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GENETIC EQUILIBRIUM IN *CHEILOSIA VERNALIS* POPULATIONS (DIPTERA: SYRPHIDAE)

ABSTRACT: Genetic equilibrium of polymorphic enzyme loci from four natural populations of *Cheilosia vernalis* (Fallén, 1817) was tested. The study populations were from different regions of the Balkan Peninsula: Mediterranean area (Morinj, Montenegro: CVMOR), low mountain in the Pannonian plain (Fruška Gora, Serbia: CVFG), and two high Dinaric mountains (Durmitor, Montenegro: CVDUR; and Kopaonik, Serbia: CVKOP). Out of twelve enzyme loci analyzed for genetic variability, only four to six were polymorphic in the studied populations. For those loci, the observed and expected values of genotype frequencies were compared with chi-square goodness-of-fit tests. Analysis of deviations of phenotypic classes from Hardy-Weinberg equilibrium revealed significant differences at polymorphic loci of all populations except in CVKOP. This implied possible important influence of evolutionary mechanisms such as low migration rates, population substructuring and natural selection in creation and maintenance of genetic variability.

KEY WORDS: allozyme, *Cheilosia vernalis*, Hardy-Weinberg equilibrium, Syrphidae

INTRODUCTION

For a species, evolution involves changes in the genetic makeup of a population from generation to generation. Thus, genetic variability of populations is a measure of the evolutionary potential of species. Assessing independence of alleles within loci, Hardy-Weinberg equilibrium (HWE), has been an objective of evolutionary biology. Nonindependence is a starting point for determining what forces are responsible for variation and changes in allele frequencies (Shoemaker et al., 1998). The HWE departures may be caused by any of the factors that promote differential reproduction. Some factors related to population genetics are: small population size that makes a population susceptible to random genetic drift, non-random mating in subdivided or fragmented populations, differential selection, mutations, and/or biological factors, inclu-

ding differences in allele frequencies between sexes, age classes or year classes (Richardson et al., 1986; Pasteur et al., 1988).

One of the most intriguing hoverfly taxa is the genus *Cheilosia*, which is among the most diverse genera of the Palaearctic Syrphidae (Vujić, 1996), with over 400 Palaearctic species (Vujić, 1992), 175 of which are European (Speight, 2003). *Cheilosia* larvae are phytophagous or fungivorous, and it has been hypothesized that adaptive radiation at the larval stage gave rise to numerous cryptic and polytypic species (Vujić, 1996). Determination of *Cheilosia* species is difficult and often inconsistent (Speight, 2003) and the taxonomic status of many *Cheilosia* species remains uncertain. One such species is *Cheilosia vernalis*, a member of the *melanura* group (Vujić, 1996), which has been the subject of scientific debate ever since its description. Great variation of the morphological traits and distinct seasonal dimorphism (Vujić, 1992) spurred the taxonomic controversy, which resulted in 7 synonyms for *C. vernalis* (Peck, 1988).

Genetic studies of diploid organisms using allozyme electrophoresis are often based on co-dominant alleles at autosomal loci. Analysis of hierarchical organization and spatial and temporal variation of taxa by electrophoresis is a reliable and commonly used technique for quantifying the genetic variability of populations. Thus far, the study of genetic variation in *C. vernalis* populations from the Balkan Peninsula was based on the nuclear allozyme gene (Milankov et al. 2002a, 2002b) and mitochondrial DNA (mtDNA) sequence data (Ståhl et al., unpublished). These data revealed a large spatial variation of genotype and allelic frequencies at allozyme loci (Milankov et al., 2002a), strong influence of low migration rates and population substructuring (Milankov et al., unpublished), and likely presence of cryptic taxa (Milankov et al., unpublished; Ståhl et al., unpublished). Yet, potential factors responsible for the genetic divergence of the conspecific populations have not been determined.

Understanding the molecular basis of adaptation and quantified genetic variation in populations of *C. vernalis* elicited further research. The goal of this paper was to analyze the gametic equilibrium in populations of *C. vernalis* from the Balkan Peninsula, and try to elucidate potential factors driving the change in genotype frequencies of the studied populations.

MATERIAL AND METHODS

Sample collection

Samples of the species *Cheilosia vernalis* were collected from four regions of the Balkan Peninsula (population code and number of collected specimens in parenthesis): Mediterranean area (Morinj, Montenegro — CVMOR: 46 specimens), hilly area of the Pannonian plain (Fruška Gora, Serbia — CVFG: 26), and two high Dinaric mountains (Durmitor, Montenegro — CVDUR: 34, and Kopaonik, Serbia — CVKOP: 6). These regions span a variety of biomes, from intermixed evergreen Mediterranean maritime woodlands, and maquis

(Morinj); isolated deciduous woodlands on the slopes of a low mountain (Fruška Gora); to deciduous woodlands at low altitudes (up to 700 m), coniferous boreal woodlands at higher altitudes and alpine and high rocky pastures and snow patches in the highest zone on the mountain peaks of the two Dinaric mountains (Kopaonik and Durmitor). A more detailed description of the collection sites is given in Milankov et al. (2002a).

Allozyme analysis

Twelve isozyme loci (for details see Milankov et al., 2002a) were analyzed using 5% polyacrylamide gel electrophoresis according to Munstermann (1979) with slight modifications (Milankov, 2001).

Extracts from different body regions were used for electrophoresis depending on metabolic function and regional distribution of an enzyme (head in 0.10 ml of loading buffer: FUM, HK, IDH, MDH, PGM; thorax in 0.15 ml loading buffer: GPD, GPI, HK, IDH, SOD). 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.

Analysis

Genotype frequencies were obtained by direct genetic interpretation of bands on gels. Genetic interpretation was done using Mendel's rule of inheritance of codominant genes. Genetic variation was analyzed using the computer program BIOSYS-2 (Swofford and Selander, 1981, modified by Black, 1997).

Chi-square goodness-of-fit test was performed using the observed and genotype frequencies expected under the Hardy-Weinberg equilibrium. Expected genotype frequencies were adjusted by Levene's coefficient for small samples (Levene, 1949). When more than two alleles were observed at a locus, genotypes were pooled into three classes (all alleles except the most common one were treated as a single allele) and tested again. Three genotype classes were calculated: (1) homozygotes for the most common allele, (2) heterozygotes for the most common allele and one of the other alleles and (3) all other genotypes.

RESULTS

Twenty eight alleles were identified at 12 analyzed loci (Milankov et al., 2002a). However, only four (CVMOR) to six loci (CVFG) were polymorphic in the studied populations (CVDUR and CVKOP had 5) (Milankov et al., 2002a). The results of the chi-square tests for deviations of observed genotypes from expected are summarized in Table 1.

Table 1. χ^2 statistic (degrees of freedom in parentheses) for deviation from Hardy-Weinberg equilibrium at polymorphic loci in four natural populations of *Cheilosia vernalis*. Levels of significance: *** = significant at $P \leq 0.001$, ** = significant at $P \leq 0.01$, or NS = $P \geq 0.05$

Locus#	CVMOR	CVDUR	CVKOP	CVFG
<i>Gpi</i>	76.73 (3) ***	29.67 (3) ***	1.97 (3) NS	35.31 (6) ***
<i>Had</i>	0.00 (1) NS	10.73 (6) NS	0.00 (1) NS	7.31 (3) NS
<i>Hk-2, Hk-3</i>	25.11 (1) ***	—	—	—
<i>Idh-2</i>	—	0.00 (1) NS	—	0.00 (1) NS
<i>Mdh-1</i>	—	31.01 (1) ***	2.33 (1) NS	—
<i>Mdh-2</i>	—	—	4.02 (1) **	20.51 (1) ***
<i>Pgm</i>	—	43.51 (6) ***	4.70 (3) NS	34.73 (3) ***
<i>Sod-1</i>	—	—	—	0.00 (1) NS

— denotes that a locus was monomorphic in the studied population;

#*Gpi* (glucose phosphate isomerase; E.C. 5.3.1.9), *Had* (2-hydroxy acid dehydrogenase; E.C. 1.1.1.99.6); *Hk-2, Hk-3* (hexokinase; E.C. 2.7.1.1), *Idh-2* (isocitrate dehydrogenase; E.C. 1.1.1.42), *Mdh-1, Mdh-2* (malate dehydrogenase; E.C. 1.1.1.37), *Pgm* (phosphoglucomutase; E.C. 2.7.5.1), *Sod-1* (superoxide dismutase; E.C. 1.15.1.1)

Analysis of deviation of pooled phenotype classes from the equilibrium, for populations with degrees of freedom larger than one, revealed that the loci *Pgm* and *Had* were in disequilibrium in CVKOP and CVFG, respectively (Tab. 2).

Table 2. χ^2 statistic (degrees of freedom in parenthesis) for deviation from Hardy-Weinberg equilibrium of pooled phenotype classes in the populations of *Cheilosia vernalis*. Levels of significance: *** = significant at $P \leq 0.001$, ** = significant at $P \leq 0.01$, or NS = $P \geq 0.05$

Locus#	CVMOR	CVDUR	CVKOP	CVFG
<i>Gpi</i>	43.57 (1) ***	19.79 (1) ***	0.86 (1) NS	19.18 (1) ***
<i>Had</i>	—	0.55 (1) NS	—	8.15 (1) **
<i>Pgm</i>	—	26.43 (1) ***	3.22 (1) **	26.05 (1) ***

#*Gpi* (glucose phosphate isomerase; E.C. 5.3.1.9), *Had* (2-hydroxy acid dehydrogenase; E.C. 1.1.1.99.6); *Pgm* (phosphoglucomutase; E.C. 2.7.5.1)

DISCUSSION

At the *Gpi* locus, the studied populations of *C. vernalis* had the heterozygous combination characteristic for other syrphid populations, with „slow” and „fast” allelomorph (Milankov, 2001). Apart from a few populations in genus *Merodon*, in all hoverflies populations surveyed so far the genotype proportions for the *Gpi* locus showed statistically significant departures from the expectations of the Hardy-Weinberg equilibrium (Milankov, 2001; Ludoški, 2002). Possible cause for the deviation of genotype frequencies from the expected values at the *Gpi* locus might be the presence of a lethal recessive allele, differential survival due to selection pressure against the „slow” homozygotes, or inability to detect the activity of the allozyme coded by alleles in the homozygous combination. Significant deviation of the observed genotype frequencies from the expected at *Mdh-2* locus in the CVKOP population might be due to the presence of a cryptic taxon with specific combination of genotypes *Gpi*^{sl/a} and *Mdh-2*^{cl/c} (Milankov et al., 2002a). In population-genetic analysis it is very important to consider effects of random drift, especially in the case of small populations which are more likely impacted by changeable environment factors, like populations of *C. vernalis*. Temporal variation between samples collected in different years was observed in the CVMOR population. Unstable genetic structure registered in the small, isolated CVMOR population could be an indicator of possible bottleneck events (severe reduction of population size), or selection „against” certain alleles (= phenotypes) during a certain period of activity. Additionally, during the short period of adult activity (only a few days) in 1995, 1996 and 1997 in CVFG, only very small numbers of active adults were registered, probably due to the high mortality, caused by sudden changes in temperature. We could assume that the weather conditions may have caused a significant decrease of the effective population size (Milankov et al., 2002a).

Excess homozygosity, indicated by the high values of the Wright's Fixation index (F_{is} ; Wright, 1951) and Selander's *D* coefficient (Selander, 1970) was observed in all populations at all loci, except at *Had* in CVMOR and CVKOP, *Idh-2* in CVDÜR and CVFG and *Sod-1* in CVFG (Milankov et al., 2002a). It could be hypothesized that heterozygote deficiency at the majority of analyzed loci was caused by non-random mating within spatially fragmented populations, small effective population size (including effect of random process), disruptive selection, the impact factor in origins, and maintenance of genetic polymorphism of species, common for species that use the environment in roughly granulated form.

In a previous study of population-genetic structure of four populations of *C. vernalis*, genetic differentiation was quantified using Wright's F_{st} coefficient (Wright, 1951) and Nei's genetic distance (Nei, 1978). A correlation between standardized allelic frequencies (F_{st}) and both geographic and genetic distance were observed. Furthermore, the analysis of genetic differentiation based on the allelic frequencies revealed that the occurrence of genetic changes during independent evolution of conspecific populations was not equal at all loci. This implies that the influence of gene flow, historical effects and genetic

drift were probably not important in genetic divergence. Contrary to this, different selective pressures on the individuals of the analyzed populations were probably a dominant mechanism of the genetic differentiation (Milankov et al., unpublished). Moreover, geographic distribution of the genotypes at *Gpi*, *Had*, *Idh-2*, *Mdh-2*, *Pgm* and *Sod-1* loci were recorded. Spatial variation was observed by registered *major*, *rare*, unique alleles and heterozygote genotypes (Milankov et al., 2002a).

In order to understand the adaptive relevance of the observed genetic polymorphism in populations of *C. vernalis* linkage disequilibrium was analyzed. The analysis of the allelic association of nine polymorphic loci in the spatially fragmented population of *C. vernalis*, the high percent of linkage alleles, ranging from 60% to 75%, was recorded. These results suggested that low migration rates, population substructuring and natural selection highly influenced the genetic divergence among the conspecific populations (Milankov et al., unpublished).

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ГЕНЕТИЧКА РАВНОТЕЖА У ПОПУЛАЦИЈАМА ВРСТЕ
CHEILOSIA VERNALIS (DIPTERA: SYRPHIDAE)

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Резиме

У раду је анализирана генетичка равнотежа полиморфних ензимских локуса четири популације врсте *Cheilosia vernalis* (Fallén, 1817). Анализиране популације воде порекло из четири различита региона Балканског полуострва: медитеранске области (Морињ, Црна Гора: ЦВМОР), ниске планине Панонске низије (Фрушка гора, Србија: ЦВФГ), и две високе планине Динарида (Дурмитор, Црна Гора: ЦВДУР; и Копаоник, Србија: ЦВКОП). Од 12 анализираних ензимских локуса четири до шест је било полиморфно. У полиморфним локусима су упоређене израчунате и очекиване вредности генотипских фреквенција χ^2 тестом. Анализом одступања фенотипских класа од очекиваних вредности према Харди-Вајнберговом принципу утврђено је значајно одступање у свим полиморфним локусима свих популација осим у ЦВКОП популацији. Резултати упућују на вероватан утицај еволуционих механизма као што су смањени проток гена, популациона структурираност и природна селекција у креирању и одржавању генетичке варијабилности.