

TRANSFERRIN POLYMORPHISM IN CARP (*CYPRINUS CARPIO* L.) POPULATION¹

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Summary. Studies on the polymorphism of transferrin totally covered 1047 individuals, including 730 dry and 317 two-year commercial carps. This material originated from production ponds of three fish-breeding estates in the vicinity of Olsztyn. The occurrence of 10 phenotypes determined by a series of four alleles were recorded. The state of genetic equilibrium retained. However, very large differences were revealed in the frequency of genes and genotypes. In the group of fry, the most frequent were *DG* heterozygotes and the frequency of *D* and *G* alleles was almost identical.

A two-year carp is characterized by significantly predominant number of *DD* homozygotes, whereas the *D* allele covers over 60% of the total gene pool. No statistically significant dependence was found between the transferrin types and the body weight. However, it was found reasonable to undertake further studies making possible the record of the dynamics of changes in the genetic structure of transferrin system and in exterior traits at different developmental stages in carp.

The monomeric structure and large specialization of functions probably constitute an evolutionally consolidated cause of a widespread differentiation of transferrin types. Genetically determined polymorphism of that protein was discovered in very many species of mammals, birds and fish. Results obtained by Valenta (1978) show that polymorphism occurs in 17 out of 23 fish species of the *Cyprinidae* family. The largest variation determined by a series of 10 alleles was found in barbel; in chub and bleak there occur 9 alleles, whereas in ide and cyprinid only two alleles. On the other hand, no differentiation in transferrin forms was found in amur, rap and vimba.

Polymorphism of transferrin in carp is determined by a series of 8 alleles. Most of the recorded so far populations had only 3 - 5 alleles (Creysse et al. 1966, Balakhin, Fomanov 1971, Balakhin, Galagan 1972, Valenta et al. 1978). More alleles were encountered very rarely and only in some breeding regions (Galagan 1973, Valenta et al. 1978). Balakhin and Galagan (1972 a), when analysing the ratios of genotypic segregations found that carp fry have irregularities expressed in doubling the expected number of heterozygotes. Kalal et al. (1975) reported very large differentiation in the values of exterior traits. Heterozygotes with the *DG* genotype

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weighed on the average 111.7 g, exceeding significantly the remaining genotypic groups (67.7 - 84.6 g). In another series of studies these regularities were confirmed. The body weight of *DG* heterozygotes was on the average 114.3 g, while in the remaining groups of fry it was within 52.1 g - 74.9 g. Predominance of the *DG* genotype was also expressed in a larger length, height and width of the body.

The widespread occurrence of favourable effects of heterozygosity of genes determining polymorphism of transferrin may be important on breeding. This paper presents genetic structure of transferrin of the mass population from north-eastern part of Poland, as well as an analysis of relationship between the occurrence of polymorphic types of that protein and the body weight of carp at various developmental stages.

MATERIAL AND METHODS

Studies on transferrin polymorphism covered totally 1047 individuals including 730 fry individuals and 317 2-year commercial carps. This material originated from production ponds of three fish-breeding estates near Olsztyn. The fish were randomly chosen for the studies.

The blood of fry was taken from the heart, and that of the adult carp — from the tail vein. Sera were separated from blood corpuscles by centrifugation; samples were stored in a frozen state (-16°C).

Polymorphism was determined by the method of horizontal starch gel electrophoresis. The composition of the applied buffers was as follows: vessel electrolyte: LiOH-0.38 g, NaOH-1.25 g, H_3BO_3 -14.15 g and 1000 ml H_2O ; — gel buffer: 50 ml vessel electrolyte 450 ml tris-citrate solution containing Tris-4.8 g, citric acid — 1.6 g and 1000 ml H_2O ;

Electrophoresis was performed according to Smithies (1955). The gel was stained with an Amido Black solution. The phenotypes of the studied individuals were identified on the basis of the number and localization of transferrin fractions. The fish body weight was determined on an automatic weight recorder with the an accuracy of 1 g.

Results of electrophoretic studies made possible determination of the genotype distribution and calculation of the gene frequency for individual groups and the whole material. The expected number of genotypic groups was calculated using Hardy-Weinberg formula; genetic equilibrium was statistically verified using the χ^2 -criterium. The relation between transferrin polymorphism and body weight was determined by a unifactorial analysis of variance.

RESULTS AND DISCUSSION

The material under study was recorded to have 10 phenotypes determined by a series of four alleles. According to the nomenclature of Valenta et al. (1976) these alleles were designated with the symbols Tf^D , Tf^E , Tf^F , Tf^G . The collection of transferrin phenotypes occurring in the studied population is presented in Fig. 1.

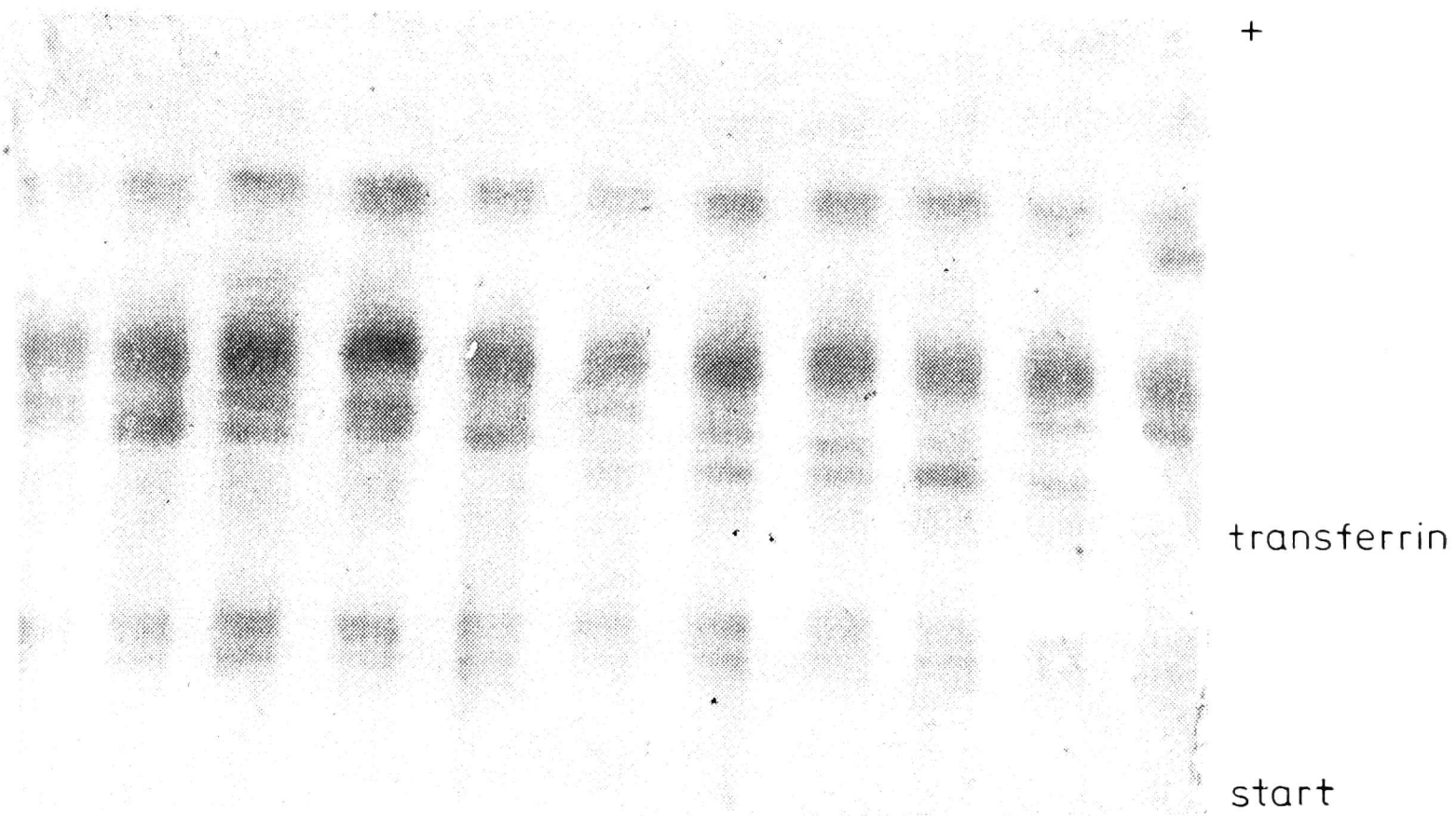


Fig. 1. Transferrin polymorphism of the blood serum in carp. Genotypes (from the left): *DE*, *EF*, *DF*, *DE*, *FF*, *DG*, *EG*, *FG*, *GG*, *DG*, *DD*

The general genetic variation expressed in the phenotype number is identical in both fry and 2-year old carp. However, large differences were observed with respect to the genotype and gene frequencies (Table 1). Fry are characterized by a predominant number of *DG* heterozygotes (29.1%), whereas in 2-year old carp the most

Table 1. Genotype distribution and gene frequency in fry and 2-year carp

Group	Genotypes (%)										Genes (%)			
	<i>DD</i>	<i>DE</i>	<i>DF</i>	<i>DG</i>	<i>EE</i>	<i>EF</i>	<i>EG</i>	<i>FF</i>	<i>FG</i>	<i>GG</i>	<i>D</i>	<i>E</i>	<i>F</i>	<i>G</i>
Fry I <i>n</i> = 303	17.8	9.6	7.9	36.6	0.3	0.7	8.6	—	4.3	14.2	44.9	9.7	6.5	38.9
Fry II <i>n</i> = 210	2.9	0.5	25.2	31.9	—	—	0.5	8.6	19.0	11.4	31.7	0.5	30.7	37.1
Fry III <i>n</i> = 217	10.6	10.6	10.6	16.1	2.3	10.1	11.1	7.4	11.1	10.1	29.3	18.2	23.3	29.3
Fry totally <i>n</i> = 730	11.4	7.3	13.7	29.2	0.8	3.3	7.0	4.7	10.5	12.2	36.5	9.6	18.4	35.5
2-year carp <i>n</i> = 198	48.0	5.6	6.6	27.3	—	1.0	4.6	1.0	3.5	2.5	67.4	5.1	7.3	20.2
2-year carp <i>n</i> = 119	27.7	12.6	19.3	12.6	1.7	2.5	3.4	5.9	8.4	5.9	49.9	11.0	21.0	18.1
2-year carp totally <i>n</i> = 317	40.4	8.2	11.4	21.7	0.6	1.6	4.1	2.8	5.4	3.8	61.1	7.5	12.0	9.14
Total population <i>n</i> = 1047	20.0	7.6	13.0	26.9	0.8	2.8	6.1	4.1	9.0	9.7	43.8	9.0	16.5	30.7

frequent are *DD* homozygotes (40.4%). The total portion of homozygotes in the group of fry (29.1%) is significantly lower than that in adult carp (47.6%). The fry are characterized by a marked dominance in the frequency of two alleles — *Tf^D* (36.5%) and *Tf^G* (35.5%), whereas in adult carp the relative portion of a single allele *Tf^D* constitutes 60% of the general gene pool of transferrin system.

As a result of the analysis of the genetic structure of fry from three ponds, some elements common for the entire studied material as well as some specific characters, distinguishing fry from different objects, could be found. In all three subpopulations the most are *DG* heterozygotes; the *GG* homozygotes are also frequently encountered, whereas the alleles *Tf^E* and *Tf^F*, as well as the genotypes involving these alleles, display large differentiation. In the both groups of adult carp, the *DD* homozygotes are the most frequent, while the *GG* genotype is encountered very rarely. The studied population should be recognized nonuniform with regard to its transferrin system, however, its variation is within the limits recorded in other breeding and production regions of carp.

Despite a large differentiation in the frequency of genotypes and genes no statistically significant abnormality was found in the genetic equilibrium of the studied population. Analysing the agreement between the observed and expected numbers of genotypic groups it should, however, be stressed that heterozygotes were dominant in fry, whereas homozygotes occurred in a larger number in 2-year old carp. Similar tendencies were also observed in other populations under study. Genetic equilibrium occurred even when heterozygous individuals exhibited a noticeable dominance over

homozygotes in respect of the body weight and other exterior characters (Kalal et al. 1975). Taking into account the results of the so-far studies, it should be believed that heterozygosity of genes determining transferrin polymorphism in carp does not cause desirable vitality increase.

The relationship between transferrin polymorphism and the body weight in the studied population also appeared statistically nonsignificant (Table 2). It is very difficult to prove the significance of differences because of a very large individual variation and small number of genotypic groups with extremely large and small body weight. The obtained results, therefore, did not support the convincing regularities recorded by Kalal et al. (1975). A certain symptom of the identity of the results is a

Table 2. The body weight of fry and 2-year carp with different transferrin genotypes

Genotypes	Groups of studied material														
	Fry I			Fry II			Fry III			2-year carp			2-year carp		
	<i>n</i>	\bar{x}	<i>s</i>	<i>n</i>	\bar{x}	<i>s</i>	<i>n</i>	\bar{x}	<i>s</i>	<i>n</i>	\bar{x}	<i>s</i>	<i>n</i>	\bar{x}	<i>s</i>
<i>DD</i>	54	30.7	8.2	6	20.8	7.8	23	56.9	20.9	95	969.5	228.2	33	774.1	193.1
<i>DE</i>	29	31.4	10.9	1	15.5	×	23	53.2	25.0	11	810.0	185.9	15	882.0	164.2
<i>DF</i>	24	28.9	7.7	53	20.5	7.4	23	48.1	22.2	13	921.5	286.2	23	926.3	227.8
<i>DG</i>	111	31.7	14.2	67	21.2	7.1	35	64.9	24.1	54	926.4	235.0	15	831.0	228.3
<i>EE</i>	1	24.0	×	—	—	—	5	64.2	31.9	—	—	—	2	755.0	×
<i>EF</i>	2	28.0	×	—	—	—	22	55.3	27.7	2	980.0	×	3	1220.0	×
<i>EG</i>	26	28.3	8.4	1	72.0	×	24	62.9	27.5	9	1034.4	324.0	4	785.0	×
<i>FF</i>	—	—	—	18	23.4	9.5	16	52.9	26.2	2	855.0	×	7	811.4	136.2
<i>FG</i>	13	24.0	5.4	40	24.2	6.6	24	57.4	22.3	7	959.3	366.1	10	825.0	210.1
<i>GG</i>	43	31.4	9.3	24	19.8	7.3	22	46.8	19.0	5	1064.0	260.8	7	682.1	165.9
Totally	303	30.6	11.1	210	21.8	8.4	217	55.9	24.3	198	944.6	245.0	119	835.9	219.0

little higher body weight of fry with the *DG* genotype as compared to the *DD* and *GG* homozygotes. However, in 2-year old carp, a very rare genotype *DG* displays the body weight similar to the population mean.

The inconsistency of the compared results may be apparent, since they characterize populations at various developmental stages. The fry in our studies, though being differentiated regarding the age and breeding conditions, is however, considerably younger, while the 2-year old commercial carp is significantly older than that in the population studied by Kalal et al. (1975).

Carp is a species, whose genetic structure underwent long selection aimed at definite useful effects. The direction of breeding selection is not always favourable to adaptational properties. A rapid growth rate of carp is not simultaneous with the natural functional efficiency of the organism. Genetic mechanisms of breeding selection may manifest themselves by desirable useful effects of the additive gene action in homozygotes, whereas heterosis effects may determine higher natural resistance and heterozygote viability. It may be expected that at different developmental stages of individuals specific genotype \times environment interactions may favour or discriminate the growth rate of homozygous and heterozygous individuals. In order to confirm this hypothesis with regard to polymorphic transferrin system studies making possible the record of dynamics of changes in the genetic structure and exterior characters in various developmental stages of carp.

CONCLUSIONS

1. The studied population was characterized by a large genetic variation of transferrin system; 10 phenotypes determined by a series of 4 alleles were recorded in it.
2. Great differentiation in the frequency of genotypes and genes was found between the groups of fry and 2-year old carp.
3. No statistically significant relationship was found between transferrin polymorphism and the body weight of carp.

REFERENCES

1. Balakhin I. A., Fomanov L. M. (1971). Raspredelenie i genneya castota tipov transferrina u bezporodnova karpa i amuskovo sazana. *Gidrobiologiceskij Zhurnal*, 3, 84 - 86.
2. Balakhin I. A., Galagan I. P. (1972 a). Raspredelenie i vyzyvaemost osobei s raznymi tipami transferina v potomstve karpa pri razlichnyh sochetaniyakh proizvoditelei. *Gidrobiologicheskij Zhurnal*, 3, 56 - 61.
3. Balakhin I. A., Galagan I. P. (1972 b). Tipy transferina *Cyprinus Carpio* L. *Gidrobiologicheskii Zhurnal*, 6, 108 - 110.
4. Creyssel R., Richard G., Silberzahn R. (1966). Transferrin variants in carp serum, *Nature*, 212, 5058.
5. Galagan I. P. (1973). O transferinakh dunajskogo sazana. *Gidrobiologicheskii Zhurnal*, 2, 94 - 99.
6. Kalal L., Valenta M., Slechtova V., Smisek J. (1975). Vyuzyti serovykh transferinu pri slachteni kapra. *Zivocisna Vyroba*, 20, 789 - 800.
7. Smithies O. (1955). Zone electrophoresis in starch gels. Group variations in the serum proteins of normal human adults. *Biochem. J.*, 61, 629 - 641.
8. Valenta M. (1978). Protein polymorphism in European fish species of the Cyprinidae family and utilization of polymorphic proteins for breeding in fish. *Procc. International Seminary on Increasing the Productivity by Selection and Hybridization in Szarvas (Hungary)*; 19 - 23 September 1978, 37 - 38.
9. Valenta M., Stratil A., Slechtova V., Kalal L., Slechta V. (1976). Polymorphism of transferrin in carp (*Cyprinus carpio* L.). Genetic determination, isolation and partial characterization. *Biochem. Genet.*, 14, 1 - 2.
10. Valenta M., Slechtova V., Kalal L., Stratil A., Janatkova J., Slechta V., Rab P., Pokorny J. (1978). Polymorfni bielkoviny kapra obecneho (*Cyprinus carpio* L.) a lina obecneho (*Tinca tinca* L.) a moznosti jejich vyuzyti v plemenarske praci. *Zivocisna Vyroba*, 23, 797 - 809.

POLIMORFIZM TRANSFERYNY U KARPIA (*CYPRINUS CARPIO* L.)

Streszczenie

Badaniami polimorfizmu transferyny objęto łącznie 1047 osobników, w tym 730 sztuk narybku i 317 dwuletnich karpki handlowych. Materiał ten pochodził ze stawów produkcyjnych trzech gospodarstw rybackich położonych w pobliżu Olsztyna. Zarejestrowano występowanie 10

fenotypów determinowanych serią czterech alleli. Stan równowagi genetycznej populacji był zachowany. Stwierdzono jednak bardzo duże różnice frekwencji genów i genotypów. W grupie narybku najczęściej występują heterozygoty *DG* a frekwencja alleli *D* i *G* jest niemal identyczna. Karp dwuletni charakteryzuje się znaczną przewagą homozygot *DD* a allel *D* obejmuje ponad 60% ogólnej puli genów. Nie stwierdzono statystycznie istotnej zależności między typami transferyny i masą ciała. Uznano jednak za celowe podjęcie dalszych badań umożliwiających rejestrację dynamiki zmian struktury genetycznej układu polimorficznego transferyny i cech eksterierowych w różnych stadiach rozwojowych karpia.

ПОЛИМОРФИЗМ ТРАНСФЕРИНА У КАРПА (*CAPRINUS CARPIO* L.)

Резюме

Исследованиями полиморфизма трансферина было охвачено 1047 особей, включая 730 мальков и 317 2-летних промышленных карпов. Исследуемый материал происходил из производственных прудов трёх рыбных хозяйств, находящихся недалеко от Ольштына. Зарегистрировано появление 10 фенотипов, обусловленных серией четырёх аллелей. Сохранено состояние генетического равновесия популяции. Обнаружены, однако, очень большие различия в частоте генов и генотипов. В группе мальков наиболее часто выступали гетерозиготы *DG*, а частота аллелей *D* и *G* была почти одинакова. 2-летний карп характеризовался значительным преобладанием числа гомозигот *DD*, а аллель *D* составляла 60% всего генного фонда. Не обнаружено статистически существенной зависимости между типами трансферина и массой тела. Однако, признано целесообразным проведение дальнейших исследований, позволяющих регистрацию динамики изменения генетической структуры полиморфической системы трансферина и признаков внешнего вида в различных стадиях развития карпа.