GENETIC DIFFERENTIATION IN TURKISH BROWN TROUT (*Salmo trutta* L.) AS REVEALED BY PROTEIN POLYMORPHISM

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ABSTRACT

GENETIC DIFFERENTIATION IN TURKISH BROWN TROUT (*Salmo trutta* L.) AS REVEALED BY PROTEIN POLYMORPHISM

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Based on 21 loci of 10 enzyme systems (AAT, CK, EST, LDH,MDH,MEP, GPI, PGM, SOD and TF). genetic structures of 8 Turkish Brown Trout (*Salmo trutta* L.) populations, namely Alakır, Gödene (Alakır-3), Eşen, Abant, Mudurnu, Sümer, Blacksea and Fırtına were determined by starch gel and cellulose acetate paper electrophoresis.Twelve of the loci were polymorphic and there were population specific fixed alleles of LDH-B2 and SOD-1 loci. Alakır, Gödene (Alakır-3) and Eşen populations showed the highest level of expected heterozygosity (0.1006). The highest percentage of polymorphic loci were observed in Blacksea (42.86) and Sümer (38.10) populations. The mean number of effective alleles for each population was between 1.1149 and 1.2125. The mean values for F_{IS} was 0.3715, for F_{IT} it was 0.4770 and for F_{ST} it was 0.2549.By combining the results belonging to some of the European countries and the result of the present study the neighbour joining dendogram based on Nei's genetic distance was constructed by using 8 loci. The results suggested that Turkish brown trout populations are close to Mediterranean and Danube phylogeographic groups.

Keywords:Turkish brown trout populations, *Salmo trutta* L., AAT, CK, EST, LDH, MDH, MEP, GPI, PGM, SOD, TF, Protein electrophoresis, neighbour joining dendogram

ÖZ

TÜRK KAHVERENGİ ALABALIK (*Salmo trutta* L.) TOPLUMLARININ GENETİK FARKLILIKLARININ PROTEİN İŞARETLERİ İLE BELİRLENMESİ

Plan, Ebru
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10 enzim sisteminin (AAT, CK, EST,LDH, MDH, MEP, GPI, PGM, SOD ve TF) 21 lokusu çalışılarak Alakır, Gödene (Alakır-3), Eşen, Abant, Mudurnu, Sümer, Karadeniz, ve Fırtına isimlerindeki 8 Türk kahverengi alabalık (*Salmo trutta* L.) populasyonunun genetik yapıları, nişasta jel elektroforezi ve selüloz asetat kağıdı elektroforezi ile belirlenmiştir. Oniki lokusun polimorfik ve LDH-B2 ve SOD-1 lokuslarının toplumlara özgü alleleri olduğu gözlenmiştir. Alakır, Gödene (Alakır-3) ve Eşen populasyonlarında yüksek düzeyde (0.1006) beklenen heterozigotluk görülmektedir ve en yüksek düzeyde polimorfik lokus yüzdesi Karadeniz (42.86) ve Sümer (38.10) populasyonlarında gözlenmiştir. Her populasyon için etkin allel sayısı 1.1149 ile 1.2125 arasında değişmektedir. F_{IS}, F_{IT} ve F_{ST} için ortalama değerler sırasıyla 0.3715, 0.4770 ve 0.2549 olarak bulunmuştur. Bazı Avrupa ülkelerinin sonuçları ile bu çalışmanın sonuçları birleştirilmiş, 8 lokus kullanılarak Nei'in genetik uzaklığına göre komşu birleştirme metoduyla dendogram oluşturulmuştur.Sonuçlar,Türk kahverengi alabalık populasyonlarının Akdeniz ve Tuna filocoğrafik gruplarına benzediğini göstermiştir.

Anahtar Kelimeler: Türk kahverengi alabalık populasyonları, *Salmo trutta* L. , AAT, CK, EST, LDH, MDH, MEP, GPI, PGM, SOD, TF, Protein elektroforezi, Komşu birleştirme dendogramı.

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LIST OF ABBREVATIONS

AAT: Aspartate aminotransferase

CK: Creatine kinase

EST: Esterase

GPI: Phosphogluco isomerase

LDH: Lactate dehydrogenase

MDH: Malate dehydrogenase

MEP: (NADP dependent) Malic enzyme

SOD: Superoxide dismutase

PGM: Phosphogluco mutase

TF: Transferrin

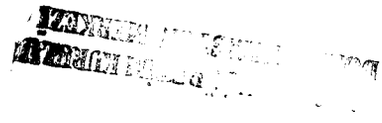
ybp: years before present

CHAPTER 1

INTRODUCTION

Brown trout, *Salmo trutta* L., is a member of the family Salmonidae, which is a relatively primitive group of teleost fish. This group is so-called "primitive" since it has soft fin rays, adipose fin, absent or incomplete oviducts, features often associated with primitive groups. However, members of this family also exhibit more "advanced" features especially in their physiology and breeding behavior (Elliot, 1994). The Salmonidae family includes three subfamilies, that are widely distributed throughout the Northern Hemisphere: Coregoninae (White fishes and Ciscoes), Thymallinae (grayling) and Salmoninae (charr,trout and salmon) with both freshwater and anadromous species (Allendorf and Waples, 1995) (Fig.1). All members of the family breed in freshwater which is used as an evidence to support the view that the family originated in freshwater (Tchernavin,1939). These species comprise a monophyletic clade descendent from a tetraploid ancestor that originated some 50 - 100 million years ago although the salmonid fossil record is extremely scarce in this period (Allendorf and Waples, 1995). The earliest fossil definitely attributed to Salmonidae is *Eosalmo driftwoodensis* from Eocene deposits (40 - 50 million years ago) in western Canada (Wilson ,1977).

Recent work on phylogenetic relationship revised the taxonomy of Salmonidae subfamily (Smith and Stearley, 1989 and Sanford, 1990). Rainbow trout, cut-throat trout, Pacific trout and salmon and their close relatives have been transferred from the genus *Salmo* to the genus *Onchorhynchus*. The genus *Salmo* is restricted only to Atlantic salmon, *Salmo salar* and brown trout, *S. trutta*.



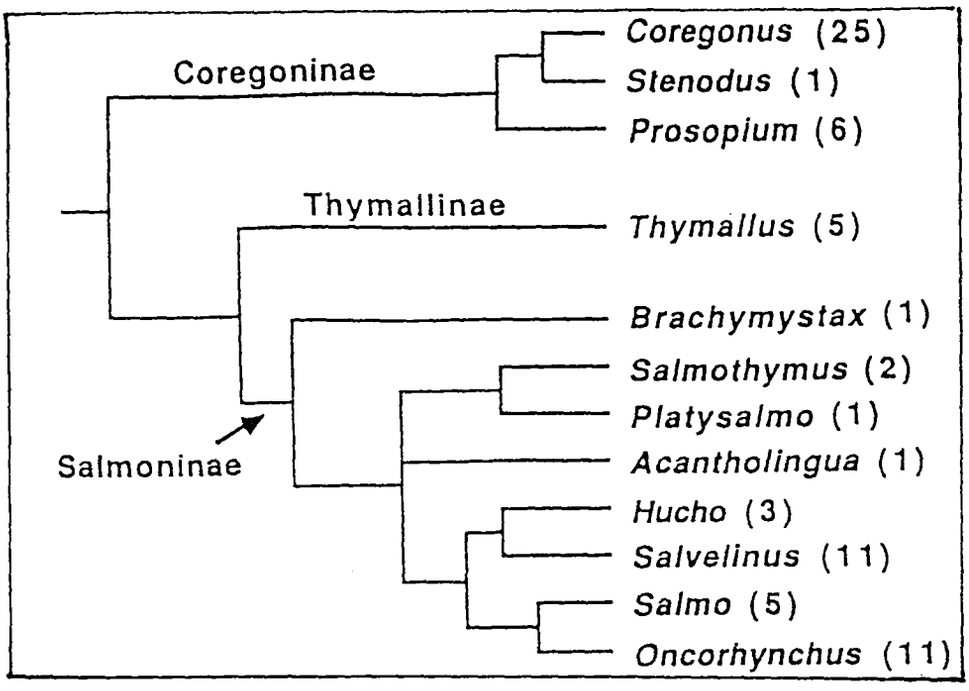


Figure 1. Phylogeny of Salmonidae family (Numbers in paranthesis indicates the number of species)(From Allendorf and Wapple, 1995)

Brown trout was first named as *Salmo trutta* by Linnaeous in 1758. Due to morphological and ecological variations in this species, it has been classified under many different common and latin names. Since then, about 50 species have been described for varieties of brown trout (Benkhe, 1986).

1.1. Distribution of brown trout:

The brown trout is a European species (see Fig. 2). Its northern limits are Iceland, northern Scandinavia and Russia, western limits are European coastline and southern limits are the northern coastline of Mediterranean Sea with the islands of Corsica, Sardinia and Sicily and the Atlas Mountains of North Africa. The eastern limits are not well known, but Ural Mountains, Caspian Sea and upper reaches of Orontes (Asi) River in Lebanon are thought to be its eastern limits (Elliot, 1994).

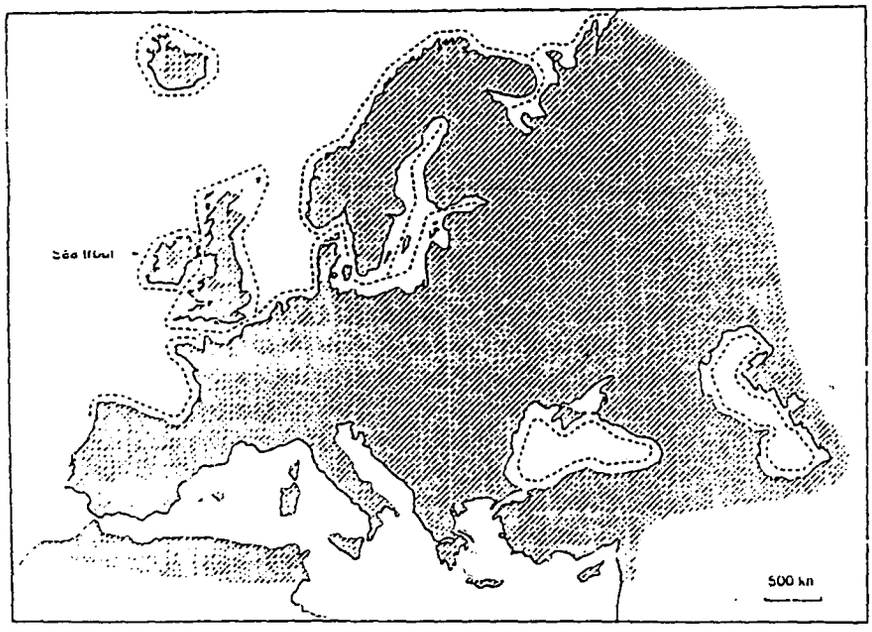


Figure 2. The geographic distribution of brown trout (From Elliot,1994)

Anadromous sea-trout populations occur in western Europe from latitude 42° northwards (dashed lines in figure 2). Sea-trout is absent in the Mediterranean Sea but it has been recorded in the Blacksea and Caspian sea (Elliot, 1994).

Brown trout have been introduced successfully into at least 24 countries outside Europe (Fig. 3).

1.2. Turkish brown trout populations:

Previous studies, as reviewed by Kuru (1975), done by employment of morphometric and meristic characters revealed that the members of *Salmo trutta* L. species is present in Turkey. Also from Seyhan river basin a salmonid species, *Salmo platycephalus* was identified from Turkey, in 1967 (Benkhe, 1986).

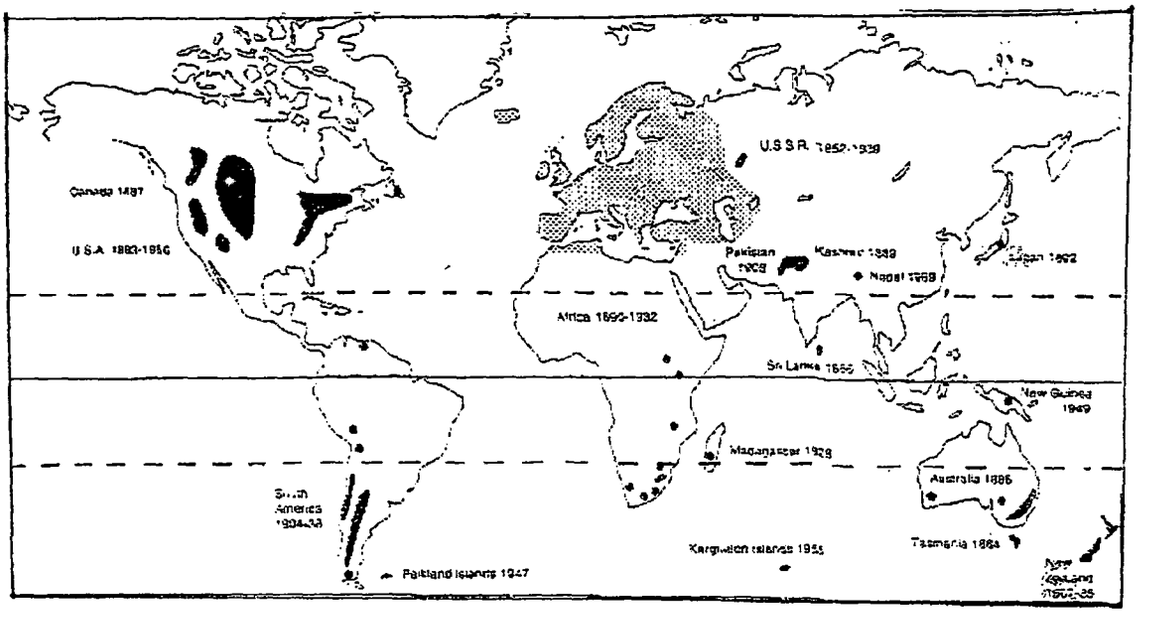


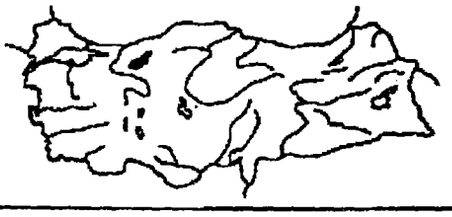
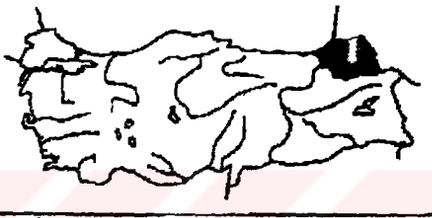
Figure 3. World distribution of Brown trout (From Elliot,1994).

According to the previous observations and studies it has been believed that there are four subspecies (or geographic forms as called by Geldiay and Balık, 1988) of *Salmo trutta* L. species in Turkey (Tortonese, 1954). The names and distributions of these subspecies are shown in Table 1 (Geldiay and Balık, 1988).

1.3. Interspecific relationships between Atlantic salmon (*Salmo salar*) and Brown trout (*Salmo trutta*)

Genetic differences between species in the same genus such as Atlantic salmon and brown trout can be easily detected because many enzyme loci are fixed for different alleles. In these two species if certain loci are examined, individual fish can be assigned to a particular species (Elliot, 1994).

Table 1. The names and distributions of subspecies of brown trout found in Turkey (Geldiay and Balık, 1988).

NAMES OF SUBSPECIES	DISTRIBUTION SUBSPECIES
S. trutta macrostigma A. DUMERIL	
S. trutta abanticus TORTONESE	
S. trutta labrax PALLAS	
S. trutta caspius KESSL	

These two species can hybridize and can have steril F1 generation. Hence, percent steril individuals can be easily identified.Presence of the hybrids can be easily detected by the protein markers PGM and GPI.

In Newfoundland, Canada the increased rate of hybridization between Atlantic salmon and brown trout had been observed in areas, where brown trout have been introduced (Verspoor, 1988). The reverse is true for brown trout hybridization with the introduced Atlantic salmon in Spain (Garcia de Leaniz and Verspoor, 1989).

1.4. Use of molecular markers in studies of natural and cultured fish populations:

Molecular markers can be used in species and hybrid identification; establishment of species and population phylogeny and phylogeographic history; determination of population (stock) structure; individual stock contribution to mixed stock fisheries; measuring level of genetic variation and the change in genetic variation in wild and cultured populations; estimation of effective population size; identification of key populations for conservation; locating source material for supplementation and restoration; determining genetic impact of deliberate and inadvertant introduction of cultured fish on natural populations; assessing performance of stocked fish; establishing gender of individuals; determination of breeding strategies; comparing relative fitness of life history variants; elimination of 'tank effect' in breeding experiments; gene mapping; quantitative trait linkage analysis and assessing success of genomic manipulations (Ferguson et al.,1995).

As indicated above, while many genetic studies of fish populations are directed towards management improvement of this natural and cultured food and recreational resource, it has been appreciated increasingly that variation within and among all fish species is an important component of biodiversity and it should be conserved for its intrinsic value (Ferguson et al., 1995).

1.5. Protein variants as genetic markers:

Molecular genetic markers are basically of two types; protein and DNA. The initial studies in the 1960s involved proteins like hemoglobin and transferrin, but by the establishment of activity staining (Hunter and Markert, 1957 cited in Ferguson et al.,1995) enzymatic proteins (allozyme variation) dominated the studies.

Over 100 protein loci have now been assayed in Salmonids (Elliot, 1994). In brown trout, one of the most polymorphic of all fish species usually two or three alleles segregate at a locus in a particular population and average heterozygosity levels are

less than 10 % (Ferguson et al. 1995). The advantages and disadvantages of protein markers are given in Table 2.

Table 2. The advantages and disadvantages of protein markers (From Ferguson et al. 1995).

Advantages of proteins	**Disadvantages of proteins**
Samples can be analyzed quickly	Tissue collection and storage (fresh or freshly frozen tissue is required)
Relatively low cost of chemicals	several tissues and relatively large amounts required most of the time killing of specimen can not be avoided)
Easy to learn technique	Low number of alleles per locus
Proteins with known functions	Only proteins detectable with histochemical stain can be examined
Some loci subject to selection	Analysis of patterns can be difficult in polyploids
Extensive database available for many species	Low funding potential since percieved by some as outdated methodology.

1.6. Genetic structure determination based on protein electrophoresis studies:

The early molecular studies of fish populations involved serology and blood groups, then in the sense of direct examination of macromolecules the real molecular studies have started. The development of starch gel electrophoresis (Smithies, 1955) coupled with histochemical staining (Hunter and Markert, 1957) allowed the detection of enzyme and other protein polymorphisms so that the first applicable simple genetic markers for large scale studies on natural populations were provided. Since 1964, electrophoretic examination of protein variants became a method for studying fish populations.

By the use of electrophoretic technique, the functionally similar but structurally different forms of enzymes that are encoded by one or more loci called isozymes, could be observed directly so that the genetic diversity of the population can be studied.

When the products of a single locus has alternative forms, it is called alleles. If the frequencies of at least two of the alleles is higher than 0.01, the locus of the gene is named as polymorphic, otherwise it is known as monomorphic.

The products of alleles at a single locus is called as allozymes. If an allozyme consists of two or four subunits, and if these are the products of different alleles of different loci, these allozymes are called heterodimers or heterotetramers. Consequently, if the alleles of the same locus are combined to form an allozyme, they are called homodimers or homotetramers.

The bands of isozymes or allozymes are observed on the gel for many individuals from a population and they are called zymogram. They can be used to calculate the allele frequencies of a particular locus or loci. Based on this information different measures of genetic variation of a population such as polymorphism or heterozygosity can be calculated. Relatively low variability found in the population may indicate the presence of higher degree of inbreeding compared to those of other populations. Allelic frequencies of two populations can be used to calculate the genetic distance between them. The most widely used genetic distance is the Nei's (1972) genetic distance.

1.7. Hypothesis on phylogeography of European brown trout populations

First studies on phylogeography of brown trout populations were based on the protein marker LDH-C (Ferguson and Flemming, 1983), had seperated brown trout populations into three populations with different alleles. LDH-C*90 marked the Atlantic populations while LDH-C*100 marked the Mediterranean populations and

LDH-C*110 marked the Po population at the Adriatic basin which is named as *Salmo marmaratus*, marbled trout.

After this preliminary classification of populations, further researches revealed more detailed phylogeographic groupings of brown trout populations (Apostolidis et al., 1996, Bernatchez and Osinov, 1995, Garcia-Marin et al. 1998).

Apostolidis et al. (1996) classified Greek populations into two groups; Mediterranean (South-Adriatic-Ionian zone) and Danube (Ponto-Aegean zone). According to their hypothesis, 2.5-6.0 million years ago divergence of Atlantic and Mediterranean stocks occured. At that time isolation of Mediterranean sea from Atlantic ocean took place and connections between Mediterranean an Paratethys were interrupted. Atlantic and Mediterranean brown trout groups were formed at this time. At the end of Messnian both Mediterranean and Paratethys were reduced to a network of lakes and named Lago Mere. Lago Mere stage played an essential role for the isolation of Mediterranean brown trout populations like West-Mediterranean, East-Mediterranean and Adriatic stocks.

Bernatchez and Osinov (1995) studying on Russian brown trout populations concentrated on the phylogeography of North-East Europe and Blacksea populations. They proposed three scenarios about the penetration of ancestral stocks to different regions. According to first scenario; brown trout had originated in Atlantic basin and penetrated the Black sea, Caspian sea and Aral sea basins recently (15000 ybp). This colonization was possible by headwater connections of the Paleo-Rhine and Danube water systems. On the other hand, second scenario suggested that brown trout might have penetrated to the Black sea from the Mediterranean basin. Third scenario stated that Caucasian trout populations consisted the primitive form which all populations of Southern sea basins radiated and expanded their range (Derzhavin, 1934 and Vladimirov, 1944, 1948).

Results based on allozyme and mtDNA markers suggested that;

1. The whole Atlantic basin was recolonized by a single southern Atlantic group which might have diverged from others more than 500000 years ago (Osinov and Bernatchez, 1996)

2. Danube populations was found to be one of the most ancient stocks according to the mtDNA studies (Osinov and Bernatchez,1996).

3. At the remote headwaters of Danube drainage in Austria, Germany, and Slovenia Danube populations might have mixed with fish from Atlantic populations (Osinov and Bernatchez, 1996).

4. It was suggested that Black, Caspian and Aral seas were scarcely a center of brown trout origin. Indeed they might have been colonized from the north according to mtDNA (Osinov and Bernatchez, 1996).

5. The mtDNA studies' results suggested that there was no gene exchange between populations of Black, Caspian and Aral sea basins and the Mediterranean sea, but there could be a possible genetic contact between populations of Blacksea and Mediterranean sea (Osinov and Bernatchez,1996).

The recent studies on the phylogeography of brown trout populations was done by Garcia-Marin et al. (1998). They divided the populations into 4 lineages according to the protein markers (see Figure 4). The discriminating markers for the lineages are in Figure 5 .According to their hypothesis the chronological events that took place in the brown trout phylogeographic group development was as follows:

18000 ybp there was Würmian ice-sheet on Eastern Europe and Western Siberia rivers. Ice-damned lakes and ice-marginal streamways had started to discharge to

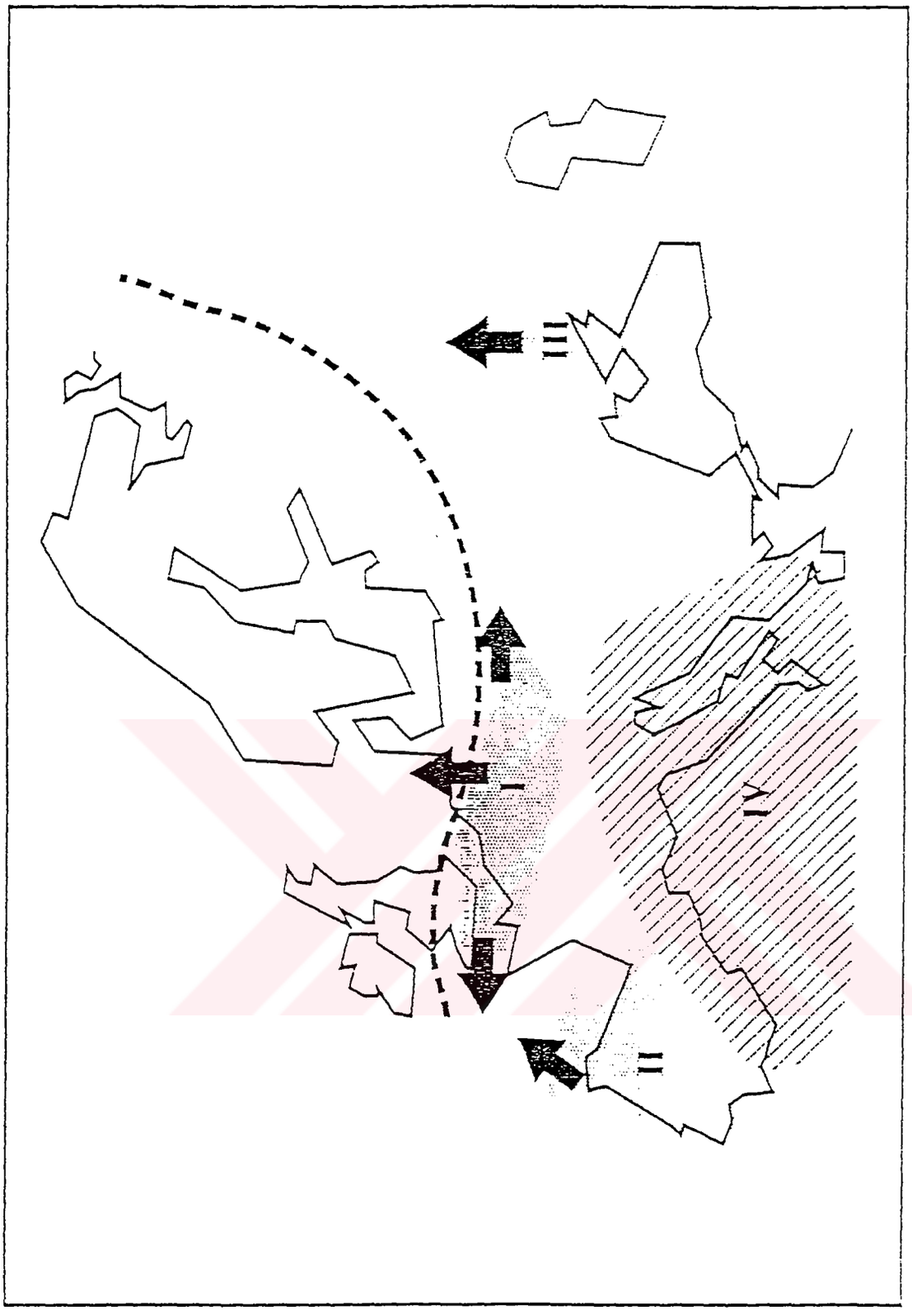


Figure 4. The map of the phylogegraphic groups constructed by using protein markers (From Garcia-Marin et al. (1998)).

south into the Aral, Caspian and Black seas and then continued into Mediterranean sea.

Lineage I (North Atlantic)	Lineage II (South Atlantic)	Lineage III (Danube)	Lineage IV (Mediterranean)
LDH-C*90 (+)	LDH-C*100 (+)	LDH-C*100 (+)	LDH-C*100 (+)
CK-A1*100 (+)	CK-A1*115 (+)	CK-A1*100 (+)	CK-A1*100 (+)
LDH-A2*QL (-)	LDH-A2*QL (-)	LDH-A2*QL (-)	LDH-A2*QL (+)

Figure 5 . The Lineages and the discriminating markers.

13500 ybp, the radial drainage system was transformed into a single marginal one that extended westwards into Norwegian sea. Those events permitted the northward migration of the lineage III and their introduction into Northern Europe in the early postglacial period. The presence of LDH-C*100 at the intermediate and high frequencies in the Norwegian samples was accepted as an evidence for the hypothesis given above (Garcia-Marin et al.,1998). From these bridgehead populations lineage III expanded to deglaciated areas which was evidenced by the presence of LDH-C*100 and CK-A1*100 alleles with fixed or high frequencies in Scottish populations (Garcia-Marin et al.,1998).

Also 13500 ybp the recolonization source was the nearest refuge, for example British Isles was colonized from unglaciated Southern Britain (Lineage I). The presence LDH-C*90 in British Isles supported this proposal. Also there was extra colonization of Britain by lineage II which was evidenced by the presence of polymorphism in LDH-C* locus and presence of CK-A1*115 at low to moderate frequencies. In Britain further colonization by lineage III was also observed.

10000 ybp Baltic sea was an ice-marginal lake with no connection to the North sea.This lake was colonized initially by lineage III invasion. After this time with a connection, the Baltic Basin became a bay of the Atlantic and colonized by lineages I and II from other deglaciated areas of Western Europe. Begining about 7500 ybp isostatic uplift caused some founded Baltic populations to become more isolated.

1.8. Aim of the present study

In the present study based on protein markers Turkish brown trout populations will be studied for the following purposes:

1. To determine the genetic structures of Turkish brown trout populations with respect to discriminator markers, such as LDH-C, CK-A1 and LDH-A2 loci and thereby define the phylogeographic status of the populations.

2. To find the priority in conservation of the populations. For this purpose:

i. Presence of the unique alleles

ii. Presence of introgression

iii. Degree of genetic variability within the populations will be examined.

3. To find out if the Abant brown trout population is an endemic one as claimed by Tortonese (1954).

4. To find out whether there was any hybridization between Atlantic salmon (*Salmo salar*) and Blacksea brown trout populations.

CHAPTER 2

MATERIAL AND METHODS

2.1. Samples

The fish used in the present study were obtained from Alakır river, near Kumluca, Antalya; from Eşen river, near Fethiye, Muğla; from Lake Abant in Bolu; Mudurnu stream tributaries in Bekdemirler village near Taşkesti, Mudurnu; from Sümer stream near Arhavi, Rize; from Yeniyol, Kapisre, Güzelyalı and Hemşin, these are small streams between Pazar (Rize) and Arhavi (Artvin) and from Fırtına river near Ardeşen, Rize. The geographic locations are shown on the map in Figure 6. The detailed information on the populations are given in Table 3 which also shows from where the rivers are born, name of subpopulations, the distances between the subpopulations and the sample sizes.

Since the number of individuals were few from Yeniyol, Kapisre, Güzelyalı and Hemşin populations samples were pooled and named as Blacksea population. Abant population consisted of two subgroups Abant-1, which was collected from amateur sportsfishers fishing around Lake Abant and Abant-2, which was collected from the hatchery of National Park in Gölcük, Bolu. Mudurnu population consisted three subgroups; Mudurnu-1 subpopulation was the first collected one consisting of two specimens from Bekdemirler village after the fish farm. Mudurnu-2 population was from the same location again consisting of two specimens which were caught approximately six months later. Also at the same time period Mudurnu-3 samples were caught from Kurşunlu near Bekdemirler and there were eight fishes in this

population. Alakır population consisted two subpopulations Alakır-1 and Alakır-2. There was 400m distance between the two sites, Alakır-2 both being close to the head water. The third population from Alakır river was Gödene (Alakır-3). Fish of this population was caught near village Altınyaka nearly 30 km away from the first two Alakır populations towards the down stream.

Table 3. The detailed information of the populations studied.

Population	Subpopulations	Sample size	Location	Additional information	Spring
Alakır	Alakır-1	28	Büyükalan village,Karaağaç, Antalya		Beydağları
	Alakır-2	15		They are 400m apart, close to head water	
Gödene (Alakır-3)		18	Altınyaka, Kumluca, Antalya		
Eşen		21	Kemer,Fethiye, Muğla	300m to source	Kızılcadağ
		4		800m to source	
Abant	Abant-1	16	Lake Abant, Bolu		Abant Mountains
	Abant-2	16			
Mudurnu	Mudurnu-1	2	Bekdemirler village, Mudurnu, Bolu		Bolu Mountains
	Mudurnu-2	2			
	Mudurnu-3	9	Kurşunlu, Bekdemirler village, Mudurnu, Bolu		
Blacksea	Yeniyol	3	Pazar, Rize		Pontic Mountains
	Kapisre	14	Arhavi, Artvin		Pontic Mountains
	Güzelyalı	1	Between Fındıklı and Arhavi	150m to sea	Pontic Mountains
	Hemşin	5	Pazar, Rize		Pontic Mountains
Sümer		4	Fındıklı, Rize	Derbent tributary	Pontic Mountains
		29		Lower-right tributary	
Fırtına		40	Ardeşen, Rize		Pontic Mountains

For sampling of Alakır, Gödene and Eşen populations electrofishing was used. Lake Abant samples were obtained by two ways: Abant 1 samples were obtained from the fishermans around the lake and Abant 2 samples were obtained from the hatchery of National Park in Gölcük, Bolu. For Mudurnu population cast-net was used. For

Sümer, Fırtına and Blacksea populations both electrofishing and cast-net was used. The detailed information about the sampling sites are given in Table 4.

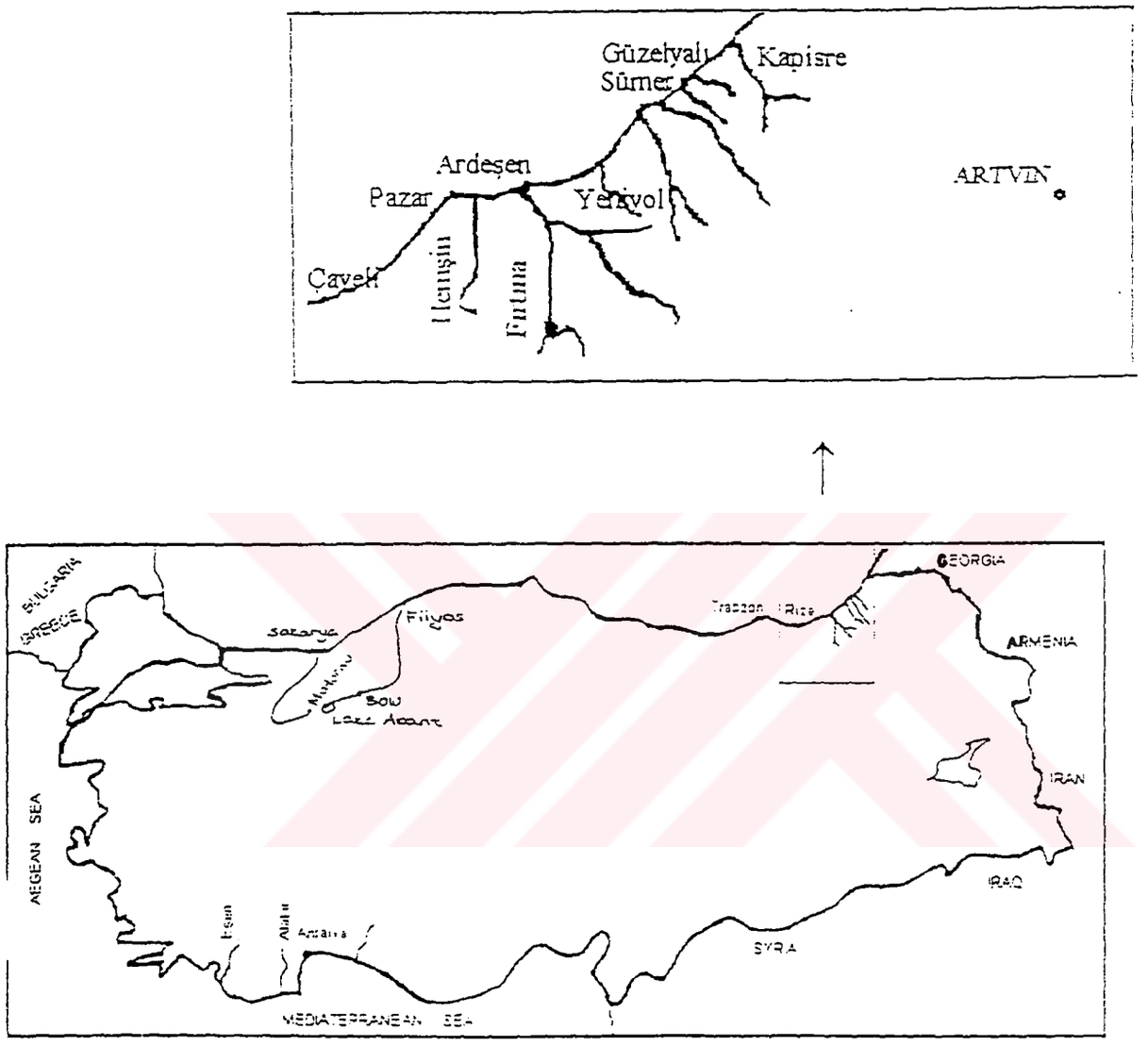


Figure 6. The geographic locations of the populations studied.

TABLE 4. Information about the presence of fish farms and types of the bottom of the stream on sampling sites.

Population	Subpopulations	Farms of Rainbow trout and Atlantic salmon	Bottom of stream	Notes
Alakır		Absent	Gravel	
Gödene (Alakır-3)		Present	Gravel	Water-mill
Eşen		Present	Gravel	
Abant		Present	Gravel	
Mudurnu		Present	Gravel	1 rainbow trout was caught . Atlantic salmon was reared in a hatchery.
Blacksea	Yeniyol	Present	Gravel	11 rainbow trouts were caught. Atlantic salmon was reared in a hatchery.
	Kapisre	Absent	Gravel	
	Güzelyalı	Absent	Gravel	
	Hemşin	Present	Gravel	
Sümer		Present	Gravel	1 rainbow trout was caught. An otter was observed.
Fırtına		Present	Gravel	

2.2. Sample preparation

Immediately after the catch before fish died blood was taken from the caudal vein of the fish and put into eppendorfs and stored at 4 °C in order to avoid hemolysis and loss of the transferrin and EST-D activities. Then the fish were quickly frozen on dry ice (-70 °C) which was supported by liquid nitrogen to inhibit the denaturation of proteins and samples were transported to the laboratory and stored at -80 °C in deep freeze.

For other protein markers the fish were dissected before they were completely thawed and approximately 1 gram of tissue samples from liver, muscle and eye (with lens) were taken. Equal volume of 0.01 M Tris-HCl (pH 7.4) buffer was added to each of the tissue samples and homogenized by a homogenizer at 4000 rpm. for 30 seconds, on ice until a good homogenate was obtained. Homogenates were stored in -80 °C

and prior to electrophoresis they were rethawed, centrifuged at 13000 g. for 20 minutes and the supernatants were stored to use for electrophoresis.

For Transferrin and EST-D, blood samples were used. For 24 hours the samples were kept at 4 °C and then they are centrifuged at 1000 g for 30 minutes. For transferrin study, the supernatants were collected and put to -80 °C for at least 1 day. The samples were then rethawed and to 50 µl blood serum (supernatant) 60 µl ethodin (6,9 diamino-2 ethoxyacridine (3 gr./100 ml H_2O)) was added and mixed with vortex. Then the mixture was placed on ice for an hour, 10 µl chloroform was added and the mixture was vortexed for 30 seconds again. The isolates were centrifuged at 12000 g for 30 minutes at 4 °C. Then the supernatants were collected and stored at -80 °C until electrophoresis was carried out. Electrophoresis of those samples were done within 2 days from isolation.

For EST-D the pellets of the original blood were used. On the pellets a few drops of distilled water was added and mixed. Then that mixture was kept at -80 °C until electrophoresis.

2.3. Electrophoresis of samples

2.3.1. Starch gel electrophoresis

2.3.1.1. Buffers:

Two buffer systems; tris-citrate-borate (TCB) and citrate-amino propyl morpholine (AM) were used during the electrophoresis. Compositions of gel buffer that was used during the preparation of gels and electrode buffer that was used to make connection between the electrodes and the gels were given below:

TCB buffer system:

Gel buffer: 0.076 M Tris (hydroxymethyl) amino methane, 0.005 M Boric acid, 0.015 M Lithium hydroxide .

Electrode buffer: 0.3 M Boric acid and 0.1 M Lithium hydroxide.

Both gel and electrode buffers were adjusted to pH 8.6 with 0.1 M Boric acid or 0.1 M Citric acid if necessary.

AM buffer system:

Gel buffer: Electrode buffer was diluted to 1:20. (result 0.002 M Citric acid).

Electrode buffer: 0.04 M Citric acid adjusted to pH 6.4 with N-(3-Aminopropyl)-morpholine.

2.3.1.2. Preparation of the gel

Horizontal starch gel was prepared with 8 % (w/v) hydrolyzed starch in the gel buffer (for further information on buffers see Appendix A) in a vacuum flask. The mixture was heated on a bunsen burner until it becomes transparent and boils. The mixture was degased with vacuum pump to ensure the mixture is free of air bubbles. The process was continued until large bubbles boiled through the solution. The starch was poured into the mold on a flat surface and the gel was left to polymerization at room temperature then placed at 4 °C in a refrigerator for cooling. Prepared gels were used on the same day.

2.3.1.3. Electrophoresis:

After the starch gel was prepared the upper layer of the gel was removed in order to have a smooth flat surface then wells were made across the width of the gel by combs. The small rectangular papers (2 x 4 mm) (Whatman Grade 17) were soaked in sample extracts and were loaded into the wells by using a pair of tweezers. It was checked that the tip of each paper strip touched the mold. Into the first and last wells small amounts of bromophenol blue solution was introduced since it migrates slightly faster than the proteins and therefore it was a useful indicator to estimate the extend of migration. The gel in the mold was placed on the cooling plate of the electrophoresis tank (LKB 2117 Multiphor II). Power supply (KRL SSA310) provided the direct current source. The connection of the electrode buffer with the gel was maintained by sponges (Vileda, Freudenberg Household Products).

The electrophoretic migration was carried out at 4 °C. Cooling circulator (Karipek, Konya) was used to cool the system. Electrophoresis was carried out at 16 V / cm when AM buffer system was used and at 14 V / cm when TCB buffer was used. Electrophoretic migration was stopped when the the bromophenol blue moved to a distance of 8 - 9 cm from the origin. This process took 3-4 hours. The picture of the system was shown in figure 7.

2.3.1.4. Staining the protein bands:

After electrophoresis, the gels were placed in appropriate staining media and incubated. For each enzyme system, the reaction mixtures and conditions for staining are indicated in appendix B and the brands and codes for the chemicals used are given in appendix C.

In total 10 enzyme systems were assayed (Table 5). The enzymes converted their substrates to products which in turn binded with the dye in the media and formed

bands that could be visualized on the gel. The reaction mechanisms of the enzymes have been given in Appendix D.

FIGURE 7. The photograph of the electrophoresis system

2.3.2. Cellulose acetate paper electrophoresis:

For EST-D enzyme system cellulose acetate paper electrophoresis was used. The cellulose acetate paper (Sartorius 11200-70-145 BBK) was incubated in gel buffer (see Appendix A) for at least 2 hours prior to electrophoresis. Then the excess buffer was blotted by a tissue paper and the cellulose acetate paper was placed in tank (Sartorius, Sartorphor electrophoresis system, US Pat. No: 4130 471; 4061561) in electrode gel (See Appendix A). The samples and bromophenol blue were applied to cathodic side of the tank. The electrophoresis was carried by 200 V (1 - 2.5 mA) for 1 hour at room temperature. After electrophoresis the cellulose acetate paper was incubated with reaction mixture (see Appendix B) at room temperature for 5 - 10 minutes then it was viewed on long wave ultra-violet light.

2.3.3. Interpretation of the bands

After the staining, the gels were examined and the genotypes of each individual was recorded based on previous information about the proteins and their alleles (Fidan (1995), Guyomard and Krieg (1983), Karakousis (1990), Largiader and Scholl (1995), Ryman et al. (1979), Taggart et al. (1981)).

2.3.4. Evaluation of the data

The data concerning the genotypes of the individuals were organized in the format of POPGENE software (Yeh et al., 1997) (Appendix E), which is a computer programme that estimates the parameters for population genetics and analysis was performed by POPGENE software. The allele frequencies calculated by POPGENE software were further organized for the NTSYS computer programme (Rohlf, 1997) and various analyses were performed. With these computer programmes the following parameters were estimated; allele frequencies, allelic richness, proportion of the polymorphic loci, heterozygosity, F-statistics, genetic distances and neighbour joining trees were constructed.

Table 5. The protein systems assayed the tissues and the buffer system used.

System studied	E. C. No .*	Loci studied	Tissue	Buffer system
AAT	2.6.1.1.	AAT -1,2 AAT -4	Muscle Liver	AM
CK	2.7.3.2.	CK -A1 CK -A2	Muscle Muscle	TCB
EST	3.1.-.-.	EST -D	Blood	Tris-Maleic
LDH	1.1.1.27.	LDH -A1 LDH -A2 LDH -B2 LDH -C	Muscle Muscle Liver Eye	TCB
MDH	1.1.1.37.	MDH -A1 MDH -A2 MDH -B1,B2	Muscle Muscle Muscle	AM
MEP	1.1.1.40.	MEP -1 MEP -2 MEP -3	Muscle Muscle Muscle	AM
GPI	5.3.1.9.	GPI -1 GPI -2 GPI -3	Muscle Muscle Muscle	TCB
PGM	5.4.2.2.	PGM -1	Liver	TCB
SOD	1.15.1.1.	SOD -1	Liver	TCB
TF			Blood	AM

* According to Shaklee et al.

2.3.4.1. Allele frequencies

After determination of the banding patterns and collection of the data, the estimation of the allele frequencies was done by POPGENE software and the following formula (Nei, 1987) was used;

$$f(A_i) = \hat{x}_i = \frac{(2N_{ii} + \sum_{j=1}^{m} N_{ij})}{2N}$$

where $f(A_i)$ represents the frequency of any allele, N represents the population size, N_{ii} and N_{ij} represent the number of A_{ii} and A_{ij} genotypes and m represents the number of alleles.

After calculation of allele frequencies, The Hardy -Weinberg equilibrium frequencies were calculated.

2.3.4.2. Measures of Genetic Diversity within populations

Measures that would allow the quantification of the genetic diversity information were needed in order to document the amount of genetic diversity in a standardized way. This quantification was done with the estimation of allelic richness, proportion of polymorphic loci and heterozygosity.

Allelic richness:

The mean number of alleles per locus (n_a) is a component of genetic diversity. It was also called as the allelic richness and was very sensitive to the sample size. The formula used to calculate n_a was;

$$\text{Mean } (n_a) = \frac{\sum_i n_{ai}}{r} \quad \text{(Nei, 1987)}$$

where n_{ai} is the number of alleles at the i^{th} locus and r is the number of loci.

Proportion of the polymorphic loci:

In this study if the most common allele frequency was 0.99 or less, the loci was accepted as polymorphic.The proportion of the polymorphic loci was calculated by;

$$\hat{p} = \frac{np}{r}$$

where np is the number of polymorphic loci, r is the number of loci (Nei, 1987).

Heterozygosity:

Genetic diversity in a population was most commonly measured by the amount of heterozygosity.

The average gene diversity (h_{avg}) over all loci was calculated by;

$$\hat{h}_{avg} = \frac{\sum_{j=1}^{r} \hat{h}_j}{r}$$

where r represents number of loci, $\hat{h}_j$ represents the value of heterozygosity for the j^{th} locus and for any locus heterozygosity is defined as follows (Nei, 1987):

$$\hat{h} = \frac{2N\,(1 - \Sigma\, \hat{x}_i^2)}{2N-1}$$

where $\hat{x}_i$ is the estimated allele frequency of x_i, N is the sample size.

The sampling variance of $\hat{h}_{avg}$ was calculated by the following formula;

$$V(\hat{h}_{avg}) = \frac{V(\hat{h}_j)}{r}$$

where $V(\hat{h}_j) = \frac{\sum_{j=1}^{r} (\hat{h}_j - \hat{h}_{avg})^2}{r - 1}$

2.3.4.3. Gene diversity statistics

Some number of genetic measures that help to understand the distribution of the genetic diversity within and among populations were calculated.

F-Statistics:

Using the measures of heterozygosity, three levels of inbreeding were defined and these were estimated by using POPGENE software.

Wright (1943,1951,1965,1969) proposed to measure the deviations of genotype frequencies in terms of three parameters F_{IS}, F_{IT} and F_{ST} for each locus, which are often called fixation indices or F-statistics.

F_{IS} measures the reduction in heterozygosity of an individual due to nonrandom mating within its subpopulation or in other words it is the estimated inbreeding coefficient due to nonrandom mating within subpopulations (Hartl and Clark ,1989).

F_{ST} is the reduction of heterozygosity of a subpopulation due to random genetic drift or in other words it is the amount of inbreeding due to the presence of population subdivision (Hartl and Clark, 1989) . F_{ST} is a measure of the genetic differentiation of subpopulations and is always positive (Hedrick, 1985).

F_{IT} is the overall inbreeding coefficient which is the amount of inbreeding due to combined effects of nonrandom mating within subpopulations and due to random genetic drift between the subpopulations (Hartl and Clark ,1989).

2.3.4.4.Genetic Distances

2.3.4.4.1.Genetic Distances

Genetic distance is the measure of gene differences between pairs of populations (or species). Distance measures are generally analogous to geometric distances, for example, zero distance is equal to no difference between groups.

The most widely used genetic distance measure was that of Nei's (Nei, 1972). Nei's (1972) standard genetic distances between the populations were calculated by NTSYS computer programme and using the calculated genetic distance matrix, a dendogram based on neighbour joining method (Saitau and Nei,1987) was drawn.

2.3.4.4.2. Computing Genetic Distances and drawing neighbour joining dendogram of Greek, Russian and other European brown trout populations together with Turkish ones

For constructing the neighbour joining dendograms of Greek, Russian, Spanish and other European brown trout populations data from these countries were used. The geographic locations and the full names of the populations, Karakousis and Triantaphyllidis (1988) and (1990), Apostolidis et al.(1996), Osinov (1988) , Osinov and Bernatchez (1996) were in Figure 8.

Using the data provided by these researchers , the genetic distance matrices and neighbour joining trees were constructed (Fig 11).

While constructing trees allele frequencies of CK-A1, CK-A2, LDH-A1, LDH-A2, LDH-B2, LDH-C, GPI-2 and SOD-1 loci were used since for all populations, data on these loci were present and these loci were reliable in a sense that the same alleles were named similarly by all researchers.

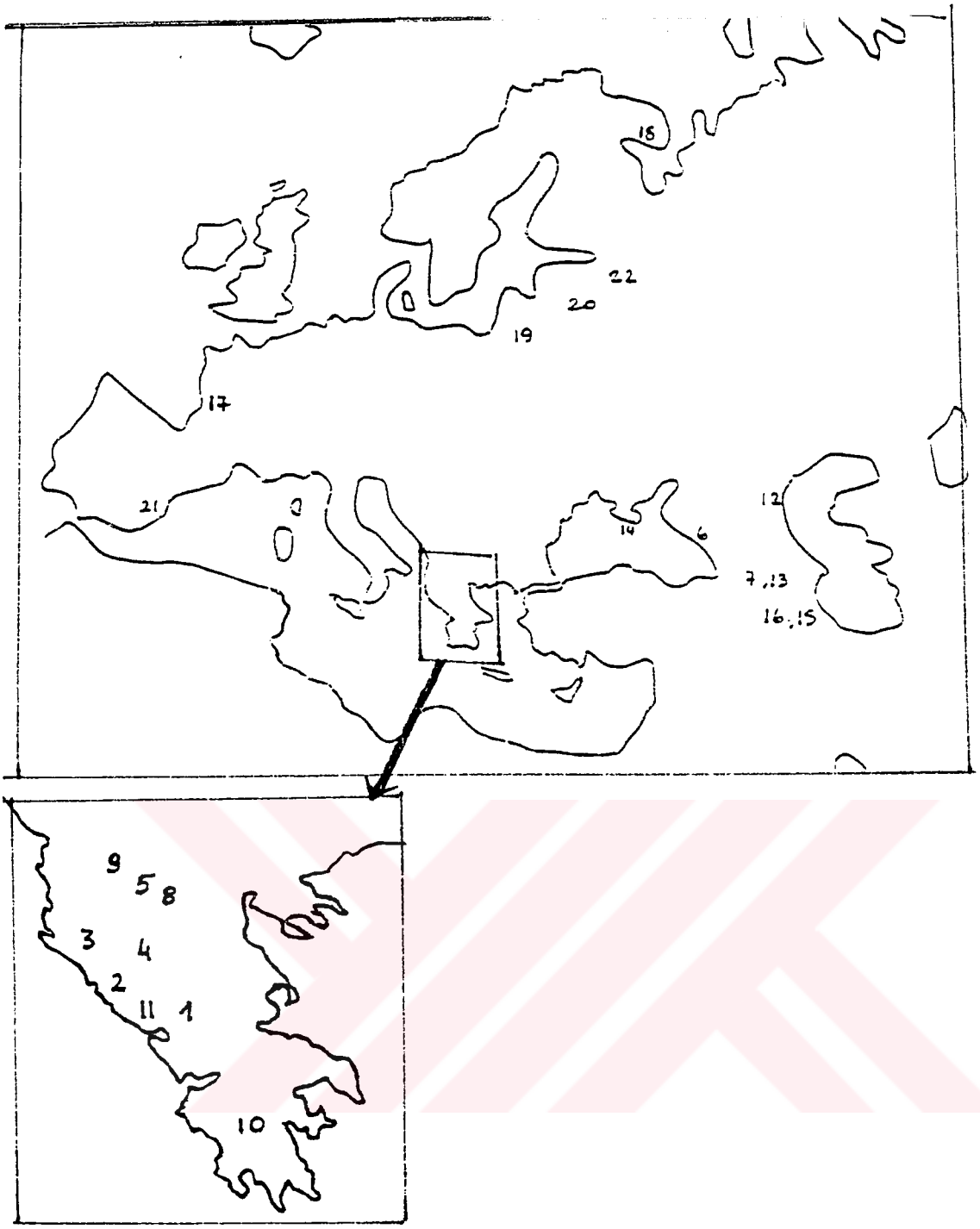


Figure 8. The geographic locations and the full names of the Greek, Russian, Spanish and other European populations (From Apostolidis et al.(1996), Osinov and Bernatchez (1996)).

(1)*Acheloos-2*, (2)*Thyamis*, (3) *Voidomatis*, (4) *Aoos*, (5) *Agios Germanos*, (6) *Bl-1* (Blacksea- Kodori river), (7) *Se-1* (Lake Sevan), (8) *Drosopigi*, (9) *Ohrid*, (10) *Alfios*, (11) *Lauros*, (12) *Ca-1* (Caspian sea-Terek river), (13) *Sfg-2* (Makenis- Lake Sevan), (14) *Bl-2* (Blacksea-Crimea), (15) *Kcm-1* (Arpa river), (16) *Ca-3* (Caspian sea- Arpa river), (17) *Garonne*, (18) *Wh-2* (Vorob'ev creek), (19) *Tachov*, (20) *Ba-1* (Baltic sea- Mezna river), (21) *Jucar-1*, (22) *Ca-6* (Caspia sea- Tsna river).

CHAPTER III.

RESULTS

3.1. Observed alleles of the enzyme systems

The genetic structures of 8 Turkish Brown Trout populations, namely, Alakır, Gödene (Alakır-3), Eşen, Abant, Mudurnu, Sümer, Blacksea and Fırtına were determined by starch gel and cellulose acetate paper electrophoresis by studying 21 loci of 10 enzyme systems.

For AAT, LDH, MDH, GPI, PGM and SOD enzyme systems the electrophoretic patterns and their interpretations were made according to the previous studies done by both Turkish researchers Fidan (1995) and Yalın (1996) and foreign researchers (Guyomard and Krieg (1983), Karakousis (1990), Ryman et al. (1979), Taggart et al. (1981)). For the other enzyme systems (CK, EST, MEP and TF) studies done by Karakousis (1990), Garcia-Marin and Pla (1996), Largiader and Scholl (1995) and Bernatchez and Osinov (1995) were used as main references. The most common allele for the loci was named as *100 (see Figure 9 for the bands observed throughout the study).

In Table 6 the alleles, their frequencies, proportion of polymorphic loci, number of polymorphic loci, effective number of alleles, mean number of alleles and average expected heterozygosities have been demonstrated.

3.1.1. AAT Enzyme System (2.6.1.1)

For this enzyme system three loci ,namely, AAT-1,2 and AAT-4 were studied.

In all of the populations AAT-1,2 loci was observed to be monomorphic and was named as *100 in accordance with the previous interpretations of this loci by Fidan (1995) and Yalın (1996).

AAT-4 locus, was polymorphic in all of the populations studied. The variant allele in this loci was called as AAT-4*74 in accordance with the sudies done by Fidan (1995) and Yalın (1996).

AAT-4 loci was determined to be in Hardy-Weinberg equilibrium except in Mudurnu population. The maximum frequency for the AAT-4*74 allele was observed in Mudurnu with a frequency of 0.5769, whereas the minimum frequency for the AAT-4*74 allele was observed in Gödene (Alakır-3) population with a frequency of 0.2941. The Fst value for this locus was observed to be 0.0272.

3.1.2. CK Enzyme System (2.7.3.2)

For this enzyme system CK-A1 and CK-A2 loci were studied.

In all of the populations, CK-A1 is found to be monomorphic with the designated allele *100.

In CK-A2 locus, CK-A2*100 and CK-A2*50 alleles were observed. CK-A2*100 allele was the common allele which was observed as monomorphic in 6 of the populations studied (see Table 6). In Mudurnu population CK-A2*50 allele was

observed with a frequency of 0.1538. Also in Fırtına population CK-A2 *50 allele was observed in only one individual out of 40 with a frequency of 0.0250.

CK-A2 loci was not in Hardy-Weinberg equilibrium in Mudurnu and Fırtına populations. The Fst value for CK-A2 was 0.1161.

3.1.3. EST Enzyme System (3.1.-.-)

For this enzyme system only EST-D locus was studied using cellulose acetate paper electrophoresis in Abant, Mudurnu, Sümer, Blacksea and Fırtına populations and it was monomorphic with the allele *100 in the studied populations.

3.1.4. LDH Enzyme System (1.1.1.27)

For this enzyme system 4 loci, namely, LDH-A1, LDH-A2, LDH-B2 and LDH-C were studied from muscle, liver and eye tissues.

In LDH-A1 locus *100 and null (called as ∅ (Karakousis and Triantaphyllidis (1988)) or called as*100QL (Garcia-Marin and Pla,1996) alleles were observed. LDH-A1 locus was observed to be polymorphic in all of the populations. The highest frequency for LDH-A1*QL allele was observed in Abant population with a frequency of 0.2414. This locus was in Hardy-Weinberg equilibrium except in Alakır and Abant populations. The Fst value for this locus was 0.0552.

In LDH-A2 locus *100 and *QL alleles were observed. This locus was polymorphic with the highest frequency of *QL allele observed in Eşen population as 0.1400. This locus was in Hardy-Weinberg equilibrium in all populations. The Fst value for this locus was 0.0126.

In LDH-B2 locus *100 and *120 alleles were observed. In Alakır and Gödene (Alakır-3) populations only LDH-B2*100 allele was observed whereas in Eşen and

Fırtına populations only LDH-B2*120 allele was observed. In Abant, Mudurnu, Sümer and Blacksea populations both LDH-B2*100 and *120 alleles were observed but hybrids for these two alleles were not observed throughout the study.

This locus was in Hardy-Weinberg equilibrium in half of the populations but not in Abant, Mudurnu, Sümer and Blacksea populations. The Fst value for this locus was 0.7144.

In LDH-C locus *90 and *100 alleles were observed.

LDH-C*90 allele was observed in Alakır, Gödene, Eşen and Blacksea populations with the highest frequency 0.3750 in Blacksea population. In Abant, Mudurnu, Sümer, Blacksea and Fırtına populations Only LDH-C*100 allele was observed with a frequency of 1.000.

This locus was in Hardy-Weinberg equilibrium in all of the populations studied except in Blacksea population.The Fst value for this locus was 0.2059.

3.1.5. MDH Enzyme System (1.1.1.37)

For this enzyme system 4 loci namely MDH-A1, MDH-A2, MDH-B1,2 were studied.

Both MDH-A1 and MDH-A2 loci were monomorphic in all of the populations with the allele *100.

In MDH-B1,2 loci polymorphism with the alleles *85,*100 and *125 were observed. The allele names are taken from the previous studies done by Fidan (1995), Yalın (1996) and Karakousis (1990).

MDH-B1,2*100 allele was observed in all of the populations with the highest frequency 1.000 in Eşen population. MDH-B1,2*85 allele was observed in all populations except in Eşen, with the highest frequency 0.4615 in Fırtına. MDH-

B1,2*125 allele was observed in Alakır and Fırtına populations with the frequencies 0.0233 and 0.0128 respectively.

This locus was in Hardy-Weinberg equilibrium except in Gödene (Alakır-3) population. The Fst value for this locus was 0.1335.

TABLE 6. Polymorphic loci, alleles and allele frequencies, number of polymorphic loci, percentage of polymorphic loci, effective number of alleles (ne), mean number of alleles (na) and average heterozygosity in the populations of *Salmo trutta* examined, with ‡ indicating the individual tests not appropriate for varifying Hardy-Weinberg equilibrium and # indicating minimum sample size for each population at the indicated enzyme system.

Enzyme	Allele	Alakır (44)(#25)	Gödene (Alakır-3) (18)(#12)	Eşen (25)(#3)	Abant (32)(#13)	Mudurnu (13)(#2)	Sümer (33)(#11)	Blacksea (23)(#8)	Fırtına (40)(#24)
AAT-4*	74	0.4722	0.2941	0.5000	0.3871	0.5769‡	0.3636#	0.4444	0.4583#
2.6.1.1	100	0.5278	0.7059	0.5000	0.6129	0.4231	0.6364	0.5556	0.5417
CK-A2*	50	0.0000	0.0000	0.0000	0.0000	0.1538‡	0.0000	0.0000	0.0250‡
2.7.3.2	100	1.0000	1.0000	1.0000	1.0000	0.8462	1.0000	1.0000	0.9750
LDH-A1*	QL	0.0769‡	0.0882	0.0200	0.2414‡	0.0385	0.1061	0.1087	0.0250
1.1.1.27	100	0.9261	0.9118	0.9800	0.7586	0.9615	0.8939	0.8913	0.9750
LDH-A2*	QL	0.1282	0.0882	0.1400	0.1034	0.1154	0.0303	0.0652	0.1000
	100	0.8718	0.9118	0.8600	0.8966	0.8846	0.9697	0.9348	0.9000
LDH-B2*	100	1.0000	1.0000	0.0000	0.0938‡	0.1667‡	0.3000‡	0.0769‡	0.0000
	120	0.0000	0.0000	1.0000	0.9062	0.8333	0.7000	0.9231	1.0000
LDH-C*	90	0.0323	0.1111	0.0909	0.0000	0.0000	0.0000	0.3750‡#	0.0000
	100	0.9677	0.8889	0.9091	1.0000	1.0000	1.0000	0.6250	1.0000
MDH-B1,2*	85	0.3605	0.4444‡	0.0000	0.1667	0.1923	0.4062	0.1136	0.4615
1.1.1.37	100	0.6163	0.5556	1.0000	0.8333	0.8077	0.5938	0.8864	0.5256
	125	0.0233	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0128
MEP-3*	90	0.0000	0.0000	0.0000	0.0000	0.0000	0.1833	0.0556	0.2875‡
1.1.1.40	100	1.0000	1.0000	1.0000	1.0000	1.0000	0.8167	0.9444	0.7125
GPI-2*	65	0.0000	0.0000	0.0625	0.0000	0.0000	0.0152	0.0278	0.0000
5.3.1.9	100	1.0000	1.0000	0.9375	1.0000	1.0000	0.9848	0.9722	1.0000
SOD-1*	50	0.0000	0.0000	1.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1.15.1.1	100	1.0000	1.0000	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000
TF*	90	0.0800‡#	0.2500‡#	0.3333‡#	-	0.0000‡#	0.0645‡	0.0000‡	0.1026‡
	100	0.3600	0.4583	0.6667	-	0.5000	0.5806	0.3095	0.4359
	102	0.5600	0.2917	0.0000	-	0.5000	0.3548	0.6905	0.4615
# of polymorphic loci		6	6	6	5	7	8	9	7
% polymorphic loci		28.57	28.57	28.57	23.81	33.33	38.10	42.86	33.33
ne		1.1849 ± 0.3865	1.2056 ± 0.4572	1.1224 ± 0.2800	1.1149 ± 0.2435	1.1656 ± 0.3067	1.2101 ± 0.3739	1.1703 ± 0.3053	1.2125 ± 0.4277
na		1.4000 ± 0.6806	1.3500 ± 0.5871	1.3000 ± 0.4702	1.2500 ± 0.4483	1.3333 ± 0.4830	1.4286 ± 0.5976	1.4286 ± 0.5071	1.4286 ± 0.6761
Average heterozygosity		0.1006 ± 0.1542	0.1006 ± 0.1542	0.1006 ± 0.1542	0.0776 ± 0.1301	0.0958 ± 0.1519	0.0958 ± 0.1519	0.0958 ± 0.1519	0.0958 ± 0.1519

AAT-1,2*, CK-A1*, EST-D*, MDH-A1*, MDH-A2*, MEP-1*, MEP-2*, GPI-1*, GPI-3*, PGM-1* were monomorphic in all populations that were examined.

3.1.6. MEP Enzyme System (1.1.1.40)

For this enzyme system 3 loci MEP-1, MEP-2 and MEP-3 were studied.

MEP-1 and MEP-2 loci were monomorphic with the allele *100 in all of the populations.

Polymorphism was observed at MEP-3 locus with the alleles *100 and *90. MEP-3*90 allele was observed in Sümer, Blacksea and Fırtına populations with the allele frequencies, 0.1813, 0.0556 and 0.2875, respectively. In other populations only MEP-3*100 allele was observed.

MEP-3 locus was in Hardy-Weinberg equilibrium except in Fırtına population. The Fst value for this locus was 0.1723.

3.1.7. GPI Enzyme System (5.3.1.9)

For this enzyme system 3 loci : GPI-1, GPI-2 and GPI-3 were studied.

GPI-1 and GPI-3 loci were monomorphic for all of the populations with the allele *100, whereas for GPI-2 locus two alleles GPI-2*65 and GPI-2*100 were observed.

GPI-2*65 allele was observed in Eşen, Sümer and Blacksea populations with the allele frequencies 0.0625, 0.015 and 0.0278.

Hardy-Weinberg equilibrium was observed in all of the populations studied for this locus. The Fst value was 0.0338.

3.1.8. PGM Enzyme System (5.4.2.2)

For this enzyme system one locus was studied. PGM-1 locus was monomorphic in all of the populations with the allele *100.

3.1.9. SOD Enzyme System (1.15.1.1)

For this enzyme system one locus was studied. SOD-1*100 and SOD-1*50 alleles were observed in the populations. In the previos study done by Fidan (1995), Sod-1*100 and Sod-1*150 alleles were observed but the *150 allele is named as *100 and *100 allele was named as *50 in this study. SOD-1*100 allele was the common allele and observed in 7 of the populations with a frequency of 1.000. SOD-1*50 was observed in only Eşen population with a frequency of 1.000.

The Fst value for SOD-1 was found to be 1.000.

3.1.10. TF

For this enzyme, TF*100, TF*102 and TF*90 alleles were observed. Both *100 and *102 alleles were named in accordance with the previous study done by Largiader and Scholl (1995), but *90 allele was observed for the first time in the present study and since the band corresponding to this allele was behind *100 band it was called *90 allele.

TF*90 showed no heterozygote bands with TF*100 and *102. Only two individuals (one from Gödene (Alakır-3), one from Blacksea) showed a heterozygote band of TF*100 and *102.

TF*90 was observed in Alakır, Gödene (Alakır-3), Eşen, Sümer and Fırtına populations with the highest frequency 0.33 in Eşen population. TF*102 had the highest frequency, 0.6905, in Blacksea population.

Hardy-Weinberg equilibrium was not observed in any of the studied populations. The Fst value for TF was 0.3329.

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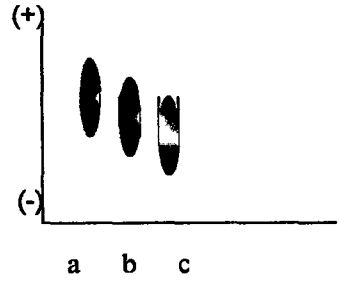
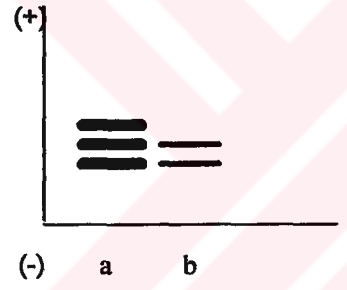
1. AAT-4 band patterns



a) AAT-4 (100/100)
b) AAT-4 (100/74)
c) AAT-4 (74/74)

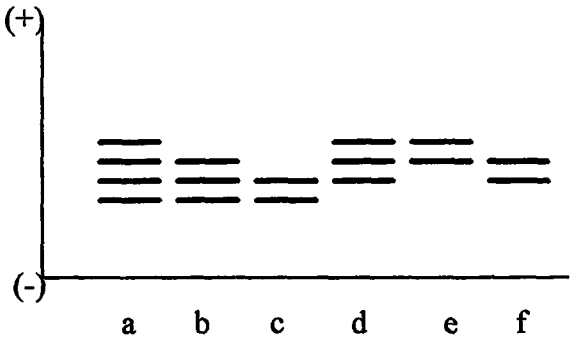
2. CK-A1 and CK-A2 band patterns



a)CK-A1 (100/100) , CK-A2 (100/100)
b)CK-A1 (100/100) , CK-A2 (50/50)

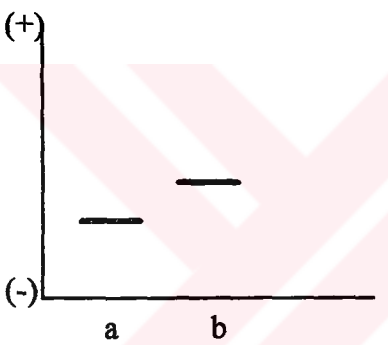
FIGURE 9. The band patterns observed throughout the study

3. LDH-A1 and LDH-A2 band patterns



a) LDH-A1 (100/100), LDH-A2 (100/100)
b) LDH-A1 (100/100), LDH-A2 (100/QL)
c) LDH-A1 (100/100), LDH-A2 (QL/QL)
d) LDH-A1 (100/QL), LDH-A2 (100/100)
e) LDH-A1 (QL/QL), LDH-A2 (100/100)
f) LDH-A1 (100/QL), LDH-A2 (100/QL)

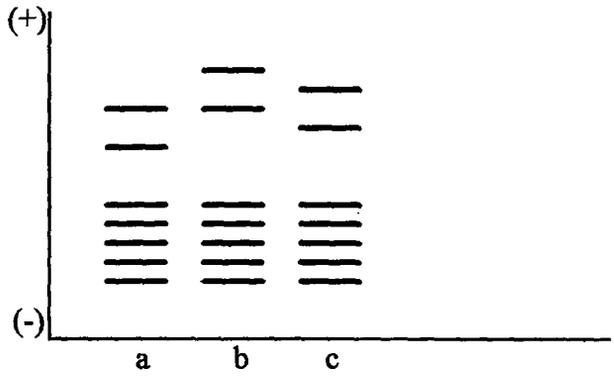
4.LDH-B2 band patterns



a)LDH-B2 (100/100)
b)LDH-B2 (120/120)

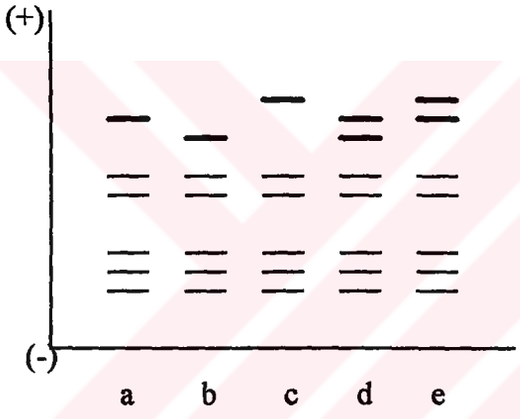
FIGURE 9 (cont.). The band patterns observed throughout the study

5. LDH-C band patterns:



a) LDH-C (90/90)
b) LDH-C (100/100)
c) LDH-C (100/90

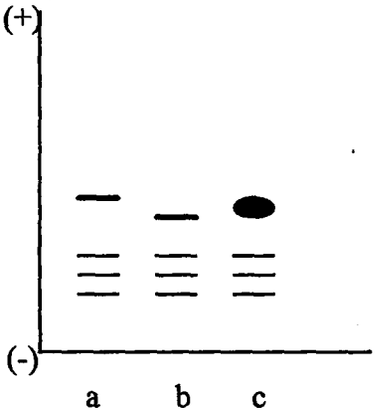
5. MDH-B1,B2 band patterns



a) MDH-A1,A2 (100/100), MDH-B1,B2 (100/100)
b) MDH-A1,A2 (100/100), MDH-B1,B2 (85/85)
c) MDH-A1,A2 (100/100), MDH-B1,B2 (125/125)
d) MDH-A1,A2 (100/100), MDH-B1,B2 (85/100)
e) MDH-A1,A2 (100/100), MDH-B1,B2 (100/125)

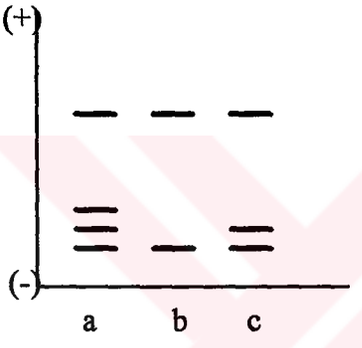
FIGURE 9 (cont.). The band patterns observed throughout the study

6. MEP-3 band patterns



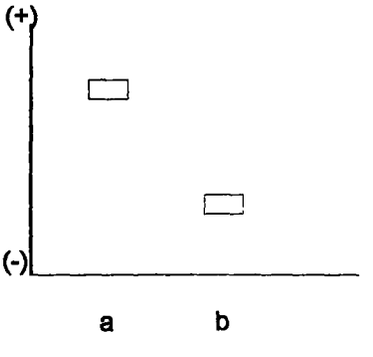
a) MEP-3 (100/100)
b) MEP-3 (90/90)
c) MEP-3 (90/100)

7. GPI band patterns



a) GPI-1 (100/100), GPI-2 (100/100), GPI-3 (100/100)
b) GPI-1 (100/100), GPI-2 (65/65), GPI-3 (100/100)
c) GPI-1 (100/100), GPI-2 (65/100), GPI-3 (100/100)

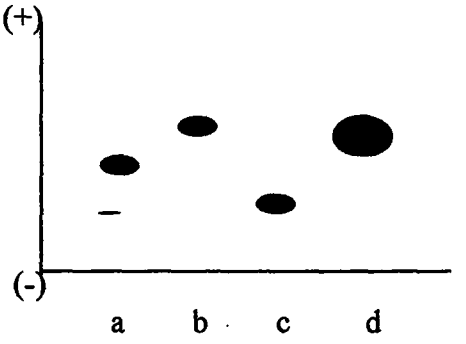
8. SOD-1 band patterns:



a)SOD-1 (100/100)
b)SOD-1 (50/50)

FIGURE 9 (cont.). The band patterns observed throughout the study

9. TF band patterns



a) TF (100/100)
b) TF (102/102)
c) TF (90/90)
d) TF (100/102)

FIGURE 9 (cont.). The band patterns observed throughout the study

3.2. Genetic Structures of the Populations

The 10 enzyme systems studied revealed a total of 21 loci with 34 alleles. 11 of the loci were (52.38 %) polymorphic.

3.2.1. Genetic Diversity

The allele frequencies determined by POPGENE (Yeh et al., 1997) are tabulated in Table 6. In several cases, an allele not detected in many populations was found to be frequent in another. For example SOD-1*50 allele was absent in all of the populations except in Eşen and in Eşen it was the only allele observed. Similarly CK-A2*50 allele was observed only in Mudurnu and Fırtına populations.

The parameters used to describe the genetic diversity of populations were calculated and the results are given in Table 6. These parameters were; number of polymorphic loci, percentage of the polymorphic loci, mean number of alleles, effective number of alleles and average heterozygosity.

Number of polymorphic loci;

The number of polymorphic loci varied from 5 in Abant population to 9 in Blacksea population. When all the populations were considered average number of polymorphic loci was 6.75 ±1.282.

Percentage of polymorphic loci;

The percentage of polymorphic loci varied from 23.81 in Abant population to 42.86 in Blacksea population. When all the populations were considered, the average percentage of polymorphic loci was 32.1425 ± 6.105

Allelic Richness;

The mean number of effective alleles for each population varied between 1.1149 (in Abant population) and 1.2125 (in Fırtına population).When all the populations were considered the mean number of effective alleles was 1.1732 ± 0.035.

Average heterozygosity;

The average heterozygosity for each population varied from 0.0776 in Abant to 0.1006 in Alakır, Gödene (Alakır-3) and Eşen populations. When all the populations were considered, the average heterozygosity was 0.0953 ± 0.0075.

3.2.2. Gene Diversity Statistics

In order to study the genetic structure of the populations comparatively Wright's F-statistics were used.

The results of the F-statistics were presented in Table 7. In the Turkish brown trout populations studied, the F_{IS} values varied from -0.0625 in LDH-A2 to 1 in CK-A2,and LDH-B2 and the mean value was 0.3715 ± 0.465. The F_{IT} varied from -0.0492 in

LDH-A2 to 1 in SOD-1, CK-A2 and LDH-B2. The mean value was 0.4770 ± 0.450. The negative values indicated the excess of heterozygotes while, the positive values indicated the excess of homozygotes (inbreeding) within populations (F_{IS}) and for the entire population (F_{IT}). Fst value varied from 0.0126 in LDH-A2 to 1 in SOD-1. The mean value for F_{ST} was 0.2549 ± 0.32. Since, the high F_{ST} value of a locus indicated the power of that locus in differentiating the populations, SOD-1 with the highest F_{ST} value exhibited the highest power to differentiate populations.

TABLE 7. Results of F-statistics analysis for the studied populations of *Salmo trutta*

Locus	F_{IS}	F_{IT}	F_{ST}
AAT-4*	0.1375	0.1610	0.0272
CK-A2*	1.0000	1.0000	0.1161
LDH-A1*	0.2500	0.2914	0.0552
LDH-A2*	-0.0625	-0.0492	0.0126
LDH-B2*	1.0000	1.0000	0.7144
LDH-C*	0.4759	0.5838	0.2059
MDH-B1,2*	-0.1998	-0.0397	0.1335
MEP-3*	0.1981	0.3363	0.1723
GPI-2*	-0.0488	-0.0134	0.0338
SOD-1*	***	1.0000	1.0000
TF*	0.9645	0.9763	0.3329
Mean	0.3715 ± 0.4650	0.4770 ± 0.4500	0.2549 ± 0.3200

3.3. Genetic Distances

3.3.1 Comparison of the results of the current study with Greek Brown trout populations' data.

In order to see the degree of genetic similarity of Turkish and Greek brown trout populations and thereby to locate the place of the studied Turkish populations within the frame of already existing phylogenetic groups, Nei's standard genetic distance was calculated using published results of 12 Greek populations (Karakousis and Triantaphyllidis (1988), (1990); Apostolidis et al. (1996)). A Neighbour joining

dendogram was constructed using eight markers (CK-A1, CK-A2, LDH-A1, LDH-A2, LDH-B2, LDH-C, GPI-2 and SOD-1) based on calculated genetic distance values which were given in Table 8.

The tree indicated existance of 2 clusters of populations.The first cluster is consisting Mediterranean and Danubian originated populations and second cluster is consisting Atlantic basin originated populations. In the first cluster Alakır,Acheloos-2, Thyamis and Voidomatis formed a sub-cluster and is combined to the other subcluster consisting Eşen, Fırtına, Blacksea, Abant, Mudurnu and Sümer. Agios Germanos and Aoos integrated to this cluster as a sub-cluster like Drosopigi and Ohrid. Louros and Gödene are the other two populations that integrated to the first cluster individually. The second cluster consisted of populations; Garonne (France), Tachov (Checz Republic) and Jucar-1 (Spain) from Atlantic drainages (Figure 10).

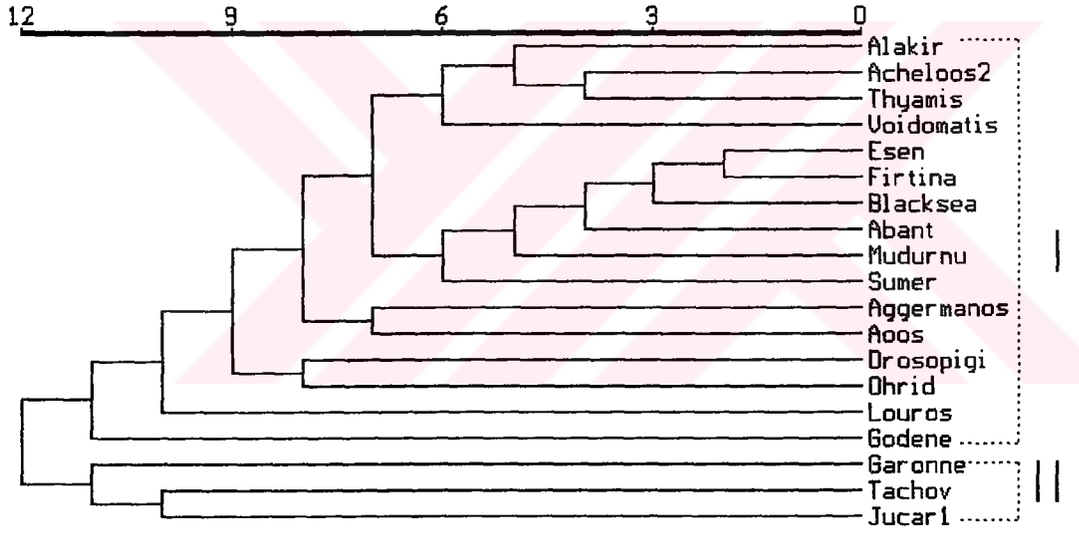


FIGURE 10. The Neighbour joining dendogram combining the data from Turkish and Greek brown trout populations based on markers CK-A1, CK-A2, LDH-A1, LDH-A2, LDH-B2, LDH-C, GPI-2 and SOD-1.

3.3.2. Comparision of the results of the current study with other countries'data

Based on the current data and data from other researcher's studies (Karakousis and Triantapyllidis (1988),(1990); Apostolidis et al. (1996); Osinov (1988); Osinov and

TABLE 8. Genetic distance of the Turkish and Greek Brown Trout populations

Populations	Alakir	Godene	Esen	Abant	Mudurnu	Sumer	Blacksea	Firtina	Louros	Voldomatis	Aggermanos	Drosopigi	Acheloos2	Thyamis	Aoos	Garonne	Tachov	Jucar 1	Ohrid	Alfios
Alakir	0.00000																			
Godene	0.00103	0.00000																		
Esen	0.31184	0.31423	0.00000																	
Abant	0.12130	0.12331	0.15726	0.00000																
Mudurnu	0.10240	0.10513	0.15723	0.00976	0.00000															
Sumer	0.06969	0.07087	0.16478	0.00912	0.00738	0.00000														
Blacksea	0.14276	0.13572	0.16187	0.02255	0.02572	0.02695	0.00000													
Firtina	0.14088	0.14350	0.14348	0.00707	0.00539	0.01332	0.02029	0.00000												
Louros	0.00209	0.00237	0.30791	0.12019	0.10099	0.06641	0.14237	0.13880	0.00000											
Voldomatis	0.00098	0.00244	0.30558	0.12292	0.09976	0.06832	0.14388	0.13771	0.00140	0.00000										
Aggermanos	0.14056	0.14189	0.49906	0.28268	0.20657	0.21760	0.30946	0.28721	0.13507	0.13618	0.00000									
Drosopigi	0.00621	0.00651	0.26390	0.12577	0.10957	0.07252	0.15150	0.14765	0.00373	0.00611	0.14350	0.00000								
Acheloos2	0.09125	0.10315	0.42600	0.23422	0.20581	0.19328	0.27118	0.24819	0.12086	0.09868	0.27271	0.12916	0.00000							
Thyamis	0.10359	0.11602	0.44022	0.24782	0.21904	0.20784	0.28591	0.26173	0.13504	0.11140	0.28811	0.14360	0.00035	0.00000						
Aoos	0.00201	0.00269	0.30495	0.12257	0.09976	0.06707	0.14247	0.13742	0.00051	0.00049	0.13418	0.00499	0.11395	0.12770	0.00000					
Garonne	0.09872	0.08153	0.43949	0.22189	0.23401	0.18016	0.17847	0.27435	0.10228	0.11287	0.26896	0.10281	0.25454	0.27031	0.11054	0.00000				
Tachov	0.35376	0.32476	0.83204	0.50888	0.54240	0.46427	0.42206	0.58514	0.35750	0.37536	0.57384	0.36090	0.55723	0.57600	0.37050	0.14884	0.00000			
Jucar 1	0.09101	0.07643	0.44129	0.24570	0.22266	0.18309	0.17840	0.26151	0.09715	0.09578	0.25806	0.10624	0.19943	0.21250	0.09594	0.05732	0.12340	0.00000		
Ohrid	0.02120	0.0224	0.21053	0.14952	0.12572	0.09080	0.17178	0.16349	0.01869	0.01872	0.15924	0.00765	0.13702	0.15111	0.01791	0.13765	0.41342	0.12324	0.00000	
Alfios	0.10837	0.10959	0.14788	0.24765	0.22257	0.18357	0.27294	0.26034	0.10333	0.10419	0.25360	0.06948	0.23800	0.25320	0.10237	0.23494	0.53380	0.22220	0.09921	0.00000

Bernatchez (1996)) neighbour joining dendogram was constructed using 30 populations from Greece, Russia and other European countries (Figure 11).

In the tree a distinct cluster was formed by the populations from Atlantic drainage (i).The second group was a mixed cluster of both Danube and Mediteranean origins. In that big cluster Alakır population was in a group together with other populations from East Mediterranean region (ii).. Eşen, Fırtına, Abant, Sümer Mudurnu and Blacksea populations were in another group(iii). On the other hand, Gödene population integrated to the outer portion of this cluster after the populations which were mainly from Danube group (iv).

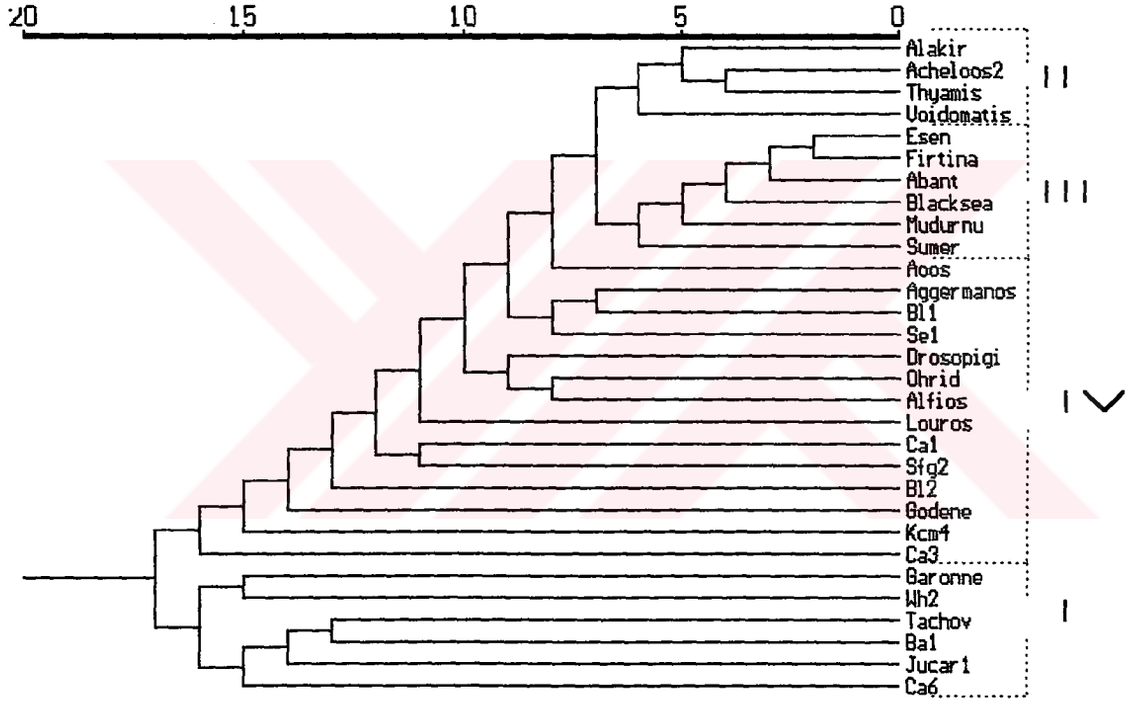


FIGURE 11. The Neighbour Joining dendogram constructed using 30 populations from Turkey, Greece and Russia.

CHAPTER IV

DISCUSSION

In this chapter, firstly, the results of the present study will be considered together with the results of two previous studies done by Fidan (1995) and Yalın (1996) on Turkish brown trout populations.

Secondly, results from Turkish populations will be evaluated together with those from other countries. There were numerous studies done on populations from various geographic regions and countries such as Denmark (Hansen et al. (1993)), Northern Ireland (Crozier and Ferguson (1986), Taggart et al. (1981)), Switzerland (Largiader and Scholl (1996a), (1996b), (1995)), Italy (Giuffra et al. (1996)) and France (Poteaux et al. (1998), Guyomard and Krieg (1983)). However comparisions will be mainly between Turkish, Greek (Karakousis (1990), Karakousis and Trianthaphyllidis (1988),(1990), Apostolidis et al.(1996)) and Russian (Osinov (1984), (1988), Bernatchez and Osinov (1995), Osinov and Bernatchez (1996)) populations because they are geographically close to each other.

4.1.1. Alleles and allele frequencies in Turkish populations

When the results of the previous studies done by Fidan (1995) and Yalın (1996) were considered the following results were obtained :

AAT-1,2*100 were monomorphic in all of the Turkish populations examined so far and in AAT-4 locus, the same alleles *74 and *100 were observed in all three studies.

In LDH-B2 locus, *100 and *120 alleles were present in Turkish populations. However *120 allele was observed only in Abant in the previous studies with a frequency of 1.00. In the present study, *120 allele was observed in Eşen, Abant, Mudurnu, Sümer, Blacksea and Fırtına populations. In Abant population, *100 allele was observed with a frequency of 0.0938 which was not observed in the previous studies.

In LDH-C locus *90 and *100 alleles were observed. In the previous studies *100 allele was observed in Abant, Üzüm and Rize populations with a frequency of 1.00. In Gödene (Alakır-3) population Yalın (1996) observed *90, *100 and *110 alleles. In the present study, LDH-C *100 was observed in Abant, Mudurnu, Sümer and Fırtına populations with an allele frequency of 1.00. LDH-C*90 was observed in Alakır, Gödene (Alakır-3), Eşen and Blacksea populations. Although Yalın (1996) observed LDH-C*90 with a frequency of 0.820 in Gödene (Alakır-3) population, in the present study the maximum frequency observed for *90 allele was 0.375 in Blacksea population. Also in the present study, LDH-C*110 allele was not observed.Observing LDH-C*110 in Gödene (Alakır-3) population which was collected in 1994 was the most interesting result in Yalın's (1996) study. This allele was known to be present exclusively in *Salmo marmoratus* (Marble trout) which is found in the northern part of Adriatic sea ,in the Po and middle part of its tributaries of the left bank (Sommani 1961 cited in Giuffra et al. (1996)) and can hybridize with *Salmo trutta*. Yalın's (1996) observation indicated the introgression of *Salmo marmoratus*.Currently Gödene (Alakır-3) population however did not exhibit any LDH-C*110 individuals . Hence, either the level of introgression is decreasing or effect of introgression is absent. Such temporal variation of genetic intogression was also observed in the studies of Poteaux et al (1998).

For MDH-B1,B2 locus in Yalın's study (1996), in only Gödene (Alakır-3) population *85, *100 and *125 alleles were observed. According to his results, Abant, Üzüm and

Rize populations were monomorphic with allele *100. In the present study, *100 allele was only monomorphic in Eşen population. The alleles *85, *100 and *125 were observed in Alakır and Fırtına populations, and in the other populations, *85 and *100 were observed.

Yalın (1996) observed GPI-1*100 in all of the populations he studied and this locus was monomorphic in the present study as well.

Both Fidan (1995) and Yalın (1996) observed GPI-2*65 in Abant population and in the other populations GPI-2*100 observed to be monomorphic. In the present study, GPI-2*100 allele was observed with a frequency of 1.00 in Abant population. The observed frequency of *65 in the previous studies was 0.038 in Abant population, which was a small frequency , so no observations of this allele in the present study was possible. On the other hand, *65 allele was observed in Eşen, Sümer and Blacksea populations and these are the first records of this allele for these populations.

PGM-1 was observed to be monomorphic in both previous and and present studies in Turkey.

In SOD-1 loci, Yalın (1996) and Fidan (1995) observed SOD-1*50 allele in Üzüm population with a frequency of 1.00 and in the other populations studied they observed SOD-1*100 allele In the present study SOD-1*50 was observed in Eşen population with a frequency of 1.00 and for the other populations, SOD-1*100 was observed. This indicates the presence of SOD-1*50 allele in some Southern populations of Turkey and in those populations this allele was fixed.

4.2.Genetic variations within and between populations

In the previous studies done by Fidan (1995) and Yalın (1996) the highest levels of heterozygosity were observed in Gödene (Alakır-3) population as 0.154 and in Rize population as 0.0523. In the present study, highest levels of heterozygosity were

observed in Alakır, Gödene (Alakır-3) and Eşen populations as 0.1006 and in Mudurnu, Sümer, Blacksea and Fırtına populations as 0.0958. In both of the studies, Abant population had the lowest level of heterozygosity. The lowest percentage of polymorphic loci and the lowest level of heterozygosity by having fixed alleles were observed in Abant population. These results were parallel to those obtained from Lake Sevan trout (*Salmo ischchan*) (Osinov and Bernatchez 1996) which was another lacustrine trout and was believed to be endemic.

Average expected heterozygosity was higher in Alakır, Gödene (Alakır-3) and Eşen populations with the value of 0.1006 and in Mudurnu, Sümer, Blacksea and Fırtına populations the expected heterozygosity was 0.0958. The lowest value of expected heterozygosity was observed in Abant population with the value of 0.0776 which was in fact an exceptable level for the brown trout populations (see Table 9), also from the Table 9 it was observed that the values of average heterozygosity for the studied populations were in a good fit with the other populations from Europe.

Table 9 . The average heterozygosity values for the brown trout populations.

Country	Range of Average Heterozygosity	Reference
Denmark	0.0710 - 0.1310	Hansen et al. (1993)
Greece	0.0000 - 0.0750	Karakousis and Triantaphylidis (1988)
Italy	0.0210 - 0.1390	Giuffra et al. (1996)
Northern Ireland	0.0820	Crozier and Ferguson (1986)
Russia	0.0020 - 0.1080	Osinov (1984),Bernatchez and Osinov (1995)
Turkey	0.0776 - 0.1006	Present study

The highest percentage of polymorphic loci was observed in Blacksea population and since this population was a composite one, it could be said that Sümer population

with the percentage of polymorphic loci, 38.10, had the highest value.However, Abant population had the lowest percentage of polymorphic loci its value could be intermediate value when it was compared with the other countries' data (see Table 10), also from the Table 10 it was observed that the percentages of polymorphic loci for the studied populations were in a good fit with the other populations from Europe.

Table 10 . The percentage of the polymorphic loci for brown trout populations

Country	Percentage of polymorphic loci (Range)	Reference
Greece	3.80 - 32.00	Karakousis and Triantaphylidis (1988)
Italy	6.40 - 42.50	Giuffra et al. (1996)
Northern Ireland	17.40 - 34.80	Crozier and Ferguson (1986)
Russia	4.00 - 35.90	Osinov (1984),Bernatchez and Osinov (1995)
Turkey	23.81 - 42.86 (38.10*)	Present study

* For Sümer population

For TF, as it was observed by Poteaux et al.(1998) no heterozygote individuals were observed in the present study. Three alternative explanations to the absence of heterozygotes can be provided.

a) Assortative-mating could generate deficincy in of heterozygotes if two different stocks excluded themselves during the reproduction period (Poteaux et al. 1998). Some special features of the mating system have been described in the literature on brown trout such as differences in spawning time or different preferences for spawning sites.

b) Behaviour mentioned above may lead to substructuring which in turn exhibits Wahlund Effect. This could explain some of the deficiency in heterozygotes if there are some substructuring in the populations.

c) The selection forces may act against hybrids.

For comparision of genetic differentiation revealed by different types of markers, F_{ST} was the most appropriate measure. If F_{ST} significantly differed from zero, then restriction of gene flow among the populations sampled was indicated and in this case observed average F_{ST} value was 0.2549 which showed a remarkable difference between populations.

If genetic drift was the dominant influence on population differentiation, then all loci should give comparable measures of F_{ST}. Significantly higher values of F_{ST} at a locus might indicate directional selection, while significantly lower values could be produced as a result of balancing selection. In this study there was unbalanced distribution of F_{ST} which might indicate directional selection. Also the high F_{IS} value (0.3715) showed the deficiency of random mating in the studied populations.

4.3. Observed alleles in Turkish populations and their distributions in a wider geographic range

CK-A1 is a diagnostic marker for Atlantic, Mediterranean and Danubian groups (Garcia-Marin et al. (1998)). CK-A1 *115 allele is present in Atlantic groups and observed in rivers which runs to Atlantic drainages. This allele was observed in neither Turkish populations or in Aral, Caspian Sea and Blacksea populations of Russia. CK-A1*100 allele is found in both Mediterranean and Danube groups and it is found in Turkish populations as well.

CK-A2*50 allele was observed only in Agios Germanos (near the Adriatic sea) population in Greece (Karakousis and Trianthapyllidis,1990). This was the first record of this allele. Yet, in Turkey it was observed both in Mudurnu and Fırtına populations.

This allele is not present in other countries but is present only in Greece and Turkey. If further populations are analysed for CK-A2 loci, there would be more populations containing *50 allele in Turkey. According to Karakousis and Trianthapyllidis (1989) Agios Germanos population was classified as a different subspecies *S. trutta peistericus* by Karaman (1937). This population also differed from the other populations in the meristic counts and in the morphometric values (Karakousis and Trianthapyllidis (1989)). Furthermore, observation of the unique allele in this population supported their view. However this "unique" allele was observed in two populations in Turkey.

SOD-1*50 allele was observed in Drosopigi and Alfios populations in Greece. Especialy in Alfios population this allele was observed with a frequency of 0.875. (Apostolidis et al 1996). This population belongs to the Ponto-Aegean geographical zone. This allele was observed as a fixed allele in Eşen (in the present study). and Üzüm (Fidan 1995) populations in Turkey. SOD-1*50 was thought to be a distinct allele for *Salmo marmaratus* populations since this allele was for the first time observed in this species (Giuffra et al 1996). However presence of this allele in both Greek and Turkish non *Salmo marmaratus* populations suggests that not only in *Salmo marmaratus* but in *Salmo trutta* populations this allele is present. Furthermore, in Turkey, it is confined in populations from the Mediteranean coast.

LDH-A1*QL is observed in Greek, Russian and Turkish populations. It is not observed in Spanish populations. Observation of this allele in Russian,Greek and Turkish populations which indicate that the genetic reservoir for this allele is in this region.

LDH-A2*QL is a diagnostic marker for differentiating Danubian and Mediterranean groups (Garcia-Marin et al., 1998) which was not observed in any of the Russian populations of Blacksea, Aral and Caspian sea. However this allele is present in both Greek and Turkish populations which supports the hypothesis of Garcia-Marin and Pla (1996). In this hypothesis presence of the LDH-A2*QL allele in non-Mediterranean regions is explained by the following arguement that those sampling

locations represent areas of merging between Danubian and Mediterranean groups which was suggested by similarities of other ichtyofauna that could be a valid explanation for the North Populations of Turkey.

LDH-B2*120 allele was observed in only Turkish populations and one population from Kura river in Russia (Osinov and Bernatchez, 1996). This indicates the presence of this allele in this region and further research on this locus can bring up a new diagnostic marker for group identification.

LDH-C is another important diagnostic marker for Atlantic, Mediterranean and Danubian groups (Garcia-Marin et al. (1998)). LDH-C*90 allele is frequently observed in Atlantic groups while the ancestral LDH-C*100 allele represents Mediterranean groups. In Danubian groups both alleles are observed. In Russian populations of Blacksea, Aral and Caspian seas LDH-C*90 allele is observed with a decreasing frequency from North to South. In Turkey frequencies were even less than those observed in Russia.

In none of the studied samples from the Blacksea region hybridization with Atlantic salmon (*Salmo salar*) was observed. If there were hybrids, they should be detected by the presence of double bands in PGM. Yet, all individuals exhibited monomorphic bands for this locus.

In the neigbour joining dendogram the special allele combinations observed in the North Atlantic populations was formed a spectrum of Atlantic-Urals, Alakır population was placed in a branch of Mediterranean groups. Although Eşen was a Mediterranean population it was placed separately in a branch between with Mediterranean-Danube groups with Blacksea, Fırtına, Sümer and Abant populations. Such a situation was observed for Aios population in Greece which was placed into to Danube group although it was geographically in Southwest of Greece. Gödene (Alakır-3) population was placed in a different branch separate from the other Turkish populations that might show the introgression effect that this population experienced.

Consequently with the light of these diagnostic genetic markers the following suggestions were made:

Firstly, as it was previously thought, Abant lake population possesing distinct phenotypes is not genetically different from the other populations in Turkey. However; it must be emphasized that, Abant population having different morphological traits has prime importance in the conservation. Stock enhancement is being done for this population and in order to retain the present level of heterozygosity in the future generations this artificial stock enhancement should be done more cautiously. Higher number of fish must be used as parents. Also due to the heavy pressure of sports-fishing the population size is decreasing. In order to establish viability of this population heavy fishing must be avoided. Also, the ecological requirements of those fish should be researched and according to the findings of this research spawning areas of the population should be conserved. Any other fish introductions to Abant lake should be avoided for not to disturb ecological food web dynamics and not to put a competitor against this fish. It would be beneficial for this population to make a stock assesment for both stock enhancement programme and further management of this population.

Secondly, according to the results of the present study, Eşen, Mudurnu and Fırtına populations should be further studied due to the presence of different alleles (in Eşen ; SOD-1*50, in Mudurnu and Fırtına; CK-A2*50 alleles) these populations should have priority in conservation.

Thirdly, the activities at the stream-beds should be minimized in order to conserve both the life-history activities and the spawning sites of brown trout populations. Also day by day the streams are becoming the waste carriers; due to pollution the brown trout, the biological indicator of clean waters, are becoming extinct in those polluted water bodies. In order to retain their survival the necessary precautions should be taken.

Lastly, the genetic markers CK-A1, CK-A2, LDH-A1, LDH-A2, LDH-B2, LDH-C, SOD-1 and TF with further addition of FBP-1 should be studied in North-western, Aegean, North-Eastern and Central-Anatolian populations in order to identify the possible transition zones in Turkey. As far it is observed that Turkey is at the junction point of Mediterranean and Danube groups and research on the suggested populations may enlighten the status of our populations further.

4.5. CONCLUSION

1.Based on discriminator protein markers determined by previous researchers: LDH-C, CK-A1 and LDH-A2, Turkish populations exhibited both Mediterrenean and Danubian characteristics

2. The dendogram based on eight markers (CK-A1,CK-A2, LDH-A1,LDH-A2,LDH-B2, LDH-C, GPI-2 and SOD-1) indicates that Alakır population is in the Mediterranean cluster; Gödene perhaps exhibiting the effects of introgression is in the far end of Danubian group but all of the rest of the populations are in between Danubian and Mediterranean Clusters. Hence, it can be said that the most of the Turkish populations examined are representing the overlap of the genomes of Danubian and Mediterranean populations

3. Furthermore, at the south-east corner of the native brown trout distribution some alleles seemed to be specific to this region.

a) LDH-A1*QL is present in Greece, Russia and Turkey.

b) LDH-B2*120 is present largely in Turkey and some in Russia.

c) CK-A2*50 is present in Greece and Turkey.

d) SOD-1*50 is present in Mediterranean coast of Turkey and in one place in Greece.

4. TF*90 must be run on the same gel with those samples from Switzerland in order to decide whether it is a unique allele for Turkish brown trout populations.

5. It was observed that the previously recorded LDH-C1*110 allele (Yalın,1996) was no longer present in Alakır population which showed that the introgression effect of *Salmo marmaratus* was at least diminishing.

6. Populations examined were generally in similar value of genetic variability. Slightly lower variability was observed in Abant.

7. Abant population was not observed to be an endemic population according to the genetic markers studied.

8. In none of the populations studied from the Blacksea region, hybridization with Atlantic salmon (*Salmo salar*) was observed.

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APPENDIX A:

A. The Tank and Gel buffer compositions used throughout the electrophoresis of the proteins

1.TCB Buffer System

1 lt. TCB gel buffer, pH: 8.6 (adjusted with 0.1 M boric acid)
Tris: 9.2036 gr.
Citric acid: 1.0507 gr.
Boric acid: 0.92745 gr.
LiOH: 0.11975 gr.

2.5 lt. TCB tank buffer, pH: 8.6 (Adjusted with 0.1 M boric acid)
Boric: 46.3725 gr.
LiOH: 5.9875 gr.

2. AM Buffer System

2.8 lt. 0.04 M AM tank buffer, pH: 6.4 (Adjusted with N-aminopropyl morpholine)
Citric acid: 23.53568 gr.

Gel Buffer is 1:20 diluted tank buffer

3. Tris-Maleic Buffer System

1 lt Tris-Maleic tank buffer, pH: 7.4 (Adjusted with 5N NaOH)
0.1 M Tris: 12.11 gr.
0.1 M Maleic acid: 11.62 gr.
0.0087 M Na_2EDTA: 3.24 gr.
0.01 M $MgCl_26H_2O$: 2.03 gr.

Gel Buffer is 1:10 diluted tank buffer

B. The compositions of the staining buffers

1.Tris-HCl Buffer
a. 1 lt. 0.2 M Tris-HCl buffer, pH: 8.0 (Adjusted with 5N HCl)
Tris: 24.22 gr.

b. 1 lt 0.1 M Tris-HCl buffer, pH: 7.6 (Adjusted with 5N Hcl)
Tris: 12.11 gr.

2. Sodium acetate Buffer
1 lt 0.05 M Sodium acetate buffer, pH: 5.2 (Adjusted with glacial acetic acid)
Sodium acetate.$3H_2O$: 5.44 gr.

3. TEA Buffer
0.15 M TEA buffer, pH:9.0 (Adjusted with pure HCl)
TEA: 20 ml/lt

APPENDIX B:

The staining buffers, reaction mechanisms and the conditions for staining of the enzyme systems studied.

Enzyme System	Staining Buffer	Reaction Mixtures	Conditions for Staining
AAT	0.1 M Tris-HCl (pH:7.6) (50 ml)	50 mg aspartic acid 5 mg pyridoxal-5-phosphate 50 mg Fast Blue BB salt 40 mg α-keto glutaric acid 200 mg agar	Incubate at 37^{0}C for 30 minutes at dark
CK	0.2 M Tris-HCl pH:8.0 (30 minutes.)	Amido black soln. 15 min. (2.5 gr. amido black , 100 ml. acetic acid)	10 % glacial acetic acid for destaining.
EST	0.05 M Sodium acetate pH:5.2 (25 ml.)	2 mg 4-methyl-umbelliferyl acetate minimal acetone 250 mg. agar	Incubate at 37^{0}C for 5 minutes at dark
LDH	TEA (50 ml.)	100 mg. lactic acid 5 mg. NAD 2.5 mg. PMS 2.5 mg. MTT 200 mg. agar	Incubate at 37^{0}C for 30 minutes at dark
MDH	0.2M Tris-HCl pH:8.0 (50 ml.)	200 mgt. malic acid 5 mg. NAD 2.5 mg. PMS 2.5 mg. MTT 200 mg. agar	Incubate at 37^{0}C for 30 minutes at dark
MEP	0.2M. Tris-HCl pH:8.0	4 mg. NADP 4 mg. PMS 8 mg. MTT 1 ml. $MgCl_2$ 5 ml. Malate 200 mg. agar	Incubate at 37^{0}C for 30 minutes at dark
GPI	0.1 M Tris-HCl pH:7.6 (50 ml.)	20 mg. Fructose-6-phosphate 4 mg. NADP	Incubate at 37^{0}C for 30 minutes at dark

		4 mg. MTT 4 mg. PMS 40 u. G6pdh (10 ml.) 1 ml. $MgCl_2$ (2 g. /100 ml.) 200 mg. agar	
PGM	0.2 M Tris-HCl pH:8.0 (50 ml.)	4 mg. NADP 4 mg. MTT 4 mg. PMS 30 mg. Glucose 1 phosphate 30 mg. $MgCl_2$ 40 u. G6pdh (10 ml.) 200 mg. agar	Incubate at 37^0C for 30 minutes at dark
SOD	0.2 M Tris-HCl pH:8.0 (50 ml.)	4 mg. MTT 4 mg. PMS 200 mg. agar	wait for 2-4 min.under sunlight and incubate for 10 minutes at $37\ ^0$C at dark
TF	0.2 M Tris-HCl pH:8.0 (30 minutes.)	Amido black soln. 15 min.	10 % glacial acetic acid for destaining

APPENDIX C:

THE BRANDS AND CODES FOR THE CHEMICALS USED:

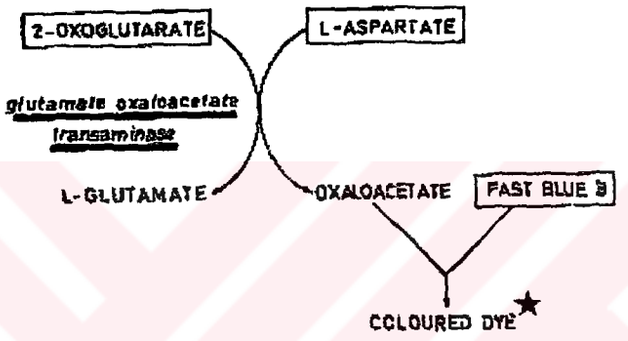
Acetic acid (glacial): Merck, 1.00056
Agar: BDH
N-aminopropyl morpholine: Sigma, A-9028
Boric acid: Sigma, B-0252
Citric acid: BDH, 1389930
Fast Blue BB salt: Sigma, F-3378
D-Fructose-6-phosphate: Sigma, F-3627
D-Fructose-1,6-diphosphate: Sigma, F-4757
D-Glucose-1-phosphate: Sigma, G-1259
Glucose-6-phosphate dehydrogenase: Sigma, G-7877
Hydrochloric acid: Merck, 1.00314
α-Ketoglutaric acid: Sigma, K-1750
L-Lactic acid: Sigma, L-2000
Lithium hydroxide: Merck, 5691
Maleic acid: Sigma, M-0375
DL-Malic acid: Sigma, M-0875
Magnesium chloride: ($MgCl_2$-$6H_2O$): Merck, 5831
Magnesium sulfate : Sigma, M-9397
4-Methlumbelliferyl acetate (MUA): Sigma, M-0883
MTT (3-[4,5-Dimethyl thiazol-2-yl]-2,5-diphenyl-tetrazolium bromide): Sigma, M-2128
Nicotinamide adenine dinucleotide (NAD): Merck,1.24542
Nicotinamide adenine dinucleotide phosphate (NADP-Na_2): Merck,1.24541
Phenazine metasulfate: Sigma, P-9625
Phosphoglucoisomerase: Sigma, P- 5381
Pyrodoxal-5-phosphate: Sigma, P-9255
Sodium acetate: BDH,10235
Starch: Sigma, S-4501
Triethanolamine: Merck, 1.08377
Tris: Sigma, T-1378

APPENDIX D:

The reaction and staining mechanisms of the proteins studied.

2.6.1.1— Aspartate amino transferase (AAT)

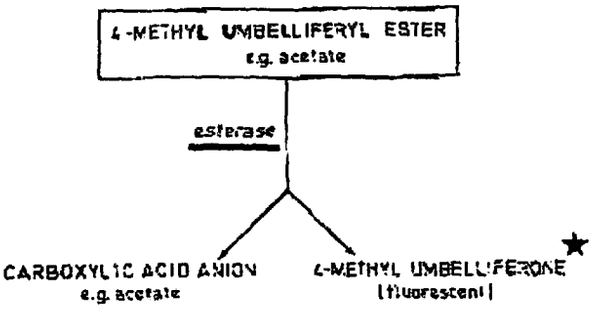
L-Aspartate + 2-oxoglutarate = oxaloacetate + L-glutamate



3.1.1.1—Esterase (EST)

also known as: carboxylesterases, ali-esterases, B-esterases.

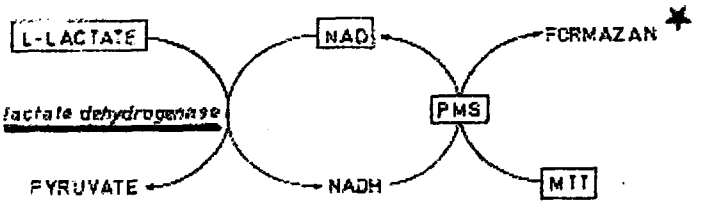
A carboxylic ester + H_2O = an alcohol + a carboxylic acid anion.



1.1.1.27—Lactate dehydrogenase (LDH)

L-Lactate + NAD = pyruvate + NADH

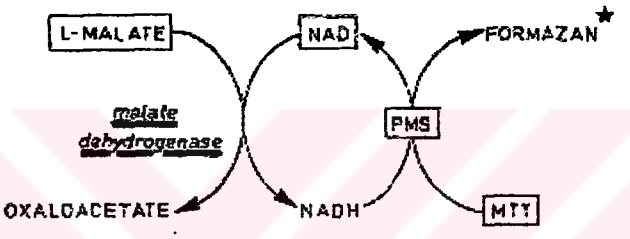
Staining system



1.1.1.37—Malate dehydrogenase (MDH)

L-Malate + NAD = oxaloacetate + NADH

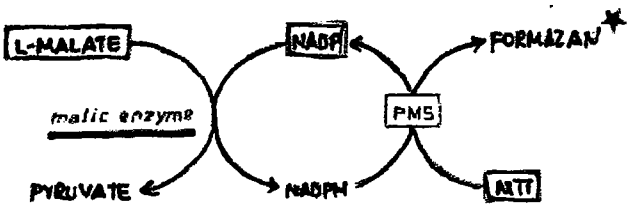
Staining system



1.1.1.40— Malic enzyme (NADP dependent)(MEP)

L-Malate + NADP = pyruvate + CO_2 + NADPH

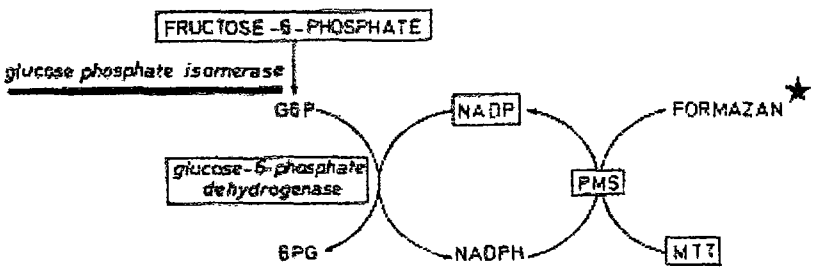
Staining system



5.3.1.9—Glucose phosphate isomerase (GPI)
also known as: phosphohexose isomerase; phosphoglucose isomerase.

Glucose-6-phosphate = fructose-6-phosphate

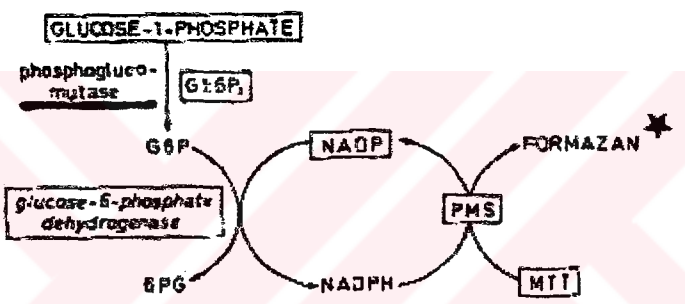
Staining system



2.7.5.1—Phosphoglucomutase (PGM)

α-D-Glucose-1-phosphate $\xrightarrow{\text{(G1:6diP)}}$ α-D-glucose-6-phosphate

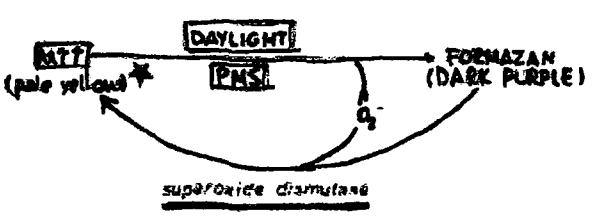
Staining system



1.15.1.1—Superoxide dismutase (SOD)
also known as: indophenol oxidase; tetrazolium oxidase; "white patch".

$$O_2^- + O_2^- + 2H^+ = O_2 + H_2O_2$$

Staining system



APPENDIX E:

Data set for the POPGENE software

/ * Türkiye için tez datası * /
Number of populations = 8
Number of loci = 21
Locus name :
PGM1 SOD1 CK1 CK2 TF MDH1 MDH2 MDH34 PGI1 PGI2 PGI3 MEP1 MEP2 MEP3 AAT12 AAT4 LDH1 LDH2 LDH4 LDH5 ESTD

name = Alakir

11	11	11	11	22	11	11	12	11	11	11	11
	11	11	11	11	11	11	11	..	..		
11	11	11	11	33	11	11	12	11	11	11	11
	11	11	11	22	11	11	..	11	..		
11	11	11	11	11	11	11	11	11	11	11	11
	11	11	..	11	11	11	11	11	..		
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