A Molecular Approach to Detect Hybridisation between Crucian Carp (*Carassius carassius*) 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*.)

R&D Technical Report W2-077/TR

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The information from this study is to be used by the Agency's Fisheries Policy and Process team to support Section 30 consenting procedures for *Carassius* spp. and their hybrids. The report will also be used to raise the profile and protect the native crucian carp

Keywords
Carassius carassius, Carassius auratus, Cyprinus carpio, crucian carp, goldfish, gibel carp, hybridisation, genetic, microsatellite, genotyping, morpholological.

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Executive Summary

The Crucian carp (*Carassius carassius*) is a medium sized cyprinid fish which mainly inhabits small still waters. There is now a strong, but as yet unsubstantiated belief that this native species is under increasing threat from hybridisation, competition and disease from other closely related non-native fishes such as common carp (*Cyprinus carpio*) and goldfish (*Carassius auratus*). These threats are considered to be Europe-wide and are compounded by difficulties in identification of pure-bred *C. carassius* and hybrids by external morphological investigation and by some unresolved taxonomic problems. Of particular note is the species in Europe commonly known as the "gibel" or "prussian" carp. It has long been discussed but is still not known whether this is a species in its own right, a subspecies of the goldfish or whether it may be of hybrid origin.

A molecular genetic protocol was established in order to identify pure-bred crucian carp, goldfish, and common carp and reliably distinguish them from their hybrids and backcrosses of at least the first two generations. A set of 5 microsatellite markers with diagnostic allele size ranges for all three species was identified and optimised for cross-amplification among the different taxa. A total of 250 individuals * were studied, these were sourced from 24 wild or semi–wild populations and 6 populations from registered fish farms or ornamental fish retailers. These were classified into pure species (crucian carp, goldfish and common carp), their respective hybrids or "gibel carp" by simple external morphological investigation and were subsequently subjected to genetic analyses.

The results showed clearly that hybrids between all three species were present in the samples and that the morphological classification into pure species or hybrids respectively was in fact correct in most cases. Differentiation between some categories of hybrid (e.g.: goldfish x crucian carp, crucian carp x common carp, etc.) based on morphological grounds proved however to be unreliable and was to some degree dependant on the experience of the sampler.

The samples from 17 of the populations or hatchery stocks studied consisted exclusively of genetically pure crucian carp. Five samples contained crucian carp hybrids and pure crucian or contained only crucian carp hybrids. Four samples contained goldfish and common carp hybrids and two samples of common carp/ crucian carp hybrids were recorded. The individuals which were morphologically identified and submitted for study as "gibel carp", fell into two genetic categories. The individuals from Germany were genetically pure but triploid goldfish, whereas the British individuals were genetically hybrids between goldfish and crucian carp.

The study identified 3 different populations containing crucian carp/ goldfish hybrids. Identification of these fish in UK waters has hitherto been presumed on the basis of their intermediate morphological traits (between crucian carp and goldfish), however, this is believed to be the first conclusive record of their presence.

The genetic data revealed first generation (F1) and second generation (F2) goldfish/ crucian carp hybrids exist in the wild, as do F1 back-crosses. Concern exists as to the readiness with which crucian carp hybridise with goldfish and common carp and the ability of these fish to continue reproducing. The impact of the competitive strengths of the crucian carp hybrids is also of major concern. 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*.

* data from a further 109 fish also became available at the end of the study, significant findings were incorporated into the report.

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Acknowledgements

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1. Introduction

The Crucian carp (*Carassius carassius*) is now considered a native species of the British Isles, though it was probably originally confined to central and eastern England. (Wheeler 1977, 2000) 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. The problem of accidental or deliberate releases of non-native species into the wild has increased drastically during the last decades mainly due to the popularity of ornamental ponds and aquaria and demand from recreational fisheries. There is now strong, but as yet unsubstantiated, belief that the crucian carp is under increasing threat from hybridisation, competition and disease through such anthropogenic activities. This is supported by many reports of once healthy crucian carp populations, now disappeared (K.Wesley, pers comm.). These threats are considered to be Europe wide and are compounded by difficulties in identification of pure-bred *C. carassius* and hybrids by external morphological investigation and by some unresolved taxonomic problems within the genus *Carassius* (see Apendix II for photographs).

Of particular note is the species in Europe commonly known as the gibel or prussian carp. It has long been discussed but is still not known whether this is a species in its own right (*C. gibelio*; Kottelat 1997), a subspecies of the goldfish (*C. auratus gibelio;* Bloch 1783) or whether it is a hybrid between the crucian carp and other related species. There are many populations of fish resembling both crucian and gibel carp present in UK waters and normal methods of morphometric examination have failed to identify their origins. Currently commercial pressures exist to allow stocking of artificially propagated crucian carp and goldfish hybrids. These fish are usually destined for stocking into inland recreational fisheries such as high stock density competition waters. Such practices might represent a significant threat to populations of pure crucian and common carp, but the degree of threat is not yet fully understood.

Genetic methods are powerful tools with which to identify cryptic species or species with few readily identifiable phenotypic characteristics and have previously also been employed to identify species-hybrids in fish. For example, allozyme electrophoresis has been successfully applied to identify hybrids between goldfish and koi carp in New Zealand (Pullan & Smith 1987). Unfortunately, the nature and amount of tissue necessary for most such protein-based methods requires sampling by destructive methods. Recent advances in DNA-based fingerprinting methods enable us now to extract sufficient genetic information from small tissue samples which can be collected in a non-invasive way (e.g. fin clippings, scales). Nuclear genetic markers, especially microsatellites, are suitable to address issues of hybridisation because they can identify both paternal and maternal contributions to the hybrid genome.

The overall aim of the project was to;

(i) identify genetic markers (microsatellites) which can be used to identify crucian carp, common carp, and their hybrids respectively

(ii) identify British populations of true crucian carp

(iii) describe the genetic characteristics of gibel carp

This information will enable better protection of native crucian carp by providing robust guidance for managing movements of *Carassius spp*. and their hybrids.

2. Material and methods

2.1. Sampling

Samples were taken from each of the three species involved (*C. carassius*, *C. auratus* and *C. carpio*) and from individuals which were identified as hybrids or gibel carp on morphological grounds (Table 1). The morphological identification was carried out using simple external investigation by Environment Agency officers as applied in the field. The sampling scheme had three components:

a) Individuals which were believed to be representatives of the pure species and were included in order provide the baseline data and genetic characteristics of each species.

b) Individuals of British *C. carassius* populations, identified on morphological criteria, to confirm their status as pure crucian carp.

c) Individuals which were identified as species hybrids or gibel carp on morphological criteria.

2.2. DNA extraction

Tissue samples (fin-clippings) were provided by Enivronment Agency officers and fishery owners and stored in 98% ethanol for DNA analysis. Total genomic DNA was extracted using a modified salting-out protocol based on Bruford *et al.* (1992). DNA-pellets were re-suspended in TE buffer and a dilution containing 10-50 ng/µl DNA was prepared from this stock solution.

2.3. Genetic methods

2.3.1 General principle and limitations of the approach

The combined impact of mutation, genetic drift and selection generates genetic differentiation between individuals, populations and species (Hartl & Clark 1989). Consequently, genetic variation is distributed not only among, but also within species. Only nuclear genetic markers which are fixed for different alleles (or groups of alleles) for the respective taxa ('diagnostic markers') can be reliably used for species identification (Avise 1994). In contrast to maternally-inherited mitochondrial DNA, nuclear markers can identify the contributions of both the father and mother to the hybrid genome. Using such nuclear markers, the characterisation of an individual as pure-bred or hybrid can be made by comparing the multi-locus genotype with theoretical expectations for a particular category. If diagnostic markers are used, pure-bred individuals are expected to have diagnostic alleles of only one species, whereas diploid F1 hybrids will possess alleles from both, species A and B at each individual locus. Theoretical expectations for subsequent generations and backcrosses are more complex because individual loci can have alleles of only species A, only species B, or be heterozygotes between A and B. The probability to be pure or heterozygote at an individual locus is different for each generation of hybrids; for example in a backcross hybrid F1 x parental species the probability is 50% for each case, but decreases through subsequent generations of backcrosses. Thus the power to detect hybrids depends on the level of backcrossing and the number of loci under investigation. Several methods have therefore been advanced to identify hybrid individuals of the different categories using maximum likelihood approaches (Nason & Ellstrand 1993, Anderson & Thompson 2002).

Table 1. Sampled taxa, sampling locations, code used in further analyses, number of individuals analysed (N) and collection date

Species	Location	Code	N	Date
Representative of pure species				
C. auratus	N.A. Hull	AU-NA	4	14/01/2003
C. auratus	F. Hull	AU-F	5	05/02/2003
C. auratus	B.M.C. Midlands	AU-BMC	1	07/02/2003
C. auratus	G.P.B. Buntingford	AU-GPB	17	18/02/2003
C. carpio	River Danube, Germany	CY-301	1	30/09/1997
C. carpio	River Elbe, Germany	CY-205	1	20/10/1997
C. carpio	S.C. Hampshire	CY-SC	4	15/01/2003
C. carpio	R.C.F. Kent	CY-RCF	9	05/02/2003
C. carpio	B.M.C. Leics.	CY-BMC	2	07/02/2003
C. carpio	M.G. Herts.	CY-MG	4	18/02/2003
C. carassius	F.F.F. Reading	CA-FFF	4	14/01/2003
C. carassius	C.F.F. Notts.	CA-CFF	8	30/01/2003
C. carassius	Kruegersee, Germany	CA-2016	3	03/11/1997
British *Carassius* populations				
C. carassius	C.C.S. Greenwich	CA-CCS	8	23/01/2003
C. carassius	T. L. High Wycombe	CA-TL	8	27/01/2003
C. carassius	B.W. Epping	CA-BW	8	29/01/2003
C. carassius	C.A.C. Bedfordshire	CA-CAC	9	29/01/2003
C. carassius	C.B.L. Corby	CA-CBL	12	29/01/2003
C. carassius	H.C. Essex	CA-HC	8	29/01/2003
C. carassius	C.F.P. Redditch	CA-CFP	4	04/02/2003
C. carassius	C.P. Wiltshire	CA-CP	5	04/02/2003
C. carassius	E.A. Devon	CA-EA	2	04/02/2003
C. carassius	S.F.S. Shaftesbury, Dorset	CA-SFS	3	04/02/2003
C. carassius	S.P. Squareshill	CA-SP	7	04/02/2003
C. carassius	M.H.F.Cheshire	CA-MHF	10	05/02/2003
C. carassius	M.N. St Helens	CA-MN	12	12/02/2003
C. carassius	W.A.C. Nr Ely, Cambs.	CA-WAC	8	18/02/2003
C. carassius	M.G. Herts.	HY-MG	3	18/02/2003
C. carassius	H.R. Stock in Pelham	CA-HR	6	18/02/2003
C. carassius	C.M. Herts	CA-CM	2	28/02/2003
Mixed samples and hybrids				
Gibel carp	River Elbe, Germany	HY-205	2	20/10/1997
Hybrids	L.L.F. Doncaster	HY-261	5	04/02/2003
Hybrids	P.G.B. Newquay	HY-PGB	2	04/02/2003
Hybrids/gibel carp	M.H.F. Cheshire	HY-MHF	3	05/02/2003
Hybrids	R.C.F. Kent	HY-RCF	10	05/02/2003
Hybrids	B.M.C. Midlands	HY-BMC	7	07/02/2003
Hybrids/ gibel carp	G.P.B. Buntingford	HY-GPB	14	18/02/2003
Hybrids	M.G. Herts.	HY-MG	8	18/02/2003
Hybrids/ gibel carp	C.M. Kent	HY-CM	21	28/02/2003
Total number of samples analysed			**250**	

2.3.2. Microsatellites

Microsatellites are DNA sequences comprising a simple repeat motif, of one to six bases (e.g.: AT or CGT) that is tandemly repeated multiple times (e.g. ATATATATAT, CGTCGTCGTCGT). Because these regions are usually non-functional they are generally not under direct selection and free of mutational constraints. Consequently these regions maintain high levels of polymorphism rendering them extremely attractive for a wide range of applications, including the investigation of genetic differentiation of populations and species (Goldstein & Schlötterer 1999). Moreover, microsatellites are distributed throughout the genome and therefore investigation of a number of randomly selected microsatellite loci will yield insights into genome-wide processes such hybridisation and introgression. Alleles at a given locus differ in their number of repeat units and thus in overall size, which enables their electrophoretic separation and identification by comparison with a ladder of known size (Jarne & Lagoda 1996). Microsatellites are co-dominantly inherited in a Mendelian fashion and therefore the number of alleles is limited by the number of homologous chromosomes; for example diploid individuals can posses a maximum of two alleles at a given locus. Hence the ploidy-level of an individual can be inferred from the average number of alleles per locus, given a sufficient number of suitably polymorphic loci.

2.3.3. Identification of universal carp markers

Primers developed to amplify microsatellite loci are often species-specific or cross-amplification is only possible in closely related species. For the present study it was necessary to identify primer pairs which could be used across all taxa under investigation. The first step was therefore to test a set of published microsatellite primers for goldfish (Zheng *et al.* 1995) and carp (Crooijmans *et al.* 1997) for cross-amplification in the respective species.

2.3.4. Genotyping

After suitable loci were identified 250 individuals were genotyped for these loci using CY5-labelled primers. PCR conditions were optimised for each locus as specified below and following amplification, fragments were separated on 6% polyacrylamide gels on an ALFexpress^{TM} automatic sequencer (Amersham-Pharmacia Biotech). Three to four internal size standards were used for each locus in order to determine the size of the amplified fragments. Additionally, the same three individuals (one from each species) were run on all gels to facilitate cross-referencing among gels.

2.3.5. Data analysis

The first step of the analysis was to determine the range of allele sizes for each species at each locus using the individuals which had been classified as pure species as base-line data. Subsequently the alleles of every individual genotype at each locus were assigned according to their origin (Goldfish: AU; crucian carp: CA, common carp: CY). The assignment of individuals as pure-bred species or hybrids was based on the relative distribution of alleles of each species among the multi-locus genotypes.

100% species specific alleles of one species:	pure species
50% alleles of species A and 50% of species B:	F1 hybrids, A x B
Alleles from 2 species, not in a 50:50 relation:	backcrosses

A multivariate analysis was conducted using a Factorial Correspondence Analysis (FCA) using the programme GENETIX version 4.01 (Belkhir *et al.* 2000). This analysis shows the genetic similarity of multi-locus genotypes and is based on the presence or absence of alleles in each multi-locus genotype.

3. Results

3.1. Identification of species specific markers

A set of 17 unlabelled primer pairs developed for goldfish (*GF1, GF11, GF17, GF22* and *GF29;* Zheng et al. 1995) and carp (*MFW1-12* Crooijmans et al. 1997) respectively was selected to be tested for their use as diagnostic markers for the three species and their hybrids.

Polymerase chain reactions (PCRs) were carried out for all 17 loci, using DNA dilutions of 4 individuals from each species (AU/NA1-4; CY/FFF1-4; CA/SC1-4) and generic amplification conditions (Mg-concentration: 1.5 mmol; annealing temperature: 48°C). Subsequently, reaction products were separated on 1.5% agarose gels and their approximate size estimated using a 100bp ladder.

Eight loci showed amplification products of an appropriate size range (100-300bp) in more than one species and are therefore potentially useful to identify hybrids (Table 2). CY5-labelled primers were ordered for these loci and the PCRs repeated for a larger number of individuals from the pure species (AU/NA1-4, AU/F1-5, AU/BMC1; CA/FFF1-4 CA/CFF1-8, CA/2016/1-3; CY/SC1-5, CY/RCF1-9, CY/BMC1-2, CY/301/1, CY205/1)**.**CY5-labelled amplification products were subsequently run on an ALF express automated sequencer in order to check for the existence of unspecific products (i.e. amplification of DNA-regions other than the target region, through unspecific binding of primers) which could hamper the analysis of the results.

Subsequently optimisation was carried out for all loci using a range of PCR annealing temperatures and cycling conditions in order to eliminate unspecific products and to guarantee reproducible results. Optimal conditions across all species could be established for all but one locus (Table 2), which was consequently excluded from further analysis (*MFW5*). After optimisation, six of the remaining loci amplified all three species. Two of these however showed low variability and were fixed for the same allele in goldfish and crucian carp (*GF11* and *GF22*), but four loci displayed alleles with a clearly distinct size range for each species and were deemed appropriate as diagnostic markers (*GF1, GF29, MFW1, MFW2*). One locus (*GF17*) did not produce amplification products for common carp but proved to be an appropriate diagnostic marker for crucian carp and goldfish (Table 2).

Table 2. Results of the optimisation of the PCR conditions.

	Reproducible amplification products			
Locus	*C. auratus*	*C. carassius*	*C. carpio*	Diagnostic size range
GF1	**X**	**X**	**X**	**X**
GF11	X	X	X	0
GF17	**X**	**X**	**0**	**x**
GF22	X	X	X	0
GF29	**X**	**X**	**X**	**X**
MFW1	**X**	**X**	**X**	**x**
MFW2	**X**	**X**	**X**	**X**
MFW5	0	0	X	0

3.2. Genotyping

Overall 257 individuals were genotyped at 5 microsatellite loci.

Firstly, alleles were numbered according to their length in basepairs (e.g. 182bp = allele 182) and individual genotypes recorded for each locus (Appendix 1). Most individual showed one or two alleles per locus suggesting a regular diploid genome. In some individuals however, 3 alleles were found for one or more loci indicating an F1 polyploidisation event.

Secondly, the allele size range and number of alleles for each species was determined on the basis of the individuals which were *a priori* classified as representatives of the pure species (Table 3). A *post-hoc* analysis of the distribution of allele size classes among species confirmed that the allele classes were species-specific for most loci (Figures 1-5). The only exception was locus MFW1 where the alleles size classes among crucian carp and common carp were overlapping (the range in crucian carp is a subset of the range in common carp, Figure 4). However, only two alleles were actually identified in both species at this locus. Therefore, at this locus, the assignment as crucian carp or common carp was in a few cases ambiguous and was based on likelihood (i.e. if an individual with allele 184 at locus MFW1 was assigned crucian carp in all other loci it was also assigned crucian in locus MFW1).

Thirdly, the remaining individuals were assigned as pure species, F1 hybrids, or backcrosses respectively, based on the specific allele ranges of each species. Most individuals fell in the first two categories and only one diploid individual (HY-BMC8) showed a deviation from a 50:50 allelic contribution of the parental species and could be classified as a backcross.

The multivariate analysis (FCA) showed that multilocus genotypes can clearly be assigned into six major groups representing the three species and their F1 hybrids (Figure 6)

Table 3. Size range of alleles (number of alleles) for goldfish, crucian carp and common carp at the the five investigated microsatellite loci.

	GF1	GF17	GF29	MFW1	MFW2
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)	168-234 (10)	173-271 (20)

Table 4. Summary of genetic species assignment, based on multilocus genotypes of 5 microsatellite loci and the agreement with morphological classification, based on the judgement of collectors.
Cases of incongruence are highlighted in bold (see also Appendix 1).

Location	Morphotype	N	Genotype
N.A.	AU	4	AU
F.	AU	5	AU
B.W.	CA	8	CA
C.F.F.	CA	8	CA
C.F.P.	CA	4	CA
C.B.L.	CA	12	CA
C.P.	CA	5	CA
C.C.S.	CA	8	CA
C.A.C.	CA	9	CA
E.A.	CA	2	CA
F.F.F.	CA	4	CA
H.P.	CA	8	CA
H.R.	CA	6	CA
Kruegersee, Germany	CA	3	CA
S.F.S.	CA	3	CA
M.N.	CA	12	CA
S.P.	CA	7	CA
T.L.	CA	8	CA
W.A.C.	CA	8	CA
River Danube, Germany	CY	1	CY
River Elbe, Germany	CY	1	CY
R.C.F.	CY	9	CY
S.C.	CY	4	CY
B.M.C.	AU	1	AU
	CY	2	**AU-CY(1), AU-CA(1)**
	AU-CY	7	**AU-CA(2), AU-CA polyploid (5)**
C.M.	CA	2	CA
	AU-CY	2	AU-CY
	GI	19	AU-CA
G.P.B	AU	17	AU
	GI	3	AU-CA
	CA-CY	11	CA-CY
L.L.F.	AU-CY	5	AU-CY(2), AU-CY polyploid (3)
M.H.F.	**CA**	10	**AU-CA**
	AU-CA	3	AU-CA
M.G.	CA	3	CA
	CY	4	CY
	CA-CY	8	CA-CY (2)**, CY (6)**
P.G.B.	AU-CY	2	AU-CY
River Elbe, Germany	GI	2	AU polyploid
R.C.F.	**CA-CY**	10	**AU-CY**

Figure 1: Distribution of allele size classes among species and hybrids at microsatellite locus GF1. The area of circles is proportional to the relative frequency of alleles within these groups. The dashed lines represent the border of the distribution of alleles for the three respective species.

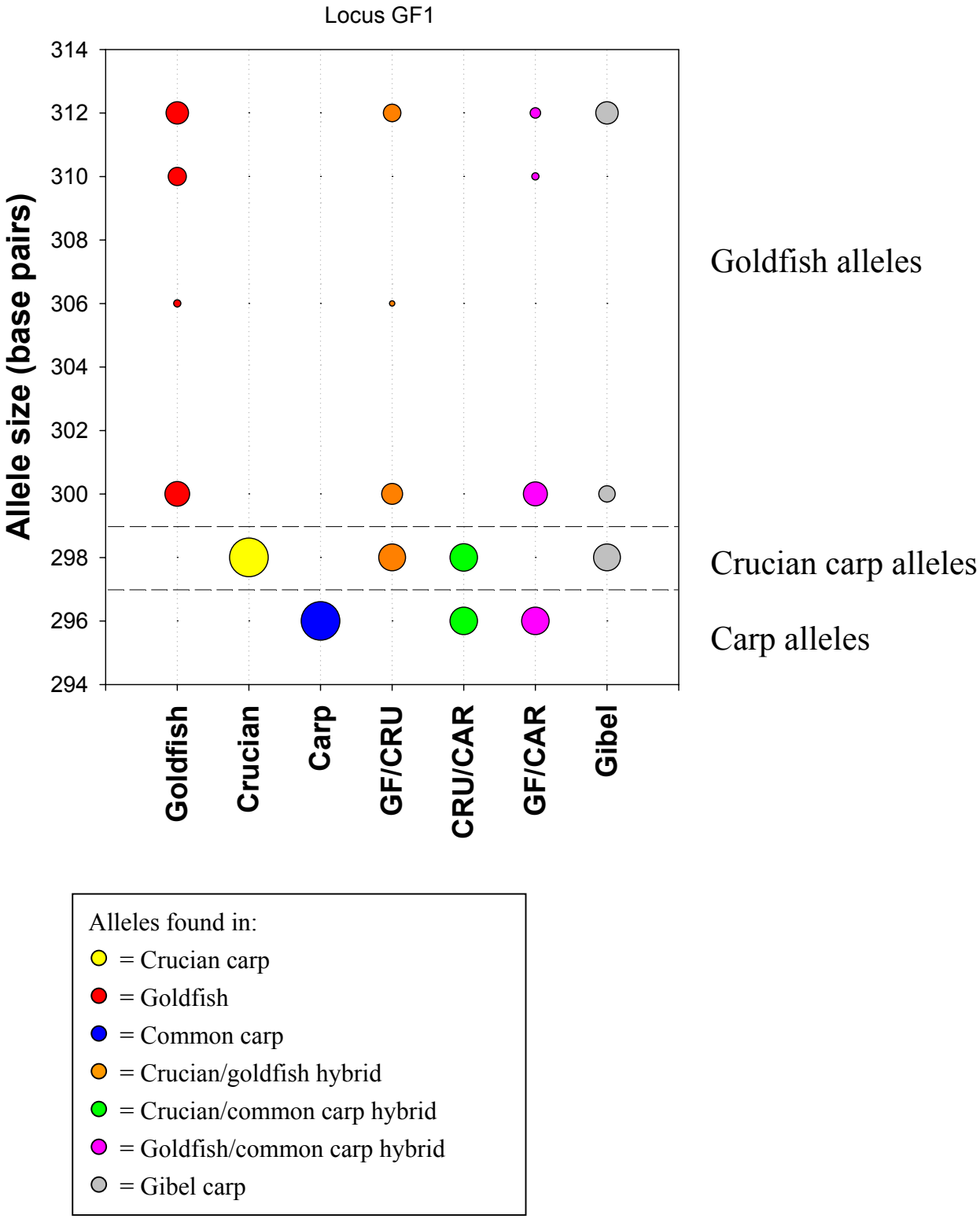


Figure 2: Distribution of allele size classes among species and hybrids at microsatellite locus GF17. The area of circles is proportional to the relative frequency of alleles within these groups. The dashed lines represent the border of the distribution of alleles for the three respective species.

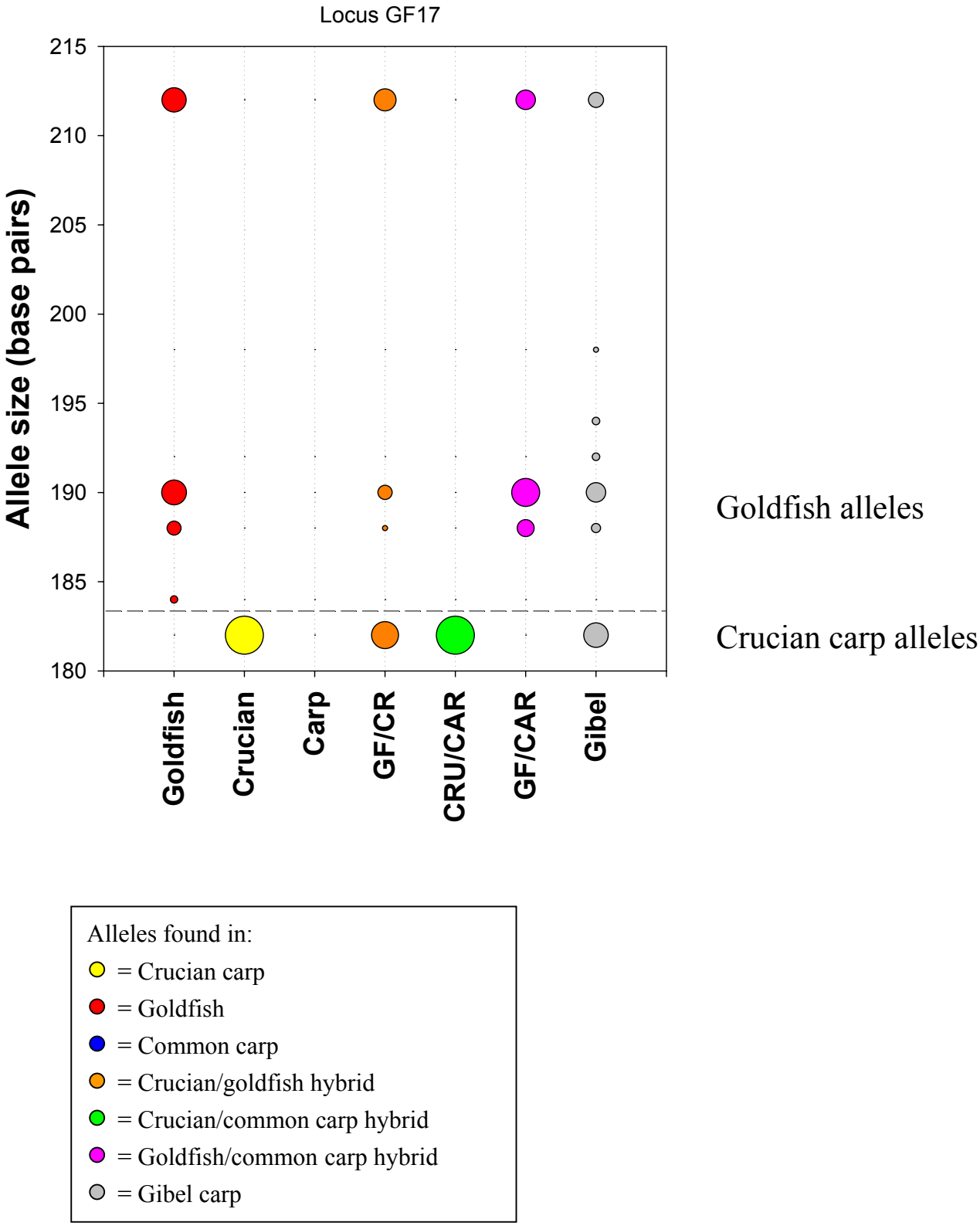


Figure 3: Distribution of allele size classes among species and hybrids at microsatellite locus GF29. The area of circles is proportional to the relative frequency of alleles within these groups. The dashed lines represent the border of the distribution of alleles for the three respective species.

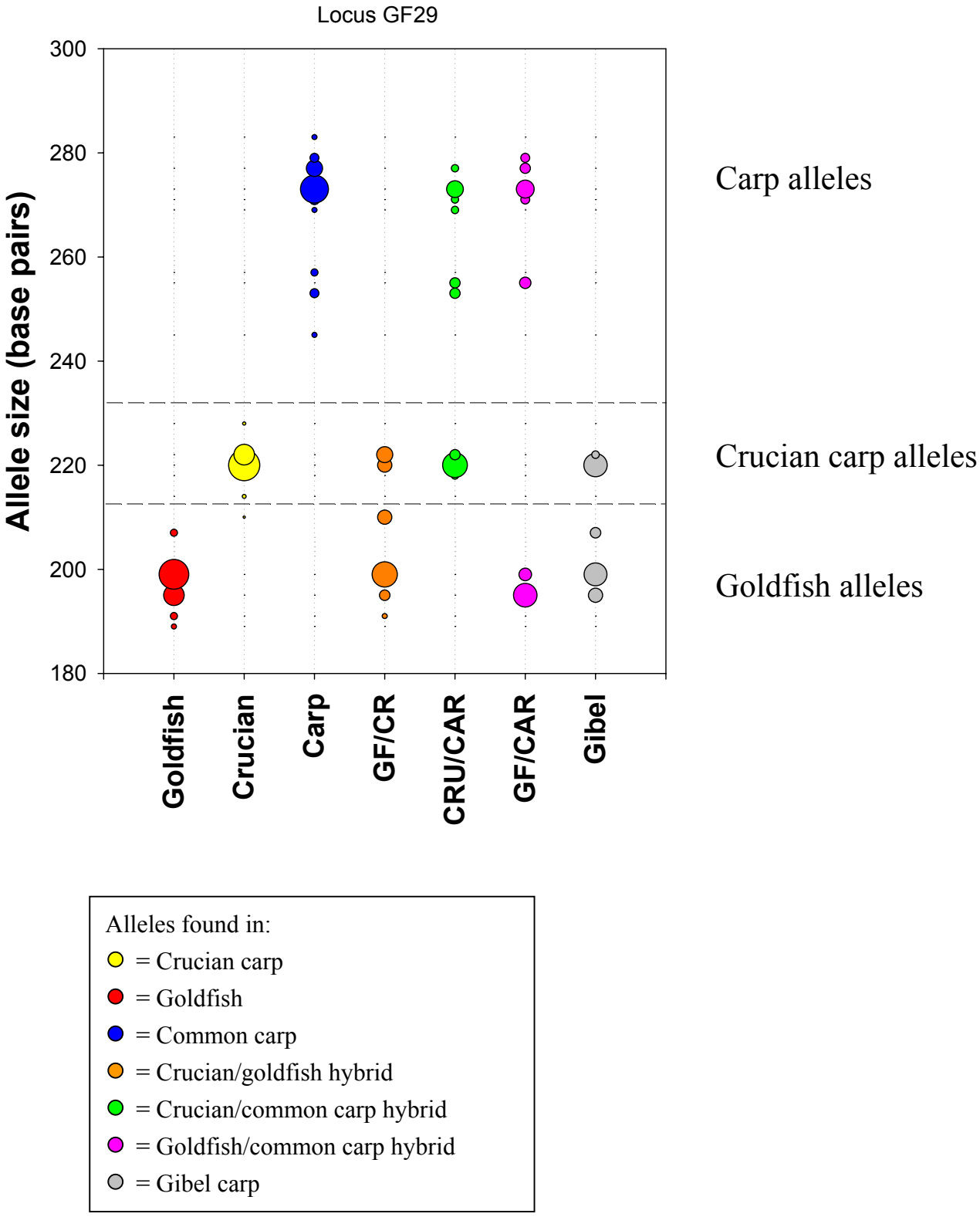


Figure 4: Distribution of allele size classes among species and hybrids at microsatellite locus MFW1. The area of circles is proportional to the relative frequency of alleles within these groups. The dashed lines represent the border of the distribution of alleles for the three respective species.

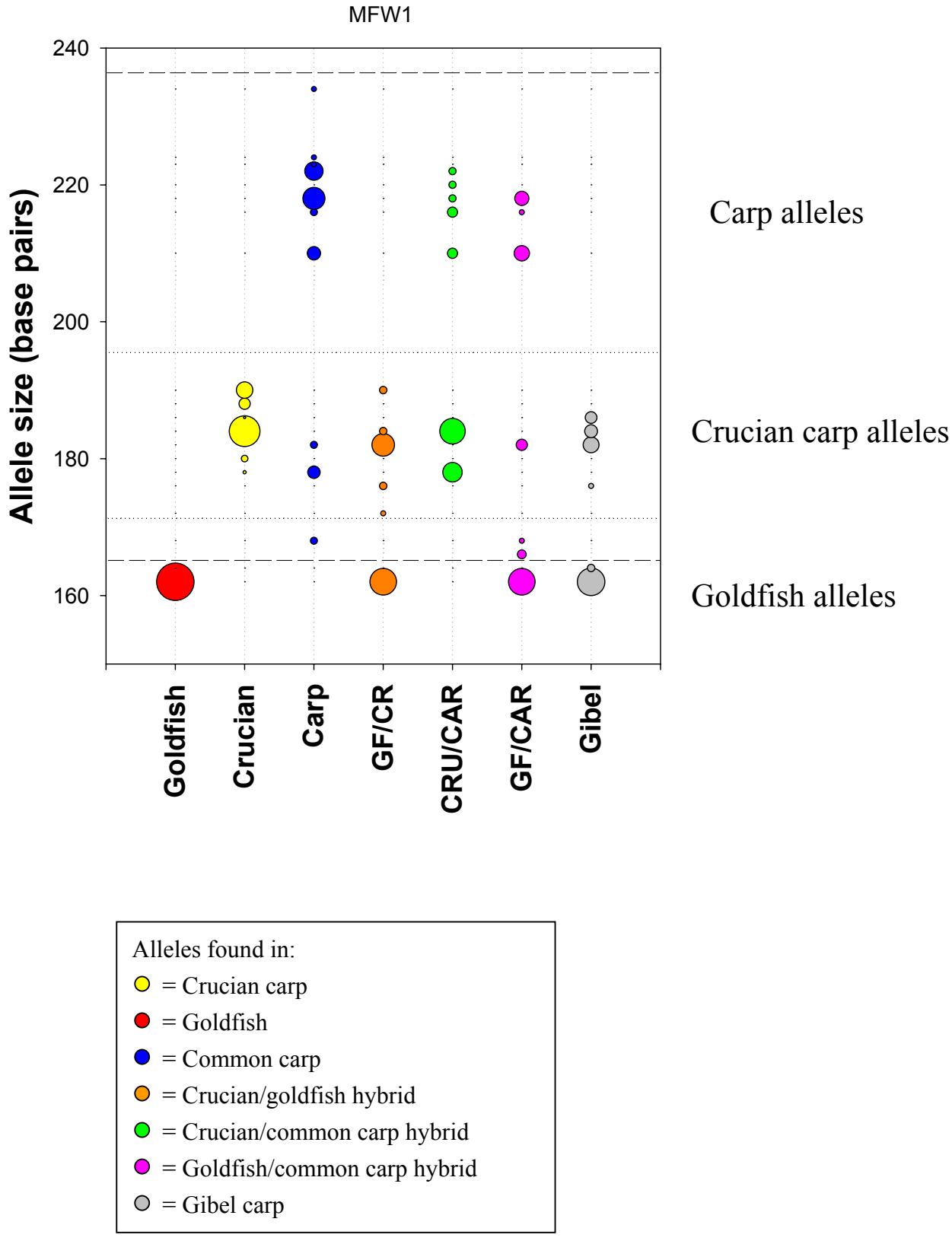


Figure 5: Distribution of allele size classes among species and hybrids at microsatellite locus MFW2. The area of circles is proportional to the relative frequency of alleles within these groups. The dashed lines represent the border of the distribution of alleles for the three respective species.

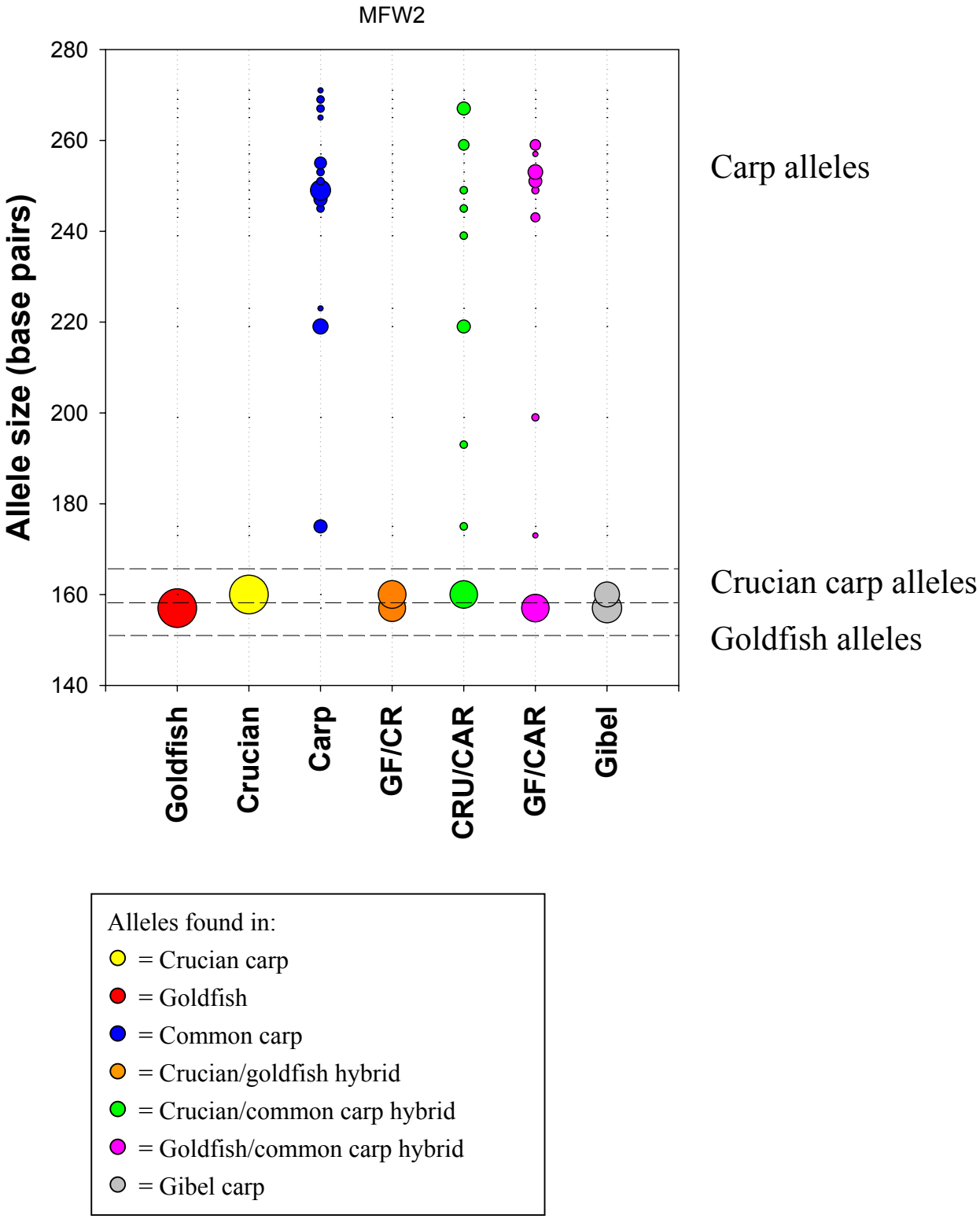
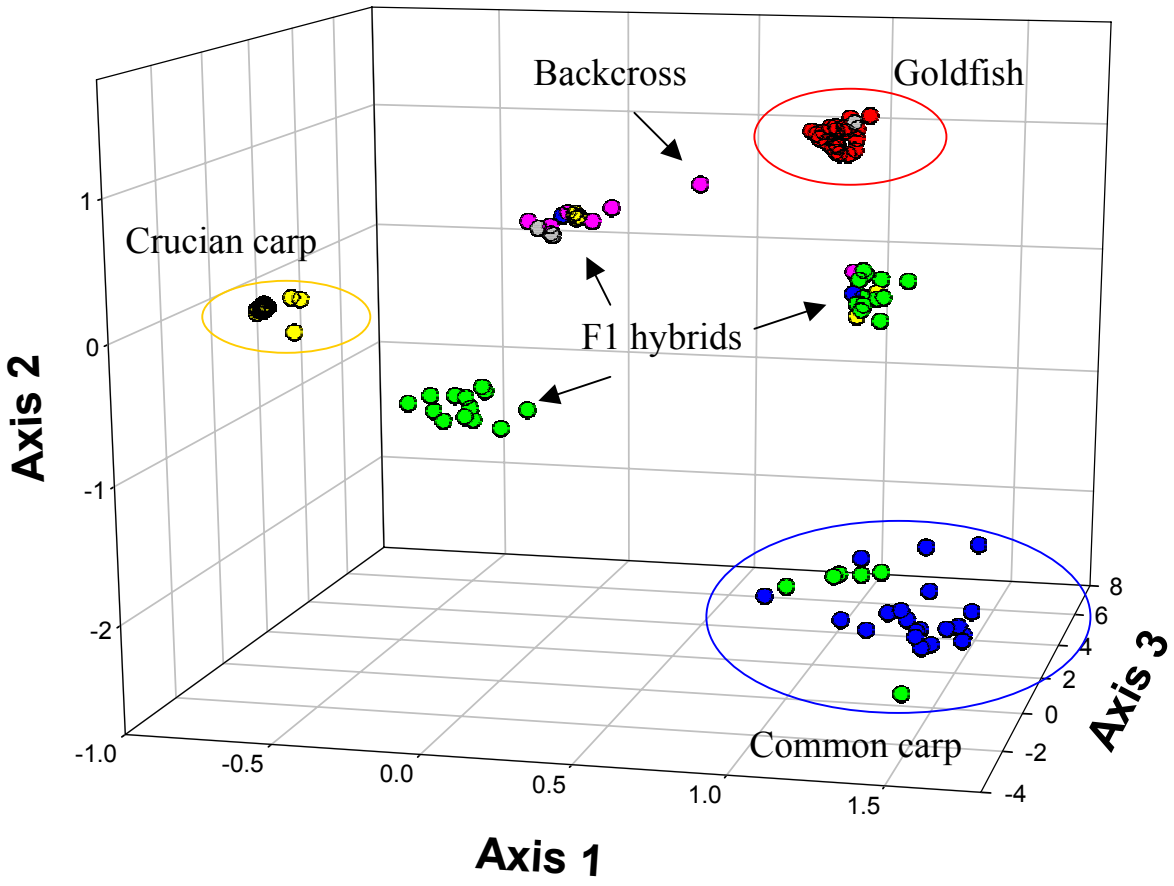


Figure 6: Factorial Correspondence Analysis of the multi-locus genotypes of all analysed individuals. The colour of the symbols indicates the morphological classification of individuals (see legend). Note that, due to overlapping data points, not all individuals are visible in this plot.



Morphological classification
- = Crucian carp
- = Goldfish
- = Common carp
- = Crucian/goldfish hybrid
- = Crucian/common carp hybrid
- = Goldfish/common carp hybrid
- = Gibel carp

4. Discussion

4.1. Identification of suitable markers

It could be demonstrated that microsatellite markers are indeed a useful tool for the identification of species and hybrids of the genus *Carassius* and *Cyprinus*. Cross-species amplification was successful for five microsatellite loci previously characterised for goldfish (*GF1, GF17, GF29*) and common carp (*MFW1, MFW2*) respectively. Furthermore, the allele size ranges of all these loci were, with just one exception, clearly distinct among all species allowing unambiguous assignment of individuals to taxonomic groups and hybrids. The results of the multivariate analysis (FCA) emphasise the power of the data to clearly separate the pure species, their F1 hybrids and even backcrosses (Figure 6). The analysis in Figure 6 also clearly allows visualisation of the numerous misclassifications made on morphological grounds.

4.2. Power and limitation of the analysis

The power to assign individuals as pure-bred or hybrid based on multi-locus genotypes depend on the number of loci analysed (Boecklen & Howard 1997). Furthermore the probability that an individual is heterozygote for at least one locus declines from the F1 hybrid generation to subsequent generations (see also Material and Methods). For example using 5 diagnostic markers the probability that a first generation backcross (F1 x parent species, BC-1) has alleles of only one species at all loci (and may therefore be misdiagnosed as a pure species) is 3% whereas it is 24% for BC-2, 51% for BC-3, 72% for BC-4 and 85% for BC-5 (Boecklen & Howard 1997). Based on these probabilities alone it can therefore be assumed that the present dataset allows a confident identification of F1 hybrids and BC1 backcrosses. The data show clearly that, with one exception (BMC-8), all hybrids fit the expected multi-locus genotype of the F1 generation (each locus has paternal and maternal alleles). Although such a genotype could theoretically also occur in low frequencies in further generations the fact that no other multi-locus genotype patterns are present in most populations makes this explanation extremely unlikely. The most likely explanation is that most analysed individuals are pure species or F1 hybrids (diploid and polyploid). Such a clear pattern made it unnecessary to apply maximum likelihood approaches to assign hybrids to the different possible categories (Nason & Ellstrand 1993, Anderson & Thompson 2002).

4.3. Status of British Carassius populations

The results showed clearly that the morphological classification into pure species or hybrids respectively was in fact correct in most cases. The classification in the exact hybrid category (e.g.: goldfish x crucian carp, crucian carp x common carp, etc.) based on morphological grounds proved however to be unreliable. The genetic data showed also that all but one hybrid individual were first generation offspring indicating that hybrid fertility is likely to be limited. Nevertheless, the detection of one backcross (goldfish/crucian carp hybrid * goldfish) provides evidence that backcrossing occurs to some degree, and even at such a low level, backcrossing raises the opportunity for introgression (Arnold 1997). The finding of several goldfish/crucian carp hybrids with well developed ovaries (CM population) also provides supporting evidence of the reproductive potential of these fish.* The presence of hybrids and goldfish in crucian carp populations represents therefore a serious threat to the genetic identity of native *C. carassius*.

* Subsequently, additional work has been carried out on a further 109 fish. This work (Hänfling and Bolton, in prep.) succeeded in recording a further single example of a goldfish/ crucian carp hybrid back-cross from a different population to the first and furthermore recorded a second generation (F2) goldfish/ crucian carp hybrid.

Overall the samples sourced from 15 wild or semi-wild populations and two fish farm populations consisted exclusively of genetically pure crucian carp whereas 5 samples contained crucian carp hybrids. The genetic analysis showed that only two of these populations, CM and MGC contained some individuals of pure crucian carp, whereas a third population, morphologically tentatively (based on frozen carcasses) identified as crucian carp, MHF, contained in fact exclusively goldfish/crucian carp hybrids. The populations from BMC, GPB and LLF contained a mixture of goldfish and/or their hybrids, many of which were mis-classified on morphological grounds

Although the focus of the report has been on crucian carp and the taxonomic status of the gibel carp there were other related findings worth mentioning.
Two populations containing common carp/crucian carp hybrids were recorded where they are believed to have been produced naturally, as were three populations containing goldfish /common carp hybrids. This has implications where crucian carp or common carp population are being managed for fishery management purposes where pure breeding populations are desired. The reproductive status of these hybrids is not fully understood, however it is reported that F_1 generation common carp/crucian carp hybrids are capable of naturally producing the F_2 generation (Skora and Erdmanski 1985) highlighting the need to regulate their introduction. One population of artificially produced (for the recreational fisheries market) hybrids were submitted as common carp/crucian carp hybrids but after genetic analysis were found to be goldfish/common carp hybrids. Identification between these two types of hybrids is particularly difficult; however the presence of common carp in the hybrid type is relatively easy due to the presence of reduced barbels.

Further information on methods of identification of crucian carp hybrids can be found in the internally produced Agency field guide.

4.4. Genetic characteristics of gibel carp

The set of samples analysed included 24 individuals from three populations (River Elbe/Germany; CM; GPB; plus 3 individuals from MHF which had similar features*) which were classified as gibel carp on morphological grounds. The genetic analyses showed that the samples fell into two categories of genotypes.

(I) Polyploid individuals exclusively with goldfish alleles. The two individuals from the river Elbe fell into this category and represented furthermore the same multi-locus genotype suggesting that they could be a clone. These characteristics are consistent with the description of gibel carp as a clonally-reproducing subspecies of goldfish, *Carassius auratus gibelio* (Zhou *et al.* 2001)and with the suggestion, based on chromosome studies on Asian *Carassius*, that gibel carp have a triploid genome (Zhou & Gui 2002).

(II) Diploid F1 generation hybrids between crucian carp and goldfish. The individuals from the three different British populations belong to category II and the multitude of multi-locus genotypes present suggests that a clonal nature of these individuals is unlikely.

At the present stage of the investigation it is impossible to verify whether only one of these two categories represents pure gibel carp or whether the term gibel carp describes in fact an assemblage of lineages with different origin. This question needs to be addressed in a large scale investigation including European *Carassius* populations.

* There was no discernable morphological difference between British fish sampled and submitted as goldfish/crucian carp hybrids and British fish submitted as gibel carp. The fish submitted as

British gibel carp were done so because of their morphological similarity to the European gibel carp, by workers familiar with European gibels. Whether British gibel carp are representative of the European gibel carp still needs to be addressed.

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Appendix 1: Genotypes of all investigated individuals (each allele consists of three digits) their morphological classification and genetic multi-locus assignment into species or hybrids (AU = goldfish; CA = crucian carp; CY = common carp).								
	GF1	GF17	GF29	MFW1	MFM2	Morphotype	Genotype	Hybrid category
F								
AU-F1	300312	212212	195199	162162	157157	AU	AU	
AU-F2	312312	190212	199199	162162	157157	AU	AU	
AU-F3	312312	188190	191195	162162	157157	AU	AU	
AU-F4	300312	188188	195199	162162	157157	AU	AU	
AU-F5	300312	188212	195199	162162	157157	AU	AU	
NA								
AU-NA1	300300	188212	195199	162162	157157	AU	AU	
AU-NA2	300312	212212	199199	162162	157157	AU	AU	
AU-NA3	300312	190212	189199	162162	157157	AU	AU	
AU-NA4	300310	190190	199199	162162	157157	AU	AU	
Kruegersee								
CA-2016/1	298298	182182	214222	180182	160160	CA	CA	
CA-2016/3	298298	182182	214228	184184	160160	CA	CA	
CA-2016/4	298298	182182	220222	184184	160160	CA	CA	
BW								
CA-BW1	298298	182182	220220	184190	160160	CA	CA	
CA-BW2	298298	182182	220220	184184	160160	CA	CA	
CA-BW3	298298	182182	220220	184184	160160	CA	CA	
CA-BW4	298298	182182	220220	184184	160160	CA	CA	
CA-BW5	298298	182182	220222	184190	160160	CA	CA	
CA-BW6	298298	182182	220220	184190	160160	CA	CA	
CA-BW7	298298	182182	220220	184190	160160	CA	CA	
CA-BW8	298298	182182	220220	190190	160160	CA	CA	
CAC								
CA-CAC1	298298	182182	220220	180184	160160	CA	CA	
CA-CAC2	298298	182182	220220	184184	160160	CA	CA	
CA-CAC3	298298	182182	222222	184184	160160	CA	CA	
CA-CAC4	298298	182182	220222	180184	160160	CA	CA	
CA-CAC5	298298	182182	220222	184184	160160	CA	CA	
CA-CAC6	298298	182182	220220	180184	160160	CA	CA	
CA-CAC7	298298	182182	220220	184184	160160	CA	CA	
CA-CAC8	298298	182182	220220	184184	160160	CA	CA	

CBL								
CA-CBL1	298298	182182	220220	188188	160160	CA	CA	
CA-CBL2	298298	182182	220220	188188	160160	CA	CA	
CA-CBL3	298298	182182	220220	188188	160160	CA	CA	
CA-CBL4	298298	182182	210222	184184	160160	CA	CA	
CA-CBL8	298298	182182	218220	188188	160160	CA	CA	
CA-CBL9	298298	182182	220220	188188	160160	CA	CA	
CA-CBL10	298298	182182	218220	188188	160160	CA	CA	
CA-CBL11	298298	182182	218220	188188	160160	CA	CA	
CA-CBL12	298298	182182	218220	188188	160160	CA	CA	
CA-CBL13	298298	182182	220220	188188	160160	CA	CA	
CA-CBL14	298298	182182	220220	188188	160160	CA	CA	
CA-CBL15	298298	182182	220220	188188	160160	CA	CA	
CCS								
CA-CCS1	298298	182182	220220	184184	160160	CA	CA	
CA-CCS2	298298	182182	220222	184184	160160	CA	CA	
CA-CCS3	298298	182182	220222	184184	160160	CA	CA	
CA-CCS4	298298	182182	220222	184184	160160	CA	CA	
CA-CCS5	298298	182182	220220	184184	160160	CA	CA	
CA-CCS6	298298	182182	220222	184184	160160	CA	CA	
CA-CCS7	298298	182182	220220	184184	160160	CA	CA	
CA-CCS8	298298	182182	220222	184190	160160	CA	CA	
CFF								
CA-CFF1	298298	182182	220222	184190	160160	CA	CA	
CA-CFF2	298298	182182	220222	184184	160160	CA	CA	
CA-CFF3	298298	182182	220222	184190	160160	CA	CA	
CA-CFF4	298298	182182	220220	184190	160160	CA	CA	
CA-CFF7	298298	182182	220222	184184	160160	CA	CA	
CA-CFF8	298298	182182	220222	184190	160160	CA	CA	
CA-CFF9	298298	182182	220220	184190	160160	CA	CA	
CA-CFF10	298298	182182	220222	184190	160160	CA	CA	
CFP								
CA-CFP1	298298	182182	222222	190190	160160	CA	CA	
CA-CFP2	298298	182182	222222	190190	160160	CA	CA	
CA-CFP3	298298	182182	222222	190190	160160	CA	CA	
CA-CFP4	298298	182182	222222	190190	160160	CA	CA	

CP								
CA-CP1	298298	182182	220220	184184	160160	CA	CA	
CA-CP2	298298	182182	220220	184184	160160	CA	CA	
CA-CP3	298298	182182	220220	184190	160160	CA	CA	
CA-CP4	298298	182182	220228	184184	160160	CA	CA	
CA-CP5	298298	182182	220220	184190	160160	CA	CA	
CA-CP6	298298	182182	220220	184184	160160	CA	CA	
EA								
CA-EA1	298298	182182	220222	184184	160160	CA	CA	
CA-EA2	298298	182182	220222	184184	160160	CA	CA	
FFF								
CA-FFF1	298298	182182	220220	184184	160160	CA	CA	
CA-FFF2	298298	182182	220220	184184	160160	CA	CA	
CA-FFF3	298298	182182	220220	184184	160160	CA	CA	
CA-FFF4	298298	182182	220220	184184	160160	CA	CA	
HC								
CA-HC1	298298	182182	222222	184184	160160	CA	CA	
CA-HC2	298298	182182	220222	184190	160160	CA	CA	
CA-HC3	298298	182182	222222	184190	160160	CA	CA	
CA-HC4	298298	182182	220222	190190	160160	CA	CA	
CA-HC5	298298	182182	220222	184190	160160	CA	CA	
CA-HC6	298298	182182	222222	184190	160160	CA	CA	
CA-HC7	298298	182182	220222	184184	160160	CA	CA	
CA-HC8	298298	182182	220222	184190	160160	CA	CA	
HR								
CA-HR1	298298	182182	222222	180180	160160	CA	CA	
CA-HR2	298298	182182	220222	180184	160160	CA	CA	
CA-HR3	298298	182182	220222	184190	160160	CA	CA	
CA-HR4	298298	182182	220222	184190	160160	CA	CA	
CA-HR5	298298	182182	220222	190190	160160	CA	CA	
CA-HR6	298298	182182	220222	184184	160160	CA	CA	
MN								
CA-MN1	298298	182182	220222	184184	160160	CA	CA	
CA-MN2	000000	182182	220222	184190	160160	CA	CA	
CA-MN3	298298	182182	222222	184190	160160	CA	CA	
CA-MN4	298298	182182	220220	184184	160160	CA	CA	

CA-MN5	298298	182182	222222	184190	160160	CA	CA	
CA-MN6	298298	182182	220222	184184	160160	CA	CA	
CA-MN7	298298	182182	220222	184190	160160	CA	CA	
CA-MN8	298298	182182	220220	184184	160160	CA	CA	
CA-MN9	298298	182182	220222	184184	160160	CA	CA	
CA-MN10	298298	182182	220220	184190	160160	CA	CA	
CA-MN11	298298	182182	222222	184184	160160	CA	CA	
CA-MN12	298298	182182	222222	190190	160160	CA	CA	
SFS								
CA-SFS1	298298	182182	220220	184184	160160	CA	CA	
CA-SFS2	298298	182182	220222	184184	160160	CA	CA	
CA-SFS3	298298	182182	220222	184184	160160	CA	CA	
SP								
CA-SP1	298298	182182	220220	184190	160160	CA	CA	
CA-SP2	298298	182182	220220	184190	160160	CA	CA	
CA-SP3	298298	182182	220220	184184	160160	CA	CA	
CA-SP4	298298	182182	220220	184184	160160	CA	CA	
CA-SP5	298298	182182	220220	184184	160160	CA	CA	
CA-SP6	298298	182182	220220	184184	160160	CA	CA	
CA-SP7	298298	182182	220220	184184	160160	CA	CA	
TL								
CA-TL1	298298	182182	220220	184190	160160	CA	CA	
CA-TL2	298298	182182	220222	184190	160160	CA	CA	
CA-TL3	298298	182182	220220	184184	160160	CA	CA	
CA-TL4	298298	182182	220222	184184	160160	CA	CA	
CA-TL5	298298	182182	220222	184184	160160	CA	CA	
CA-TL6	298298	182182	220222	184190	160160	CA	CA	
CA-TL7	298298	182182	220222	184190	160160	CA	CA	
CA-TL8	298298	182182	220222	184184	160160	CA	CA	
WAC								
CA-WAC1	298298	182182	220220	184184	160160	CA	CA	
CA-WAC2	298298	182182	220222	184184	160160	CA	CA	
CA-WAC3	298298	182182	220220	184184	160160	CA	CA	
CA-WAC4	298298	182182	220220	184184	160160	CA	CA	
CA-WAC5	298298	182182	220222	184190	160160	CA	CA	
CA-WAC6	298298	182182	220220	184184	160160	CA	CA	

CA-WAC7	298298	182182	220220	184184	160160	CA	CA	
CA-WAC8	298298	182182	220220	184184	160160	CA	CA	
River Elbe								
CY-205	296296	000000	257273	210218	249271	CY	CY	
River Danube								
CY-301	296296	000000	269273	210234	223255	CY	CY	
RCF								
CY-RCF1	296296	000000	273273	218223	249249	CY	CY	
CY-RCF2	296296	000000	271273	218222	175175	CY	CY	
CY-RCF3	296296	000000	271273	218222	249249	CY	CY	
CY-RCF4	296296	000000	253273	178218	249249	CY	CY	
CY-RCF5	296296	000000	273273	222222	249249	CY	CY	
CY-RCF6	296296	000000	253273	218222	249249	CY	CY	
CY-RCF7	296296	000000	273273	218218	251251	CY	CY	
CY-RCF8	296296	000000	273277	218218	249249	CY	CY	
CY-RCF8	296296	000000	273277	218218	247269	CY	CY	
CY-RCF9	296296	000000	271273	218218	175175	CY	CY	
HY-RCF1	296300	000190	195255	162210	157253	**CA-CY**	**AU-CY**	F1
HY-RCF2	296300	000188	195255	162210	157249	**CA-CY**	**AU-CY**	F1
HY-RCF2	296300	000188	195255	162210	157253	**CA-CY**	**AU-CY**	F1
HY-RCF3	296300	000188	195255	162210	157253	**CA-CY**	**AU-CY**	F1
HY-RCF4	296300	000212	195271	162218	157199	**CA-CY**	**AU-CY**	F1
HY-RCF5	296300	000190	195273	162210	157253	**CA-CY**	**AU-CY**	F1
HY-RCF6	296300	000212	195273	162166	157253	**CA-CY**	**AU-CY**	F1
HY-RCF7	296300	000190	195277	162166	157253	**CA-CY**	**AU-CY**	F1
HY-RCF8	296310	000190	195279	162218	157173	**CA-CY**	**AU-CY**	F1
HY-RCF9	296300	000188	195273	162216	157199	**CA-CY**	**AU-CY**	F1
HY-RCF10	296300	000212	199273	162218	157243	**CA-CY**	**AU-CY**	F1
SC								
CY-SC2	296296	000000	273273	222222	247255	CY	CY	
CY-SC3	296296	000000	273273	218222	247255	CY	CY	
CY-SC4	296296	000000	273279	218222	255269	CY	CY	
CY-SC5	296296	000000	273277	222222	247255	CY	CY	
River Elbe								
Gi-205/1	300300	190192194	199207	162162	157157	GI	AU	F1 polyploid
Gi-205/2	300300	190192194	199207	162162	157157	GI	AU	F1 polyploid

LLF								
HY-261/1	296300	000190	195271	162182	157251	AU-CY	AU-CY	F1
HY-261/2	296300310	000190	195273	162168	157257	**CA-CY**	**AU-CY**	F1 polyploid
HY-261/3	296312	000188	195273	162218	157251	**CA-CY**	**AU-CY**	F1
HY-261/4	296300	190212	191195273	162182	157227251	**CA-CY**	**AU-CY**	F1 polyploid
HY-261/5	296300	190212	191195273	162182	157227251	**CA-CY**	**AU-CY**	F1 polyploid
BMC								
HY-BMC1	312312	182190	195199	162176182	157160	**AU-CY**	**AU-CA**	F1 polyploid
HY-BMC2	298300312	182190212	199210	162182184	157160	**AU-CY**	**AU-CA**	F1 polyploid
HY-BMC3	298312	182190212	199210	162172176	157160	**AU-CY**	**AU-CA**	F1 polyploid
CY-BMC4	298300	182212	199210	162176	157160	**CY**	**AU-CA**	F1
AU-BMC5	312312	212212	191199	162162	157157	AU	AU	
HY-BMC6	298312	182212	191220	162190	157160	**AU-CY**	**AU-CA**	F1
HY-BMC7	298306	182190	195220	162182190	157160	**AU-CY**	**AU-CA**	F1 polyploid
HY-BMC8	298312	182188	199210	162182	160160	**AU-CY**	**AU-CA**	backcross
HY-BMC9	298300	182190	195220	162190	157160	**AU-CY**	**AU-CA**	F1
CY-BMC10	296312	212212	199277	162218	157243	**CY**	**AU-CY**	F1
CM								
HY-CM1	298298	182212	199222	162176	157160	GI	AU-CA	F1
HY-CM2	298312	182212	199220	162182	157160	GI	AU-CA	F1
HY-CM3	298312	182190	195220	162186	157160	GI	AU-CA	F1
HY-CM4	298312	182190	199220	162182	157160	GI	AU-CA	F1
HY-CM5	298312	182198	199220	162184	157160	GI	AU-CA	F1
HY-CM6	298312	182212	199220	162182	157160	GI	AU-CA	F1
HY-CM7	298312	182190	195220	162184	157160	GI	AU-CA	F1
HY-CM8	296300	190190	199273	162218	157253	AU-CY	AU-CY	F1
HY-CM9	298298	182182	220220	184184	160160	CA	CA	
HY-CM10	298312	182212	199220222	162182	157160	GI	AU-CA	F1 polyploid
HY-CM11	298312	182188	199220	162186	157160	GI	AU-CA	F1
HY-CM12	298312	182190	195220	162182	157160	GI	AU-CA	F1
HY-CM13	298312	182190	195220	162186	157160	GI	AU-CA	F1
HY-CM14	298312	182190	195220	162186	157160	GI	AU-CA	F1
HY-CM15	298312	182190	199220	162182	157160	GI	AU-CA	F1
HY-CM16	298312	182212	199220	162182	157160	GI	AU-CA	F1
HY-CM17	298312	182212	199220	162182	157160	GI	AU-CA	F1
HY-CM18	296300	190190	199277	162210	157259	GI	AU-CY	F1

HY-CM19	298298	182212	199220	162184	157160	GI	AU-CA	F1
HY-CM20	298312	182212	199220	162182	157160	GI	AU-CA	F1
HY-CM21	298298	182190	195220	162186	157160	GI	AU-CA	F1
HY-CM22	296300	190190	199271	162210	157259	AU-CY	AU-CY	F1
HY-CM23	298298	182182	220220	184186	160160	CA	CA	
GPB								
HY-GPB1	298312	182188	199220	162184	157160	GI	AU-CA	F1
HY-GPB2	298312	182188	199220	162184	157160	GI	AU-CA	F1
HY-GPB3	298300	182190	195220	162184	157160	GI	AU-CA	F1
AU-GPB4	312312	184188	199199	162162	157157	AU	AU	
AU-GPB5	312312	190190	199199	162162	157157	AU	AU	
HY-GPB6	296298	000182	220222	178178	160219	CA-CY	CA-CY	F1
AU-GPB7	310310	190212	195199	162162	157157	AU	AU	
HY-GPB8	296298	000182	220271	178184	160239	CA-CY	CA-CY	F1
HY-GPB9	296298	000182	220269	184218	160245	CA-CY	CA-CY	F1
HY-GPB10	296298	000182	220273	184220	160249	CA-CY	CA-CY	F1
HY-GPB11	296298	000182	220273	184222	160267	CA-CY	CA-CY	F1
HY-GPB12	296298	000182	220273	178184	160259	CA-CY	CA-CY	F1
HY-GPB13	296298	000182	220255	178184	160175	CA-CY	CA-CY	F1
HY-GPB14	296298	000182	220273	184210	160259	CA-CY	CA-CY	F1
HY-GPB15	296298	000182	220255	184210	160219	CA-CY	CA-CY	F1
AU-GPB16	300300	190190	195199	162162	157157	AU	AU	
AU-GPB17	310310	212212	195199	162162	157157	AU	AU	
AU-GPB18	300312	190212	195199	162162	157157	AU	AU	
AU-GPB19	310312	190212	199199	162162	157157	AU	AU	
AU-GPB20	306310	190212	199207	162162	157157	AU	AU	
AU-GPB21	310310	190190	199207	162162	157157	AU	AU	
AU-GPB22	300310	190190	195199	162162	157157	AU	AU	
AU-GPB23	300310	190190	199199	162162	157157	AU	AU	
HY-GPB24	296298	000182	220253	178184	160193	CA-CY	CA-CY	F1
AU-GPB25	300300	190212	195195	162162	157157	AU	AU	
AU-GPB26	310312	190190	199199	162162	157157	AU	AU	
AU-GPB27	300300	190212	195199	162162	157157	AU	AU	
AU-GPB28	300306	190190	195195	162162	157157	AU	AU	
AU-GPB29	300300	190212	195199	162162	157157	AU	AU	
HY-GPB30	296298	000182	220253	178184	160219	CA-CY	CA-CY	F1

AU-GPB31	300300	212212	199199	162162	157157	AU	AU	
MG								
CY-MG1	296296	000000	273277	168216	219253	CY	CY	
CY-MG2	296296	000000	273277	178218	249265	CY	CY	
CY-MG3	296296	000000	253273	178178	175219	CY	CY	
CY-MG4	296296	000000	273277	168216	219253	CY	CY	
HY-MG5	296296	000000	277277	210210	219267	**CA-CY**	**CY**	
HY-MG6	296298	000182	222273	184216	160267	CA-CY	CA-CY	F1
HY-MG7	296298	000182	218277	184216	160267	CA-CY	CA-CY	F1
HY-MG8	296296	000000	277283	210224	249249	**CA-CY**	**CY**	
HY-MG9	296296	000000	257273	182218	219219	**CA-CY**	**CY**	
HY-MG10	296296	000000	245273	178210	219267	**CA-CY**	**CY**	
HY-MG11	296296	000000	271277	182218	175247	**CA-CY**	**CY**	
HY-MG12	296296	000000	273279	178210	219247	**CA-CY**	**CY**	
CA-MG13	298298	182182	220220	178184	160160	CA	CA	
CA-MG14	298298	182182	220220	178184	160160	CA	CA	
CA-MG15	298298	182182	220220	180184	160160	CA	CA	
PGB								
HY-PGB1	296312	000188	195279	162210	157253	AU-CY	AU-CY	F1
HY-PGB2	296300	000212	195279	162218	157259	AU-CY	AU-CY	F1
MHF								
CA-MHF1	298312	182212	199220	162182	157160	**CA**	**AU-CA**	F1
CA-MHF2	298300	182212	199222	162182	157160	**CA**	**AU-CA**	F1
CA-MHF3	298312	182212	199222	162182	157160	**CA**	**AU-CA**	F1
CA-MHF4	298300	182212	199222	162182	157160	**CA**	**AU-CA**	F1
CA-MHF5	298300	182212	199222	162182	157160	**CA**	**AU-CA**	F1
CA-MHF6	298312	182212	199222	162182	157160	**CA**	**AU-CA**	F1
CA-MHF7	298300	182212	199222	162182	157160	**CA**	**AU-CA**	F1
CA-MHF8	298300	182212	199222	162182	157160	**CA**	**AU-CA**	F1
CA-MHF9	298300	182212	199222	162182	157160	**CA**	**AU-CA**	F1
HY-MHF10	298300	182212	199220	162182	157160	AU-CA	AU-CA	F1
HY-MHF11	298300	182212	199222	162182	157160	AU-CA	AU-CA	F1
HY-MHF12	298300	182212	199220	162182	157160	AU-CA	AU-CA	F1
CA-MHF13	298300	182212	199220	162182	157160	**CA**	**AU-CA**	F1

Appendix II: Images of the investigated species, their hybrids and gibel carp.

Image 1: *Carassius carassius*, crucian carp

Image 2: *Carassius auratus*, brown and red colour morph of the goldfish

Image 3

Image 4

Image 5

Image 6

Images 3, 4, 5 and 6: British gibel carp, morphologically identified and genetically confirmed as goldfish/crucian carp hybrids

Image 5: *Carassius sp*., from France

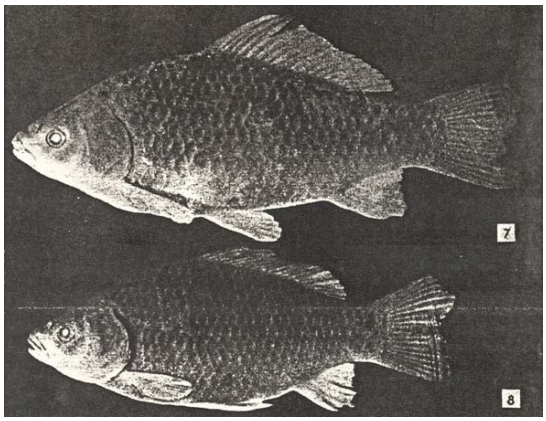

Image 8: Specimens of *Carassius auratus gibelio*, gibel carp, from the river Danube in Czechoslovakia, 1968 (Hensel 1971). – Note the similarity to the goldfish/crucian carp hybrids, particularly image 3.

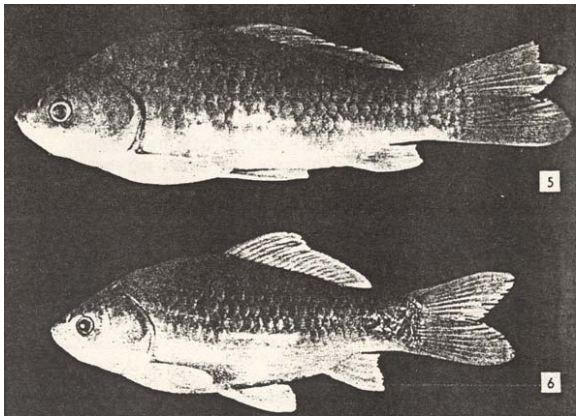

Image 9: Specimens of Carassius auratus gibelio, gibel carp, from the river Danube in Czechoslovakia, 1968 (Hensel 1971). Note the similarity to the goldfish in image 2.

Appendix III: Glossary of specific terms used in the text. Modified from Lawrence (1995) and The On-line Medical Dictionary (© CancerWEB 1997-2003; http://cancerweb.ncl.ac.uk/omd/)

allele	one of a number of alternative forms of a gene or non-coding region which can occupy a given genetic **locus** on a **chromosome** (see also **mutation**)
allozyme electrophoresis	method to differentiate between **allelic** variations of enzymatic proteins from homogenised tissue (e.g. muscle or liver) by exploiting their different electrophoretic mobilities.
amplification	multiplication of a gene or DNA region from a larger template (e.g. total **genomic** DNA) usually by **PCR**
annealing temperature	temperature at which the binding of primer and single stranded template DNA is possible during **PCR**
base pair (bp)	a single pair of complementary **nucleotides** from opposite strands of the double helix. The number of base pairs is a measure of length of a double-stranded DNA
chromosome	small, rod shaped body, consisting of a single very long molecule of DNA. Chromosomes are located in the cell nucleus and make up the nuclear **genome**.
clones	Group of genetically identical individuals derived by repeated asexual cell division.
codominant genetic marker	the **alleles** of all homologous **chromosomes** of each individual that can be identified at any **locus**
diploid	Having two sets of homologous **chromosomes** and therefore two copies of the basic genetic complement of a species
DNA	The molecule that encodes genetic information in the nucleus of cells. It determines the structure, function and behaviour of the cell. DNA is a double-stranded molecule held together by weak bonds between **base pairs** of **nucleotides**. The four nucleotides in DNA contain the bases: adenine (A), guanine (G), cytosine (C), and thymine (T). In nature, **base pairs** form only between A and T and between G and C (so-called complementary nucleotides), thus the base sequence of each single strand can be deduced from that of its partner.
F1 hybrid	the first filial generation of a cross between two pure breeding lines
gene	the basic unit of inheritance, by which hereditary characteristics are transmitted from parent to offspring. At the molecular level a single gene consists of a length of DNA which encodes and directs the synthesis of a protein.
genetic drift	Changes in gene frequencies in a population due to stochastic, random processes rather than **natural selection**

genetic variation	Heritable variation in a population as a result of the presence of different **alleles** of any gene and their shuffling into new combinations by sexual reproduction
genome	the genetic complement of a living organism or single cell
genotype	the genetic constitution of an organism. In the context of molecular markers the genotype describes the alleles present in an organism at a single locus or at a set of **loci** (multilocus genotypes)
locus	the position on a **chromosome** occupied by a particular gene or non-coding region (such as **microsatellites**)
microsatellites	DNA region composed of a very short nucleotide sequence repeated in tandem many times. Usually very variable.
mitochondrial DNA	small circular DNA located in the mitochondria and maternally inherited
molecular marker	molecular approach to detect genetic variation among individuals, populations and species using techniques on the level of DNA and proteins
mutation	change in the composition of the basic building blocks (nucleotides) of DNA. Such changes in DNA sequence can occur spontaneously and are the source of new **alleles** at any **locus** if inherited to the next generation.
natural selection	non-random reproduction of certain **genotypes** due their differential fitness (survival and reproduction) under certain environmental conditions
nucleotides	the basic building blocks of nucleic acids. They are made up of a nitrogen-containing purine or pyrimidine base linked to a sugar (ribose or deoxyribose) and a phosphate group. **DNA** of higher organisms is made up of only four nucleotides. See also **DNA**.
oligonucleotides	linear sequence of a small number of **nucleotides** joined by phosphodiester bonds.
phenotypic	the visible or otherwise measurable physical and biochemical characteristics of an organism; a result of the interaction of **genotype** and environment
polymerase chain reaction (PCR)	technique for selectively replicating a certain DNA region *in vitro* to produce a large amount of copies of a certain **locus** from a larger template (e.g. total genomic DNA).
polymorphic loci	genetic loci with two or more **alleles**

polyploid	Having more than two sets of homologous **chromosomes** and therefore multiple copies of the basic genetic complement of a species
primer	A pair of synthetic oligonucleotides complementary to the flanking regions of the DNA region to be copied, which are bound to the DNA during **PCR** (**annealing**) before the reaction commences to ensure that DNA replication is initiatied at the required points.

Appendix IV: Schematic representation the structure of nuclear DNA in a diploid organism.