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Variability of Intermuscular Bones, Vertebrae, Ribs, Dorsal Fin Rays and Skeletal Disorders in the Common Carp*

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Summary. The number of intermuscular bones, vertebrae, ribs, dorsal fin rays and an index of bone disorders were determined from x-ray photographs of over 1000 common carp. These carp represented a broad genetic range, including five distinct lines of the domesticated European carp, one group of the Chinese race *Big-Belly* and 12 crossbreds. The genetic, and even the phenotypic, variation in intermuscular bones were much smaller than those found in earlier experiments. Variation of other bone characters was also analyzed and the relationship of intermuscular bones and ribs to vertebrae was determined.

Introduction

The number of intermuscular bones has been counted in many fish species, either by cooking the fish and dissecting out the intermuscular (henceforth IM) bones (Lieder 1961a,b; Anwand, 1962; Kandler, 1971; Jahnichen, 1971; Jahnichen and Ball, 1972), or by counting the IM bones from x-ray photographs (v. Sengbusch, 1963; v. Sengbusch and Meske, 1967; Meske, 1968; Kossmann, 1972).

According to Lieder (1961 a,b), who examined 11 specimens, the number of IM bones in the common carp is 99, with a range of 95 to 104. Lieder was apparently the first to consider the possibility of reducing the number of IM bones in the common carp by selective breeding, but he was not optimistic because of the small phenotypic variation that he found. He also stated the need for x-ray photographs of live fish for work of this type. Kandler (1971) found a mean number of 80 IM bones (range: 76-84), in four carp from Holstein, while in three wild carp (one from the river Elbe and two from a lake in Turkey) the number of IM bones ranged from 100 to 102.

The first worker to use large samples with the object of selecting carp with a reduced number of IM bones was v. Sengbusch (1963). He and his coworkers took x-ray photographs of hundreds of carp, counted their IM bones and found a relatively wide phenotypic variation (v. Sengbusch, 1967, v. Sengbusch and Meske, 1967, Meske, 1968). For example, in one population they found a range of 70 to 135 IM bones. Kossmann (1972) separated a genetic component for IM bones by studying 8 full-sib families belonging to the German domesticated carp. In spite of the narrow genetic basis of his population, he found relatively wide differences between the family means. In view of the above results we decided to search for genetic variability in IM bones of carp, starting from a broad genetic basis including five lines of the European domesticated carp, a strain of the Chinese race *(Big-Belly)*, three inter-race F_1 crossbreds and nine crossbreds among the European lines. We expected to find in our populations at least as wide a range of IM bones as that found by Kossmann.

The x-ray photographs served to study several other bones, i.e., vertebrae, ribs, dorsal fin rays and visible bone disorders. Over 1000 fish were photographed, but, contrary to expectations, the range of variation in IM bones was far to narrow to permit a selection program.

The Fish Stocks

Altogether 18 groups were studied. These included six closed lines and 12 crossbreds (Table 1). The lines were the following (Hulata et al., 1974; Moav et al., 1964):

(a) *Gold;* an inbred line called *Gold* was initiated with mutant *Gold* individuals (homozygous recessives) found in a fish farm in Israel in 1963.

(b) *Blue-Grey;* a second inbred line called *Blue-Grey* was founded by individuals homozygous for the two recessive body colouration mutations, *Blue* and *Grey*. These were found in another fish farm in Israel in 1960.

(c) *Našice;* a selected strain developed in the fish farm Našice in Yugoslavia, and introduced to Israel in 1970 (Fijan, personal communication).

(d) *Holland-B;* this strain was introduced to Israel

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Table 1. The genetic groups, their parents and numbers of fish studied

Tested group	Parents strains and numbers				No. of fish studied in	
	1971		1972			
	female	male	female	male	1971	1972
Big-Belly (BB)	BB (16)	BB (16)	BB (6)	BB (6)	7	114
BB × Nas	BB (16)	Nas (5)			9	
BB × Gold	Gold (4)	BB (16)			9	
BB × Dor	Dor (19)	BB (18)			9	
Našice (Nas)	Nas (16)	Nas (4)	Nas (17)	Nas (17)	15	73
Nas × Gold	Gold (4)	Nas (4)	Nas (15)	Gold (7)	26	58
Nas × Dor	Dor (14)	Nas (5)	Nas (16)	Dor (15)	13	17
Nas × Blue			Nas (6)	Blue (7)		43
Nas × Hol-B			Nas (14)	Hol-B (10)		113
Gold	Gold (4)	Gold (10)	Gold (11)	Gold (10)	15	48
Gold × Dor	Dor (15)	Gold (7)			13	
Gold × Blue	Blue (5)	Gold (9)			13	
Dor-70 (Dor)	Dor (15)	Dor (18)	Dor (38)	Dor (37)	16	39
Dor × Blue			Dor (21)	Blue (4)		60
Blue-Grey (Blue)			Blue (13)	Blue (4)		59
Blue × Hol-B			Hol-B (34)	Blue (18)		93
Holland-B (Hol-B)			Hol-B (10)	Hol-B (9)		103
Hol-B × T	T (29)	Hol-B (12)	T (31)	Hol-B (64)	17	32
Total	157	124	232	208	162	852

from the Netherlands ten years ago and kept since then as a closed line.

(e) *Dor-70;* this Israeli line is a product of a selection experiment for higher growth rate (unpublished). It has been kept as a closed line since 1965.

(f) *Big-Belly;* this race of Chinese common carp was introduced to Israel from Taiwan in 1970 (Lin, personal communication).

All the tested groups are listed in Table 1 according to the origin of their parents, number of parents that participated in their reproduction by multiple spawns (Moav and Wohlfarth, 1973) and number of fish studied in 1971 and in 1972.

Methods

Each group was spawned in a separate pond in the last week of April. Nursing to mean weights of 25 g (Wohlfarth et al., 1965) was also carried out in separate ponds. The nursed fingerlings were brand-marked (Moav et al., 1960a,b), and stocked into communal (mixed) ponds serving part of our experiments for studying genetic differences in growth rate (Wohlfarth and Moav, 1972; Moav and Wohlfarth, 1973). When these experiments terminated in mid November, samples of fish were x-ray photographed for the present study.

The x-ray photographs. Counts of IM (intermuscular) and other bones were made on negatives of x-ray photographs. In 1971 the camera used was Softex E-3, and in 1972 it was Softex E-2. The photographic plates were Agfa structurix D7p FW. Small sheets of 20 × 10 cm were cut and inserted into black envelopes. During exposure the fish were laid on the envelopes and were marked by metal numbers. In 1971 the negatives were developed with an automatic Exomat instrument at the Hadassa Hospital in Jerusalem, and in 1972 they were developed with similar equipment at the Rambam Hospital in Haifa. The developed negatives were fixed, washed and dried. For bone counting the negatives were magnified and illuminated by a Heliograph instrument.

During exposure the fish were laid 20-30 cm from the source of radiation (185-200 KV at 9 mAmp), for between 5 and 60 seconds depending on the size of the fish. To stop the fish moving during exposure, they were cold-shocked in a container filled with a mixture of ice and water.

The thick front part of the carp body required longer exposure than the thinner tail. Large fish (over 400 g) required three exposure durations - longest at the front (up to 60 seconds), shorter in the mid-body, and shortest for the tail region. The IM bones of the thick front were the most difficult to count. Occasionally the end of the tail was slightly over-exposed, so that the posterior IM bones were also not completely clear.

Experimental Results

The numbers of vertebrae, ribs, dorsal IM bones, ventral IM bones, dorsal fin rays and index of bone disorders were determined, and from these counts, total number of IM bones (dorsal plus ventral), total number of

Table 2. Measurements on the studied characters (R=range; M=mean; SE=Standard error; CF=Coefficient of variation).

A. Number of vertebrae, ribs and ventral (ribs plus ventral intermuscular) bones

	Vertebrae								Ribs				Ventral bones			
	1971				1972				1972				1972			
Group	R	M-30	SE	CV %	R	M-30	SE	CV %	R	M-29	SE	CV %	R	M-55	Se	CV %
Big-Belly (BB)	2	4.1	0.4	3.1	6	3.6	0.1	2.3	4	0.0	0.1	3.8	11	3.6	0.2	3.8
BB × Nas	2	4.1	0.2	1.8												
BB × Gold	0	4.0	0.0	0.0												
BB × Dor	1	4.4	0.2	1.8												
Našice (Nas)	2	5.5	0.2	1.9	5	5.3	0.1	2.0	4	1.2	0.1	3.6	11	5.6	0.3	3.8
Nas × Gold	1	5.0	0.0	0.6	2	5.3	0.1	1.4	4	2.5	0.1	3.1	9	7.8	0.3	3.0
Nas × Dor	1	4.9	0.1	1.1	3	5.5	0.2	2.3	4	2.4	0.3	3.8	8	5.9	0.6	3.8
Nas × Blue					3	4.4	0.1	1.9	4	1.0	0.1	3.3	8	4.7	0.2	3.4
Nas × Hol-B					3	5.2	0.1	1.8	6	2.3	0.1	3.7	12	5.3	0.3	3.5
Gold	1	5.1	0.1	1.0	2	5.1	0.1	1.3	4	1.8	0.2	4.2	8	5.7	0.3	3.3
Gold × Dor	1	5.5	0.1	1.4												
Gold × Blue	2	5.1	0.2	1.6												
Dor-70 (Dor)	3	4.4	0.2	2.3	2	4.7	0.1	1.6	2	1.5	0.1	2.8	7	5.1	0.3	2.9
Dor × Blue					3	4.6	0.1	1.6	4	1.5	0.1	3.0	13	4.8	0.3	4.1
Blue-Grey (Blue)					2	4.7	0.1	0.1	4	1.1	0.1	2.6	8	5.5	0.3	3.4
Blue × Hol-B					3	5.5	0.1	1.7	6	2.1	0.1	3.5	11	6.7	0.2	3.4
Holland-B (Hol-B)					3	5.5	0.1	1.6	4	2.0	0.1	3.5	12	4.4	0.2	3.8
Hol-B × T	1	5.3	0.1	1.3	2	5.2	0.1	1.6	2	1.9	0.2	3.3	13	4.7	0.2	3.9
Mean (total)	3	4.9	0.1	2.2	8	4.9	0.0	2.5	6	1.6	0.1	4.3	16	5.3	0.1	4.0

B. Number of dorsal and ventral intermuscular bones

	Dorsal InterM bones								Ventral InterM bones							
	1971				1972				1971				1972			
Group	R	M-63	SE	CV %	R	M-63	SE	CV %	R	M-26	SE	CV %	R	M-26	SE	CV %
Big-Belly (BB)	5	1.1	0.7	3.1	13	0.7	0.2	3.4	5	0.7	0.7	7.4	12	3.6	0.2	6.8
BB × Nas	4	3.0	0.4	2.1					5	1.6	0.5	5.8				
BB × Gold	4	1.6	0.4	1.8					4	1.2	0.4	4.7				
BB × Dor	6	2.0	0.7	3.2					5	1.3	0.5	5.5				
Našice (Nas)	8	3.6	0.6	3.4	13	3.6	0.3	3.4	7	1.1	0.5	6.4	11	4.4	0.3	7.7
Nas × Gold	8	3.6	0.3	2.9	8	4.1	0.2	2.6	8	1.7	0.4	6.6	7	5.2	0.2	5.2
Nas × Dor	7	3.2	0.6	3.1	9	3.3	0.5	3.4	7	1.8	0.5	6.5	6	3.5	0.5	7.1
Nas × Blue					7	2.4	0.3	2.5					7	3.7	0.3	5.8
Nas × Hol-B					14	3.2	0.2	2.9					11	3.0	0.2	6.5
Gold	4	2.7	0.3	1.9	7	2.9	0.2	2.5	9	1.1	0.6	8.6	8	4.0	0.3	5.6
Gold × Dor	9	5.1	0.7	3.4					7	1.3	0.5	6.2				
Gold × Blue	6	3.5	0.5	2.5					4	1.6	0.4	5.2				
Dor-70 (Dor)	7	2.8	0.5	3.3	8	2.8	0.3	3.0	13	0.5	0.8	11.9	8	3.7	0.3	5.4
Dor × Blue					20	2.8	0.3	4.0					12	3.3	0.3	7.7
Blue-Grey (Blue)					9	3.0	0.2	2.8					7	4.4	0.3	6.3
Blue × Hol-B					11	3.9	0.2	2.7					10	4.5	0.2	6.4
Holland-B (Hol-B)					11	3.3	0.2	2.8					9	2.4	0.2	6.6
Hol-B × T	7	3.5	0.5	3.1	12	3.7	0.5	4.1	7	1.8	0.4	6.5	13	2.8	0.4	7.7
Mean (total)	10	3.2	0.2	3.1	20	2.9	0.1	3.4	14	1.3	0.2	7.0	16	3.7	0.1	7.1

C. Number of total intermuscular bones, dorsal fin rays and total intermuscular bones divided by number of vertebrae

Group	Total InterM bones 1971 R	M-90	SE	CV %	1972 R	M-90	SE	CV %	InterM/Vertebrae 1971 Mean	1972 Mean	Dorsal fin rays 1972 R	M-18	SE	CV %
Big-Belly (BB)	7	0.9	1.0	2.9	16	3.4	0.3	3.3	2.66	2.78	6	0.6	0.1	5.7
BB × Nas	5	3.7	0.5	1.6					2.75					
BB × Gold	4	1.8	0.5	1.6					2.70					
BB × Dor	10	2.3	1.0	3.2					2.68					
Našice (Nas)	9	3.8	0.7	3.0	22	7.0	0.4	3.8	2.64	2.75	5	2.1	0.1	5.1
Nas × Gold	14	4.2	0.6	3.3	12	8.4	0.4	2.9	2.69	2.79	4	2.1	0.1	4.3
Nas × Dor	8	3.8	0.6	2.2	13	5.8	0.9	3.8	2.69	2.70	5	0.8	0.3	7.6
Nas × Blue					11	5.0	0.4	2.7		2.77	4	2.4	0.1	4.3
Nas × Hol-B					15	5.2	0.3	2.9		2.71	5	2.0	0.1	5.0
Gold	12	2.7	0.9	3.4	12	5.8	0.4	2.7	2.64	2.73	6	1.9	0.1	5.3
Gold × Dor	12	5.3	0.9	3.5					2.69					
Gold × Blue	8	4.3	0.6	2.3					2.68					
Dor-70 (Dor)	16	2.3	1.2	5.1	11	5.5	0.5	3.3	2.68	2.75	5	1.2	0.1	4.2
Dor × Blue					29	5.0	0.5	4.3		2.75				
Blue-Grey (Blue)					12	6.4	0.3	2.7		2.78				
Blue × Hol-B					13	7.4	0.3	2.6		2.75				
Holland-B (Hol-B)					14	4.7	0.3	2.9		2.67				
Hol-B × T	12	4.2	0.8	3.4	14	5.1	0.7	3.9	2.67	2.71	4	2.7	0.2	5.6
Mean (total)	20	3.5	0.3	3.4	29	5.6	0.1	3.5	2.68	2.74	9	1.8	0.0	5.8

D. Bone disorders and a χ^2 test for independence of light and severe disorders (100P% = percentage of fish showing bone disorders; the four classes of disorders were defined in the text)

Group	Proportion and Index of bone disorders 1971 100P %	M	SE	1972 100P %	M	SE	No. of fish at four classes of disorders (1972) 0	1-4	5	6-9	χ^2
Big-Belly (BB)	57	1.6	0.9	22	0.8	0.2	89	16	3	5	11.9**
BB × Nas	78	1.6	0.5								
BB × Gold	78	2.1	0.9								
BB × Dor	67	0.7	0.2								
Našice (Nas)	100	5.2	0.5	63	2.0	0.3	27	33	3	10	2.1
Nas × Gold	96	2.1	0.4	41	1.1	0.3	34	18	3	3	0.5
Nas × Dor	100	2.9	0.5	65	2.1	0.5	6	8	0	3	2.0
Nas × Blue				61	1.4	0.3	17	20	5	1	2.9
Nas × Hol-B				86	2.2	0.2	16	88	4	5	4.8*
Gold	87	1.9	0.5	60	2.3	0.4	19	16	5	8	6.9**
Gold × Dor	92	2.3	0.4								
Gold × Blue	62	0.9	0.3								
Dor-70 (Dor)	88	2.9	0.7	33	0.7	0.3	26	11	1	1	0.4
Dor × Blue				57	2.1	0.4	26	21	3	9	3.5
Blue-Grey (Blue)				46	1.7	0.3	32	16	2	9	8.6**
Blue × Hol-B				40	0.9	0.2	56	31	2	4	2.3
Holland B (Hol-B)				89	2.0	0.1	11	85	2	5	1.2
Hol-B × T	84	1.5	0.3	63	2.3	0.5	12	12	2	6	3.4
Mean (total)	85	2.2	0.2	57	1.6	0.1	371	375	35	69	9.5**

* Significant at the 5 % level

** Significant at the 1 % level

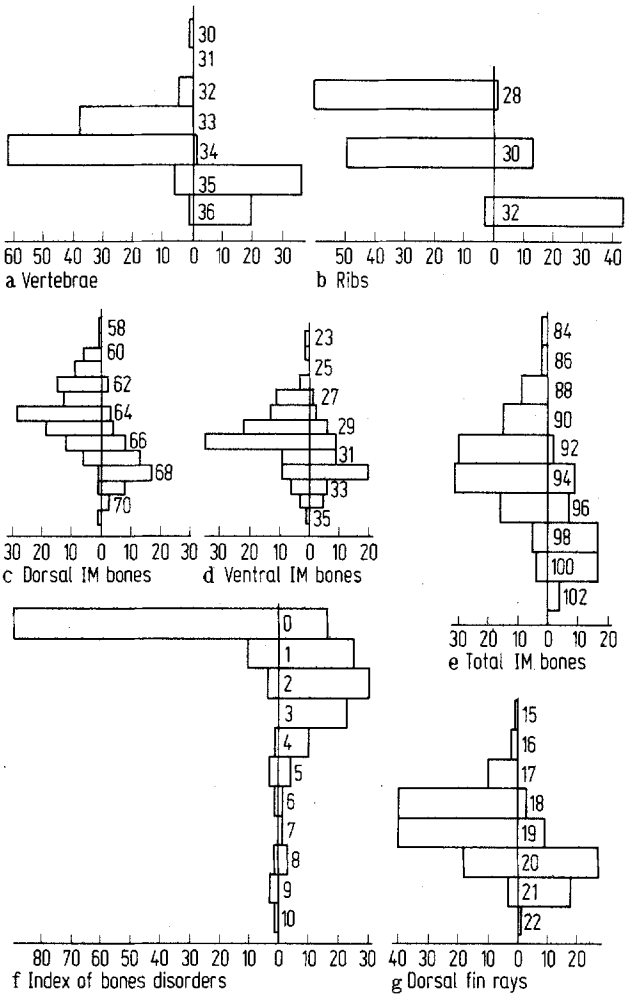

Fig. 1. The frequency distributions of several bone characters in the two groups *Big-Belly* (BB) and *Našice* × *Gold* (BB) (n = 114) is presented by the left hand columns and *Našice* × *Gold* (n = 58) by the right hand columns

ventral bones (ribs plus ventral IM bones), and the ratio: total number of IM bones to number of vertebrae, were computed. The means (M), ranges (R), Standard errors (SE) and Coefficients of variation (CV-Standard deviation divided by the mean) of the above characters are presented in Tables 2A, 2B, 2C, and 2D. For the index of bone disorders the proportion of fish having any kind of disorder was computed (Table 2D, the χ^2-test of this table will be discussed later). The intra-group frequency distributions of the 1972 samples of the two extreme groups *Big-Belly* and *Našice* × *Gold* are presented in Fig. 1.

Dominance relationships. The dominance ratio was measured by d/a, when d is the difference between the mean of an F_1 crossbred and the mid-point (unweighed mean) of its two parents and a is half the difference between the mean of the two parents. The ratio d/a serves as a measure of the relative degree and direction of dominance (Falconer, 1960). Ten F_1 crossbreds were tested simultaneously with their two parents. Three of these ten were inter-racial crossbreds of the Chinese *Big-Belly* and a European line, and the remaining seven were crossbreds among the five European lines. The dominance ratios of the various characters were computed for each crossbred population (Table 3).

For most of the studied characters and crossbreds the inter-parental range (2a) was relatively small, so that the relative error associated with the dominance ratio was large.

Variation between individuals within groups and between the groups means. Table 4 shows, for all the studied characters, the means (over all the tested groups), the within groups Standard deviations, the between groups Standard deviations and the intra-class correlation coefficients (the between variance component divided by the sum of the within plus between variance components). This coefficient is similar to heritability, and in the present case it estimated the proportion of intergroup genetic variance in the total variance. Table 4 also shows a reasonably good agreement between the years 1971 and 1972. The between group variances (or SD) of all the traits, though small in absolute terms and in relation to the within variances, all varied significantly from zero. However, statistical significance does not necessarily mean usefulness to the breeder, i.e., although the between groups SD of total IM bones is highly significant, it is negligibly small (1.3) and practically useless in a breeding scheme aimed at reducing the number of IM bones.

Correlation between the results of 1971 and 1972, and between crossbreds and their parents means. Differences between means of genetic groups (lines, strains, families, etc.) may serve for estimating genetic variance provided that the contribution of the "common environment" component to the intra-class correlation (i.e., to the relative similarity between group mates) is negligible. Our data contain two lines of evidence to this effect: (a) the correlation between performance of the same groups in the two years 1971 and 1972, and (b) the correlation between crossbreds and their parents. In both cases, only genetic factors contribute to the covariation. Two of the four primary characters measured in 1971 and 1972 showed a high between-years correlation. These were: (i) number of vertebrae, with a cor-

relation coefficient of 0.8; and (ii) dorsal IM bones with an extremely high correlation of 0.98. For the remaining two characters - ventral IM bones and index of bone disorders - the correlations were close to nil. The zero correlation of ventral IM bones reduced the inter-years correlation of total IM (dorsal plus ventral) bones to 0.74.

The correlation coefficients of crossbreds with their parents means for the 1972 results were all positive, ranging between 0.15 for the index of bone disorders to over 0.5 for vertebrae, ribs and dorsal IM bones (right-hand column, Table 4). Thus, the evidence of the two correlations (between years, and between crossbreds and their parents) leads to the conclusion that genetic differences constitute the major source of inter-group variation in number of vertebrae, number of dorsal IM bones, and total IM bones.

Correlations between characters. Two kinds of correlations between characters were computed, the correlation between individuals within groups and the correlation between group means. These correlations, based on the 1972 results only, and excluding the Chinese *Big-Belly* group, are presented in Table 5. The within-group correlations (means over all the groups) are located above, and the between-groups correlations below the main diagonal.

The sources of within-, and between-groups covariation are identical to those of the within-, and between-groups variances. Thus, the between-group correlation are largely due to genetic covariation, while the within-groups correlations are strongly affected by environmental factors. Table 5 shows that for most character combinations the between-groups correlation was higher than the within-group correlation. This fits the common situation where environmental sources tend to reduce, rather than to enhance, inter-character correlations. Table 5 also shows that practically all the high correlation values are restricted to characters that are, by definition, part-whole pairs, i.e., total IM bones (whole) and ventral IM bones (part), as well as to characters that are strongly associated with number of vertebrae, i.e., vertebrae and ribs are highly correlated (0.7) and so are vertebrae and dorsal IM bones (0.8). Consequently, ribs are also correlated with dorsal IM bones (0.6).

Number of vertebrae. The Chinese *Big-Belly* had significantly fewer vertebrae than the European carp and the inter-race crossbreds were similar to their Chinese parent. Within the European race, the inter-group (genetic) range was less than one vertebrae (when means over the two years are considered). One fish of the *Našice* group had the highest number of vertebrae (38), and one *Big-Belly* had the lowest number (30). The average dominance ratio, over the two years, of this character was zero (Table 3).

Number of ribs. Ribs were counted only on one side of the body and the counted number was multiplied by two. Hence the exclusively even numbers (Fig. 1b). The Chinese *Big-Belly* had two ribs less than the European, and the intra-European range was rather small.

Number of Intermuscular bones. Dorsal and ventral IM bones appear to behave like two different characters (Tables 2B and 2C, and Fig. 1c and 1a). The coefficient of variation (CV) of number of ventral IM bones was more than twice that of dorsal IM bones (7.1% versus 3.4%, Table 2B), but its relative genetic (between-groups) variance was considerably smaller. A partial explanation for this difference may be the higher error in counting ventral IM bones. The significant difference of almost 2.5 ventral IM bones between the means of 1971 and 1972 (27.3 ± 0.1 in 1971 and 29.1 ± 0.1 in 1972) may be partially due to the same reason. The dorsal IM bones, on the other hand, showed almost identical means in the two years (66.2 ± 0.2 in 1971 and 65.9 ± 0.1 in 1972, Table 2C).

The correlation between dorsal IM bones in the two years was 0.98, but practically zero (-0.01), for ventral IM bones. The latter low value may be another reflection of the greater difficulty in counting ventral IM bones, but it may also be due to higher sensitivity of these bones to environmental variation.

Total number of ventral bones. Ribs and ventral IM bones were defined as ventral bones. Since ventral IM bones are located only posterior to the ribs, "competition" between the two types of bones in the border region should cause negative correlation between the two types of bones. However, our results show zero correlation between the two (Table 5); therefore, the hypothesis of "competition" should be rejected, and we must conclude that the borderline between the two is firmly determined.

The mean number of ventral bones was 60.3 ± 0.1 (Table 2A) compared with 65.9 ± 0.1 dorsal IM bones. This difference shows that three vertebral segments containing dorsal IM bones have neither ribs nor ventral IM bones.

Table 3. Dominance ratios of the studied characters (a=half the range between the parental means; d=dominance deviation=the difference between the mean of the crossbred and the mid-parental value)

Group	Vertebrae				Ribs		Intermuscular bones										Dorsal fin rays		Index of bone disorders			
							Total				Dorsal				Ventral							
	1971		1972		1972		1971		1972		1971		1972		1971		1972		1972		1971		1972	
	a	d/a	a	d/a	a	d/a	a	d/a	a	d/a	a	d/a	a	d/a	a	d/a	a	d/a	a	d/a	a	d/a	a	d/a
BB × Nas	0.7	-1.0					1.5	0.9			1.2	0.5			0.2	3.7					1.8	-1.0		
BB × Gold	0.5	-1.3					0.9	0.0			0.8	-0.5			0.2	1.8					0.2	2.0		
BB × Dor	0.1	1.0					0.7	1.0			0.8	0.0			0.1	6.9					0.7	-2.3		
Mean: BB × Eur	0.4	-0.9					1.0	0.7			0.9	0.0			0.2	3.7					0.9	-1.1		
Nas × Gold	0.2	-1.5	0.1	1.3	0.3	3.4	0.5	1.8	0.6	3.2	0.5	1.0	0.4	2.2	0.0		0.2	5.0	0.1	0.5	1.6	-0.8	0.2	-6.3
Nas × Dor	0.5	-0.2	0.3	1.6	0.1	8.9	0.7	1.0	0.8	-0.6	0.4	0.1	0.4	0.2	0.3	3.5	0.4	-1.5	0.5	-1.8	1.2	-1.0	0.6	1.2
Nas × Blue			0.3	-2.2	0.1	-2.7			0.3	-4.9			0.2	-2.8			0.0		0.4	1.8			0.1	-3.5
Nas × Hol-B			0.1	-2.3	0.4	1.9			1.2	-0.6			0.2	-1.4			1.0	-0.4	0.1	-3.3			0.0	16.3
Gold × Dor	0.3	1.9					0.2	13.3			0.1	33.4			0.3	1.8					0.5	-0.2		
Dor × Blue			0.0	-27.0	0.2	1.5			0.5	-2.0			0.1	-1.4			0.4	-2.2	0.1	7.8			0.5	1.9
Blue × Hol B			0.4	1.0	0.4	1.4			0.8	2.2			0.1	5.1			1.0	1.1	0.5	-0.2			0.1	-6.4
European mean	0.4	0.7	0.2	0.0	0.2	2.4	0.5	3.0	0.7	0.0	0.3	3.1	0.3	0.3	0.2	11.1	0.5	-0.1	0.3	0.1	1.1	-0.8	0.3	-0.3
Overall mean	0.4	-0.2	-	-	-	-	0.2	2.4	-	-	0.6	0.8	-	-	0.2	3.7	-	-	-	-	1.0	-1.0	-	-

Table 4. The between- and within-groups standard deviations, intra-class correlations and parents-crossbreds correlations

	SD between groups means (SD_b)		SD between individuals within groups (SD_w)		Intraclass correlation coefficient	Mid-parents-crossbreds correlation coefficient
	1971	1972	1971	1972	1972	1972
A) Present Results						
Vertebrae	0.55	0.55	0.51	0.60	0.44	0.55
Ribs		0.70		1.12	0.27	0.51
Total IM bones	1.28	1.31	2.78	3.30	0.12	0.39
Dorsal IM bones	1.06	0.83	1.86	2.02	0.12	0.51
Ventral IM bones	0.41	0.79	1.85	1.94	0.12	0.35
Total ventral bones		1.05		2.14	0.17	0.17
Dorsal fin rays		0.62		1.01	0.25	0.22
Index of disorders	1.39	0.61	1.81	2.23	0.05	0.15
B) Kossmann's Results						
Vertebrae		0.32		0.55	0.23	
Total IM bones		4.48		4.90	0.44	
Dorsal IM bones		2.32		2.91	0.32	
Ventral IM bones		2.54		2.94	0.41	

Table 5. Between- and within-groups correlation coefficients of the studied characters (only 1972 results. The within and between correlations are located, respectively, above and below the main diagonal)

	Vertebrae	Ribs	Total IM bones	Dorsal IM bones	Ventral IM bones	Total ventral bones	Dorsal fin rays	Index of bone disorders
Vertebrae		0.4	0.3	0.2	0.0	0.4	-0.1	-0.1
Ribs	0.7		0.1	0.2	0.0	0.4	0.0	0.0
Total IM bones	0.4	0.3		0.8	0.8	0.7	0.1	-0.2
Dorsal IM bones	0.8	0.6	0.8		0.2	0.3	0.0	-0.1
Ventral IM bones	0.4	0.0	0.9	0.5		0.9	0.1	-0.2
Total ventral bones	0.4	0.5	0.5	0.7	0.8		0.0	-0.2
Dorsal fin rays	-0.4	-0.1	-0.1	0.3	-0.2	-0.3		-0.1
Index of disorders	0.1	0.4	-0.5	-0.2	-0.5	-0.4	0.3	

Dorsal fin rays. Dorsal fin rays were counted because we thought that they might be correlated with IM bones. Table 5 shows that this was not the case. The range of variation of dorsal fin rays was very small, the Chinese *Big-Belly* being at the lower end of the range with almost two rays less than the European mean.

Skeletal disorders. Inspection of the x-ray photographs revealed a high incidence of bone disorders of varying types and severity. The most common disorder was curling of ribs and IM bones. Less frequent were misshaped, misplaced, undersized, or fused vertebrae. In the latter case, the number of neural spines emerging from the mass of fused vertebrae was used to determine the number of vertebrae. The most severe disorder which affected two of the studied fish was twisted spinal columns (hunchbacks). The skeletal disorders were quantified by the following index:

zero: normal (no disorders).
1 to 4: increasing degrees of disorders not including vertebrae fusion or twisted spinal column.
5: vertebrae fusion.
6 to 9: vertebrae fusion plus disorders of category 1 to 4.
10: twisted vertebral column.

Within the context of the present study skeletal disorders are of interest for two reasons: (i) possible correlations with IM bones; (ii) correlation with degree of inbreeding.

The index of disorders was considerably higher in 1971 (mean = 2.2) than in 1972 (mean = 1.6). This discrepancy might be a result of environmental differences during embryonic development in the two years, or to

Table 6. Organization of the ribs and intermuscular bones along the vertebral column (1972 results)

Group of fish	Total no.	Dorsal InterM bones		Ventral (ribs or InterM) bones			
		With	Without	With ribs	With ventral InterM	With ribs or ventral InterM	Without
Big-Belly (BB)	33.6	31.9	1.7	14.5	14.8	29.3	4.3
Našice (Nas)	35.3	33.3	2.0	15.1	15.2	20.3	5.0
Nas × Gold	35.3	33.6	1.7	15.7	15.6	31.3	4.0
Nas × Dor	35.5	33.2	2.3	15.7	14.7	30.4	5.1
Nas × Blue	34.4	32.7	1.7	15.0	14.8	29.8	4.6
Nas × Hol-B	35.2	33.1	2.1	15.7	14.5	30.2	5.0
Gold	35.1	32.9	2.2	15.4	15.0	30.4	4.7
Dor-70 (Dor)	34.7	32.9	1.8	15.2	14.8	30.0	4.7
Dor × Blue	34.6	32.9	1.7	15.3	14.6	29.9	4.7
Blue-Grey (Blue)	34.7	33.0	1.7	15.1	15.2	30.3	4.4
Blue × Hol-B	35.5	33.4	2.1	15.6	15.3	30.9	4.6
Holland-B (Hol-B)	35.5	33.1	2.4	15.5	14.2	29.7	5.8
Hol-B × T	35.2	33.1	2.1	15.4	14.4	29.8	5.4
Mean (Without BB)	35.1	33.3	2.0	15.4	14.9	30.3	4.8

differences in the intensity of natural selection and mortality during nursing. The absence of inter-year and crossbred with parents correlations of this character does not favour the hypothesis that its variance was caused by genetic factors. The hypothesis that light and severe disorders were determined by independent genetic or environmental factors was tested by dividing the fish into the following four classes: (a) no disorders of either kind (index 0); (b) only light disorders (index 1 to 4); (c) only severe disorders (index 5); (d) both light and severe disorders (index 6 to 9); two fish had index 10 and they were not included in the test.

χ^2-tests for independence of the two kinds of disorder were performed on the 1972 results (Table 2D). Of the 13 groups, three had a significant (at the 1% level) excess of fish with the two kinds of disorder. One group *(Nas × Hol-B)* had a significant (at the 5% level) excess of the zero class, and the total of all the groups also had a small but highly significant excess of the class with the two disorders (69 instead of the expected 54). These χ^2 tests suggest that the two kinds of disorder are only partially correlated, and are partially determined by independent factors. Alternatively, it is possible that the two are caused by the same factors, but higher mortality of individuals with the two kinds of disorder created the impression of partial independence.

Inbreeding did not appear to increase the rate and severity of bone disorders (Table 3). Four crossbreds actually showed heterosis in the wrong direction, i.e., more disorders than their parents, and the average dominance ratio was close to zero (Table 3).

The relationship of intermuscular bones and ribs to vertebrae. Both ribs and IM bones are arranged in pairs, each dorsal and ventral pair being associated with a vertebral segment (Kandler, 1971). The number of vertebrae associated with ribs, dorsal, and ventral IM bones was determined by dividing these auxiliary bones by two, and inspection of the x-ray photographs helped to determine their arrangement according to vertebrae. Approximately two vertebrae have neither ribs nor IM bones (group means, Table 6, fourth row from left). One of these is located next to the head and the second may be the last tail vertebrae. Approximately 33 vertebrae have dorsal IM bones (31.9 in the *Big-Belly* to 33.6 in *Nas × Gold*). Of these, 15 also have ribs and 15 have ventral IM bones. Thus, about 30 vertebrae have dorsal IM bones and ventral bones (ribs or ventral IM bones). This leaves around 3 vertebrae with dorsal, but without ventral bones. One of these is the second from the head, and the remaining two are either at the end of the tail, or in midbody between the ribs and the ventral IM bones.

Table 6 helped to determine that the vertebra missing in the Chinese *Big-Belly* belongs to the group of vertebrae having both dorsal IM bones and ribs.

Discussion

The present study showed a low genetic, as well as phenotypic, range of IM bones. On these results, the chances of reducing the number of IM bones by means of a conventional selection program are nil. The wide genetic basis covered by the experiment strengthens this conclusion, since it reduces the likelihood of broadening the

Table 7. A summary of Kossmann's results

Group of fish	No. of fish	Vertebrae R	Vertebrae M-30	Vertebrae SE	Vertebrae CV %	Dorsal Inter-M bones R	Dorsal Inter-M bones M-60	Dorsal Inter-M bones SE	Dorsal Inter-M bones CV %	Ventral Inter-M bones R	Ventral Inter-M bones M-22	Ventral Inter-M bones SE	Ventral Inter-M bones CV %	Total Inter-M bones R	Total Inter-M bones M-85	Total Inter-M bones SE	Total Inter-M bones CV %
9013	18	2	3.1	0.1	1.4	9	6.9	0.4	2.8	8	6.9	0.6	8.2	13	10.7	0.8	3.5
9012	15	1	3.9	0.1	1.0	5	7.2	0.3	1.9	7	7.5	0.6	8.0	11	11.7	0.7	3.1
9010	15	3	2.9	0.2	2.3	16	1.1	1.3	7.9	16	4.1	1.2	17.6	31	2.5	2.8	10.5
9009	37	2	3.2	0.1	1.9	14	2.2	0.5	5.3	11	1.5	0.4	11.0	19	0.7	0.8	5.7
9006	21	1	3.5	0.1	1.5	7	6.5	0.5	3.3	7	3.7	0.3	6.3	10	7.1	0.6	3.0
0059	26	2	3.6	0.1	1.9	15	3.2	0.7	5.9	10	1.2	0.6	12.5	21	1.2	1.1	6.4
0040	14	1	3.4	0.1	1.5	9	2.9	0.7	3.9	12	0.8	1.0	16.2	15	0.6	1.4	6.0
0034	11	1	3.6	0.1	1.5	10	4.4	1.1	5.7	12	4.7	1.0	12.8	17	6.1	1.4	6.0
Mean (total)	157	4	3.5	0.1	2.0	20	4.1	0.3	5.8	20	3.3	0.3	14.6	36	4.4	0.5	7.3
Present results																	
Mean 1971	162	3	4.9	0.1	2.2	10	6.2	0.2	3.1	14	5.3	0.1	7.0	20	8.5	0.3	3.4
Mean 1972	852	8	4.9	0.0	2.5	20	5.9	0.1	3.4	16	7.7	0.1	7.1	29	10.6	0.1	3.5

range with new carp stocks. However, Kossmann's (1972) results, summarized in Table 7 and in the last part of Table 4, show an average within-group SD of total IM bones 1.5 times higher than that of the present counts (4.9 versus 3.3), and inter-group SD over 3.3 times higher than the present counts (4.48 versus 1.31). This last difference in what we assume to be genetic variability is the most striking difference between the two studies. Kossmann's results justify selection for reducing IM bones in carp, whereas the present results lead to the opposite conclusion. The apparent contradiction between the results of the two experiments requires an explanation. Differences in competence level, in methods and techniques, cannot account for the different results because, with the exception of the variance magnitudes, the two sets of results are very similar and both appear to have strong internal consistencies. The eight groups tested by Kossmann were full-sib offsprings of single-pair matings, all originating from what appears to be a single breed of carp. On the other hand, all the groups of the present experiment were reproduced by mass-spawns (Table 1), and they consisted of separate lines (breeds or races) and their crossbreds. Therefore, a wider total range of variation and larger within-group variances were expected in the present experiment compared with Kossmann's.

We may consider the hypothesis that Kossmann's population segregated in one or two loci with a relatively strong effect on IM bones, so that his genetic differences were essentially mono-genic rather than polygenic. This hypothesis has some support in Kandler's (1971) finding of only 80 IM bones in four Holstein domesticated carp, and around 100 IM bones in other German carp. If Kossmann's carp population originated from a cross between a Holstein-like line and another line with a high number of IM bones, Kossmann's observed segregation could be the result. Otherwise, it is difficult to conceive of such a wide segregation among the offspring of Kossmann's 16 parents when it was completely absent among the offspring of the hundreds of parents of the present experiment. The above hypothesis also requires that some of Kossmann's progenies have clear bi-modal distribution and others very small variance. This does not appear to be the case (Table 7).

Another hypothesis is that IM bones have genetic sensitivity to some environmental factors. In other words, wide genotype-environment interactions may cause an increase in the genetic variance in some environments but not in others. This hypothesis is based on the fact that different sets of environments impose different limiting factors , so that one environment may reveal genetic variation which remains hidden in another. For example, temperature variation during the critical stage for IM bone development might influence the variance of IM bones. The degree of scaliness (proportion of body covered with scales) of mirror carp has, as a rule, a strong genetic determination, but occasionally it changes drastically due to unknown environmental reasons (unpublished results). It is possible that IM bones behave in a similar way. The last hypothesis may be tested by hatching random samples of the same spawn under varying controlled conditions. Experiments along this line could be a first step in further attempts to investigate the genetic variation in IM bones.

v. Sengbusch (1967) suggested inspection on a television screen of tens of thousends of live fish in order to pick up very rare "boneless" mutants. This method requires expensive instruments, and the probability of success might be increased if the fish to be inspected were grown under environmental conditions enhancing genetic variation (assuming that the last hypothesis is correct).

Another approach could be to induce genetic variation artificially by chemical or radiation mutagens. This requires preliminary research to establish mutagenic procedures that are effective in inducing point mutations in carp. In either case, the search for carp with a reduced number of IM bones should be carried out on populations having a wide genetic basis and parents that underwent at least one generation of inbreeding (brother-sister mating) to increase to proportional expression of rare recessive mutants.

The apparent negligible variation in IM bones in wild as well as domesticated carp suggests that this character is strongly correlated with reproductive fitness, that the optimal number is between 90 to 100 IM bones, and that attempts to reduce the number drastically would be counteracted by the opposing force of natural selection.

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