

A molecular approach to detect hybridisation between native crucian carp (*Carassius auratus*) and non indigenous carp species (*Carassius auratus* and *Cyprinus carpio*) in UK waters, including a consideration of the taxonomic status of gibel carp (*Carassius spp.*)

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Simplex taken and study funded by Environment Agency. Technical analysis completed by Dept of Molecular Biology, Hull University.



Native and introduced *Carassius*



Hybridisation in *Carassius*: Background

The Crucian carp (*Carassius auratus*) is now considered a native species of the British Isles, though it was probably originally confined to central and eastern England. In contrast, other closely related cyprinid fishes such as common carp (*Cyprinus carpio*) and goldfish (*Carassius auratus*) have been introduced into British waters by human activities.

There is now strong, but as yet unsubstantiated, belief that the crucian carp is under increasing threat from hybridisation, competition and disease principally brought about by anthropogenic activities.

Hybridisation in *Carassius*: Background

These threats are considered to be Europe wide and are compounded by difficulties in identification of pure-bred *C. auratus* and hybrids by external morphological investigation and by some unresolved taxonomic problems within the genus *Carassius*.

This study is broken down to two main components: The genetic study of crucian carp and their respective hybrids and a simple field guide for identifying true crucian carp.

Hybridisation in *Carassius*: Aim

Overall aim of the project:

- to identify genetic markers (microsatellites) which can be used to identify crucian carp, goldfish, common carp, and their hybrids respectively
- to identify British populations of true crucian carp
- to describe the genetic characteristics of gibel carp

Hybridisation in *Carassius*: Sampling

Fish clips from 250 individuals* sampled from 24 wild or semi-wild populations and 6 populations from registered fish farms or ornamental fish retailers

External morphological investigation

=> Classification into pure species (crucian carp, goldfish and common carp), their respective hybrids or 'gibel carp'

* results from a further 109 fish became available towards the end of the study.

Hybridisation in *Carassius*: Methods

Diagnostic codominant markers: Alleles can be assigned unambiguously to one of the three species

Predictions for relative paternal and maternal contribution to the hybrid genome:

F1 hybrids A x B
50% alleles of species A and 50% of species B

Backcrosses: Alleles from 2 species, not in a 50:50 relation e.g. backcross F1 with species A: contribution species A: 50-100% contribution species B: 0-50%

Hybridisation in *Carassius*: Methods

PCR* were carried out on 17 unlabelled primer pairs developed for goldfish and carp in order to check their suitability for diagnostic markers.

Size range of alleles (number of alleles) for goldfish, crucian carp and common carp are shown below at the five investigated microsatellite loci.

	GF1	GF17	GF29	MFW1	MEW2
Goldfish	300-312 (7)	184-212 (7)	189-207 (5)	162 (1)	157 (1)
Crucian carp	298 (1)	182 (1)	210-228 (6)	178-190 (7)	160 (1)
Common carp	296 (1)	-	245-283 (10)	168-214 (10)	173-221 (20)

Hybridisation in *Carassius*: Methods

Microsatellites:

Short tandem repeats of DNA

New mutations delete or insert one repeat.

Allele 1: AGAGAGAGAG (12bp)
Allele 2: AGAGAGAG (10bp)

Alleles at a given locus differ in number of repeat units and thus in overall size which enables separation.

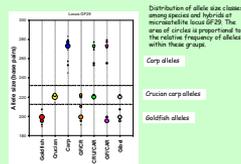
Electrophoretic detection of size differences:

Hybridisation in *Carassius*: Methods

Electrophoretic detection of size differences: locus GF29

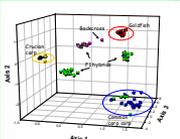


Hybridisation in *Carassius*: Results



Hybridisation in *Carassius*: Results

Factorial Correspondence analysis of multi-locus genotypes



- Crucian carp
- Goldfish
- Common carp
- Crucian, goldfish hybrid
- Goldfish, common carp hybrid
- Gibel carp

Hybridisation in *Carassius*: Results

Crucian carp were found to be present in 15 different wild or semi-wild British populations.

Hybrids between all 3 sampled species were identified. These were mainly F1 hybrids.

F1 back cross crucian/goldfish hybrids were recorded from 2 wild fish populations.

A single F2 crucian/goldfish hybrid was recorded from a wild population.

The investigated samples of 'gibel carp' fell in two genetic categories: i) triploid goldfish from Germany ii) crucian carp x goldfish hybrid from UK.

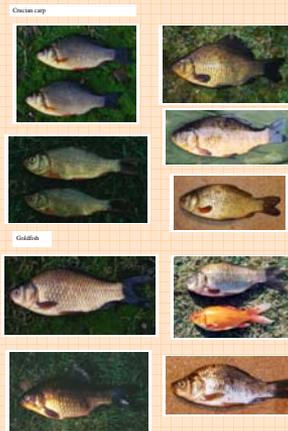
Hybridisation in *Carassius*: Discussion

Many British populations of true *C. auratus* exist in the wild. Therefore there is still something to protect!

Identification of F1 crucian carp/goldfish hybrids in UK waters has previously been presumed on the basis of their intermediate morphological traits, however, this is believed to be the first conclusive validated record of their presence.

Natural occurrence of F1 hybrids is of significant concern whilst the Agency is concerned with protecting crucian populations. Even more concerning is the occurrence of F1 back crosses and F2 individuals.

F1 and F2 hybrids will create competitive pressures and have further reproductive potential.



Hybridisation in *Carassius*: Discussion

Backcrossing raises the opportunity for introgression (Arnold 1997), this may ultimately lead to species evolution. The presence of hybrids and goldfish in crucian carp populations represents therefore a serious threat to the genetic integrity of native *C. auratus*.

The gibel carp were classified as such comparing images, body morphology and meristic counts provided by Hensel (1970) and Skora (1977). At the present stage of the investigation it is impossible to verify whether samples from both Germany and UK represent gibel carp or whether the term gibel carp describes in fact an assemblage of lineages with different origin.

Hybridisation in *Carassius*: Discussion

Results from this project will be used to support Environment Agency policy on fish introductions. Further work is planned, including gathering more detailed and geographically extensive information on waters holding *Carassius* populations; investigating the taxonomic status of 'gibel carp' from other European countries; and assessing further the reproductive capabilities of hybrid *Carassius*.

Crucian Carp Field Guide

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The crucian carp is a native fish species of the UK, originally probably confined to the eastern and central regions. It lives in still waters, from very small streams and woodland ponds, where it is commonly found in several populations in southern and central England. It can attain sizes of four pounds and above. Body shape of the crucian carp varies greatly between sites, so much so, that the 'type' form has been recognised, a deep bodied form with a shallow bodied form associated with it.

The crucian carp is under increasing threat from direct competition with goldfish and carp, hybridisation with these species and competition among from brown fishbait, habitat degradation and releases by non genetic and non-reproductive parasites. Due to the difficulties in identification between the crucian carp and the broad gilded hybrid of the two, assessment of the present distribution of the true gibel carp. This field guide has been developed to enable quick correct identification of these species.

Please note that there are very few definitive morphological or meristic traits which can positively identify a species, however, using a number of characteristics together gives a better judgement to be made.

External Features	Crucian Carp	Feral Goldfish	Crucian Carp/Goldfish Hybrid
Body length	to 23"	to 23"	to 23"
Number of lateral line scales	35	28-32	30-32
Overall form	Deep bodied	Shallow bodied	Intermediate
Head fin position	Close to the body	Further from the body	Intermediate
Public fin colour	Orange, usually with dark spots	Yellow from pink to orange	Intermediate
Body coloration	Dark	Light	Intermediate
Operculum	Crucian form	Goldfish form	Intermediate
Opercular flap	Crucian form	Goldfish form	Intermediate
Anal fin position	Close to the body	Further from the body	Intermediate
Number of scales	to 23"	to 23"	to 23"
Number of scales on the gill arch	27 mean ± 2	24-25 mean ± 2	24-25 mean ± 2
Number of gill rakers	18-20 mean ± 2	18-20 mean ± 2	18-20 mean ± 2
Number of gill rakers on the gill arch	18-20 mean ± 2	18-20 mean ± 2	18-20 mean ± 2
Number of gill rakers on the gill arch	18-20 mean ± 2	18-20 mean ± 2	18-20 mean ± 2

Samples collected in 1981 and 1982, genetic verification of identity was made on all samples collected in 1981. A further set of samples collected in 1981 and 1982, and all fish from the present study, were also of the first year were used for genetic analysis. Figures in this table are their genetic relationships to different from the combined data, because it was the most that the sample sizes are significantly smaller.

