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Heterosis in the Growth Rate of Hungarian-Israeli Common Carp Crossbreeds and Evaluation of their Sensitivity to Koi Herpes Virus (KHV) Disease

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Abstract

Koi herpes virus (KHV) disease first occurred in Israel in spring 1998. In search of genetic solutions to the disease, two strains of domesticated common carp (Szarvas-22 and Dinnyes) were imported from Hungary in 2002 to test their genetic contribution to KHV resistance and heterosis in growth parameters when crossed with a local strain (Dor-70). During 2004, six genetically different groups were reared, including the three above-mentioned strains and three crossbreeds (Szarvas-22 x Dor-70, Dinnyes x Dor-70, and Nasic x Dor-70). Fish were vaccinated by injection of an inoculum containing attenuated virus, individually marked, stocked in communal earthen ponds, and cultured to market size. Data on resistance to the disease and growth rates were collected. The two Hungarian strains and their crossbreeds had no advantage in specific resistance to KHV but contributed to heterosis when crossed with Dor-70. This was expressed by an increase of more than 10% and 20% in growth rate, compared to the commercial Israeli crossbreed (Nasic x Dor-70).

Introduction

The first industry to confront an outbreak of koi herpes virus (KHV) disease, also known as CNGV (carp nephritis and gill necrosis virus), was the Israeli common carp (*Cyprinus carpio* L.) industry. Huge losses during spring and fall 1998 generated a quick response and intensive search for ways to control the disease (Perelberg et al., 2003). Together with immediate veterinary solutions, tools to deal with the disease by increasing general genetic resistance were sought.

The first postulate was that fish vitality is closely related to their level of heterozygosity and mono or polygenic heterosis (Kirpichnikov,

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1957, 1958, 1967, 1981). The most frequently-used and rapid methods of increasing heterozygosity are hybridization between wild and/or domesticated forms of the same species (intraspecific crossing) or through interspecific crossing. A special case is commercial hybridization, i.e., crossbreeding of two races or strains for the purpose of commercial scale rearing of the first heterosis generation.

In Israel, the commercial hybrid is produced by crossing the Israeli domesticated *Cyprinus carpio carpio* strain (Dor-70) with the Yugoslavian strain (Nasic, brought to Israel in 1970). In 1999, Dor-70 was crossed with sperm of wild *C. carpio haematopterus* from the Amur River, Czech Republic (Zak et al., 1999). Such crossings enrich dominant genes leading to heterosis, which is expressed as greater vitality and faster growth (Kirpichnikov, 1959). Our offspring were relatively resistant to KHV, but this trait was manifested only in the F1 population, as had been found by Kirpichnikov (1962, 1967) and Andriyasheva (1966, 1971). When backcrosses were performed with the domesticated carp, the disease resistance disappeared (Zak et al., 2000; Shapira et al., 2005). In spite of the high growth rate of this crossbreed, their scaly appearance and uneconomical culture prevented successful marketing.

The next step was intergeneric hybridization between the local domesticated *C. carpio* and another Cyprinidae, *Carassius auratus* (comet). The hybrids were sterile allodiploids with total resistance to the disease, but their growth rate was lower than that of the commercial Israeli hybrid (Zak et al., 1999). Backcrossing the only two fertile females from that population with domesticated common carp males produced allotriploid hybrids with total resistance to KHV and a growth rate similar to that of common carp (Zak et al., 2002) but these hybrids were also scaled and unattractive to the local market.

In spring 2002, two strains of domesticated carps were imported from Hungary. The strains were produced during the previous summer at the Research Institute for Fisheries, Aquaculture and Irrigation (HAKI) in Szarvas. The present paper compares crosses between Dor-70 and the imported Hungarian strains with the commercial Israeli hybrid in terms of growth performance and KHV resistance.

Materials and Methods

Experimental fish. Six genetically different groups were compared for heterosis in growth rate and resistance to KHV disease. One group was the Hungarian Dinnyes strain, obtained by crossbreeding the highly inbred Hungarian domestic carp (gynogenesis) with the Nasic strain. This population was mass-selected for a round body shape, regularity of scales, and high growth rate (Bacos and Gorda, 2001). The second Hungarian strain, Szarvas-22, was derived from individual selection within the Sumony mirror strain, inbreeding (gynogenesis), and strict selection through four generations. Quantitative traits such as strong formation and high fecundity were used as selection criteria (Bacos and Gorda, 2001). A third group was the Israeli strain, Dor-70, a domestic carp of European origin, developed at the Dor Research Station (Israel) by selection for fast growth rate in local conditions. Dor-70 is characterized by a high growth rate in a wide range of conditions and good quality crossbreed progenies (Wohlfarth et al., 1980), although a moderate inbreeding level might have affected its performance during the last two decades.

The three hybrid groups consisted of Dinnyes x Dor-70, Szarvas-22 x Dor-70, and the commercial Israeli hybrid, Nasic x Dor-70. Nasic is a Yugoslavian strain of common carp that was genetically improved for round body shape, high fecundity, and growth rate. In Israeli conditions, its growth rate is low and it is used mainly to produce a commercial hybrid with Dor-70 (Wohlfarth et al., 1984).

Nasic x Dor-70 was used as a reference for comparing growth performance of the Hungarian-Israeli hybrids while Dor-70, a common parent of all three hybrids, was used to assess heterosis.

Fish crossing and nursing. All crossings were performed on the same day (Table 1). Spawning was induced by injecting females with calibrated carp pituitary extract (Yaron et

Table 1. Six genetic groups used in this study.

Genetic group	*Female*	*Male*	*Description*
1	Dinnyes	Dinnyes	Hungarian strain
2	Szarvas-22	Szarvas-22	Hungarian strain
3	Dor-70	Dor-70	Israeli strain
4	Dinnyes	Dor-70	Hungarian-Israeli hybrid
	Dor-70	Dinnyes	Israeli-Hungarian reciprocal hybrid
5	Szarvas-22	Dor-70	Hungarian-Israeli hybrid
	Dor-70	Szarvas-22	Israeli-Hungarian reciprocal hybrid
6	Nasic	Dor-70	Israeli commercial hybrid

al., 1984) and males with synthetic GnRH (Dagin) hormone. More than ten specimens were involved in each cross. The numbers of females available from the Szarvas-22, Dor-70, and Nasic strains were low and a rather large number of males was used to compensate for the reduction of genetic variability.

A similar number of eggs was selected from each female within a genetic group and carefully mixed. The egg blend was divided into equal batches. Each batch was inseminated with milt from one male of the necessary genetic group. Fertilized eggs of each cross were transferred to a separate incubation chamber. Reciprocal crosses between the Hungarian and Dor-70 strains were performed.

Four-day-old larvae were stocked on March 21, 2004, in 350-m^2 earthen ponds at a stocking density of 26,000 larvae per pond, one genetic group in each pond. In the case of reciprocal crossbreeds, 13,000 larvae of each cross were stocked in a single pond. The larvae were reared until 10 g when samples of each group were transferred to an indoor facility for the KHV resistance trial and vaccination procedure in controlled conditions.

Sensitivity to KHV and the attenuated virus vaccine. Sensitivity to KHV was measured by cohabitation with experimentally infected sick fish. Fifty fish of each genetic group were held in 60-l plastic tanks together with five infected koi carp. Water at a constant 23°C flowed through the tanks at 20 l/h. When the koi began to die, they were removed from the tanks. The remaining fish continued in the same conditions for another 21 days during which clinical signs and mortality were monitored. When mortality occurred, kidney samples from moribund fish were taken for PCR analysis to identify the disease agent.

Sensitivity to the attenuated virus vaccine was tested by individually injecting inoculum (Kovax Ltd.) into 500 fish of each genetic group. The fish were anesthetized and injected intraperitoneally with 0.2 ml of the inoculum containing 250 pfu/ml, so that each fish was injected with 50-100 pfu. During the injection and the following 30 days, the fish were held in plastic tanks with an independent water supply at a constant 23°C. After 30 days, three replicated samples of 50 fish from each genetic group were challenged by exposure to sick koi carp, as described above.

The vaccinated fish were cultured for another month to reduce weight differences among groups. A day before stocking the fish in earthen ponds for growout, a sample of 50 fish of each genetic group were individually weighed and 150 fish of each group were marked with distinctive symbols.

Growth rate testing. To test the growth rate, fifty marked fish from each of the six genetic groups were stocked on September 20, 2004, into each of three 350-m^3 replicate earthen ponds (i.e., 300 fish in each pond). Fish were fed *ad libidum* during the two first months. From late November onwards, fish were fed at 1% of the fish biomass in the pond. The ponds were drained on April 7, 2005, and the fish were collected, sorted into genetic groups according to mark, counted, and weighed. On the same day, the fish were re-stocked with the rest of the fish in their replicate into an identical pond for further growout. During the summer, feed was given *ad libidum* and fish samples were weighed monthly. The ponds were drained on July 17, 2005, and fish were harvested, sorted, counted, and weighed.

Statistical analyses. Survival after experimental infection and vaccination was analyzed using the Chi-square test for equality of distributions (Papoulis, 1990). Growth performance data were analyzed by one-way-ANOVA, followed by the Duncan multi-comparison of means test. Since the initial weights of the genetic groups were not homogeneous, the following correction factor was used: b = 4.54 - 0.113X' + 0.00671Y', where b is the predicted correction factor, X' is the mean initial weight of all tested groups, and Y' is the observed weight gain (Wohlfarth and Milstein, 1987). The corrected weight gain Y was calculated as Y = Y' - b(Xi - X'), where Xi is the initial weight of a given genetic group i (Wohlfarth et al., 1983).

Results

Sensitivity to KHV and the attenuated virus vaccine. All groups were susceptible to KHV disease, with a cumulative mortality of over 80% following controlled exposure to the wild type pathogenic virus. The highest survival (20%) occurred in the Dor-70 strain and was significantly higher than in all other groups (Table 2).

The first groups to reach a suitable size for vaccination were Dor-70, Dinnyes x Dor-70, and Szarvas-22. These groups were injected with an attenuated virus dose of 100 pfu/fish. Following injection, the Szarvas-22 group had an unexpected reaction including exophthalmia and massive mortality of 50%, while the other two groups reacted normally. Because of this, another 500 fish of the Szarvas-22 group and the remaining experimental groups were vaccinated with only 50 pfu/fish. Response to the vaccine was determined by the survival rate and clinical signs one month after vaccination. The three crossbreeds responded better than the three parent strains. Szarvas-22 had the lowest survival

Table 2. Survival (%) following experimental infection with wild type koi herpes virus (n = 50) and vaccination with attenuated virus (n = 500).

Genetic group		*Avg wt (g) (%)*	*Survival after infection (%)*	*Survival after vaccination*
1	Dinnyes	15	4^{b}	79^{d}
2	Szarvas-22	12	10^{ab}	68^{e}
3	Dor-70*	22	20^{a}	89^{c}
4	Dinnyes x Dor-70*	12	2^{b}	97^{a}
5	Szarvas-22 x Dor-70	15	4^{b}	93^{b}
6	Nasic x Dor-70	14	6^{b}	97^{a}

Different superscripts within a column indicate significant differences at $p<0.05$ for survival after infection and $p<0.001$ for survival after vaccination.
* Vaccinated with a 100 pfu/fish of attenuated virus. Other groups were vaccinated with 50 pfu/fish.

after vaccination, even when the dose was reduced.

Growth rate. Growth rates were estimated for autumn-winter (September 20-April 7) and spring-summer (April 7-July 17). During autumn-winter, the two Hungarian-Israeli crossbreeds had better growth rates than the other four groups (Table 3). Among the parent lines, the higher the initial weight, the higher the weight gain. Although the commercial Israeli hybrid had a lower initial weight than Dinnyes, its mean weight gain was higher than that of Dinnyes. The weight gain relationships between groups were maintained in the corrected weight gain relationships, i.e., the Hungarian-Israeli crossbreeds had significantly higher corrected weight gains than the other groups.

The spring-summer growout period lasted 102 days. The mean yield in the three ponds was 9 t/ha (range 8.6-9.4). Survival and corrected weight gains were significantly better in the crossbreeds than in the parent strains (Table 4).

To compare growth between the commercial Israeli hybrid and the two Hungarian-Israeli hybrids, their weight increases were compared to two reference groups, the common parent Dor-70 and the commercial Israeli hybrid (Table 5). In autumn-winter, Dinnyes x Dor-70 performed better than the commercial Israeli hybrid by 34% and Szarvas-22 x Dor-70 performed better by 29%. During summer-spring, this advantage was reduced to 16% and 3%, respectively, in spite of the fact that the commercial Israeli hybrids were at least 100 g smaller than the Hungarian-Israeli hybrids. The advantage over Dor-70 was even more convincing, especially during summer when growth in Dinnyes x Dor-70 was 52% greater than in Dor-70 and growth in Szarvas-22 x Dor-70 was 34% greater. This pattern was observed in each of the three replicates, strengthening the hypothesis that the observed differences were genetic and not environmentally generated.

Discussion

In the first stage of this study, the genetic groups were produced. In the second stage, susceptibility of the offspring to the wild CNGV (also referred to as KHV disease) and their resistance to attenuated virus vaccine were tested. Lastly, vaccinated fingerlings were cultured to market size and growth rates were compared. In each stage, performance was compared.

Survival during early developmental stages in ponds is usually related to egg quality (maternal effect) and environmental factors

Table 3. Weight gains in autumn-winter.

Genetic group	*Mean initial wt (g)*	*Mean final wt (g)*	*Mean wt gain (g)*	*Corrected wt gain (g)*	*Survival (%)*
Dinnyes	37.8±10.2	335±23	297	292[c]	85
Szarvas-22	43.4±13.3	419±16	376	358[b]	91
Dor-70	50.4±13.1	434±27	384	347[bc]	85
Dinnyes x Dor-70	31.9±9.1	458±39	426	441[a]	87
Szarvas-22 x Dor-70	32.2±10.0	443±34	411	423[a]	95
Nasic x Dor-70	27.2±6.7	328±21	302	328[bc]	92

Different superscripts in corrected wt gain column indicate significant differences at $p<0.0004$.

Table 4. Weight gains and survival in summer and overall growout periods.

Genetic group	*Mean final wt (g)*	*Mean summer wt gain (g)*	*Mean general wt gain (g)*	*Calculated summer growth rate (g/day)**	*Corrected overall wt gain (g)*	*Survival (%)*
Dinnyes	983±46	651	945	6.3^{c}	940^{d}	66^{c}
Szarvas-22	1057±34	639	1014	6.0^{cd}	966^{d}	79^{b}
Dor-70	1078±35	644	1028	5.7^{d}	928^{d}	77^{b}
Dinnyes x Dor-70	1314±91	856	1282	8.6^{a}	1321^{a}	81^{ab}
Szarvas-22 x Dor-70	1194±44	753	1162	7.6^{b}	1199^{b}	90^{a}
Nasic x Dor-70	1038±36	710	1011	7.4^{b}	1085^{c}	91^{a}

Different superscripts within a column indicate significant differences at $p<0.001$ for calculated summer wt gain, $p<0.0001$ for corrected overall wt gain, and $p<0.007$ for survival.
* Calculated summer growth rate = [(corrected overall wt gain) - (corrected winter wt gain)]/102 days

Table 5. Weight gains of the crossbreeds, expressed as percentages of the weight gains of the common parent (Dor-70) and of the commercial Israeli crossbreed (Nasic x Dor-70).

Pond	*Group*	*Compared to Dor-70 (%)*			*Compared to Nasic x Dor-70 (%)*		
		Winter	*Summer*	*Overall*	*Winter*	*Summer*	*Overall*
1	Nasic x Dor-70	89	124	111	-	-	-
	Szarvas-22 x Dor-70	113	131	125	127	106	112
	Dinnyes x Dor-70	115	143	133	130	116	120
2	Nasic x Dor-70	90	135	117	-	-	-
	Szarvas-22 x Dor-70	124	134	130	138	100	111
	Dinnyes x Dor-70	125	150	140	139	111	119
3	Nasic x Dor-70	106	132	123	-	-	-
	Szarvas-22 x Dor-70	129	135	133	121	102	108
	Dinnyes x Dor-70	142	162	155	133	122	126
Avg	Nasic x Dor-70	95	130	117	-	-	-
	Szarvas-22 x Dor-70	122	134	129	129	103	110
	Dinnyes x Dor-70	127	152	143	134	116	122

such as availability of natural food or presence of predator insects (e.g., Kirpichnikov, 1961; Hulata et al., 1976; Hepher, 1978). Among the tested groups, Dor-70 larvae had the lowest fertility in the egg stage and lowest survival in the larvae ponds, resulting in very low density of fish that reached a larger size faster than in other groups. Fish size is positively correlated to disease resistance (Perelberg et al., 2005), possibly explaining the relatively high survival of the Dor-70 group after induced infection.

At final harvest in July, many fish of the Dinnyes and Szarvas-22 groups had malformations in the rostrum and operculum, the most severe being irreversible reduction of the operculum. These malformations, together with the relatively low final survival, suggest that peptides and/or enzymes in these strains have low resistance to high temperature. This can be explained from a biochemical polymorphism point of view. Various forms of a peptide and/or enzyme are present in an organism, each is active in specific environmental conditions during fish ontogenesis (Kirpichnikov, 1981). The presence of the different peptides and/or enzyme isoforms is related to the geographic origin of the fish population (Johnson, 1976). The variety of alleles that code the peptides and/or enzymes in each population is the result of natural selection in the genome to enable the fish to adjust to life in its temperature range. Deliberate acclimatization of fish to conditions beyond their evolutionary temperature range may lead to extreme phenomena (Van Dijk et al., 1999; Smirnova et al., 2002). Hence, the malformations and low survival of the Hungarian strains may be explained by a lack of adaptive response to the typically high water temperatures of the Israeli summer. Kirpichnikov (1981), following Lerner (1954), called similar phenomena 'phenodeviants', indicating that they may result from inbreeding depression.

The crossbreeds had higher weight gains than their parents (Fig. 1). This can be attributed to heterosis resulting from the successful combination of alleles of the local and imported strains, including alleles responsible for proteins and/or enzymes with different levels of heat resistance. The heterosis counteracted the negative effects of inbreeding depression by increasing the level of general heterozygosis. The expression of heterosis in different strain combinations is related to the genetic distance between the parent strains (Pak and Tsoi, 1986).

Data on the expression of transferrin alleles in the studied strains were collected from the literature to evaluate the genetic distance between the strains. Transferrin is a glycoprotein that binds an iron ion into the organism. The presence of polymorphism in the transferrin locus of carps allows us to use transferrin alleles as genetic markers. The distribution of transferrin alleles may explain the difference in heterosis in the crossbreeds. Table 6 shows the relative frequency of six alleles in the four common carp strains used in this study. Data on Dor-70 and Nasic were collected in 1993, five years before the KHV disease outbreak in Israel. We assume that the disease reduced genetic variability within these populations, changing the relative fre-

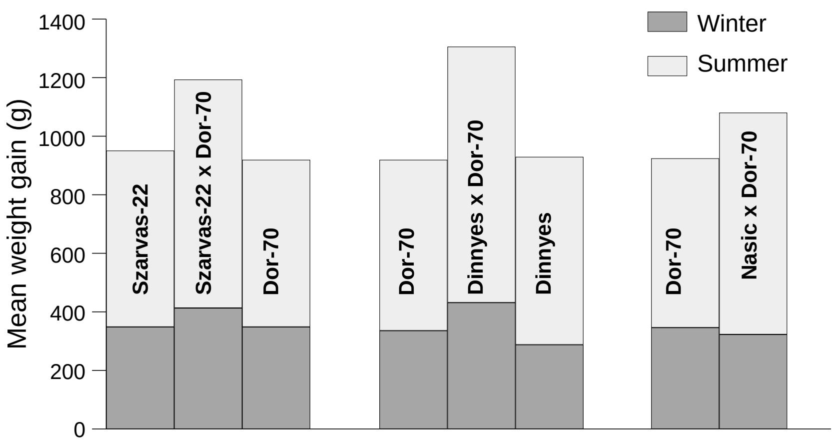

Fig. 1. Comparison of the corrected mean weight gains of the crossbreeds with those of their parents in the autumn-winter, summer-spring, and overall culture periods.

Table 6. Frequency (%) of six transferrin alleles in four *Cyprinus carpio* strains.

Allele	*Dor-70*[1]	*Nasic*[1]	*Szarvas-22*[2]	*Dinnyes*[2]
A	-	9.8	-	71.7
B	-	0.9	-	14.2
C	-	-	-	14.1
D	62.9	71.4	44.6	-
E	5.8	-	27.0	-
G	31.3	17.9	28.4	-

[1] Ben-Dom et al., 2000
[2] Bacos and Gorda, 2001

quency of some alleles according to their selective value for survival but not changing the qualitative allele structure. Nevertheless, the data emphasize the differences between the strains: Nasic differs from Dor-70 in three alleles of the transferrin locus that have low frequencies; Szarvas-22 and Dor-70 share three alleles but in different frequencies; the greatest genetic distinction is between Dinnyes and Dor-70, which share no allele. Crossing these two should result in full heterozygosis in the transferrin locus and, indeed, this fits our results in that the best growth performance was obtained in the Dinnyes x Dor-70 crossbreeds. The differences in transferrin confirm the assumption that our results were due to genetics.

There are several biochemical and molecular methods of determining genetic polymorphism in populations. Using them, it is possible to follow genetic changes in broodstocks and to select genetically distant strains for crossbreeding to obtain greater heterosis in the offspring (e.g., Nei, 1972).

In conclusion, the Hungarian strains and their crossbreeds had no advantage in relation to resistance to KHV disease. Thus, all attempts up to now to find a genetic solution to this disease through intraspecific crossings have been ineffective while genetic solutions were found in interspecific crosses. On the other hand, crossbreeds of Dor-70 and either Hungarian strain (more so Dinnyes) had significantly better growth performance than the commercial Israeli crossbreed and the parent strains, especially in autumn-winter. This advantage may be due to the genetic distance between the strains that probably combines the benefits of alleles suitable to both cold (Hungarian) and warm (Israeli) conditions.

Most of the advantages of the Hungarian-Israeli crossbreeds were expressed during the winter, while the growth rate of the commercial Israeli crossbreed was higher in the summer, suggesting that the Israeli crossbreed has adapted to local summer temperatures. If the Nasic strain were replaced by the Dinnyes in production of the commercial crossbreed, the culture season could possibly be expanded to include the cold period, and fish would reach market size earlier. Using a Dinnyes x Dor-70 crossbreed would be advantageous on farms that can stock and feed carps earlier in the winter, while the Nasic x Dor-70 crossbreed would have an advantage on farms that cannot raise them until the warm season.

A further explanation of the lower growth performance of the Israeli commercial crossbreed might be depletion of genetic richness in the local broodstock since the first occurrence of KHV disease in 1998. No vaccine was available at that time, so natural selection for resistance to the disease might have had a negative effect on genetic diversity related to growth performance. The annual reoccurrence of the disease requires special broodstock management using only fish vaccinated before exposure to the disease as broodstock to preserve genetic diversity within the breeding strains. Non-vaccinated survivors of the disease might be parents with poor genetic characteristics and their progeny might have decreased heterosis.

Another management possibility is the use of cryopreserved sperm of preferred strains. Sperm of Nasic and Dor-70 were cryopreserved in Israel in 1999, one year after the disease outbreak. In view of our results, using Dinnyes instead of Nasic to produce fry for commercial growout should be considered. In which case, it is recommended to cryopreserve its sperm as soon as possible.

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