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Allozyme variation in the winter moth, *Operophtera brumata* (Lepidoptera: Geometridae), in isolated populations

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Abstract. Allozyme variation was investigated in winter moth adults from four isolated localities in South Bohemia. Ten gene loci, five of which were polymorphic, were studied. Significant deviations of genotype frequencies from Hardy-Weinberg equilibrium were observed at most of polymorphic loci within the populations. The average heterozygosities for winter moth populations from four ecologically distinct localities ranged from 0.146 to 0.279. The winter moth F_{ST} mean value of 0.171 was relatively higher than that found in most other Lepidoptera, indicating fragmented population structure. The Červené Blato population had the greatest genetic distance (D = 0.265) from the other three populations. The genetic distance and F_{ST} values reflect geographic distance of the studied populations.

INTRODUCTION

The winter moth, *Operophtera brumata* (L.) (Lepidoptera: Geometridae), a species with wingless females, is a polyphagous pest of deciduous and fruit trees in the Palaearctic Region (Varley & Gradwell, 1958). It is found throughout most of Europe and parts of western and eastern Asia (Kozhanchikov, 1950).

Outbreaks of the winter moth have been recorded in England, in Scandinavian countries, and in British Columbia, Canada (Cuming, 1961; Varley & Gradwell, 1963; Embree, 1965; Tenow, 1972). During 1961–1964 there was a population explosion of the winter moth in the former Czechoslovakia, particularly in Moravia and Slovakia, causing extensive damage to oak and mixed forest stands (Mrkva, 1968). In recent years the winter moth has become the most important pest on fruit trees in southern Bohemia causing serious defoliation, particularly on apple and cherry trees along the roads (Rejmánek & Spitzer, 1980). However, the literature relating to the winter moth has been typically concerned with the problem of its distribution, phenology, ecology and biological control (Kozhanchikov, 1950; Embree, 1965; Cuming, 1961; Holliday, 1977, 1985; Hassell, 1980), and does not integrate information from population ecology with population structure inferred from allozyme analyses.

To date nothing has been known about the patterns of genetic variability in the winter moth. The aim of this study is the description of the genetic variability in several winter moth populations in South Bohemia in 1991–1992. The intraspecific variation can reflect different ecological characteristics in the populations studied.

MATERIALS AND METHODS

STUDY SITES. The winter moth collection sites were selected to represent ecologically distinct localities in South Bohemia.

They include the following places: 1. Horská Kvilda, a mountain spruce forest and a subalpine peat bog, located in the Šumava mountains of southwestern Bohemia, 2. Červené Blato, a transient raised peat bog near Salmanovice in Třeboň Basin, 3. Plástovice, and 4. České Budějovice, both in an agricultural landscape. Samples of the winter moth were collected on the bilberry, *Vaccinium myrtillus* L. at H. Kvilda and Č. Blato, and on apple trees along the main road near Plástovice and Č. Budějovice. More detailed information on sampling sites can be found elsewhere (San & Spitzer, 1993).

ELECTROPHORETIC ANALYSIS. Samples for enzyme analyses consisted routinely of about 50 males collected in the field. They were frozen and stored at –20 or –70°C until electrophoresis could be performed. The homogenates were prepared from an individual's thorax and abdomen in 10% glycerol in 0.05 M Tris – HCl buffer, pH 7.2, using a glass homogenizer with teflon pestle. They were then centrifuged at 10,000 g for 20 minutes at 4°C. All the samples were electrophoresed on vertical 7% polyacrylamide slab gels according to the procedure of Williams & Reisfeld (1964) at 60 V for the first 15 minutes and at 150 V for 5 hours. Enzyme activities were visualized by methods according to Shaw & Prasad (1970) or Harris & Hopkinson (1976).

The following enzyme systems were tested: Esterase (*Est*, E.C. 3.1.1.1); phosphoglucomutase (*Pgm*, E.C. 5.4.2.2); malate dehydrogenase (*Mdh*, E.C. 1.1.1.37); hexokinase (*Hk*, E.C. 2.7.1.1), malic enzyme (*Me*, E.C. 1.1.1.40); xanthine dehydrogenase (*Xdh*, E.C. 1.2.1.37); glucose-6-phosphate dehydrogenase (*G-6-pdh*, E.C. 1.1.1.49); lactate dehydrogenase (*Ldh*, E.C. 1.1.1.27); α-glycerophosphate dehydrogenase (*α-Gpd*, E.C. 1.1.1.8); leucine aminopeptidase (*Lap*, E.C. 3.4.1.1); glutamic-oxaloacetic transaminase (*Got*, E.C. 2.6.1.1); adenylate kinase (*Ak*, E.C. 2.7.4.3); 6-phosphogluconate dehydrogenase (*6-Pgd*, E.C. 1.1.1.44) and phospoglucose isomerase (*Pgi*, E.C. 5.3.1.9).

Allelic variants or allozyme bands for each locus were numbered in the order of increasing anodal mobility, number 1 being the allele with slowest mobility. The average heterozygosity H (average proportion of heterozygotes per locus), genetic identity I (the proportion of electrophoretically identical proteins over an analyzed set of loci between two related populations or species) and genetic distance D ($-\log_e I$) were calculated according to Nei (1987). The genetic differentiation among populations and fixation indices F_{ST} (which measures the extent of genetic differentiation of subpopulations), F_{IS} and F_{IT} (they measure the deviations of genotype frequencies from Hardy-Weinberg proportions in the subpopulations and in the total population, respectively) have been analyzed by Wright's (1965) F-statistics as modified by Nei (1977). The relationships between localities were summarized in the form of a dendrogram derived from an unweighted-pair-group method using arithmetic averages (UPGMA; Sneath & Sokal, 1973).

RESULTS

Fourteen enzyme systems were tested in the winter moth. Five enzymes, *Est*, *Pgm*, *Mdh*, *Hk* and *Me* were polymorphic – the frequency of the common allozyme was less than 0.95. Five other enzymes, *Xdh*, *G-6-pd*, *Ldh*, *α-Gpd* and *Lap*, were monomorphic. *Pgi*, *Ak*, *Got* and *6-Pgd* were not included in the analysis because of a lack of colour reaction with the stain. The *Est* was the most polymorphic enzyme having 7 allozymes. *Pgm* was polymorphic in Červené Blato, Plástovice and České Budějovice, but not in the Horská Kvilda moths. *Me* was polymorphic in Červené Blato and Plástovice, but its activity was too low to be detected in moths from the Horská Kvilda and České Budějovice populations.

Significant deviations of genotype frequencies from Hardy-Weinberg expectations were observed at some polymorphic loci within populations (Tab. 1). The allozyme frequencies, heterozygosities for single loci and average heterozygosities are also presented in Tab. 1.

TABLE 1. Allozyme frequencies, number of moths sampled (n), heterozygosity (h) for single loci, average heterozygosity (H), and X^2 comparisons of observed to expected genotype numbers (Hardy-Weinberg) for four populations of the winter moth in South Bohemia.

		Localities			
Gene locus	Alleles	Kvilda	Č. Blato	Plástovice	Č. Budějovice
Polymorphic loci					
Est	1	0.081	0.036	0.115	0.104
	2	0.108	0.167	0.063	0.083
	3	0.270	0.310	0.021	0.250
	4	0.351	0.143	0.427	0.135
	5	0.149	0.202	0.052	0.220
	6	0.041	0.071	0.228	0.135
	7	0	0.071	0.094	0.073
	n	46	42	48	48
	h	0.77	0.813	0.744	0.838
	X^2	40.64*	45.13*	21.52^{ns}	26.99^{ns}
Pgm	1	0	0.135	0.135	0
	2	0	0.459	0.271	0
	3	0	0.354	0.104	0.208
	4	0	0.052	0.083	0.313
	5	1.000	0	0.344	0.292
	6	0	0	0.063	0.188
	n	12	48	48	24
	h	0	0.650	0.776	0.754
	X^2	—	31.57*	32.58*	12.06ns
Mdh	1	0.854	0.063	0.854	0.750
	2	0.146	0.937	0.146	0.250
	n	48	48	48	36
	h	0.252	0.119	0.252	0.380
	X^2	1.69^{ns}	0.78^{ns}	3.80^{ns}	0.019^{ns}
Hk	1		0.396	0.479	0.500
	2		0.604	0.521	0.500
	n	0	24	24	12
	h		0.488	0.509	0.521
	X^2		2.34^{ns}	20.24**	11.001**
Me	1	0	0.521	0.500	0
	2	0	0.479	0.500	0
	n	48	24	24	12
	h		0.509	0.510	
	X^2	—	20.24**	24.00**	—
Monomorphic loci					
Xdh	n	0	48	48	48
G-6-pd	n	24	48	48	48
Ldh	n	24	48	48	48
α-Gpd	n	48	50	48	48
Lap	n	48	48	48	48
H		0.146	0.258	0.279	0.277
Variance V(H)		0.010	0.010	0.011	0.014

ns = non-significant at $P \leq 0.05$
* = significant at $P \leq 0.05$
** = significant at $P \leq 0.01$

Results of the estimates of Nei's (1987) fixation indices are shown in Tab. 2. Among the subpopulations analysis of the variance in allele frequency (F_{ST}) indicated significant differentiation (mean $F_{ST} = 0.171$) at four of the five loci for the winter moth. The fixation index F_{IS}, which measures the deviation from the proportion of heterozygotes expected for each locus, was 0.425, 0.424 and 0.472 for *Est*, *Pgm* and *Mdh*, respectively, and –0.850 and –0.300 for the *Hk* and *Me* loci, respectively. A negative value of F_{IS} indicated there were more heterozygotes than expected. The differences between the inbreeding coefficients (F_{IS}) were significant at three of the five loci (Tab. 2).

TABLE 2. Fixation indices for polymorphic loci in four populations of the winter moth.

Gene locus	n	F_{IT}	F_{ST}	X^2	F_{IS}	X^2
Est	184	0.456	0.055	121.44**	0.425	33.235**
Pgm	132	0.588	0.285	376.20**	0.424	23.730^{ns}
Mdh	192	0.718	0.465	178.56**	0.472	42.775**
Hk	60	–0.780	0.040	4.80*	–0.850	43.350**
Me	48	–0.280	0.010	0.96^{ns}	–0.300	4.320^{ns}
Mean	—	0.140	0.171	—	0.034	—

** = P < 0.01
* = P < 0.05
ns = non-significant

A comparison of populations for all enzyme systems was made to establish genetic identity and genetic distance values among sites and populations. Data from 10 enzyme loci were employed. Genetic identity values (Tab. 3, above diagonal) showed that marked levels of overall genetic differentiation occurred among all groups, but that the Červené Blato moths showed the least similarity (I = 0.767) to those from Horská Kvilda, České Budějovice and Plástovice. Moths from České Budějovice and Plástovice were most similar to each other (I = 0.972). Genetic distance values (Tab. 3, below diagonal) showed the greatest genetic distance between the Červené Blato and all the other populations and those from České Budějovice and Plástovice showed the closest distance. Fig. 1 plots the results of the unweighted-pair-group method using arithmetic averages based on genetic distances.

TABLE 3. Genetic identity (above diagonal), and genetic distance (below diagonal) among four populations of the winter moth.

Population	H. Kvilda	Č. Blato	Plástovice	Č. Budějovice
H. Kvilda	*	0.767	0.943	0.934
Č. Blato	0.265	*	0.883	0.895
Plástovice	0.059	0.124	*	0.972
Č. Budějovice	0.068	0.111	0.028	*

DISCUSSION

One of the main objectives of population genetics is to describe the amount of genetic variation in populations and to understand mechanisms that maintain variability. In general, the mean heterozygosities for *O. brumata* populations (0.146–0.279) are higher than the values determined for most other invertebrates (Selander & Kaufman, 1973). For instance, in *Drosophila* the value 0.145 was found (Wagner & Selander, 1974) and 0.083 ± 0.044 for the nine *Yponomeuta species* (Menken, 1982). The values of mean heterozygosity in *O. brumata* populations are similar to that (0.270) for *Heliothis zea* (Sluss et al. 1978) and to values commonly found in some forest Lepidoptera, 0.22–0.30 (Mitter & Futuyma, 1979). The average heterozygosities for the populations from Červené Blato, Plástovice and České Budějovice (0.258, 0.281 and 0.277, respectively) differed from that from Horská Kvilda (0.116), where the number of loci examined was lower.

Some enzyme loci are regularly polymorphic while others are rarely so (Wagner & Selander, 1974). A tendency for the amount of variation in particular enzymes to remain constant within species and among species groups suggests that the degree of variability is related to physiological functions or biological properties of the enzyme (Ayala & Powell, 1972). Enzymes involved in glucose metabolism were postulated to be less variable than other non-glucose-metabolizing enzymes (Gillespie & Kojima, 1968; Kojima et al., 1970; Johnson, 1974). Data for *Est* and *Hk* alleles agree with this hypothesis. Esterases, many of which have very broad substrate specificities, are usually the most polymorphic enzymes in insects and other organisms. Over a dozen alleles have been identified in local populations of butterflies (Burns & Johnson, 1967, 1971). In this study seven alleles were obtained at the *Est* loci. Stauffer et al. (1992) reported in *Ips typographus* that the number of alleles observed for *Est* locus was between 8 and 18. *Pgm*, though belonging to the group of glucose-metabolizing enzymes, was found to be rather polymorphic in some *O. brumata* populations. Similarly, the number of *Pgm* alleles in two sympatric populations of *Yponomeuta padellus* was 7 (Menken, 1982).

On average, approximately 17.1% (Tab. 2) of the total variance of allele frequencies was due to genetic differences among the winter moth populations (mean F_{ST} = 0.171). This left about 82.9% of the total gene diversity (heterozygosity) to be found in moths within any given population. This estimate can be compared with estimates determined in other studies for a variety of species with differing population structures, 0.485 in *Collops tricolor* S. (Coleoptera: Melyridae), an allopatric beetle (King, 1988), and 0.154 for the dingy cutworm, *Feltia jaculifera* (Gn.) (Lepidoptera: Noctuidae) (Gooding et al. 1992). The winter moth F_{ST} value of 0.171 was much higher than values reported for other moths and butterflies; e.g. 0.009 in the *Danaus plexippus* (Lepidoptera: Danaidae) (Eanes & Koehn, 1978) and 0.027 in *Yponomeuta* (Lepidoptera: Yponomeutidae) moths (Menken, 1982).

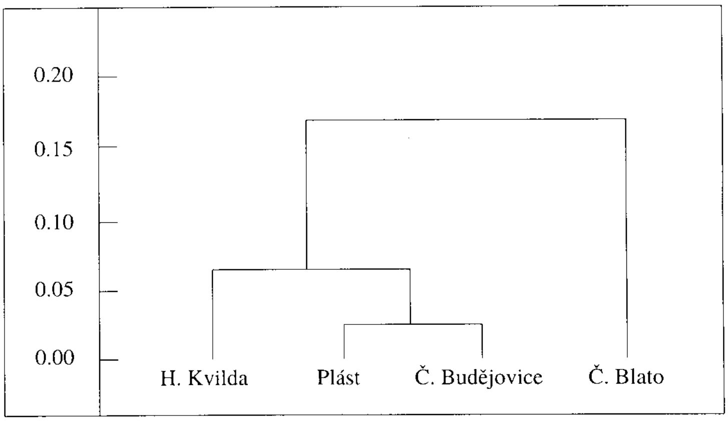

Fig. 1. Dendrogram (UPGMA) of the genetic distance among populations of the winter moth.

The fixation indices (F_{IT} and F_{IS}) computed for each individual locus showed persistent heterozygote deficiency at three loci (*Est*, *Pgm* and *Mdh*, both F_{IT} and $F_{IS} > 0$) and heterozygote excess at loci of *Hk* and *Me* (both F_{IT} and $F_{IS} < 0$). Thus, genetic variability at the five polymorphic enzyme loci indicates significant deviation from expected number of heterozygotes. In this case the deviations may be caused by selection, subdivision of populations (Wahlund's principle) and/or inbreeding effect. The simultaneous deficit and excess of heterozygotes at various polymorphic loci hints at both substructuring and selection in winter moth. The degree of genetic differentiation between the various populations of the winter moth is relatively high (mean D = 0.109) compared with other species. The mean genetic distance between 11 populations of the moth *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) was 0.034 (Sluss & Graham, 1979), the genetic distances in conspecific populations of *Yponomeuta* species range from 0.000 to 0.023, the mean being only 0.006 (Menken, 1981).

The genetic similarity coefficient is indicative of genetic divergence between populations (mean I = 0.899, Tab. 3). Conspecific populations (including local populations or subspecies) have generally similar coefficients, usually above 0.75 on a scale of 0–1 (Avise, 1974; Ayala, 1975). A value of 0.63 was found for Douglas-fir beetles, *Dendroctonus pseudotsugae* (Coleoptera: Scolytidae) (Stock et al. 1979), and 0.92 for several of the populations of a mountain pine beetle, *Dendroctonus ponderosae* in the Pacific Northwest (Stock & Guenther, 1979).

These similarities and differences between the various populations of the winter moth appear to have a geographic basis. In the case of České Budějovice and Plástovice populations, environmental conditions are similar – predominantly cultivated landscapes with small, wet hay meadows and fragments of deciduous forest. The main road connecting České Budějovice to Plástovice may aid in the transportation of males between these two populations. Thus, the geographic proximity may provide evidence to explain the high levels of genetic similarity of winter moths between these two populations. The Červené Blato moths, being geographically more distant and isolated from the other three sites, would be expected to be somewhat different genetically. The data on genetic similarity and genetic distance (Tab. 3) in this study supported this hypothesis.

In some oligophagous insects populations are structured along host plant lines: e.g. in mountain pine beetle populations the genetic differentiation was associated more closely with host tree species than with geographic distances among sites (Stock & Amman, 1980). Moths from Horská Kvilda and from Červené Blato were collected on *Vaccinium myrtillus* L., and those from Č. Budějovice and Plástovice on apple trees. The heterozygosities of polymorphic enzymes and genetic distances between populations do not indicate an influence of host plants. This result is similar to that found in several geometrid species taken from different host plants where genetic differentiation occurred only in one of three species and only in one of seven polymorphic loci (Mitter & Futuyma, 1979). Menken (1981) obtained similar results in the case of *Y. padellus* populations where the influence of different host plants had been studied.

The genetic variability of a population determines its ability to adapt to changing environmental conditions (Frati et al., 1992). From a practical point of view, recognizing such variation among populations of a pest insect species is essential for developing sound management practices (Wellington, 1977). Since the winter moth is of considerable economic importance, the genetic differences described here underline the necessity for the use of caution in extrapolating information obtained from one population and applying it to others, when control measures are being planned. No correlation between allozyme variability pattern at one or more loci and the ability of a population to be harmful has been found in this paper. Thus future studies are necessary to define in greater detail the unique features of the allozyme variation related to host plant use, pest status and stability of populations in variable environments. Additional studies of winter moth populations in zones where Horská Kvilda, Červené Blato, Plástovice and České Budějovice converge are required to elucidate the type and extent of genetic differentiation occurring in this species.

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