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Genetic structure of populations of *Ochlerotatus dorsalis* (Meigen, 1830) (Diptera: Culicidae)

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Abstract

Allozyme variability at nine loci of Palaearctic and Nearctic populations of *Ochlerotatus dorsalis* was determined. Estimates of genetic variability showed that the Palaearctic population ranked higher than the Nearctic population in percentage of polymorphic loci (*P:* 0.77; 0.33), the mean number of alleles per locus (*A:* 3.3; 1.8) and value of mean heterozygosity (H_o: 0.263; 0.059). In addition to the lack of identical alleles at *Est-6* locus, genetic differentiation of Palaearctic and Nearctic populations was due to differences of allele frequency variances at *Had, Idh-2* and *Gpi* loci.

Introduction

Polyacrylamide gel electrophoresis (PAGE) allows precise, efficient and rapid analysis of enzyme variability as a basis for studying biochemical adaptations. Within the genus *Ochlerotatus* investigation of the *dorsalis* group has been hindered by disagreement regarding the taxonomic status of its constituent species. Relationships between the Holarctic *Oc. dorsalis* and the Nearctic *Oc. melanimon* (Dyar) (Freeborn, 1926; Barr, 1955), and the Palaearctic *Oc. caspius* (Pallas) (Gutsevich, 1985; Bozicic-Lothrop, 1988) have long been a cause of controversy. Larvae of *Oc. dorsalis* may be found in inland saline ponds, lakes and marshes, as well as sea shores of California, Canada, Britain, north, central, and southern Europe, Siberia, China and Mongolia (Milankov *et al.*, unpublished). The adults have a medical importance as vectors of arboviruses causing California encephalitis (Wood *et al.*, 1979), of the California serogroup Hart Park and Cache Valley viruses (Clark *et al.*, 1986) and St. Louis encephalitis virus (Emmons *et al.*, 1987).

This paper reports an investigation of the genetic structure of Palaearctic and Nearctic populations of *Oc. dorsalis,* using allozyme variability at nine isozyme loci.

Material and Methods

Sample Collection. Samples of *Oc. dorsalis* were collected from northern Yugoslavia (Rusanda, Zrenjanin), (AD1; 131 samples) and from California (Salton Sea), (AD2; 66 samples). Third and fourth instar larvae of the Palaearctic population were collected, reared to adults, and then frozen. Species identification was based on characteristic mesonotal and abdominal tergite scale patterns.

Allozyme Analysis. Genetic variation was studied by standard 5% polyacrylamide gel electrophoresis (Munstermann, 1979). Tris-Boric-EDTA (pH 8.9) buffer was used to assay glucose phosphate isomerase (E.C. 5.3.1.9. GPI), esterase (E.C. 3.1.1.1. EST), phosphoglucomutase (E.C. 2.7.5.1. PGM), malic enzyme (E.C. 1.1.1.40. ME), octanol dehydrogenase (E.C. 1.1.1.73. ODH); and Tris-Citric (pH 7.1) buffer was used to assay α-glycerophosphate dehydrogenase (E.C. 1.1.1.8. GPD), 2-hydroxy acid dehydrogenase (E.C. 1.1.99.6. HAD) and isocitrate dehydrogenase (E.C. 1.1.1.42. IDH).

Statistical Analysis. Statistical analysis of electrophoretic variability data was performed using the computer program BIOSYS-2 (original version: BIOSYS-1, Swofford & Selander, 1981; as modified by Black, 1997). The tests included: genotype and allele frequencies, the percentage of polymorphic loci (*P*), mean observed and expected heterozygosity (H_o, H_e) for small samples corrected using Levene's (1949) formula.

Deviation between H_o and H_e of separate variable loci was evaluated using Wright's inbreeding coefficient (*F*) (Wright, 1951) with the mean *F* statistics calculated by a jack-knifing procedure over loci (Weir, 1990) and Selander's (1970) *D* statistics. Wright's *F* statistics, the difference between the frequency of heterozygotes observed and those expected, three levels of genetic differentiation: F_{is} (variation within subpopulations), F_{it} (homozygosity of an individual relative to total population), and F_{st} (amount of subdivision relative to the limiting amount under complete fixation) were calculated.

Results

The allozyme analysis of *Est-1, Est-6, Gpd-2, Gpi, Had, Idh-2, Me, Pgm,* and *Odh* loci revealed the presence of 28 alleles in AD1 population and 16 in AD2. Genotype frequencies were calculated (Table 2). Loci *Gpd-2* and *Odh* in AD1 population were monomorphic, while *Me, Pgm* and *Est-1* of AD2 were polymorphic (based on the 95% criterion).

In contrast to AD2 population differences in observed (H_o) and expected (H_e) heterozygosity based on Hardy-Weinberg, values in the Palaearctic (AD1) population of *Oc. dorsalis* were statistically significant for *Est-6, Gpi* and *Pgm* loci. The genotype fixation index, *F*, indicated excess homozygosity ($F_{is}>0$) in both populations at most loci, except at *Gpi* (AD1), *Had* (AD2), *Idh-2* (AD1), *Pgm* (AD2) and *Odh* (AD1). These results were in accordance with values calculated by Selander's *D* statistics. *D* was 0 at *Had* locus in the AD2 population.

F statistics was used to describe the genetic structure in Palaearctic and Nearctic populations of *Oc. dorsalis*. The mean fixation index in the total population was high (F_{IT}=0.674). F_{IT} values for *Est-1, Est-6, Had* and *Me* loci were considerably higher (from 0.748 to 1.000) followed by *Pgm* (0.356) and *Idh-2* (0.326) loci. Negative F_{IT} values were registered at *Odh* (F_{IT}= -0.018) and *Pgm* (F_{IT}= -0.058) loci.

28 alleles at 8 variable loci were registered in populations of the species *Oc. dorsalis.* No significant differences in the allele frequencies at *Me, Pgm* and *Odh* loci were observed. F_{st} used as a measure of genetic differentiation between populations indicated high genetic subdivision (F_{st}=0.329). This population subdivision of *Oc. dorsalis* was due to differences of allele frequency variance (F_{st}) at *Had, Est-6, Gpi* and *Idh-2*. Value of *F* parameters by jack-knife estimates over loci were in concurrence with previous results.

Analysis of population genetic structure parameters showed many differences between the two populations. All parameters indicated higher genetic variability in the Palaearctic population (Table 1).

Discussion

Protein-enzyme polymorphism can serve as an indicator of changes in genes occurring during evolution. These processes can be complex since they involve co-adaptive changes in systems of genes.

Analyses of isozyme variability have shown that various loci have different levels of variability. Thus, locus *Gpd-2* was monomorphic in four Nearctic populations (Shultz *at al.,* 1986) and in a Palaearctic population (Lambert *et al.*, 1990) which agrees with the results in this study. Furthermore, the *Idh-2* locus in all Nearctic populations was monomorphic (Shultz *et al.*, 1986) as we found to be the case in the Nearctic AD2 population, but not in the Palaearctic AD1 population (Milankov *et al.*, 2000).

Unlike populations from New Mexico, Michigan, Utah and Wyoming where the *Me* locus was monomorphic (Shultz *et al.*, 1986), we found 2 and 3 alleles, respectively, in the Salton Sea (AD2) and Rusanda (AD1) populations.

Shultz *et al.,* (1986) and Lambert *et al.*, (1990) found the *Pgm* locus to be polymorphic in all analysed populations of *Oc. dorsalis.* No heterozygotes have previously been registered at locus *Pgm* in Nearctic populations (Shultz *et al.*, 1986), but we found one homozygote and one heterozygote in the Salton Sea (AD2) population. One homozygote and three heterozygotes were found at the *Pgm* locus in a Palaearctic population of *Oc. dorsalis* from France (Lambert *et al.*, 1990), and we observed three homozygotes and two heterozygotes in the population from Rusanda (AD1).

Lambert *et al.* (1990) reported two homozygotes and one heterozygote at the *Gpi* locus in a population from France; we observed one homozygote and one heterozygote in the Rusanda (AD1) population. The *Gpi* locus is monomorphic in the Salton Sea (AD2) population (Milankov *et al.*, 2000). In contrast to the Palaearctic population, the *Idh-2*, *Had* and *Est-6* loci were monomorphic in the Nearctic population (AD2). Locus *Odh* is monomorphic in both populations (Milankov *et al.*, 2000), except that the rare allele Odh^{108} occurs in the Nearctic population.

The *Est-6* locus is diagnostic for separating the conspecific AD1 and AD2 populations. At this locus the largest number of genotypes (5 homozygotes and 7 heterozygotes) was found in the Rusanda population, and the allele $Est\text{-}6^{84/84}$ was also found. In sympatric populations of *Oc. dorsalis* and *Oc. caspius* from Rusanda identical alleles were observed at the *Est-6* locus (Milankov & Vapa, unpublished).

In contrast to the polymorphic loci *Est-6, Gpi, Had, Idh-2, Pgm* and *Odh* (99% criterion) in the Palaearctic population, and *Had* and *Pgm* (99% criterion) in the Nearctic population, no heterozygotes were registered in the *Me* and *Est-1* loci. Malic enzyme (NADP-Malate dehydrogenase), coded by alleles of two (Richardson *et al.*, 1986) or three loci (Munstermann, 1979), is a tetrameric protein of the glycolytic pathway. In some aedine mosquitoes two *Me* loci are visible (Matthews & Munstermann, 1983; Foley & Bryan, 1991), probably corresponding to the mitochondrial (*Me-1* or slow locus) and the cytoplasmic forms (*Me-2* or fast locus). Absence of heterozygotes in the polymorphic *Me* locus could be the result of a number of factors, such as selection against heterozygotes (i.e. disruptive selection), the presence of null alleles, assortative mating, population substructuring and presence of cryptic species in sympatry, or some non-genetic, post-translational modification and electrophoretic insufficiency. The presence of heterozygotes in other polymorphic loci showed that inbreeding, non-random mating and genetic drift (which affect all polymorphic loci in a similar fashion) could not be the cause of the deficiency of heterozygotes in the *Me* and *Est-1* loci. Also, null alleles are unlikely since they are very rare for the enzymes of intermediary metabolism (such as glycolytic enzymes). It is possible that the analysed *Me* locus was not mitochondrialy located (which could also cause the absence of heterozygous genotypes) since ME allozymes in *Oc. dorsalis* populations showed similar electrophoretic mobility as the allozyme coded by *Me-2* in the *Aedes aegypti* laboratory strain used as a standard. The lack of heterozygosity could be the result of sub-sampling and non-random mating (a sample set consists of individuals from different subpopulations or genetically distinct groups such as cryptic species) since *Oc. dorsalis* is well known for its capacity to migrate long distances. Additionally, it has been proven that the lack of heterozygosity in the *Me-1* and *Acon-1* loci, and the *Odh* locus in the populations of *Anopheles quadrimaculatus* species C (Narang *et al.*, 1990) and *An. minimus* (Green *et al.*, 1990), respectively, indicated genetic substructuring - division of analysed species into subpopulations or cryptic species.

Although the comparison of enzyme maps revealed that numerous inversions (mostly paracentric) and translocations caused subgeneric genetic divergence, it was also found that certain linkage associations were conserved in *Aedes* evolution (Matthews & Munstermann, 1994). Since linkage mapping results showed that the changes in *Aedes* (recently the sub-genus *Ochlerotatus* has been elevated to generic level) occurred at a much lower rate than in other examined organisms (Rai *et al.*, 1982), it could be assumed that the registered pattern is present in *Oc. dorsalis* although the linkage map has not yet been constructed for this species. Therefore, it could be assumed that the cytoplasmic *Me* locus in *Oc. dorsalis* is associated with the sex-determining locus on chromosome 1, as in *Aedes aegypti* (Munstermann, 1990), *Ae. triseriatus* (Matthews & Munstermann, 1990) and *Ae. scutellaris* group (Pashley & Rai, 1983). In this study, however, no difference between males and females in the alleles of the *Me* locus was observed that would support this hypothesis.

Esterases (EST) exist as molecular forms possessing a large number of isoenzymes. Two types of esterases have been identified in mosquito species, preferentially hydrolysing either α- or β- naphthyl acetate. Two esterase loci (*Est-1, Est-6*) were analysed in this study using the substrate α- naphthyl acetate. At the *Est-1* locus no heterozygotes were registered. The absence of heterozygotes might be due to the presence of null alleles (quite common for multiple-locus enzyme complexes, e.g. esterases). Null alleles, causing the excess of phenotypic homozygotes, were registered in the *Est-1, Est-2* (Stordeur, 1976) and *Est-3* loci (Urbanelli *et al.*, 1980) in populations of *Culex pipiens*; and *Est-4* and *Est-5* in *Aedes albopictus* (Tadano, 1987). Esterase loci are mostly a part of the linkage group II, as in the case of the *Est-6* locus in *Aedes aegypti* (Munstermann & Craig, 1979) and in the *Ae. scutellaris* group (Pashley & Rai, 1983) as well as *Est-5* in the *Ae. triseriatus* group (Munstermann *et al.*, 1982).

The presence of major and rare alleles indicates genetic divergence among populations of *Oc. dorsalis*. The same major alleles (>0.5) at *Gpi, Pgm* and *Odh* were registered in both populations. On the other hand, those at *Gpd-2* (which was monomorphic), alleles at monomorphic *Est-6, Idh-2* and *Had* loci, as well as a major allele at the *Me* locus in the AD2 population, were not major alleles in the AD1 population. In accordance with these results, no statistically significant differences in allele frequencies at *Est-1, Pgm* and *Odh* loci were observed. Apart from the lack of identical alleles at the *Est-6* locus, genetic differentiation of Palaearctic and Nearctic populations was due to differences of allele frequency variances at *Had* (F_{st}=0.711), Est-6 (F_{st}=0.536) and *Idh-2* (F_{st}=0.344).

Genetic divergence among populations was, to a lesser extent, affected by allelic frequency variance at *Gpi* (F_{st}=0.243), *Est-1* (F_{st}=0.055) and *Odh* (F_{st}=0.095) loci.

Estimates of genetic variability showed that the Palaearctic population ranked higher than the Nearctic population in percentage of polymorphic loci, the mean number of alleles per locus, and the value of mean heterozygosity. Observed differences in values of genetic structure parameters were probably due to association of genetic variability, level of ecological heterogeneity, effective size of populations and historical events, as well as independent evolution of geographically distant populations. However, analysis of allozyme variability at

17 isozyme loci in Nearctic populations showed that polymorphism, heterozygosity and mean number of alleles per locus were uniform (5.88; 0.035-0.059; 1.06 respectively) (Shultz *et al.*, 1986) and similar to corresponding values calculated for the AD2 population (*A*: 1.8; H_o: 0.059), except for frequency of polymorphic loci (*P:* 33.3). The observed differences can be attributed to different numbers of analysed loci and the fact that not the same enzymes were assayed for both populations. Of 17 isozyme loci analysed by Shultz *et al.* (1986), only 4 loci (*Gpd-2, Idh-2, Me* and *Pgm*) were included in our study. Higher values of all genetic structure parameters were registered in the Palaearctic population than in the Nearctic population, and also when the Rusanda population was compared with a population from France (Lambert *et al.*, 1990). Frequency of polymorphic loci and heterozygosity were slightly higher in the AD1 population than in the population from France (*P:* 0.714; H_o: 0.178), which was probably due to there being only three common loci (*Gpd-2, Gpi* and *Pgm*) analysed.

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References

Barr, R. (1955) The resurrection of *Aedes melanimon* Dyar. *Mosquito News* **15,** 170-172.

Bozicic-Lothrop, B. (1988) Comparative ecology of *Aedes dorsalis* Complex in the Holarctic. *56th Annual Conference of the California Mosquito and Vector Control Association* **56,** 139-145.

Clark, G., Crabbs, C., Bailey, C., Calisher, C. & Craig, G. (1986) Identification of *Aedes campestris* from New Mexico: with notes on the isolation of western equine encephalitis and other arboviruses. *Journal of the American Mosquito Control Association* **2,** 529-534.

Emmons, R.W., Dondero, D.V., Chan, C.S., Milby, M.M., Walsh, J.D., Reeves, W.C., Bayer, E.V., Hui, L.T. & Murray, R.A. (1987) Surveillance for arthropod-borne viral activity and disease in California during 1986. *55th Annual Conference of the California Mosquito and Vector Control Association* **55,** 1-11.

Foley, D.H. & Bryan, J.H. (1991) *Anopheles annulipes* Walker (Diptera: Culicidae) at Griffith, new south Wales. 1. Two sibling species in sympatri. *Journal of the Australian Entomological Society* **30,** 109-112.

Freeborn, B. (1926) The mosquitoes of California. *University of California Technical Bulletin Entomology* **3,** 368-374.

Green, C.A., Gass, R.F. & Munstermann, L.E. (1990) Population-genetic evidence for two species in *Anopheles minimus* in Thailand. *Medical and Veterinary Entomology* **4,** 25-34.

Gutsevich, A.V. (1985) A problem of subspecies in bloodsucking Diptera. pp 27-29. In: Skarlato O.A. (Ed.) *Systematics of Diptera (Insecta). Ecological and morphological principles.* Oxonian Press, PVT. Ltd, New Delhi.

Lambert, M., Pasteur, N., Rioux J-A., Delabre-Belmonte, A. & Balard, Y. (1990) *Aedes caspius* (Pallas, 1771) et *A. dorsalis* (Meigen, 1830) (Diptera: Culicidae). Analyses morphologique et genetique de deux populations sympatriques. Preuves de l'isolement reproductif. *Annles de la Societé entomologique de France* **26,** 381-398.

Levene, H. (1949) On a matching problem arising in genetics. *Annals of Mathematical Statistics* **20,** 91-94.

Matthews, T. & Munstermann, L. (1983) Genetic diversity and differentiation in northern populations of the tree-hole mosquito *Aedes hendersoni* (Diptera: Culicidae). *Annals of the Entomological Society of America* **76,** 1005-1010.

Matthews, T. & Munstermann, L. (1990) Linkage maps for 20 enzyme loci in *Aedes triseriatus. Journal of Heredity* **81,** 101-106.

Matthews, T. & Munstermann, L.E. (1994) Chromosomal repatterning and linkage group conservation in mosquito karyotypic evolution. *Evolution* **48,** 146-154.

Milankov, V., Vapa, Lj. & Petric, D. (2000) Genetic markers for the identification of *Aedes caspius* (Pallas, 1771) and *Aedes dorsalis* (Meigen, 1830) (Diptera: Culicidae). *Biological Journal of the Linnean Society* **71,** 53-60.

Munstermann, L. (1979) *Isozymes of Aedes aegypti: phenotypes, linkage, and use of genetic analysis of sympatric populations in East Africa.* Ph.D. Dissertation, University of Notre Dame. 176 pp.

Munstermann, L. (1990) Gene map of the yellow fever mosquito *Aedes (Stegomyia) aegypti* (2N=6). 3.179-3.183 pp. In S.J. O'Brien (Ed.) *Genetic maps.* 5th ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Munstermann, L.E. & Craig, G. (1979) Genetics of *Aedes aegypti*. Updating the linkage map. *Journal of Heredity* **70**, 291-296.

Munstermann, L.E., Taylor, D. & Matthews, T. (1982) Population genetics and speciation in the *Aedes triseriatus* Group. pp 433-453. In W.W.M. Steiner, W.J. Tabachnick, K.S. Rai, S. Narang, (Eds.) *Recent developments in the genetics of insect disease vectors.* Champaign. IL.

Narang, S., Seawright, J. & Kaiser, P.E. (1990) Evidence for microgeographic genetic subdivision of *Anopheles quadrimaculatus* species C. *Journal of the American Mosquito Control Association* **6**, 179-187.

Pashley, D.P. & Rai, K.S. (1983) Linkage relationships of eleven enzyme loci in the *Aedes scutellaris* group. *Biochemical Genetics* **21**, 1195-1201.

Rai, K.S., Pashley, D.P. & Munstermann, L.E. (1982) Genetics of speciation in aedine mosquitoes. 84-129 pp. In: W.W.M. Steiner, W.J. Tabachnick, K.S. Rai, S. Narang, (Eds.) *Recent developments in the genetics of insect disease vectors.* Champaign. IL.

Richardson, B., Baverstock, P., Adams, M. (1986) *Allozyme electrophoresis and population structure.* Academic Press. Sydney. 395 pp.

Schultz, J.H., Meier, P.G. & Newson, H.D. (1986) Evolutionary relationships among the salt marsh *Aedes* (Diptera: Culicidae). *Mosquito Systematics* **18**, 145-170.

Selander, R.K. (1970) Behaviour and genetic variation in natural populations. *American Zoologist* **10**, 53-66.

Stordeur de, G. (1976) Esterases in the mosquito *Culex pipiens pipiens* L.: formal genetics and polymorphism of adult esterases. *Biochemical Genetics* **14**, 481-493.

Swofford, D.L. & Selander, R.K. (1981) BIOSYS-1: A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *Journal of Heredity* **72**, 281-283.

Tadano, T. (1984) A genetic linkage map of the mosquito *Aedes togoi*. *Japanese Journal of Genetics* **59**, 165-176.

Tadano, T. (1987) Genetic studies on two carboxylesterase loci in *Aedes albopictus*. *Journal of the American Mosquito Control Association* **3**, 137-141.

Urbanelli, S., Cianchi, R., Petrarca, V., Sabatinelli, G., Coluzzi, M. & Bullini, L. (1980) Adaptation to the urban environment in the mosquito *Culex pipiens* (Diptera: Culicidae). *Ecologia*, S.IT.E/ATTI 1, Parma, 305-316.

Weir, B.S. (1990) *Genetic data analysis*. Sunderland, Massachusetts, Sinauer Associates, Inc. 445 pp.

Wood, D.M., Dang, P.T. & Ellis, R.A. (1979) *The insects and arachnids of Canada.* Part 6. The mosquitoes of Canada (Diptera: Culicidae). Quebec, Canadian Government Publishing Centre. 390 pp.

Wright, S. (1951) The genetical structure of populations. *Annals of Eugenics* **15**, 323-354.

Table 1. Estimates of genetic structure in populations of *Ochlerotatus dorsalis* species

Population	n (SE)	A	H_e (SE)	H_o (SE)	Private allele	$P_{(0.95)}$
AD1	39.0 (3.6)	3.3 (0.7)	0.463 (0.092)	0.263 (0.086)	14	0.778
AD2	24.2 (3.2)	1.8 (0.4)	0.162 (0.085)	0.059 (0.055)	1	0.333

n = Mean sample size per locus, SE = standard error

A = Mean number of alleles per locus

H_e = Expected heterozygosity averaged over all loci, SE = standard error

H_o = Average frequency of observed heterozygosity, SE = standard error

$P_{(0.95)}$ = Frequency of polymorphic

Table 2. Genotype frequency at variable loci in populations of *Ochlerotatus dorsalis* species

Locus	Genotype	AD1	AD2
Est-1	*92/92*	0.023	0.056
	96/96	0.139	0.389
	98/98	0.442	0.333
	100/100	0.349	0.111
	102/102	0.047	0.111
	H_o	0.000	0.000
	H_e	0.501	0.351
Est-6	*84/84*	-	1.000
	88/88	0.133	-
	93/93	0.133	-
	96/96	0.100	-
	98/98	0.167	-
	100/100	0.033	-
	86/93	0.033	-
	88/98	0.033	-
	88/93	0.067	-
	88/96	0.167	-
	96/100	0.067	-
	96/102	0.033	-
	102/104	0.033	-
	H_o	0.433	0.000
	H_e	0.801	0.000
*Gpi**	*88/88*	0.419	1.000
	88/100	0.581	-
	H_o	0.581	0.000
	H_e	0.412	0.000
*Had**	*90/90*	0.077	0.970
	100/100	0.564	-
	90/100	0.205	0.030
	100/110	0.154	-
	H_o	0.359	0.000
	H_e	0.409	0.000
*Idh-2**	*83/83*	0.127	-
	90/90	0.237	1.000
	83/90	0.309	-
	83/97	0.127	-
	90/97	0.200	-
	H_o	0.636	0.000
	H_e	0.613	0.000
Me	*102/102*	0.625	0.773
	104/104	0.325	0.227
	106/106	0.050	-
	H_o	0.000	0.000
	H_e	0.501	0.351
*Odh**	*100/100*	0.952	1.000
	100/108	0.048	-
	H_o	0.048	0.000
	H_e	0.046	0.000
Pgm	*100/100*	0.187	-
	140/140	0.375	0.500
	160/160	0.125	-
	100/140	0.250	0.500
	140/160	0.062	-
	H_o	0.313	0.500
	H_e	0.596	0.375

*Genotype frequency at *Idh-2, Had, Odh* and *Gpi* loci were presented in Milankov *et al.*, 2000