, *Cyprinus carpio*, can be divided into at least two subspecies: *C. c. carpio* in Europe and *C. c. haematopterus* in East Asia. In order to get a better understanding on the still controversially debated origin and spread of European common carp, the sequence variation of the complete mitochondrial D-loop was examined in 248 individuals from 24 populations representing a geographical range from Western Europe (Spain) to Central Asia (Uzbekistan). Two SNPs and one (AT) microsatellite motif were detected by sequencing 35 individuals (1–3 per population) resulting in nine haplotypes ranging from 928 to 940 bp in length. Seven of these haplotypes were described for the first time. All nine European/Central Asian haplotypes were closely related to each other and clustered into a single group with 94% bootstrap support if compared to four representatives of East Asian common carp. The centre of origin of modern European common carp could be located in or close to Central Asia (Ponto-Caspian Sea basin) considering that the total number as well as the number of endemic haplotypes was highest in that region. Two D-loop haplotypes, H2 and H5, were shared by most of the European/Central Asian populations. However, a remarkable prevalence shift of haplotypes from H2 and H5 being equally frequent in wild and wild/feral to H2 dominating or being fixed in domesticated populations indicated a bottleneck/founder effect at early stages of common carp cultivation and domestication in Europe.

1. Introduction

The common carp (*Cyprinus carpio* L.) is amongst the economically most important freshwater fishes with a global fishery and aquaculture production reaching approx. 3.3 million tonnes in 2009 (FAO, 2011). During the long history of common carp domestication (about 4000 years in China and several hundred years in Europe; Wohlfarth, 1984) a lot of local strains have been developed by classical methods of selection and crossbreeding throughout its distribution range (Hulata, 1995). Within the last two decades several studies on the evolution, phylogeography and population genetic structure of wild and domesticated common carp have been carried out both in Europe (e.g. Czech Republic, France, Germany, Hungary, Poland, and Russia) and Asia (e.g. China, Japan, and Vietnam) (for reviews see Chistiakov and Voronova, 2009; Gui and Zhu, 2012; Vilizzi, 2012). Genetic differentiation into two subspecies, *C. c. carpio* in Europe and *C. c. haematopterus* in East Asia, was clearly evident, and the existence of a third subspecies *C. c. rubrofuscus* that might have diverged from *C. c. haematopterus* in China has been suggested (Zhou et al., 2004). However, some controversy on the origin and spread of European common carp still exists.

Our own studies based on allozymes, mitochondrial *ND-3/4* and *ND-5/6* gene regions, and microsatellite loci strongly supported a common ancestor of European and Central Asian common carp, and simultaneously demonstrated a deep divergence of these two groups from East Asian common carp including River Amur wild carp (Kohlmann et al., 2003, 2005). In contrast, Froufe et al. (2002) suggested an Asian ancestry and single introduction of common carp into the River Danube basin based on the identity of a partial, 565 bp long D-loop sequence in 21 European individuals from the upper River Danube in Austria and Hungary with four Japanese ornamental Koi carp, although all five Asian River Amur wild carp displayed unique haplotypes differing from one to 12 base substitutions from the European haplotype. Finally, Zhou et al. (2003) found indications for different ancestors of European domesticated common carp: PCR-RFLP analysis of the mitochondrial *ND-5/6* gene regions (approx. 2.4 kb) and D-loop sequences (928 bp) clustered German mirror carp with the European subspecies but Russian scattered scaled mirror carp with the Asian subspecies. When evaluating the conclusions of Froufe et al. (2002) and Zhou et al. (2003) it has to be considered, however, that both studies did not include any common carp from Central Asia. Moreover, these conflicting results might have been caused by confounding effects of natural range expansions with large-scale human-mediated translocations/introductions and/or breeding activities including hybridisation of subspecies. For example, Russian common carp breeds could be divided with only rare exceptions into two main groups corresponding to their breeding history by RAPD and microsatellite markers: one group originated from European common carp, whilst the second one showed substantial admixture with Asian River Amur wild carp (Ludanny et al., 2006, 2010).

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Microsatellite loci proved to be very efficient in detecting significant differentiation between European common carp populations even if they originated from the same country (Desvignes et al., 2001; Hulak et al., 2010; Kohlmann et al., 2005; Lehoczky et al., 2005; Ludanny et al., 2010). On the other hand, PCR-RFLP analyses of the mitochondrial *ND-3/4* and *ND-5/6* gene regions did not reveal any variation in 10 out of 11 European common carp populations studied; the occurrence of two individuals with an East Asian haplotype amongst 27 wild/feral common carp from the German part of the River Danube could be attributed to artificial, human-mediated mixing/hybridisation (Kohlmann et al., 2003). Also, in accordance with Froufe et al. (2002), no variation could be detected by Thai et al. (2005, 2006) in a 745 bp long D-loop segment of European common carp despite the fact that East Asian populations from Vietnam, China and Indonesia displayed considerable haplotype diversity. In contrast, Zhou et al. (2003, 2004) described three haplotypes of complete D-loop sequences from German mirror carp and River Volga wild carp, and Mabuchi et al. (2008) found two D-loop haplotypes amongst Japanese common carp which were supposed to be of European origin based on their similarity with the European haplotypes previously reported by Zhou et al. (2003, 2004) and Thai et al. (2005). Recently, Tsipas et al. (2009) detected two haplotypes for a partial D-loop sequence of 567 bp and Imsiridou et al. (2009) found six haplotypes for a partial D-loop sequence of 646 bp in European common carp caught in lakes and rivers of Greece.

Even if mitochondrial DNA can only reconstruct the maternal phylogeny but cannot detect hybridisation between phylogeographic lineages or subspecies, these latter findings suggest that the D-loop sequence might be a suitable marker to get a better understanding on the origin and spread of European common carp. To test this hypothesis, we re-examined the large DNA collection originating from our previous studies and included newly obtained tissue samples of common carp from the Ukraine and Kazakhstan.

2. Material and methods

2.1. Fish

In order to examine the D-loop sequence variability 24 common carp populations representing a geographical range from Western Europe (Spain) to Central Asia (Uzbekistan) were chosen (Table 1). Twelve of them were characterised as wild or wild/feral, nine as domesticated and three as unknown genetic status. The usual sample size was 10 individuals (min.: 8; max.: 16). Genomic DNA was either already available from previous studies or newly isolated from ethanol preserved tissue samples using the peqGOLD Tissue DNA Mini Kit (Peqlab Biotechnologie). The majority of these populations had already been analysed for variation in allozymes (Kohlmann and Kersten, 1999; Murakaeva et al., 2003), mitochondrial *ND-3/4* and *ND-5/6* gene regions (Gross et al., 2002; Kohlmann et al., 2003; Memiş and Kohlmann, 2006) and microsatellite loci (Kohlmann et al., 2005; Memiş and Kohlmann, 2006). Details on population designation and sampling locations (including regional maps) can be found in these papers.

2.2. Detection of D-loop sequence polymorphisms

Initially, 30 individuals were randomly chosen from the populations mentioned above to search for D-loop sequence polymorphisms. Later on, in order to cover all complete D-loop haplotypes detected by the routine population screening methods described below, another five individuals had to be sequenced resulting in a total number of 35 individuals (1 to 3 per population; Table 1).

Due to technical restrictions of the available DNA sequencer the complete common carp D-loop sequence had to be split into two overlapping segments. Primers for PCR amplification of both segments (Table 2) were designed with the Primer3, v. 0.4.0 software (http://frodo.wi.mit.edu/primer3/) based on alignments of a complete common carp mtDNA sequence (Mabuchi et al., 2006) with already known complete common carp D-loop sequences (Mabuchi et al., 2008). The composition of reaction mixes and protocols for PCR amplification were identical for all applications of the present study with the only exception that double volumes were used for PCRs preceding cycle sequencing.

Each reaction mix was composed of 2.5 μl of 10× PCR buffer (Fermentas), 2.0 μl of 25 mM $MgCl_2$, 2.0 μl of 1.25 mM dNTPs, 0.5 μl of each primer (10 pmol/μl), 5.0 μl template DNA, 0.1 μl of *Taq* DNA-polymerase (5 units/μl; Fermentas) and sterile water up to a final volume of 25.0 μl. The hot start PCR programme consisted of an initial denaturation at 94 °C for 3 min followed by 34 cycles of denaturation at 94 °C for 10 s, annealing at 55 °C for 10 s, extension at 72 °C for 50 s, and a final extension at 72 °C for 10 min. PCR products were purified using the peqGOLD Cycle-Pure Kit (Peqlab Biotechnologie), and DNA concentrations were measured with a BioPhotometer (Eppendorf). Cycle sequencing was performed using the CEQ DTCS-Quick Start Kit

Table 1
Information on the origin and genetic status of the 24 European/Central Asian common carp populations examined.

Population name	Genetic status	Country of origin	Number of individuals sequenced	Population code in Kohlmann et al. (2003, 2005)
Badajoz	Domesticated	Spain	1	EU18d
River Rhine	Wild/feral	Germany	2	EU4wf
River Danube	Wild/feral	Germany	1	EU2wf
Maier	Farmed wild R. Danube	Germany	1	EU1wf
Scaly pond carp	Domesticated	Germany	1	EU5d
Fiedler	Domesticated	Germany	2	EU6d
Petershain	Domesticated	Germany	1	EU15d
Kauppa	Domesticated	Germany	1	EU7d
Glinzig	Domesticated	Germany	2	EU12d
Zator	Domesticated	Poland	2	EU21d
Pohorelice	Domesticated	Czech Republic	1	EU20d
River Tisza	Wild/feral	Hungary	1	EU3wf
Tata	Domesticated	Hungary	1	EU19d
Lake Bafra Cernek	Wild	Turkey	3	-
Lake Iznik	Wild	Turkey	1	-
Lake Sapanca	Wild	Turkey	2	-
Central Ukraine	Unknown	Ukraine	2	-
Eastern Ukraine	Unknown	Ukraine	1	-
Kazakhstan	Unknown	Kazakhstan	1	-
Lake Arnasaiskie	Wild	Uzbekistan	1	CA4w
Lake Tuzkan	Wild	Uzbekistan	2	CA1w
River Kli	Wild	Uzbekistan	2	CA6w
Lake Aidar	Wild	Uzbekistan	2	CA5w
River Syr-Darya	Wild	Uzbekistan	1	CA2w

Table 2
PCR primers used to sequence the complete common carp D-loop (F = forward; R = reverse).

Target	Primer sequence (5′–3′)
D-loop, sequencing of 1st segment	F: TCC CAA AGC CAG AAT TCT AAA R: CAC ATA CAA GGA AAA TGT TCA ACC
D-loop, sequencing of 2nd segment	F: TGG CAT CTG GTT CCT ATT TCA R: TAA TAA GGT CGG GAC CAT GC
SNP1, PCR-RFLP	F: see D-loop, 1st segment R: see D-loop, 1st segment
SNP2, PCR-RFLP	F: see D-loop, 2nd segment R: CGT TCT TGA GTC CTC CTT GG
Microsatellite PCR	F[a]: CCA AGG AGG ACT CAA GAA CG R: see D-loop, 2nd segment

[a] 5′ labelled with Cy5.