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Morphometric and molecular characterisation of *Cyprinus carpio* × *Carassius auratus* hybrids

Remigiusz Panicz · Paulina Hofsoe · Jacek Sadowski · Bartosz Mysłowski · Mirosław Półgęsek

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Abstract Among members of the Cyprinidae family, hybridisation is more widespread than in any other group of freshwater fish. Therefore, the aim of this work has been to use two different approaches, biometric and genetic, to assess differences between common carp (*Cyprinus carpio*) × goldfish (*Carassius auratus*) hybrids and their parents. Twenty-three length measurements of 10 fish from each group have been performed. Statistical analyses of results were conducted using Levene's, ANOVA, NIR and Kruskal–Wallis tests. Genetic analysis has been conducted by means of amplified fragment length polymorphism (AFLP) technique. The AFLP allowed us to distinguish groups of fish and showed that hybrids had more in common with *C. auratus* than with *C. carpio*.

Keywords AFLP · Diversity · Goldfish · Hybrids · ITS1 · Morphometry

Introduction

Among members of the Cyprinidae family, hybridisation is more widespread than in any other group of freshwater fish, which has resulted in creation of numerous natural and artificial hybrids (Scribner et al. 2000). Factors that contribute to hybridisation among the Cyprinidae species include their common and widespread distribution, similarity of preferred spawning habitats, as well as temporal overlaps of their spawning activities and artificial activities (Hubbs 1955). Natural hybrids of the Cyprinidae have mainly been identified as crosses between roach (*Rutilus rutilus* L.) and bream (*Abramis brama* L.) and observed in large numbers in various water bodies (Kennedy and Fitzmaurice 1973; Wyatt et al. 2006; Toscano et al. 2010). Additional examples were described as hybrids of koi (*C. carpio*) and goldfish, bream and silver bream (*Blicca bjoerkna* L.), common nase

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(*Chondrostoma nasus* L.) and *Parachondrostoma toxostoma* (L.) or barbus (*Barbus barbus* L.) and *Barbus meridionalis* (L.) (Smith and McVeagh 2005; Briolay et al. 1998; Durand et al. 2003; Crespin et al. 2002). Natural hybridisation takes place probably because it is one of the ways of species evolution (Scribner et al. 2000). In the course of human breeding programmes, the possibilities of many crosses between species, genera and even families have been tested. Some of them, mainly crosses between species with large differences in numbers of chromosomes or karyotypes, failed, as in the cross between common carp and grass carp (*Ctenopharyngodon idella*) (Wu 1990). Other combinations were more successful, and their unique features include a higher resistance to diseases, ability to grow in cool water, colour variants or a higher growth rate (Scribner et al. 2000). However, hybrid production by human activities should be under control, because the impact of natural hybrids on natural environment is considerably lower compared to artificial hybrids (Scribner et al. 2000).

Measurements supported by statistical analysis had been applied successfully in earlier studies to characterise carp strains (Corti et al. 1988), hybridisation and cross-breeding of species within the Cyprinidae family (Hulata 1995), as well as parents and their hybrids in the natural environment (Toscano et al. 2010). Molecular analyses have significantly improved and shortened the hybrid identification process and are currently applied in many studies focusing on this problem (Hashimoto et al. 2010).

In the present study we have decided to use morphometric data and AFLP method (amplified fragment length polymorphism) to find differences between parents and their potential hybrids. AFLP is reliable, stable and reproducible, and the analysis may be performed without any knowledge of nucleotide sequence that is going to be examined (Vos et al. 1995; Bagley et al. 2001). Recently, this technique has been applied to many fish research projects concerning the development of genetic linkage maps (Liu et al. 2003), assessment of genetic diversity in wild and captive populations (Kubota et al. 2008), evolutionary analysis of tetraploids (Liu et al. 2009) and the analysis of common carp and goldfish hybrids (present study).

The aim of this work has been to use two different approaches, biometric and genetic, to assess differences between *C. carpio* × *C. auratus* hybrids and their parents.

Materials and methods

Material used for measurements and genetic analysis was obtained from FRS of West Pomeranian University of Technology, Szczecin. Two-year-old live specimens of common carp ($n = 30$), goldfish ($n = 30$) and their hybrids ($n = 30$) were anaesthetised with 2-phenoxyethanol and measured according to a modified method developed by Brylińska (2000, p. 56). As a result, two morphometric (number of rays in dorsal and anal fin) and twenty-one meristic values were obtained. Measurements were performed using callipers with 0.01 mm accuracy (Fig. 1).

Morphometric approach

A statistical analysis of measured values was performed using Statistica 9.0 software package (Statsoft 2009). For every variable, Levene's test for equality of variances has been performed. Then the one-way ANOVA and post hoc NIR tests were applied to homogenous variables in order to assess whether the differences between their averages

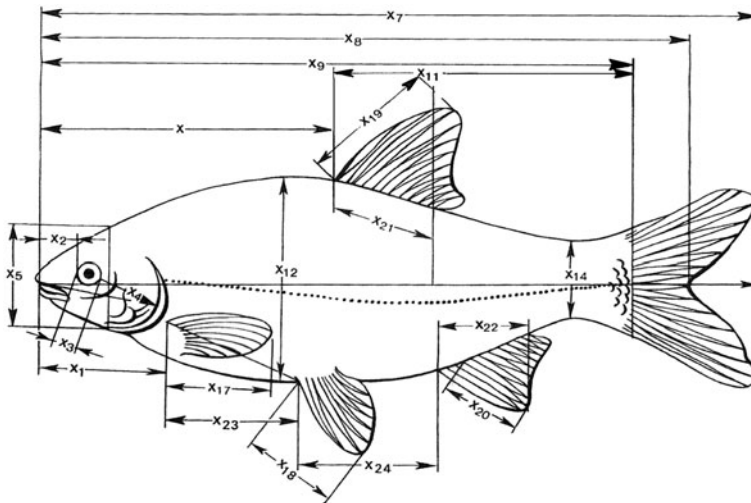


Fig. 1 Body measurements of fish according to Brylińska scheme. X predorsal distance (pD), x_1 lateral length of head (lc), x_2 preorbital distance (prO), x_3 horizontal diameter of eye (O), x_4 postorbital distance (poO), x_5 head depth (hc), x_7 total length (TL), x_8 fork length (FL), x_9 body length (SL), x_{11} postdorsal distance (poD), x_{12} maximum body depth (H), x_{14} minimum body depth (h), body width (laco), x_{17} pectoral fin length (IP), x_{18} ventral fin length (IV), x_{19} dorsal fin height (hD), x_{20} anal fin height (hA), x_{21} dorsal fin length (ID), x_{22} anal fin length (lAbs), x_{23} distance between pectoral and anal fin (P–V), x_{24} distance between ventral and anal fin (V–A)

were statistically significant. The remaining variables were studied using nonparametric Kruskal–Wallis and multiple (bilateral) comparison tests.

Genomic DNA preparation

From each group (common carp, goldfish and their hybrids) 5 fin clips have randomly been excised, and DNA extraction was performed using peqGOLD Tissue DNA Kit (PEQLAB Biotechnologie). Purity and concentration of DNA eluates were assessed on a 1 % agarose gel, using Nanodrop ND-1000 (Thermo Fisher Scientific Inc.) spectrophotometer, and then stored in frozen form until needed for further analysis.

AFLP procedure

AFLP with slight modifications was followed according to the procedure developed by Vos et al. (1995). To circumvent the problem of mismatching, stricter conditions were applied during the PCR according to suggestions proposed in earlier papers (Krauss 1999; O’Hanlon and Peakall 2000). Separation and binning of AFLP fragments were performed using the CEQ™ 8000 DNA Analysis System (Beckman Coulter). Running conditions had been reported in Papa et al. (2005). The CEQ 8000 scoring algorithm automatically produced AFLP fragments in binary mode, which were further used in FAMD 1.2 to calculate Jaccard’s coefficient, perform principal coordinate analysis (PCoA) and estimate population–population distances (chord distance from allele frequency) based on Bayesian and square root methods (Schlüter and Harris 2006).

Results

Morphometric approach

Comparison of morphometric features of goldfish, carp and their hybrids has shown that, depending on the analysed variable, mean distribution of their values differed according to applied statistical tests (Table 1). In some cases (lAbs, hD, O and L.pr.A) averages did not differ statistically, in other (Tl, Fl, Sl, IP and hC) between goldfish and *C. carpio* × *C. auratus* there was no difference, whereas in the group of *C. carpio* and *C. carpio* × *C. auratus*, no difference was found for h, V–A and L.pr.D averages. Nevertheless, six values of variables, that is, pD, laco, IV, P–V and lc, differed from one of the analysed groups to another. In the *C. carpio* × *C. auratus* hybrid group, the average values of three variables, namely pOD, ha and poO, were positioned between average values characteristic of *C. carpio* and *C. auratus*.

A high variability of morphometric features indicated hybridisation without domination of one of the parents. Other features observed by authors, such as the presence of shortened barbells, general body shape, scales and skin colour, further confirmed the obtained results.

Genetic analysis

Population–population distances from allele frequency estimates based on Bayesian and square root methods have shown that hybrids were more similar to *C. auratus* rather than to *C. carpio* (Table 2). Matrix size calculated using matrix statistics feature in FAMD 1.2 software had 705 with 47 loci (polymorphic bands) produced without any missing data in loci and individuals. The software count band statistics calculated 20 private bands for common carp, 11 for goldfish, none private bands for hybrid groups and none fixed private bands for any group. The calculation of Jaccard's similarity coefficient and building PCoA plot showed that three populations had produced their own groups, except one sample of the hybrid and one of *C. carpio* which had formed their own outstanding group, marked by a small dashed line circle (Fig. 2). It proves that AFLP technique applied to distinguish two parental lineages and their potential hybrids was applied successfully. However, in further studies more numerous samples should be analysed in each of the groups to check whether more separate samples will appear.

Discussion

Hybrid production with desired traits is profitable; however, this practice should be supported by appropriate methods to unequivocally identify parental organisms and their offspring. Both methods of analysis, that is, biometric and genetic, have been applied to determine characteristic hybrid features in the Cyprinidae family (Briolay et al. 1998). Natural hybrids and those produced by artificial breeding between carp and goldfish have been reported from many countries. Some of them were described as sterile; however, Russian triploid hybrids had been considered as fertile, although stages of gonadal development in hybrids were not analysed (Pullan and Smith 1987; Zelinskij et al. 1992). Nevertheless, the problem of testes and ovary maturity must be tackled in future studies, paying special attention to the quality and quantity of sperm and eggs.

An analysis of biometric features showed that hybrids possessed a mixture of parental features; however, based on authors' observations, it is difficult to indicate typical body elements entirely characteristic of either carp or goldfish. During studies on the

Table 1 Mean distribution of values obtained with different statistical tests

Group/Feature	TI	FI	SI	pD	pOD	h	H	IABs	hA	Iaco	IV	IP
Carp	290 ^a	259 ^a	237 ^a	116 ^a	111 ^a	30 ^a	69 ^a	21 ^a	38 ^a	44 ^b	43 ^a	48 ^a
Goldfish	249 ^b	227 ^b	203 ^b	90 ^c	107 ^{ab}	34 ^b	86 ^b	23 ^a	29 ^b	51 ^c	40 ^b	39 ^b
Hybrid	252 ^b	225 ^b	196 ^b	96 ^b	97 ^b	32 ^a	73 ^a	24 ^a	30 ^{ab}	40 ^a	38 ^c	39 ^b
	AN	AN	AN	AN	KW	AN	AN	AN	KW	AN	KW	KW
Group/feature	V-A	P-V	hD	ID	lc	pro	O	poO	hC	L.pr.D	L.pr.A	Body weight [g]
Carp	64 ^a	59 ^a	34 ^a	83 ^a	68 ^a	27 ^c	16 ^a	32 ^a	54 ^a	20 ^a	7 ^a	349 ^a
Goldfish	72 ^b	52 ^b	29 ^a	79 ^{ab}	50 ^b	14 ^a	12 ^a	25 ^b	46 ^b	18 ^b	7 ^a	399 ^a
Hybrid	59 ^a	47 ^c	31 ^a	76 ^b	57 ^c	19 ^b	12 ^a	29 ^{ab}	45 ^b	19 ^a	7 ^a	272 ^b
	AN	AN	KW	AN	KW	AN	KW	KW	AN	AN	KW	AN

Values in columns marked by identical letters do not differ statistically $p > 0.05$. Statistical method: AN ANOVA and LSD test, KW Kruskal–Wallis test and multiple comparison of mean ranges

Table 2 Chord distance from allele frequency estimates based on the two tests

	Hybrids	<i>C. auratus</i>	<i>C. carpio</i>
<i>Bayesian method</i>			
Hybrids	0.0000000000		
<i>C. auratus</i>	0.1357414742	0.0000000000	
<i>C. carpio</i>	0.1662020208	0.2430554100	0.0000000000
<i>Square root method</i>			
Hybrids	0.0000000000		
<i>C. auratus</i>	0.2096936973	0.0000000000	
<i>C. carpio</i>	0.2465484508	0.3510386854	0.0000000000

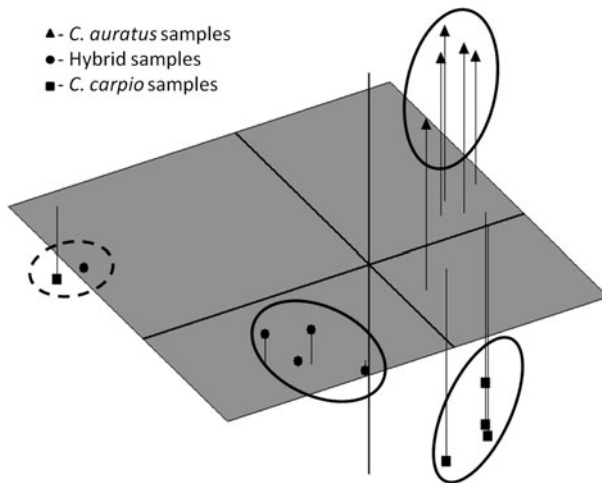


Fig. 2 Principal coordinate analysis plot for all samples produced by means of FAMD 1.2 software

identification of hybrid populations in New Zealand, scientists also found mixed traits (Smith and McVeagh 2005). Hybrids reared at Fisheries Research Station at our university possessed shorter barbells, mixed body shape, scaling and colour, which clearly indicate mixed features in the offspring. Additionally, pOD, ha and poO features have shown that *C. carpio* × *C. auratus* hybrids are a mixture of their parents. The offspring obtained from hybridisation is hard to identify due to blending of parental characters (Scott and Crossman 1973). Some authors have suggested counting scales on lateral lines; however, the reliability of this feature might be overestimated (Hume et al. 1983). The present study has shown that pD, laco, IV, P–V, lc and pro feature might be used as a discriminative one for hybrids identification.

External characters depend on the kind of environment and might change according to interactions between biotic and abiotic factors (Olsson et al. 2007; Chizinski et al. 2010). Therefore, to identify hybrids of carp and goldfish, we have applied AFLP technique to analyse similarity among whole genomes of *C. carpio*, *C. auratus* and their hybrids. It is a challenging approach that has successfully been applied to analyse phylogenetic relationships of Japanese crucian carps (Yamamoto et al. 2010). Mainly, all samples were separated into three groups, which was illustrated by a MEGA5 dendrogram or PCoA plot.

Only two samples, one from the hybrid group and one from the carp group, were placed outside all groups. Chord distances from allele frequency based on Bayesian and square root methods revealed that samples of hybrids showed greater similarity to goldfish (13.5 and 21 % of differences, respectively, depending on the utilised methods) than to common carp specimens (16.6 and 24.7 % of differences, respectively, depending on the utilised methods).

Morphological characters have been described and may be used in morphological discrimination of *C. carpio* × *C. auratus* hybrids. Genetic analysis performed by assessing polymorphic sites of genome (AFLP) has successfully been applied in this study. On the basis of the obtained results, the authors suggest that further studies might use more numerous samples from each population and analyse them with the AFLP technique, which produces reliable results.

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