Acta Zoologica Academiae Scientiarum Hungaricae 48 (4), pp. 255–267, 2002

GEOGRAPHIC VARIATION OF CHEILOSIA VERNALIS (FALLÉN, 1817) (DIPTERA: SYRPHIDAE)

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Natural populations of *Cheilosia vernalis* (FALLÉN, 1817) from village Morinj, Mediterranean area (Montenegro); low mountain Fruska Gora in the Pannonian plain (Serbia) and two high Dinaric mountains Durmitor (Montenegro) and Kopaonik (Serbia) on the Balkan Peninsula were analyzed for genetic variability at 12 enzyme loci. Geographic variation was analyzed based on the presence of specific genotypes, rare and private alleles at *Gpi, Had, Idh-2, Mdh-2, Pgm* and *Sod-1* loci. Difference in genetic structure parameters was also observed.

Key words: *Cheilosia vernalis*, Syrphidae, allozyme, geographic variation

INTRODUCTION

Greatly variable species *Cheilosia vernalis* (FALLÉN, 1817) belongs to the *melanura* group of the genus *Cheilosia*, the family Syrphidae. The lack of mimetic characters, otherwise present in hoverflies, is specific for the tribe Cheilosiini. The species in the genus *Cheilosia* are dark with shiny thorax and abdomen. Comparing with other species groups in this genus, the group *melanura* comprises closely related species with the least differentiated male genitalia diagnostic features (VUJIC 1992) – considered the most important character in the systematics and taxonomy of hoverflies. Population genetic analyses of the species *Cheilosia vernalis* have not been performed, and taxonomy and systematics were based solely on the analysis of morphological traits.

The species *Cheilosia vernalis* has been described 8 times under different names (PECK 1988, VUJIC 1992). This suggests that there had been problems in defining its taxonomic status. Based on interpopulation variability of morphological characters (face in profile – facial tubercula and mouth edge, the shape, size and colour of antennae, distribution and colour of body hairs, cuticular punctuation), it has more than once been suggested that *Ch. vernalis* includes several closely related species (SPEIGHT & LUCAS 1992, VUJIC 1992). However, no satisfactory basis for subdividing the species has yet been demonstrated and the male terminalia of the various different variants appear identical (SPEIGHT & LUCAS 1992).

The species *Ch. vernalis* shows distinct seasonal dimorphism. Spring brood specimens are typically entirely, or predominantly, brown haired and frequently have the third antennal segment orange, while summer specimens tend to be pre-

dominantly dark-haired, with the third antennal segment dark brown (SPEIGHT & LUCAS 1992).

Ch. vernalis is extremely widespread species. It has been registered in most European countries, on Caucasus, in Siberia and the Oriental region. In the northern part of its areal preferred environments are dry meadows with short vegetation, old pasture, dune systems and grassy clearings in woodland, while it becomes increasingly montane in the south (SPEIGHT & LUCAS 1992, TORP 1994). It has been registered in lower altitudes in the north of the Balkan Peninsula and in the Mediterranean zone (MARCOS-GARCIA 1990, VUJIC 1992). *Cheilosia vernalis* can be found in urban biotopes as well: parks, gardens and ruderal environments (BARKEMEYER 1994).

The objective of this study was to analyze the population genetic structure, geographic variation and genetic differentiation among populations of Palaearctic species *Cheilosia vernalis* by allozyme electrophoresis.

MATERIALS AND METHODS

Sample collection

Samples of the species *Cheilosia vernalis* were collected from four geographic regions: Morinj, Montenegro (CVMOR; 46 specimens), Adriatic sea in the Mediterranean area; Fruska Gora Mts, Serbia (CVFG; 26), in hilly area of the Pannonian plain; and two high Dinaric mountains Durmitor, Montenegro (CVDUR; 34) and Kopaonik, Serbia (CVKOP; 6) (Fig. 1).

Investigated areas included different biogeographic landscapes on the Balkan Peninsula. The analyzed populations originating from specific habitats, geographically distant with no gene flow, were a good model for the analysis of genetic diversity and molecular mechanisms of adaptation in *Ch. vernalis*. Collection of the insect material was hindered by the very short period of activity (adults of this species are active only a few days in early spring). In addition, unstable weather conditions often caused the reduction of the population size (CVKOP was very small). However, the inclusion of the population from Kopaonik was important not only for enzyme polymorphism analysis, but also for taxonomic reasons. Morphologically distinct individuals, and unique combination of genotypes at *Gpi* and *Mdh–2* loci were registered (see Results and Discussion), suggesting the presence of cryptic taxa. Good zymogram resolution that enables correct identification of genotypes was an additional challenge. The species had a specific gene expression, and in spite of successful analyses of a large number of isoenzyme loci in other syrphid species and groups (MILANKOV 2001, MILANKOV *et al.* 2001, MILANKOV *et al.* 2002*a, b*), electrophoregrams for the following enzymes could not be obtained: AAT (aspartate amino transferases), AO (aldehyde oxidase) and ME (malic enzyme). Genotype identification for some enzyme systems (GPI, MDH, HAD and SOD) further decreased the number of specimens included in the analysis. Due to the low success in the analysis of *Sod-1*, the allele frequencies at this locus were not included in the analysis of hierarchical structure of the species (Wright's *F* statistics). Further investigation of the biology of *Ch. vernalis* might help explain the difficulties encountered in the analyses, especially for the individuals originating from Kopaonik.

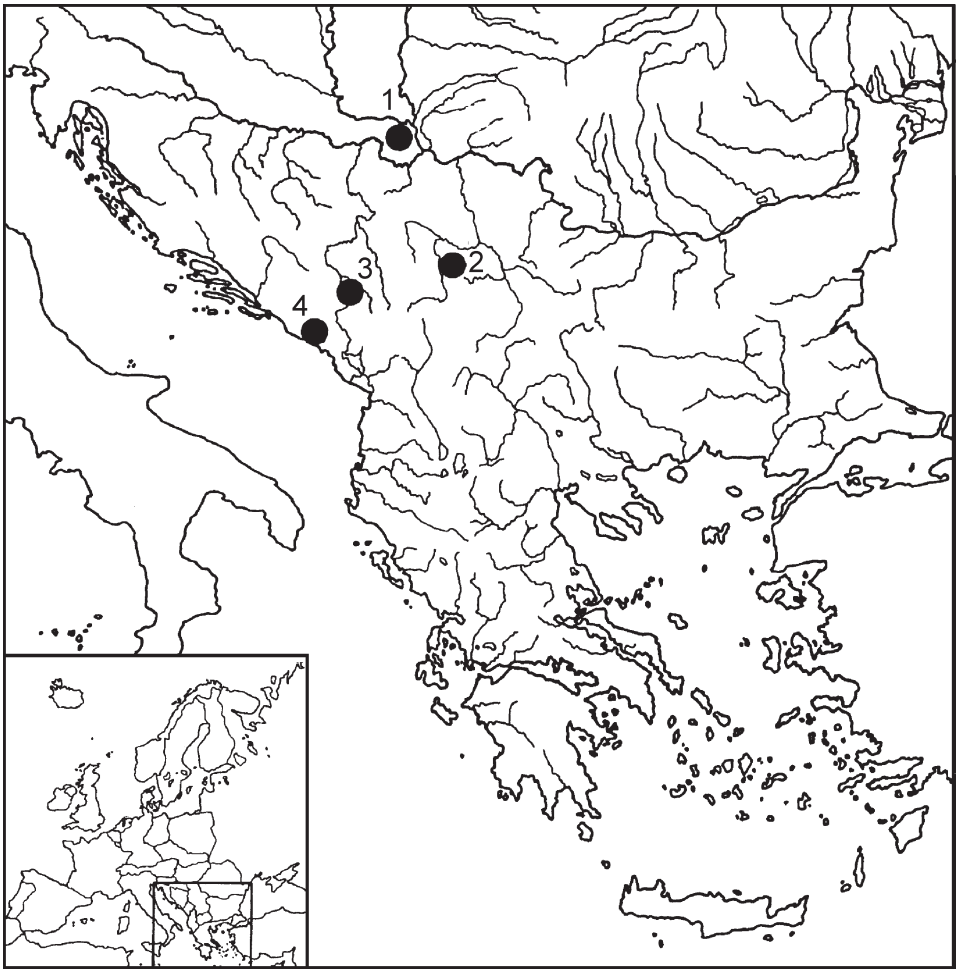

Fig. 1. Map of the Balkan Peninsula. Origin of the analyzed populations of *Cheilosia vernalis*: 1. Fruska Gora, 19°50'E, 45°10'N (Serbia); 2. Kopaonik, 20°40'E, 43°15'N (Serbia); 3. Durmitor, 19°00'E, 43°11'N (Montenegro); 4. Morinj, 18°40'E, 43°29'30''N (Montenegro). Locality Morinj is the territory with intermixed evergreen Mediterranean maritime woodlands and maquis and Submediterranean oak woodlands (*Lauro-Castanetum sativae*; *Rusco-Carpinetum orientalis*; *Orno-Quercetum ilicis*). Mountain Fruska Gora is a low mountain on the south border of the Pannonian plain, mostly covered with South European deciduous woodlands (*Fagus* and different *Quercus*) isolated from other autochthonous forests. Kopaonik and Durmitor are two high Dinaric Mts with different types of biomes, with deciduous woodlands (predominantly *Fagus*) in low altitudes (up to 700 m), European coniferous boreal woodlands (*Picea* and *Pinus*) in the higher altitudes, and the biome of alpine and high Nordic rock-grounds pastures and snow patches in the highest zone on the mountains peaks. The populations of *Ch. vernalis* were collected in *Fagus*, *Picea* and intermixed *Picea* and *Fagus* forests

Temporal variation of the CVMOR population was analyzed based on the genetic differentiation of the 46 specimens collected in years 1995 (CM1: 16), 1997 (CM2: 11) and 1998 (CM3: 19). CVMOR specimens from all three seasons were pooled together for geographic variability analysis of the species. Prior to electrophoresis, species were identified based on the shape of face, colour of legs and the length and colour of hairs on mesonotum and scutellum.

Allozyme analysis

The genetic variation was studied by standard 5% polyacrylamide gel electrophoresis (MUNSTERMANN 1979) with slight modifications (MILANKOV 2001). Tris-Boric-EDTA (pH 8.9) buffer was used to assay fumarate hydratase (E.C. 4.2.1.2. FUM; *Fum*), glucose phosphate isomerase (E.C. 5.3.1.9. GPI; *Gpi*), hexokinase (E.C. 2.7.1.1. HK; *Hk-2, Hk-3*), phosphoglucomutase (E.C. 2.7.5.1. PGM; *Pgm*), and superoxide dismutase (E.C. 1.15.1.1. SOD; *Sod-1*). Tris-Citric (pH 7.1) buffer was used to assay α-glycerophosphate dehydrogenase (E.C. 1.1.1.8. GPD; *Gpd-2*), 2-hydroxy acid dehydrogenase (E.C. 1.1.99.6. HAD; *Had*); isocitrate dehydrogenase (E.C. 1.1.1.42. IDH; *Idh-1, Idh-2*), and malate dehydrogenase (E.C. 1.1.1.37. MDH; *Mdh-1, Mdh-2*).

Insect specimen electrophoresis was performed in the same gel for direct interpopulation comparison. Loci were numbered and alleles marked alphabetically with respect to increasing anodal migration. Extracts from different body regions were used for the analysis of isozyme variability depending on metabolic function and regional distribution of enzyme (head + 0.10 ml: FUM, HK, IDH, MDH, PGM; thorax + 0.15 ml: GPD, GPI, HK, IDH, SOD). Duration of electrophoretic run at 90 mA (141–210V) was 2.00–4.00 hrs.

Analysis

Geographic variation of the species *Ch. vernalis* was investigated by analysis of specific homozygous genotypes and private (unique) alleles. Statistical analysis of electrophoretic variability data was performed using BIOSYS-1 (SWOFFORD & SELANDER 1981). The tests included: genotype and allele frequency, the percentage of polymorphic loci (*P*), mean observed and expected heterozygosity (H_o, H_e) for small samples corrected using Levene's (1949) formula. Difference between H_o and H_e at 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 deviation of H_o from H_e, three levels of genetic differentiation F_{is} (variation within subpopulation), F_{it} (homozygosity of individual relative to the total population), and F_{st} (amount of subdivision relative to the limiting amount under complete fixation) were calculated. Genetic identity (NEI 1972) was used to perform hierarchical cluster analysis using arithmetic averages (UPGMA) and the relationships were summarized in the form of dendrogram.

RESULTS

Nine enzyme systems coded by alleles of 12 loci were assayed in populations of the *Cheilosia vernalis*. *Fum*, *Gpd*-2 and *Idh*-1 loci were monomorphic in all populations. *Hk-2* and *Hk-3* loci were polymorphic only in CVMOR, *Idh*-2 in

CVDUR and CVFG, *Mdh*-1 in CVDUR and CVKOP, *Mdh*-2 in CVKOP and CVFG, while *Pgm* was monomorphic only in CVMOR. The greatest number of alleles (22) was registered in CVDUR and CVFG populations, followed by CVKOP (19) and CVMOR (17) (Table 1).

Heterozygotes *Gpi*$^{b/h}$ (except CVMOR), *Had*$^{k/o}$ (CVMOR, CVDUR), *Had*$^{o/s}$ (except in CVMOR), *Idh-2*$^{e/g}$ (CVDUR, CVFG), *Pgm*$^{a/e}$, *Pgm*$^{b/c}$ (CVDUR) and *Sod-1*$^{a/c}$ (CVFG) showed geographic distribution. Six out of seven registered het-

Table 1. Genotype frequencies at variable loci in the populations of *Cheilosia vernalis*

Locus	Genotype	CVMOR	CVDUR	CVKOP	CVFG
Gpi	*b/b*	–	–	0.400	–
	f/f	0.043	–	–	0.038
	g/g	0.239	0.333	0.200	0.231
	h/h	0.718	0.576	0.200	0.693
	b/h	–	0.091	0.200	0.038
Had	*o/o*	0.769	0.818	0.750	0.200
	p/p	–	0.045	–	0.500
	k/o	0.231	0.045	–	–
	o/s	–	0.092	0.250	0.300
Hk-2	*e/e*	0.214	–	–	–
	f/f	0.786	1.000	1.000	1.000
Hk-3	*e/e*	0.214	–	–	–
	f/f	0.786	1.000	1.000	1.000
Idh-2	*e/e*	1.000	0.889	1.000	0.960
	e/g	–	0.111	–	0.040
Mdh-1	*a/a*	1.000	0.794	0.800	1.000
	b/b	–	0.206	0.200	–
Mdh-2	*a/a*	–	–	–	0.167
	b/b	1.000	1.000	0.667	0.833
	c/c	–	–	0.333	–
Pgm	*a/a*	–	–	0.200	–
	b/b	–	0.119	–	0.040
	c/c	1.000	0.735	0.400	0.440
	e/e	–	0.088	0.400	0.520
	a/e	–	0.029	–	–
	b/c	–	0.029	–	–
Sod-1	*a/a*	1.000	1.000	1.000	0.889
	a/c	–	–	–	0.111

erozygotes at five loci were found in CVDUR, two of which were unique. Four heterozygotes were registered in CVFG (1 unique), two in CVKOP and one in CVMOR. High-mobility alleles at *Sod-1*, *Had* and *Idh-2* loci were not registered as homozygotes (Table 1).

Three unique homozygotes were registered in CVKOP (*Gpi*$^{b/b}$, *Mdh-2*$^{c/c}$, *Pgm*$^{a/a}$), 2 in CVMOR (*Hk-2*$^{e/e}$, *Hk-3*$^{e/e}$) and 1 in CVFG (*Mdh-2*$^{a/a}$) (Table 1). Two private alleles were detected in CVMOR (*Hk-2*^{e}, *Hk-3*^{e}) and CVFG (*Mdh-2*^{a}, *Sod-1*^{c}), one in CVKOP (*Mdh-2*^{c}), while none were registered in CVDUR (Tables 1 & 2).

The presence of major (≥0.5) and rare (<0.05) alleles indicated genetic divergence among populations of *Ch. vernalis*. The same major alleles at *Hk-2, Hk-3, Idh-2, Mdh-1, Mdh-2* and *Sod-1* were registered in all investigated populations. However, different major alleles were found at *Gpi* (in CVKOP) and *Had* (CVFG). The majority of rare alleles was registered in CVDUR (5), CVFG (4) and CVMOR (1). The largest number (3) of rare alleles was registered at *Had* in CVDUR (Table 2).

Difference between observed (H_o) and expected (H_e) heterozygosity based on Hardy-Weinberg values was statistically significant for all variable loci in all populations except *Had* (CVMOR and CVKOP), *Idh-2* (CVDUR, CVFG) and *Sod-1* (CVFG) (Table 3). Genotype fixation index, *F*, indicated excess homozygosity ($F_{is} > 0$) in all populations at all loci, except at *Had* in CVMOR and CVKOP populations, at *Idh-2* in CVDUR and CVFG, and *Sod-1* in CVFG (Table 3). These results were in

Table 2. Allelic frequencies at heterozygous loci in the populations of *Cheilosia vernalis*

Locus	Allele	CVMOR	CVDUR	CVKOP	CVFG
Gpi	*b*	–	0.045	0.500	0.019
	f	0.043	–	–	0.038
	g	0.239	0.333	0.200	0.231
	h	0.718	0.621	0.300	0.712
Had	*k*	0.115	0.023	–	–
	o	0.885	0.886	0.875	0.350
	p	–	0.045	–	0.500
	s	–	0.045	0.125	0.150
Idh-2	*e*	1.000	0.944	1.000	0.980
	g	–	0.056	–	0.020
Pgm	*a*	–	0.015	0.200	–
	b	–	0.132	–	0.040
	c	1.000	0.750	0.400	0.440
	e	–	0.103	0.400	0.520
Sod-1	*a*	1.000	1.000	1.000	0.944
	c	–	–	–	0.056

accordance with Selander's *D* statistics, since *D* was 0 only for *Had* in CVKOP and *Idh-2* in CVFG (Table 3).

F statistics was used to describe temporal variation among CVMOR samples collected in three seasons: 1995 (CM1), 1997 (CM2) and 1998 (CM3). Standardized variance of allele frequency, F_{st} value, indicated high genetic substructuring (F_{st}=0.377). The comparison of pairs of subpopulations showed that 33% of the allele frequency variance at *Gpi, Had, Hk-2* and *Hk-3* was due to genetic differentiation between CM1 and CM2 subpopulations. *F* statistics for CM2 and CM3 showed the lack of substructuring (F_{st}=0.063). Estimates of genetic variability showed that CM1 subpopulation ranked the highest in percentage of polymorphic loci (33.3%) and in the mean number of alleles per locus (1.4), followed by CM3 (25%; 1.3) and CM2 with the lowest values (16.7%; 1.2). Contrary to the above

Table 3. Deviation from Hardy-Weinberg equilibrium of genotype frequencies at nine polymorphic loci in the populations of *Cheilosia vernalis*

Locus	Population	H_o	H_e	F_{is}	*D*	*P*
Gpi	CVMOR	0	19.824	1.000	–1.000	***
	CVDUR	3	16.785	0.819	–0.821	***
	CVKOP	1	3.444	0.677	–0.710	*
	CVFG	1	11.627	0.912	–0.914	***
Had	CVMOR	6	5.412	–0.130	0.109	ns
	CVDUR	3	4.721	0.350	–0.365	*
	CVKOP	1	1.000	–0.143	0.000	ns
	CVFG	3	6.368	0.504	–0.529	*
Hk-2,3	CVMOR	0	9.600	1.000	–1.000	***
Idh-2	CVDUR	2	1.943	–0.059	0.029	ns
	CVFG	1	1.000	–0.020	0.000	ns
Mdh-1	CVDUR	0	11.284	1.000	–1.000	***
	CVKOP	0	1.778	1.000	–1.000	*
Mdh-2	CVKOP	0	2.909	1.000	–1.000	**
	CVFG	0	6.809	1.000	–1.000	***
Pgm	CVDUR	2	14.119	0.856	–0.858	***
	CVKOP	0	3.556	1.000	–1.000	***
	CVFG	0	13.633	1.000	–1.000	***
Sod-1	CVFG	2	1.943	–0.059	0.029	ns

H_o = Observed heterozygosity; H_e = Expected heterozygosity over all loci; F_{is} = Fixation index (WRIGHT, 1951); *D* = Selander's coefficient; *P* = Level of significance (ns = not significant, * = significant at $P \leq 0.05$, ** = significant at $P \leq 0.01$, *** = significant at $P \leq 0.001$)

Table 4. Summary of Wright's F statistics at eight polymorphic loci in the populations of *Cheilosia vernalis*

Locus	F_{is}	F_{st}	F_{it}
Gpi	0.905	0.027	0.907
Had	0.260	0.262	0.454
Hk-2	1.000	0.163	1.000
Hk-3	1.000	0.163	1.000
Idh-2	–0.021	0.012	–0.009
Mdh-1	1.000	0.166	1.000
Mdh-2	1.000	0.222	1.000
Pgm	0.937	0.319	0.957
Mean	0.799	0.185	0.836
Jack-knife estimates over loci			
Mean	0.801	0.183	0.834
S.D. (x)	0.133	0.069	0.106

mentioned parameters, the highest value of mean heterozygosity was observed in CM2 (0.036) followed by CM1 (0.017) and CM3 (no heterozygotes).

The mean fixation index at total population was high (F_{it}=0.798), but the loci did not contribute equally. F_{it} values at *Gpi, Hk-2, Hk-3, Mdh-1, Mdh-2* and *Pgm* loci were high (from 0.907 to 1.000), except *Had* (F_{it}=0.454). Negative F_{it} values were registered at *Idh-2* locus. Fixation index (F_{is}) showed persistent heterozygosity excess only at *Idh-2* ($F_{is} < 0$). Except at *Had*, local inbreeding was more important ($F_{is} > F_{st}$). F_{st} as a measure of genetic differentiation between populations indicated genetic subdivision (F_{st}=0.185). Value of *F* parameters by Jack-knife estimates over loci (0.183) were in accordance with previous results (Table 4).

Analysis of population genetic structure parameters showed small differences in the mean number of alleles per locus (*A*) and average H_o. Average H_o in all populations was smaller than H_e. The mean number of alleles per locus, and the frequency of polymorphic loci were the smallest in CVMOR, and higher in the other three populations. Based on the 0.99 criterion, the highest percent of polymorphic loci was registered in CVFG (Table 5).

Genetic identity by locus among conspecific populations was almost complete ($I > 0.95$) at 81% of the analyzed loci, while no loci showed complete genetic difference ($I < 0.05$). Based on the average genetic identity (*I:* Nei, 1972), CVMOR and CVDUR were more similar to each other then to the other populations. Genetic identity among these populations and CVKOP (next cluster) ranged from 0.928 (between CVMOR and CVKOP) to 0.960 (between CVKOP and CVDUR). The

Table 5. Estimates of genetic structure parameters in the populations of *Cheilosia vernalis*

Population	*n* (SE)	*A*	H_e (SE)	H_o (SE)	Private allele	$P_{(0.95)}$
CVMOR	36.3 (3.8)	1.42 (0.19)	0.110 (0.049)	0.019 (0.019)	1	0.333
CVDUR	27.3 (2.9)	1.83 (0.34)	0.132 (0.054)	0.033 (0.015)	0	0.417
CVKOP	4.5 (0.5)	1.58 (0.23)	0.208 (0.082)	0.037 (0.025)	1	0.417
CVFG	21.3 (1.8)	1.83 (0.30)	0.172 (0.070)	0.041 (0.025)	2	0.417 (0.500)
Mean	22.35	1.675	0.156	0.033	–	0.375 (0.396)

n = Mean sample size per locus, SE = standard error; *A* = Mean number of alleles per locus; H_e = Expected heterozygosity averaged over all loci; H_o = Average frequency of observed heterozygosity; $P_{(0.95)}$ = Frequency of polymorphic loci based on the 0.95 (0.99) criterion

third cluster was formed by CVFG. Genetic identity among CVFG and the other 3 populations ranged from 0.933 (CVFG and CVMOR; CVFG and CVKOP) to 0.954 (CVFG and CVDUR) (Fig. 2).

DISCUSSION

The use of morphological characters in defining cryptic taxa and evolutionary relationships of closely related species has been proven insufficient in many groups of organisms (SHARMA *et al.* 1999), especially in insects (FOLEY *et al.* 1995, NARANG *et al.* 1993, MILANKOV *et al.* 2000, 2001). The reasons for this are contribution of ecological variance to the overall phenotype variance, complicated defining of polygene genetic control of morphological characters and pleiotropic effect. Also, the difference in selection pressures on different traits and speed of

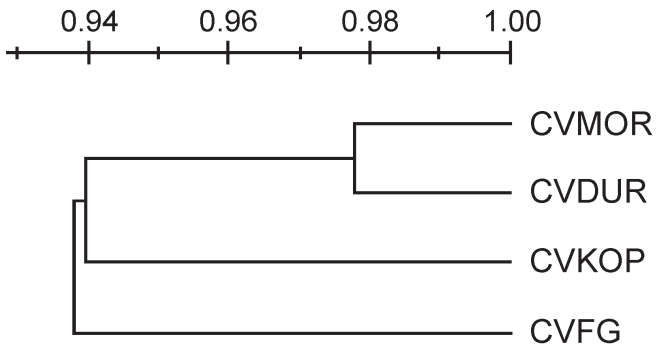

Fig. 2. Dendrogram of genetic relationships among the populations of *Cheilosia vernalis* species using unweighted pair group clustering of NEI's (1972) identity

evolutionary changes in genes at certain loci might cause the absence of correlation between morphological and gene-enzyme variability.

There had been problems in defining the taxonomic status of widely distributed Palaearctic species *Ch. vernalis* based on the analysis of morphological characters. Due to high variability of the studied parameters and distinct seasonal dimorphism it has more than once been suggested that *Ch. vernalis* comprises several closely related species (SPEIGHT & LUCAS 1992, VUJIC 1992). The results of gene-enzyme analysis and descriptions of morphological characters were compared for each specimen. No correlation was found between morphological characters and particular genotypes or alleles. The specimens with distinct morphological traits did not have unusual genotypes, while specimens with unique genotypes had no particular morphological differences comparing to specimens with the most frequent genotypes. No correlation between sex and certain alleles or genotypes was registered. However, specific combination of rare alleles in *Gpi* and *Mdh-2* loci was found. Only in two specimens from CVKOP homozygote $Gpi^{b/b}$ was registered, and in the both instances combined with a unique homozygote $Mdh\text{-}2^{c/c}$. This unique combination of specific homozygotes suggests possible presence of cryptic taxa, but further investigation is necessary. It is important to point out that the allele Gpi^{b} (<0.05) was present only in heterozygous combinations in CVDUR and CVFG.

The reason for the significant deviation (Table 3) between observed and expected genotype frequencies at *Gpi* locus is probably the observed specific pattern of GPI zymogram. The populations of *Ch. vernalis* had the heterozygous combination characteristic for other syrphid populations (MILANKOV 2001), with "slow" and "fast" allelomorph. Unique for two individuals from CVKOP was the genotype $Gpi^{b/b}$. No other syrphid population had the homozygote formed by the "slow" allelomorphs (MILANKOV 2001). Possible explanation for the deviation of genotype frequencies from the expected values for *Gpi* locus might be the presence of the lethal recessive allele, as well as inability to detect the activity of the allozyme coded by alleles in the homozygous combination. Also, individuals might have differential survival due to selection pressure against the "slow" homozygotes. Significant deviation of the observed genotype frequencies from the expected at *Mdh-2* in CVKOP population might be due to the cryptic taxon with specific combination of genotypes $Gpi^{a/a}$ and $Mdh\text{-}2^{c/c}$. Random changes (drift) in genotype frequencies can be very important in small populations, thus it has to be included in population – genetic analysis. It is also important to bear in mind that the period of activity is very short (only a few days) for adults of *Ch. vernalis* and is strongly impacted by environment (unstable weather conditions in early spring cause the reduction of the effective population size). Registered differences in values of ge-

netic structure parameters were probably due to association between genetic variability, level of ecological heterogeneity and effective size of populations. The small population of changeable size, originating from Morinj, had the smallest mean number of alleles per locus and average frequency of observed heterozygosity. The highest values of these parameters were registered in the population from Fruska Gora. Very small numbers of active adults were registered in 1995, 1996 and 1997 in CVFG population. This might have been due to the high mortality caused by sudden changes in temperature, which might have affected the genetic structure of the population. The way in which the weather conditions can significantly influence the effective population size is illustrated by the registered differences among the three samples of CVMOR collected in different years. Comparison of genetic variability parameters and F_{st} estimates among samples collected in different years showed that the small population CVMOR had unstable genetic structure. The highest value of the standardized variance (F_{st}) between CM1 and CM2, the lowest polymorphism, and the average number of alleles per locus calculated for CM2 are indicators of possible population bottleneck (severe reduction of population size), or selection "against" certain alleles (that is, phenotypes). Thus, the allele Hk^{e}, and the rare allele Gpi^{r} [allozymes of this locus show thermal stability differences (WATT *et al.* 1996, KAIN *et al.* 1997) as well as latitudinal clines (MILANKOV *et al.* 2001)] were registered in CM1 and CM3, but not in CM2. Differential selection might have caused the presence of the registered heterozygote $Had^{k/o}$ in CM1 and CM2, but not in CM3 sample.

The analysis of allozyme variability and interpopulation genetic divergence among Mediterranean population from Morinj, two montane populations from Durmitor and Kopaonik and population from the low Pannonian mountain Fruska Gora, presented in this paper, showed that this extraordinary variability could presently be interpreted as geographic variability. The presence of major alleles indicated genetic divergence of the analyzed populations of *Ch. vernalis*. Spatial distribution of genotypes, rare and major alleles caused distinct structuring of the species. F_{st} as index of genetic differentiation was 0.185 among analyzed populations. Population subdivision of *Ch. vernalis* was due to differences of allele frequency variances at *Pgm*, *Had* and *Mdh-2*. Genetic divergence among populations was, to a lesser extent, affected by allelic frequency variance at *Mdh-1*, *Hk-2* and *Hk-3*. Besides the decreased gene flow between geographically distant populations, elimination or favorization of alleles and genotypes through natural selection, and coadaptation of gene combinations to specific habitat conditions, historic factors played a role in genetic divergence. Rare alleles suggest the possibility of population bottlenecks in the past (MUNSTERMANN 1994).

Genetic differentiation of investigated populations falls within local population differentiation values of fruitflies *Drosophila willistoni* sibling species (AYALA *et al.* 1974) and hoverflies *Merodon avidus* A and *M. avidus* B of the *avidus* group (MILANKOV *et al.* 2001). The small number of analyzed specimens, and the possible presence of cryptic taxa, caused CVKOP to have the lowest calculated genetic identity values. Genetic divergence of CVKOP and CVFG was due to specific genotype $Gpi^{b/b}$ and the difference of $Gpi^{h/h}$ and $Gpi^{b/h}$ genotype frequencies. Spatial variability of the genotypes at *Had* and *Pgm* loci caused genetic differentiation of CVFG from other analyzed populations of *Ch. vernalis*.

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Acknowledgements – We would like to thank for the collected material to D. MILENKOVIC, P. RADIŠIC and S. RADENKOVIC. Our gratitude goes to LÁSZLÓ BARSI for his help with the technical matters. This work was supported by the Ministry for Science, Technologies and Development of the Republic of Serbia, Grant No 1770.

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Revised version received November 11, 2002, accepted December 2, 2002, published February 28, 2003