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Morphological recognition of artificial F1 hybrids between three common European cyprinid species: *Rutilus rutilus*, *Blicca bjoerkna* and *Abramis brama*

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Abstract　The F1 hybrids of roach *Rutilus rutilus*, silver bream *Blicca bjoerkna*, and common bream *Abramis brama* from experimental reciprocal cross-breedings were identified at 18 months of age in relation to the parental species. The morphological analysis, including quantitative (plastic and meristic) and nonmetric features differing in the roach, the silver bream and the common bream, revealed that roach × common bream and roach × silver bream hybrids were easier to distinguish from their parents than silver bream × common bream hybrids. These roach hybrids had many morphological similarities such as the upper iris coloured in red as in the roach, and they were morphologically intermediate to the two parents. This contrasted with the silver bream × common bream hybrids, in which intermediate characteristics were also observed, but with some parental variants. Roach × silver bream hybrids were distinguishable from roach × common bream hybrids by its large eye, its lower scale numbers along the lateral line and its two rows of pharyngeal teeth. Silver bream × common bream hybrids, compared to the two other types of hybrids studied, had higher anal fin soft ray numbers and a clear eye iris with a median black line. In all interspecific crosses of these three cyprinid species fish, the reciprocal hybrids were generally indistinguishable [*Acta Zoologica Sinica* 54 (1): 144－156, 2008].

Key words　Quantitative, Nonmetric features, Hybrids, Cyprinid

三种普通欧洲鲤科鱼类（拟鲤、粗鳞鳊和欧鳊）人工杂交 F1 代形态学观察

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摘　要　对反交试验获得的18个月的拟鲤、粗鳞鳊和欧鳊F1代进行了鉴定。对不同于拟鲤、粗鳞鳊和欧鳊三种鱼的可量和可数形态特征分析表明，拟鲤×欧鳊和拟鲤×粗鳞鳊杂交种比粗鳞鳊×欧鳊杂交种与它们的双亲更容易区分。拟鲤杂交种间具有很多形态上的相似性，例如，上侧虹膜在拟鲤为红色，杂交种则介于双亲之间。在粗鳞鳊×欧鳊杂交种中也可以观察到居间类型。拟鲤×粗鳞鳊和拟鲤×欧鳊各自的杂交种可以通过眼睛的大小、较少的侧线鳞数和2行咽喉齿等特征相区分。粗鳞鳊×欧鳊杂交种与另外两个杂交种相比，具有更多的臀鳍分支鳍条数和一个中央具一黑线的虹斑。三个种进行杂交再获得的互交种通常不易区分［动物学报 54（1）：144－156，2008］。

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关键词　可量特征　非可量特征　杂交种　鲤科鱼类

Cyprinid hybrids collected in natural environments have already been described morphologically (Regan, 1911; Wheeler and Easton, 1978; Penczak, 1978; Witkowski and Blachuta, 1980; Bianco, 1982; Das and Nelson, 1988), karyologically (Pervozvanskiy and Zeinskyi, 1981), and using allozyme variation (Brassington and Ferguson, 1976; Golubstsov and Il' in Ⅱ, 1983; Golubstsov et al., 1990) in terms of assumed parental species. Unfortunately, these descriptions rarely specify male and female parents of hybrids (direction of hybridization). These descriptions are therefore incomplete because they omit information that is relevant to the identification of the hybrid.

Roach *Rutilus rutilus* (L.) and common bream *Abramis brama* (L.) hybrids are probably the most frequent in nature among cyprinid hybrids in the Old World (Golubstsov et al., 1990). During the large-scale hybridization between roach and common bream, the species producing the highest abundance of hybrids, from 37% to 90% of the parental populations, was recorded by Fahy et al. (1988) in Irish Reservoir. Natural hybrids of roach × common bream have been described in many European water bodies and remain the most widely studied. On the other hand, the hybrids of roach × silver bream *Blicca bjoerkna* (L.) and common bream × silver bream have only very rarely been studied, probably because the roach × silver bream hybrids are much less abundant (Wheeler, 1969) or because the common bream × silver bream hybrids are very similar to the parental species and consequently are difficult to identify in the wild.

The best approach to identifying natural hybrids is probably the characterization of pilot hybrids produced in a controlled environment. This strategy was adopted by Pitts et al. (1997) to check the identity of common bream × roach hybrids collected in Forty Foot Drain in England. Their study was focused on the meristic characteristics (the number of anal fin rays and the number of scales along the lateral line, between the lateral line and the dorsal fin, and between the lateral line and the anal fin) and genetics (evaluated by electrophoresis) to confirm the identity of natural hybrids.

For identifying the hybrids of the three cyprinid species fish, the standard profile expressed as the length ratios of morphometric characteristics, the iris colour, the fin placement and the number of teeth, taking into account the direction of hybridization, have not been used to characterize the hybrids in Western Europe. However, this type of study is important because it could help to detect the hybrids among the parents and also to differentiate these hybrids from other hybrids from other species colonizing the same natural habitat.

In this study, we aimed to further examine the morphological difference between the F1 reciprocal hybrids and between F1 hybrids and parents. The success of this examination could contribute to the recognition of the F1 hybrids in European waters in which the roach, the silver bream and the common bream usually live in sympatry in the same type of rivers (Huet, 1949; Philippart, 1989) and naturally hybridize (Wheeler, 1969; Penczak, 1978; Swinney and Coles, 1982), even if the precise definition of hybrid generation from the natural environment requires genetic analysis (Pacheco et al., 2002) when the hybrids are fertile. Since the quantitative (plastic and meristic) and nonmetric characteristics differ in roach, silver bream and common bream, they were used as the criteria for the morphological analysis of the hybrids.

1 Materials and methods

1.1 Production of intra- and interspecific generations

Roach, silver bream, common bream and their reciprocal F1 hybrids identified in this study were produced in the laboratory at Tihange Aquaculture Station in Belgium from producers (Table 1) collected in a natural population in a fish pass at the Lixhe dam (Belgian Meuse River, 50°45′N; 5°40′E), during spring 2002. Four interspecific crosses (male × female: roach × common bream = R × A; common bream × roach = A × R; roach × silver bream = R × B; silver bream × roach = B × R) and three intraspecific crosses (roach × roach = R × R; silver bream × silver bream = B × B; common bream × common bream = A × A) were produced from six producers (a male and a female from each of the three species) in experiment 1. In experiment 2, four producers (a male and a female from two species) were used to produce two interspecific crosses (silver bream × common bream = B × A; common bream × silver bream = A × B) and two intraspecific crosses (silver bream × silver bream = B × B; common bream × common bream = A × A) (see also Nzau Matondo et al., 2007).

Spawning of the producers was induced by injection of Ovaprim, a synthetic hormone made up of an analogue of salmon GnRH and a dopamine inhibitor (Syndel Laboratories Ltd, Canada). Sperm was individually collected in a syringe by stripping the mature male and was kept on ice until fertilization. Eggs from each female were divided into two parts; one part was mixed with the sperm of the male of the other species (hybrids) and the other part with the sperm of the conspecific male (intraspecific crossbreeding). For roach, eggs were divided into three parts which were individually fertilized with sperm from common bream, silver bream and roach. Fertilization was carried out using a dry technique at 1 ml of sperm per 100 g of spawn. The fertilized eggs were incubated in 1-l Zoug bottles at 18℃; the larvae were bred in 0.42 m × 0.42 m × 0.12 m trays and allowed to grow in 1.04 m × 1.04 m × 0.41 m basins at 20℃, in a recirculating system at the Tihange Aquaculture Station. Two replicates were maintained for each cross during embryogenesis and breeding. The photoperiod was set at 16 h of light and 8 h of darkness, dissolved oxygen was above 6 mg/l, nitrites and ammonium below 0.3 mg/L and pH 7.9 ± 0.8. The fish were fed exclusively with *Artemia nauplii* (50% protein) for the first 2 weeks after hatching, then with a mixture of *Artemia* and dry food (54% protein, initial fish feed, Lucky Star, Taiwan) for the following 2 weeks, and thereafter with dry food (52% protein) only. After 7 weeks, daily food (Nutra food, Skretting Trow, France) was readjusted per fish biomass weekly and was identical in all breeding experiments. The hybrids and the parental species were morphologically examined at 18 months of age.

1.2 Plastic and meristic characteristics

The morphological analysis was conducted for the quantitative (plastic and meristic) and nonmetric characteristics differing in the roach, the silver bream and the common bream (Spillmann, 1961; Maitland, 1972; Muus and Dahlström, 1991). A minimum of ten fish were sampled per hybrid and parental species in each replicate. Each fish was scored for the quantitative traits described below. The plastic quantitative traits measured (Fig.1A) and the meristic characteristics examined (Fig.1B, 1C and 1D) are summarized in Table 2. Figure 1 was made of hand drawings from images taken with a digital camera and visual observation of fish.

Table 1 Identifications of producers in experiments 1 and 2

Fish (♀-♂)	Fork length (mm)	Weight (g)	Age (years)	Soft rays in anal Fin (number)	Scales along lateral line (number, left/right)	Gill rakers on first arch (number, left/right)	Pharyngeal-tooth formula (left/right)
Experiment 1							
A-A	390 – 430	1 092 – 1 251	7 – 9	25 – 26	56/56 – 53/54	23/23 – 24/24	5/5 – 5/5
R-R	196 – 250	115 – 251	5 – 5	10 – 11	43/42 – 44/44	13/12 – 12/12	5/5 – 5/5
B-B	216 – 230	226 – 228	4 – 5	20 – 20	47/46 – 47/47	16/16 – 18/17	2.5/5.2 – 2.5/5.2
Experiment 2							
A-A	400 – 455	1 154 – 1 625	7 – 9	26 – 26	55/55 – 53/53	23/22 – 23/23	5/5 – 5/5
B-B	252 – 260	362 – 348	5 – 5	21 – 22	47/48 – 47/47	16/17 – 17/17	2.5/5.2 – 2.5/5.2

A: Common bream; B: Silver bream; R: Roach. For each parameter, the first and second numbers indicate numbers of female (♀) and male (♂) fish, respectively.

Table 2 The list of 23 quantitative morphological traits observed in the F1 hybrids and parental species

Quantitative traits			
No.	Plastic traits	No.	Meristic characteristics
1	Aspect ratio of caudal fin ($height^2/height \times length$)	16	Number of scales along the lateral line
2	Eye diameter as percentage of head length	17	Number of scales between the dorsal fin origin and the lateral line
3	Anteeye distance as % head length	18	Number of scales between the pelvic fin origin and the lateral line
4	Antepelvic distance as % antedorsal	19	Number of scales between the ventral midline of abdomen and the lateral line
5	Length of dorsal fin base as % anal base	20	Number of scales between the anal fin origin and the lateral line
6	Distance from snout to postdorsal as % anteanal	21	Number of soft rays in the pelvic fin
7	Anteeye distance as % eye diameter	22	Number of soft rays in the anal fin
8	Head length as % fork length	23	Number of soft rays in the dorsal fin
9	Antedorsal distance as % fork length		
10	Antepelvic distance as % fork length FL		
11	Anteanal distance as % fork length		
12	Length of anal fin base as % fork length		
13	Body depth as % fork length		
14	Head depth as % fork length		
15	Body diameter as % body dept		

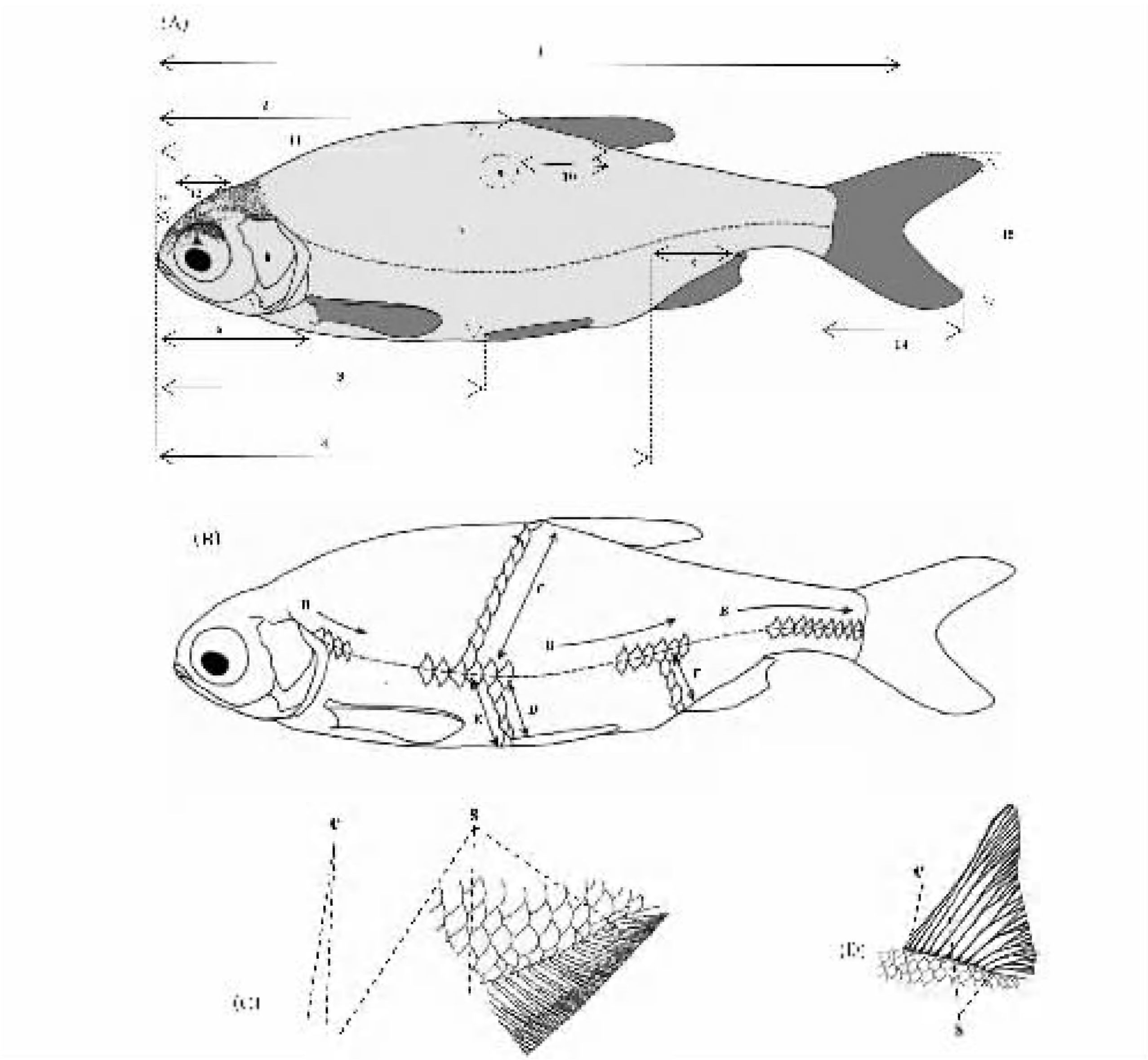

Fig.1 Indications on measurements taken (A), scale count (B), anal fin rays (C) and dorsal fin rays (D)

(A) and (B). Roach × silver bream specimens. (C) and (D). Common bream × Common bream specimen fins. e: Spine rays. S: soft rays; male × female.

Measurements in Fig.1 (A) are as follows: 1) fork length; 2) antedorsal length measured horizontally from anterior of snout to level of articulation of first dorsal fin ray; 3) antepelvic length measured horizontally from anterior of snout to level of articulation of first ray of pelvic fin; 4) anteanal length measured horizontally from anterior of snout to level of articulation of first ray of anal fin; 5) length of anal fin base measured horizontally between level of anterior and posterior of anal fin base; 6) length of head measured horizontally from anterior of snout to level of posterior edge of operculum; 7) maximum body depth (fins not included); 8) maximum depth of head; 9) horizontal diameter of body taken midway between first ray of dorsal fin and lateral line; 10) length of dorsal fin base measured horizontally between level of anterior and posterior of dorsal fin base; 11) snout to postdorsal length, measured horizontally from anterior of snout to level of posterior end of dorsal fin; 12) horizontal diameter of eye; 13) length of snout measured horizontally from anterior of upper jaw to level of anterior margin of eye; 14) length of caudal fin measured horizontally from base of caudal fin to level of posterior tip; 15) maximum height of caudal fin. Scale counts in Fig.1 (B) are: B) number of scales along lateral line; C) number of scales between dorsal fin origin and lateral line; D) number of scales between pelvic fin origin and lateral line; E) number of scales between ventral midline of abdomen and lateral line; and F) number of scales between anal fin origin and lateral line.

1.3 Pigmentation of the upper iris

The colouring of the upper iris of the eye was quantified based on the techniques used in ornamental carp (koi) *Cyprinus carpio* L., and goldfish *Carassius auratus auratus* L. by Gomelsky et al. (1995), Gouveia et al. (2003), and Hancz et al. (2003). A minimum of ten fish per hybrid type and parental species from two replicates were collected and photographed with a digital camera in light produced by two neon tubes. The colour intensity was measured from three regions, each measuring 0.12 × 0.12 cm, in the upper part of iris (centre, left, and right side) using Photoshop software

(Adobe Photoshop® version 7.0) installed on a computer. The intensity of red, green, and blue was evaluated on the left eye using a scale with values ranging from 0 to 255.

1.4 Nonmetric characteristics

The alternative nonmetric characteristics such as the pharyngeal-tooth formula, mouth position, fin colours, scale forms, and upper iris description were observed. The shape of the anal fin was drawn by hand from digital camera images and visual observation of the fish. For the dental examination, the pharyngeal bones dissected from fresh fish were cleaned by maceration in hot water and analysed under a microscope. The figure illustrating the pharyngeal teeth was drawn by hand from microscope images.

1.5 Data analysis

For these 23 quantitative morphological traits, the F1 hybrid specimens were identified using the method of the principle component based on a Euclidian genetic distance matrix. The iris colour between hybrids and parental species was compared using the nonparametric Mann-Whitney U-test in which the level of significance was accepted at $P < 0.05$. For the most distinctive quantitative characteristics between the species such as the eye diameter as a percentage of head length, the length of the dorsal fin base as a percentage of the anal base, the length of the anal fin base as a percentage of fork length (FL), and the number of scales along the lateral line (Lli) and between the dorsal fin origin and the Lli, a hybrid index was calculated from the average values of hybrids and their parents, using the following formula (Witkowski and Blachuta, 1980; Crivelli and Dupont, 1987): $HI = 100 \times (H_i - M_{i1}) / (M_{i2} - M_{i1})$, where for a characteristic I, H_i = average of hybrid for characteristic I; M_{i1} = average of species representing the female parent for characteristic I; and M_{i2} = average of species representing the male parent for characteristic I. For a characteristic I, the value of HI was interpreted as follows: from 45 to 55, intermediate characteristic to two species; < 45, characteristic close to female parent's species; > 55, characteristic close to male parent's species; and > 100 and < 0, characteristic specific to the hybrid.

2 Results

2.1 Multivariate space of quantitative traits

In the multivariate space of the quantitative traits (Fig.2) produced from all individuals to identify the F1 hybrids and its parents, the nonoverlapping quantum position was observed between the F1 hybrids of roach × common bream and roach × silver bream and the parental species. This contrasted with the F1 hybrids of silver bream × common bream in which the overlapping positions were observed with one or two parental species. The distribution of phenotypes in the F1 hybrids (Table 3) showed no overlap (100%) in the roach × common bream and roach × silver bream hybrids. However, the F1 hybrids of silver bream × common bream revealed some degree of overlap, i. e. 27% with the paternal parent for B × A hybrids and 6% and 42% with the paternal and maternal parent, respectively, for A × B hybrids. Between the roach, the silver bream and the common bream, no overlap was observed, whereas between the silver bream and the common bream, a high degree of overlap was observed. Between the reciprocal F1 hybrids, some overlap was observed in all crosses. No overlap was observed between the silver bream × common bream hybrids and the roach × common bream or roach × silver bream hybrids. The hybrid and parental species specimens used in the experimental analysis are shown in Fig.3.

Table 3 Distribution of phenotypic groups in the F1 hybrid progenies

F1 generation (male × female)	Total number of fish	Morphotype					
		Paternal cluster		Maternal cluster		Intermediate cluster	
		n	%	n	%	n	%
R × A	36	0	–	0	–	36	100
A × R	30	0	–	0	–	30	100
R × B	30	0	–	0	–	30	100
B × R	20	0	–	0	–	20	100
B × A	37	10	27	0	–	27	73
A × B	31	2	6	13	42	16	52

2.2 Distinctive characteristics

For the most distinctive characteristics (Table 4), the roach × common bream or roach × silver bream F1 hybrids showed that the length of the anal fin was intermediate to the length in the parents, as also demonstrated by hybrid index analysis. In these hybrids, the anal fin base was longer than that of the dorsal fin and its origin was always posterior to vertical passing through the last ray of the dorsal fin, and pelvic fins were inserted closer to vertical, passing through the first ray of the dorsal fin. The shape of their anal fin was also intermediate to the shape in the parents' anal fin (Fig.4). Between these hybrids, the roach × silver bream hybrids had larger eyes (35% – 45% of head length) than the roach × common bream hybrids (23% – 32%). Hybrid index analysis revealed that the eye size of the roach × silver bream hybrids was closer to the silver bream. In breams, the anal fin base was longer than the dorsal fin base. The origin of the anal fin for the silver and the common bream was beneath the posterior ray of the dorsal fin, but the origin of the pelvic fin was anterior to the vertical, passing through the first ray of the dorsal fin. The silver bream could be distinguished from the

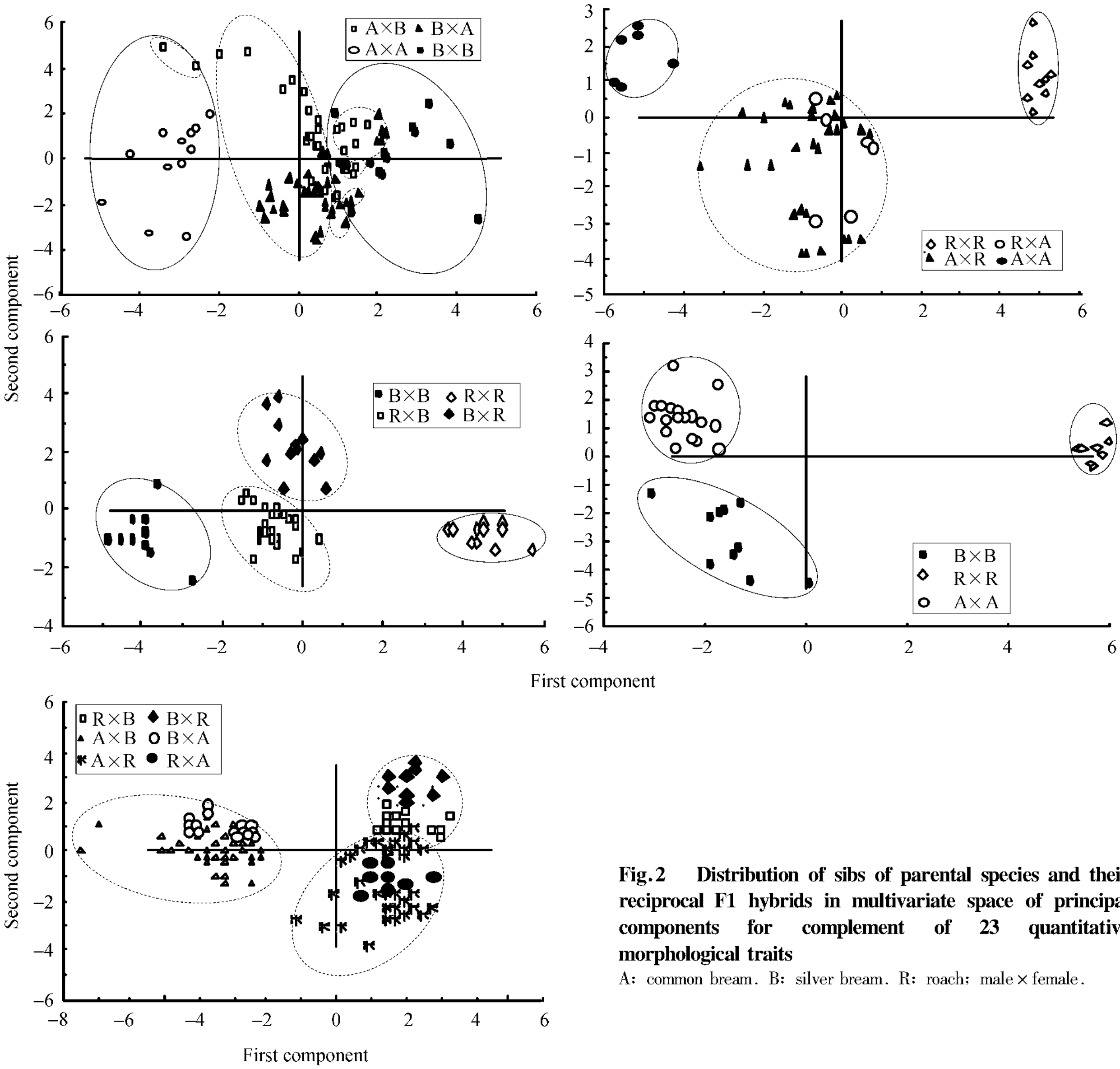

Fig.2　Distribution of sibs of parental species and their reciprocal F1 hybrids in multivariate space of principal components for complement of 23 quantitative morphological traits

A: common bream. B: silver bream. R: roach; male × female.

common bream by its large eyes. In roach, the pelvic fin was inserted under vertical, passing through the first ray of the dorsal fin, its anal fin originated very posterior to the last ray of the dorsal fin, and its dorsal fin base was longer than the anal fin base. The silver bream × common bream hybrids showed the same profile as their parental species: the anal fin base was nearly double the length of the dorsal fin base and the eye diameter intermediate or close to the silver bream. In all interspecific crosses of the three cyprinid species, no difference was found between the reciprocal F1 hybrids.

2.3　Meristic characteristics

The meristic characteristics such as the number of soft rays and scales along the lateral line distinguished the roach × common and roach × silver bream F1 hybrids from their parental species but only the number of scales differentiated these two types of hybrids. The roach × common bream hybrids had 16 – 18 soft rays in the anal fin versus 24 – 26 rays in the common bream and 9 – 12 in the roach. Similarly, the roach × silver bream hybrids had 14 – 16 soft rays in the anal fin versus 19 – 22 in the silver bream and 9 – 12 in the roach. These hybrids had 40 – 42 scales along the lateral line versus 44 – 50 in the roach × common bream hybrids. The silver × common bream hybrids had higher numbers of soft rays (22 – 25) in the anal fin than both the roach × common bream and the roach × silver bream hybrids, and the number of scales along the lateral line were also greater (47 – 50) than the roach × silver bream hybrids, but an overlap was observed with the roach × common bream hybrids. The reciprocal hybrids of the three cyprinid species were also indistinguishable.

The hybrid index analysis revealed that the number of scales in roach × common bream hybrids was intermediate or closer to roach but the number of anal fin soft rays was closer to the common bream. In roach × silver bream hybrids, the number of scales between the dorsal fin and the lateral line and the number of anal fin

Fig.3 Specimens of hybrids and parental species used in experimental analysis
A: common bream; R: roach; B: silver bream; male × female. Scale in mm.

soft rays were closer to the silver bream. In silver bream × common bream hybrids, the number of scales along the lateral line was nearer to the number of scales found in silver bream, but the number of soft rays was closer to the common bream.

2.4 Pigmentation of the upper iris

As for the pigmentation of the upper iris of the eye, all roach hybrids showed an iris coloured in red. However, the centre region of the iris in the hybrids and the parental species was less red, green and blue than the lateral parts of the iris (Fig.5). The intensity of the red colour in all parts of the upper iris of the roach × common bream hybrids was intermediate to the same trait in the parents, while these hybrids showed significantly higher levels of intensity (Mann-Whitney U-test, $P < 0.05$) than the common bream but rarely significantly lower levels than the roach. In the roach × silver bream hybrids, the red colour in all parts of the upper iris was also intermediate to the red colour in the parents but not statistically different (U-test, $P > 0.05$) from the roach and rarely less significantly diffrerent (U-test, $P < 0.001$) from the silver bream. Within these species, the roach had more red colouration (U-test, $P < 0.001$) than both the silver bream and the common bream in all parts of the upper iris except the centre, where the silver bream was not significantly different (U-test, $P = 0.0933$) from the roach. The silver bream had a higher red colouration (U-test, $P < 0.05$) than the common bream, but on the left side, these two species were not significantly different (U-test, $P = 0.1139$). For green and blue colouration, the hybrids in all interspecific crosses were more intensely coloured than their parents. In each colour and iris part, the reciprocal hybrids were generally indistinguishable to a significant degree.

2.5 Pharyngeal teeth and bones

Data on the alternative nonmetric characteristics, dental examination, showed a higher variability of pharyngeal-tooth phenotypes in the F1 hybrids than in the parental species (Table 5) and a hybrid pharyngeal bone shape intermediate to the that of the parents (Fig.6). The roach × common bream hybrids' teeth were arranged in a single row, like their parents, whereas the roach × silver bream and the silver bream × common bream hybrids always had two rows of teeth on one or both the pharyngeal bones. Teeth arranged in two rows were only observed in the silver bream. The teeth forming the second row were generally less robust than those in the same row of silver bream. The pharyngeal bones in this species and in the roach were much stockier in appearance than the bones in the common bream. The roach ×

Table 4 Comparison of the most distinctive morphometric and meristic characteristic ratios in the hybrids and parental species

Male × female	n	Fork length (mm)		Number of scales: Along the lateral line			Number of scales: Between the base of dorsal fin and the lateral line			Number of soft rays in the anal fin			Length of anal fin base as % fork length			Length of dorsal fin base as % anal fin base			Diameter of the eye as % head length		
		Mean ± SD	Range	Mean ± SD	Range	HI	Mean ± SD	Range	HI	Mean ± SD	Range	HI	Mean ± SD	Range	HI	Mean ± SD	Range	HI	Mean ± SD	Range	HI
Experiment 1																					
A × A	30	195.0 ± 5.6	190.0 – 197.0	53.1 ± 1.8	50.0 – 55.0		12.2 ± 0.2	12.0 – 12.5		24.6 ± 0.7	24.0 – 26.0		20.4 ± 0.5	19.9 – 20.9		51.1 ± 6.8	45.9 – 58.8		18.0 ± 0.8	17.5 – 18.9	
R × A	36	155.7 ± 12.4	141.0 – 185.0	48.3 ± 1.8	47.0 – 50.0	45	11.1 ± 0.7	10.0 – 12.5	35	16.4 ± 0.5	16.0 – 18.0	58	14.4 ± 1.6	12.7 – 16.5	53	75.0 ± 9.9	60.0 – 94.4	24	25.7 ± 2.7	23.3 – 30.0	128
A × R	30	148.2 ± 3.5	142.0 – 152.0	46.3 ± 1.8	44.0 – 48.0	36	10.7 ± 0.7	10.0 – 12.0	52	16.3 ± 0.5	16.0 – 17.0	42	15.2 ± 1.2	13.5 – 17.3	54	80.9 ± 7.6	75.0 – 91.7	70	26.6 ± 3.7	23.3 – 32.1	−43
R × R	30	160.2 ± 9.5	150.0 – 172.0	42.4 ± 0.8	41.0 – 44.0		9.1 ± 0.5	8.5 – 9.5		10.4 ± 1.1	9.0 – 12.0		9.0 ± 1.1	7.3 – 9.9		150.5 ± 14.0	128.6 – 166.7		24.0 ± 0.9	23.3 – 25.0	
R × B	34	108.2 ± 6.1	92.0 – 113.0	41.8 ± 0.7	41.0 – 42.0	11	9.4 ± 1.0	8.0 – 10.5	79	14.4 ± 0.7	14.0 – 16.0	60	14.5 ± 1.6	11.1 – 16.1	50	74.6 ± 17.7	50.0 – 100.0	22	38.5 ± 2.7	34.8 – 45.0	6
B × R	20	113.6 ± 8.1	101.0 – 125.0	41.7 ± 0.7	40.0 – 42.0	−15	9.7 ± 0.9	8.0 – 10.5	43	15.9 ± 0.3	15.0 – 16.0	58	13.4 ± 1.3	12.0 – 16.1	40	85.5 ± 11.6	65.0 – 100.0	67	39.2 ± 2.8	34.8 – 45.0	97
B × B	32	102.0 ± 13.8	95.0 – 138.0	47.3 ± 1.1	46.0 – 49.0		10.5 ± 1.2	9.5 – 12.0		20.3 ± 1.0	19.0 – 22.0		20.0 ± 1.7	17.5 – 22.9		57.6 ± 11.1	44.7 – 69.5		39.7 ± 9.1	33.0 – 51.6	
Experiment 2																					
A × A	30	119.1 ± 7.4	108.0 – 132.0	52.7 ± 2.3	50.0 – 54.0		12.2 ± 0.3	12.0 – 12.5		24.7 ± 0.6	24.0 – 25.0		20.7 ± 1.2	18.9 – 22.9		46.8 ± 8.0	40.0 – 62.5		27.1 ± 3.2	23.3 – 34.4	
A × B	31	115.7 ± 13.3	87.0 – 138.0	48.5 ± 1.2	47.0 – 50.0	25	12.3 ± 0.8	12.0 – 14.0	106	23.7 ± 0.6	22.0 – 25.0	77	19.0 ± 1.6	17.2 – 22.1	−34	58.3 ± 10.9	44.4 – 80.0	−69	35.2 ± 3.8	30.0 – 44.0	36
B × A	37	109.7 ± 9.8	94.0 – 132.0	49.1 ± 1.2	47.0 – 50.0	60	11.5 ± 0.6	11.0 – 12.5	41	23.7 ± 0.6	22.0 – 25.0	22	21.3 ± 2.8	19.0 – 26.7	−400	53.1 ± 10.9	36.4 – 77.8	252	36.8 ± 3.8	30.3 – 45.0	45
B × B	41	103.0 ± 13.8	85.0 – 125.0	47.1 ± 1.3	46.0 – 49.0		10.5 ± 1.1	9.5 – 12.0		20.3 ± 1.1	19.0 – 22.0		20.0 ± 1.9	16.7 – 23.8		53.6 ± 10.6	37.0 – 68.2		39.7 ± 7.7	30.0 – 53.3	

A: common bream; B: silver bream; R: roach; male × female; HI: hybrid index; SD: standard deviation; n: number of fish

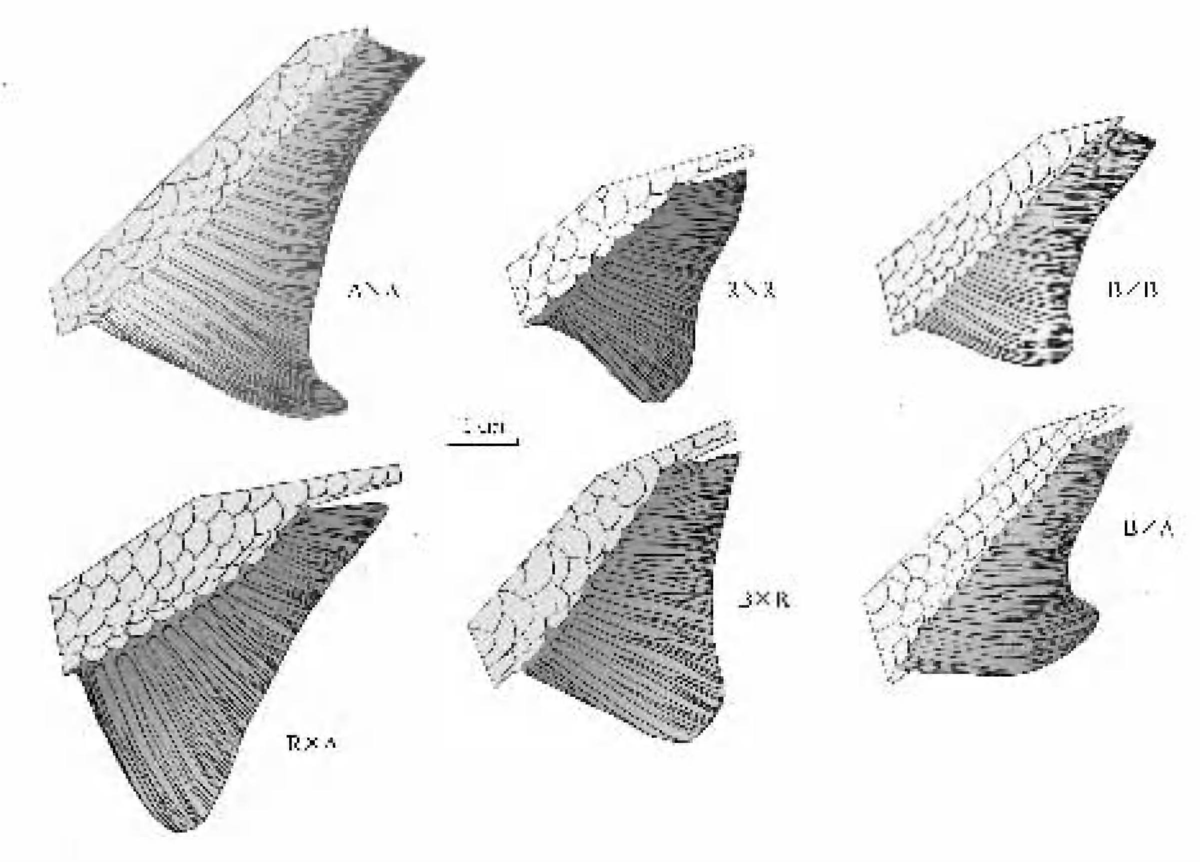

Fig.4 Shape of anal fin at the hybrids and parental species
A: common bream; R: roach; B: silver bream; male × female.

common bream hybrids had four to five teeth on the right pharyngeal bone versus five to six on the left side. The roach × silver bream and the silver bream × common bream hybrids had one or two teeth on one row as opposed to four to six versus four to five on the other row in the first and the second types of hybrids, respectively, although one fish in the silver bream × common bream hybrids had two series of four teeth on one pharyngeal bone. In the parental species, the common bream had only five teeth on each bone. The roach had five to six and four to six teeth on the left and the right sides of pharyngeal bones, respectively. The silver bream had two teeth on the second row as opposed to four to five on the first.

3 Discussion

3.1 Parental morphology and quantum position of hybrids

The morphological and meristic data of the parental species described in this study correspond well with the description reported by Regan (1911), Spillman (1961), Wheeler (1969), and Maitland (1972). The identification of these species could therefore be considered correct. The narrow range of the characteristics analysed and the strict intermediary aspect of the quantum position observed, particularly in the roach × common bream and roach × silver bream F1 hybrids confirm that the parental species in the present study are pure-bred specimens. This intermediate position of the hybrids conforms to the Mendelian theoretical model postulating that all the F1 individuals are intermediate to the parents for characteristics expressed by co-dominant alleles from parents. In contrast, the parental variants in the silver bream × common bream F1 hybrids observed could be promoted by many similarities of the morphological characteristics between its parental species and by problems standardizing all the environmental conditions such as the sex ratio, because in our experiments, the fish observed were sexually immature. Indeed, the morphological characteristics could be related to growth and growth itself could be influenced by individual factors (sex, age), genetics (species, heterosis), and environmental factors (ration and food balance, temperature, disease) (Vieven et al., 1998; Barriga-Sosa et al., 2004). An abundant food supply, for example, generally produced more fish with a deeper body in the roach, whereas deep-bodied fish are rarer when food is scarce (Muus and Dahlström, 1991). In morphological characteristics analysed, the morphometric characteristics are more variable than meristic characteristics with regard to ontogeny and to mitigate what, we have expressed them as the length ratios. The

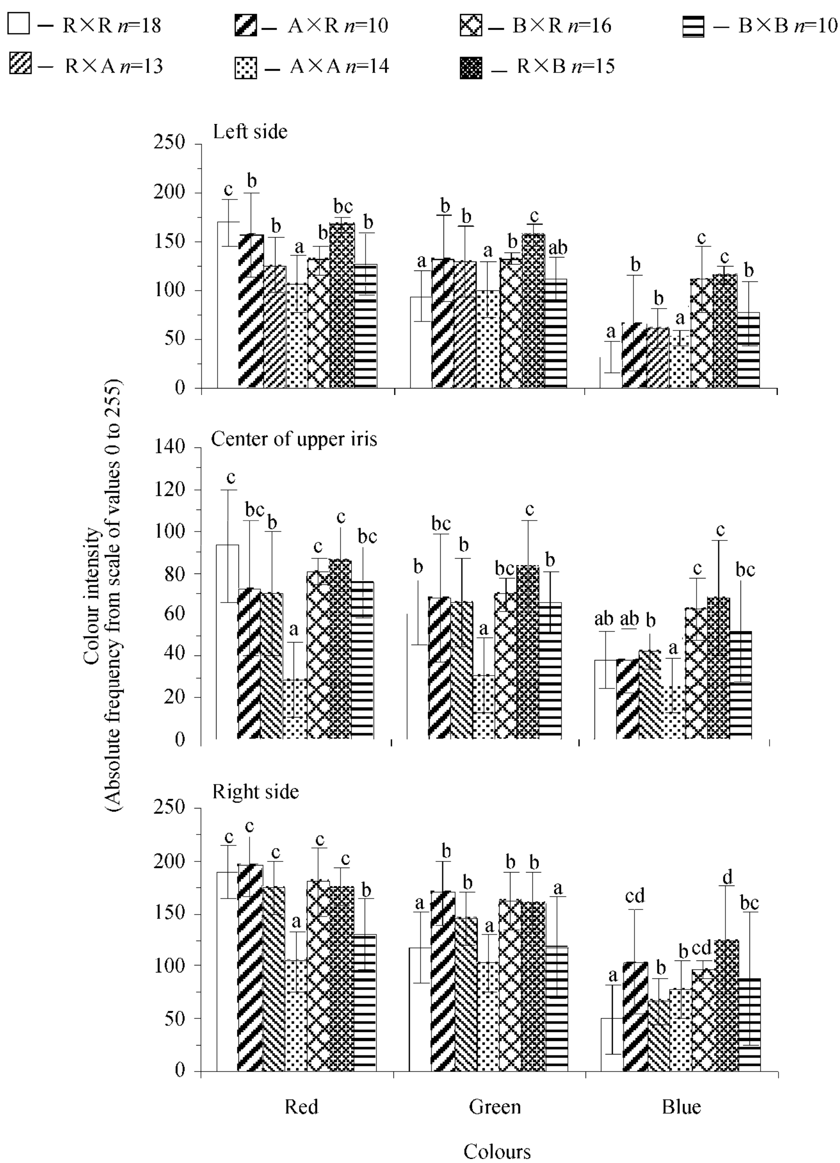

Fig.5　Colour intensity (absolute frequency from value scale) in the three regions of the eye's upper iris (center, left, and right side) in the roach hybrids

Means and standard deviations; for each region and colour, the bars sharing at least one common script are not significantly different, whereas other comparisons differ at $P < 0.05$ (Mann-Whitney U-test). A: common bream; B: silver bream; R: roach; male × female.

meristic characters are stable for smaller and larger fish.

3.2　Intermediate anal fin and large eye in hybrids

In the roach × common bream and roach × silver bream F1 hybrids, the intermediate morphology of the anal fin observed in the hybrids (intermediate base, black with red highlight colours) between the roach (short base, red colour) and the silver bream or the common bream (long base, black colour) demonstrated that our hybrids resulted from a fusion of the genetic material of the parental species. This hypothesis could be confirmed by genetic analysis using the quantitative trait loci. Legendre et al. (1992) also observed an intermediate morphology of the adipose fin in the hybrids (small adipose fin) between *Heterobranchus longifilis* Valenciennes (large adipose fin) and *Clarias gariepinus* (Burchell) (no adipose). This intermediate morphological characteristic was confirmed by the chromosomic chart of hybrids (Teugels et al., 1992a) and by their enzymatic polymorphism (Teugels et al., 1992b). The morphology of the anal fin thus seems to be a good criterion for rapidly identifying roach × common bream or roach × silver bream hybrids from the parents in the field where these species colonize nearly the same habitats. Economidis and Wheeler (1989) previously showed that the base length of the anal fin was an effective means for differentiating the roach × common bream hybrids (19% standard length), the roach (12%), and the common bream (27%) in Lake Volvi in

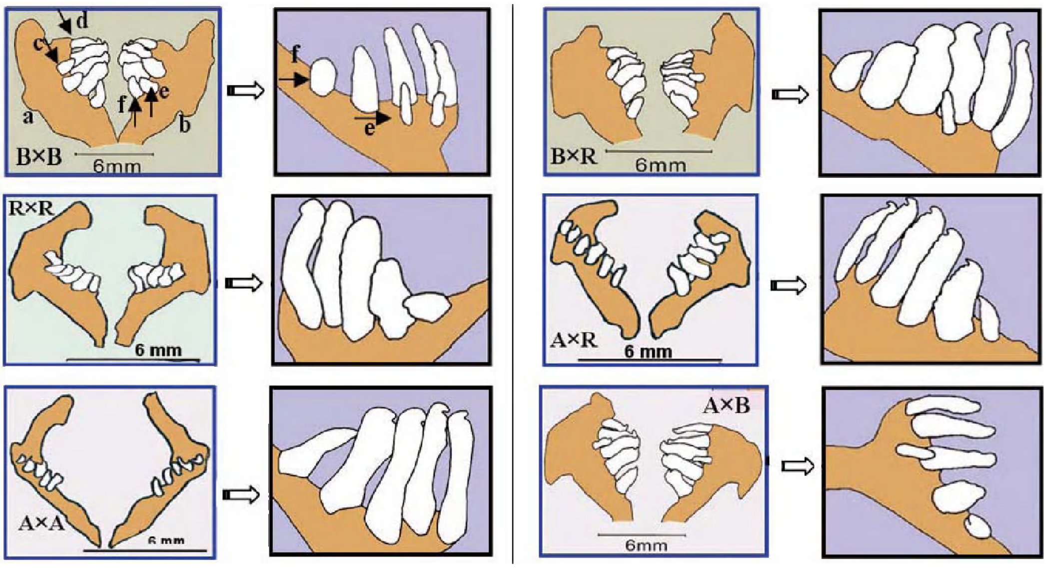

Fig.6 Pharyngeal teeth and bones in the hybrids and parental species

a: the right side of pharyngeal bones; b: the left side of pharyngeal bones; c and d: the second and first dental rows on the right side; e and f: the first and second dental rows on the left side; A: common bream; R: roach; B: silver bream; male × female.

Greece. This characteristic is reinforced by the red colour of the upper iris of the eye, a characteristic inherited from the roach and observed in all hybrids involving the roach. This suggests that a dominant gene may control the development of this characteristic in progenies. Moreover, the silver bream had a large eye and this characteristic was observed in its hybrids. It could thus be used to distinguish roach × common bream from roach × silver bream hybrids in the wild. Like iris colour, eye size in the silver bream seems to be a dominant characteristic.

3.3 Prevalence of silver bream characteristics in hybrids

The analysis of the phenotypic distributions in the silver bream × common bream hybrids revealed the prevalence of the characteristics of the parental variant silver bream over those of the common bream. This observation may match the results reported by Wheeler (1969), who observed that the silver bream × common bream hybrids were intermediate in most characteristics, but generally resembling the silver bream in body shape. Moreover, a single British specimen of these hybrids caught in Great Ouse and identified by Wheeler strongly resembled the silver bream in all characteristics except the length of the anal fin. Wheeler examined the adult fish collected in a natural environment, whereas the present study investigated young fish produced in captivity. This suggests that the parental species in this study were correctly identified.

3.4 Anal fin rays and number of lateral line scales

The number of soft rays on the anal fin and the number of scales on the lateral line in the roach × common bream hybrids in this study corresponds to those defined by other studies in both Eastern (Golubstsov et al., 1990) and Western Europe (Wheeler, 1969; Cowx, 1983; Wood and Jordan, 1987). Wood and Jordan (1987) reported the range of 15 – 19 anal fin rays, 15 – 16 for Golubstsov et al. (1990) in these hybrids versus 16 – 18 in this study. Wheeler (1969) counted 42 – 54 scales along the lateral line in these hybrids versus 44 – 50 in this study. Little information was available on roach × silver bream and silver bream × common bream hybrids. This might be due to the scarcity in European waters of roach × silver bream hybrids (Penczak, 1978; Swinney and Coles, 1982) and the high level of similarity between silver bream × common bream hybrids and their parents. However, Swinney and Coles (1982) analysed three specimens of natural roach × silver bream hybrids and they also found that their meristic characteristics were generally intermediate between those of the parental species. They noted 15 – 17 anal fin rays versus 14 – 16 in this study. Nikoliukin (1952), cited by Backiel and Zawisza (1968), counted 21 – 25 anal fin rays in artificial silver bream × common bream hybrids versus 22 – 25 in this study.

3.5 Pharyngeal teeth organization

The number and organization of the pharyngeal teeth were reliable identification criteria for the hybrids, but this required sacrificing fish for identification. A high variability in pharyngeal-tooth phenotypes in the F1 hybrids could be due to the different combinations of variant alleles of the two parents (Kampton, 1991 cited by Yakovlev and al., 2000). Identification of the hybrids resulting from the reciprocal crossbreeding in captivity based on dental examination was a significant contribution

Table 5　Frequencies of pharyngeal-tooth formulae (f) in the roach (R), the silver bream (B), the common bream (A) and their F1 hybrids

Generation	*n*	Pharyngeal teeth	f, %
Experiment 2 (male × female)			
A × A (5/5 × 5/5)	30	5/5	100
A × B (5/5 × 2.5/5.2)	1	1.4/5.1	4
	1	2.4/5.1	4
	1	4.4/5.1	4
	1	1.5/5	4
	20	1.5/5.1	80
	1	2.5/5.1	4
B × A (2.5/5.2 × 5/5)	4	5/5.1	17.4
	18	1.5/5.1	78.3
	1	1.5/5.2	4.3
B × B (2.5/5.2 × 2.5/5.2)	3	2.5/4.2	12.5
	21	2.5/5.2	87.5
Experiment 1 (male × female)			
A × A (5/5 × 5/5)	36	5/5	100
R × A (5/5 × 5/5)	27	5/5	81.8
	1	6/4	3.0
	5	6/5	15.2
A × R (5/5 × 5/5)	11	5/5	36.7
	19	6/5	63.3
R × R (5/5 × 5/5)	12	5/5	31.6
	2	5/6	5.3
	4	6/4	10.5
	20	6/5	52.6
R × B (5/5 × 2.5/5.2)	15	1.5/5.1	50
	3	2.5/5.1	10
	3	6/5.1	10
	6	1.6/5.1	20
	3	6/5.2	10
B × R (2.5/5.2 × 5/5)	2	1.5/5	6.5
	9	1.5/5.1	29
	2	6/5.1	6.5
	2	1.6/4.2	6.5
	16	1.6/5.1	51.6
B × B (2.5/5.2 × 2.5/5.2)	5	2.4/5.2	14.3
	3	2.5/4.2	8.6
	27	2.5/5.2	77.1

In the first column, the pharyngeal-tooth formulae of mating males and females are given; the pharyngeal-tooth number on the left and right sides of bones; the pharyngeal-tooth number on the second vs the first dental row on the left side and the first vs the second dental rows on the right side.

of this study. There were always two rows of teeth on one or both pharyngeal bones in the hybrids, resulting from one parental species that has one row of teeth (roach or common bream) and the other species with two rows (silver bream). However, Wood and Jordan (1987) found two common bream with a single tooth on the second row. This dental observation was also reported by Zhukov (1958) in natural silver bream × common bream hybrids in Russia and by Wheeler (1969) and Swinney and Coles (1982) in both natural roach × silver bream and silver bream × common bream hybrids from British waters. In addition, this feature has already been observed in the hybrids of other species of cyprinids from Lake Mikri Prespa: *Alburnus alburnus belvica* Karaman (one row of teeth) crossbred with *Rutilus rubilio* Bonaparte (two rows) (Crivelli and Dupont, 1987). It therefore appears that the presence of two rows of pharyngeal teeth may be a dominant characteristic. Double rows of teeth in roach × silver bream hybrids could serve to distinguish them from the morphologically similar roach × common bream hybrids, as also announced by Swinney and Coles (1982). Moreover, hybrids of two parental species with only one row of teeth always had only one row, as noted by Pushkina (1964) on the natural roach × common bream hybrids.

3.6　Numbers of pharyngeal teeth

As for the number of pharyngeal teeth per row, our results were similar to those from other studies conducted on specimens captured in the natural environment (Zhukov, 1958; Wheeler, 1969; Swinney and Coles, 1982; Cowx, 1983; Wood and Jordan, 1987; Golubstsov et al., 1990). Eight specimens of the natural roach × common bream hybrids from the Mozhaysk Reservoir showed 5 – 6 teeth on each pharyngeal bone (Golubtsov et al., 1990), versus 4 – 6 in this study. Swinney and Coles (1982) observed 11 specimens of the natural silver bream × common bream hybrids with 0 – 2 teeth (second row) and five (first row) in each bone, versus 0 – 4 teeth (second row) and 4 – 5 (first row) in this study, and three specimens of natural roach × silver bream hybrids with 0 – 2 teeth (second row) and 5 – 6 teeth (first row) versus 0 – 2 teeth (second row) and 4 – 6 teeth (first row) in this study. In the artificial silver bream × common bream hybrids, Nikoliukin (1952), cited by Backiel and Zawisza (1968), also observed the pharyngeal-tooth formula 1.5∶5.1, which accounted for over 70% of the hybrids in this study.

In spite of the problems identifying the reciprocal hybrids (direction of hybridization) at the present stage, a number of criteria successfully identified the F1 hybrids from their parents. Roach can be distinguished from breams by their short anal fin, low numbers of rays on the anal fin and the iris coloured in red. Roach × bream hybrids have larger anal fins and higher numbers of anal fin rays than roach, and roach × silver bream hybrids have a low number of scales along the lateral line and two rows of pharyngeal teeth versus a high number of scales on this line and only one row in roach × common bream hybrids. Breams have the large anal fin and high ray numbers on the anal fin, and silver bream can be distinguished from common bream by their large eye and two rows of pharyngeal teeth, as in the hybrids between these breams. From an ecological point of view, these morphological parameters could contribute to the recognition of F1 hybrids compared to the parental species in a natural population.

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