Genetic Variation and Microevolution at Allozyme Loci in Eastern Mediterranean Populations of the Geometrid Moth *Cyclophora Puppillaria* (Lepidoptera)

Geometrid Bir Güve olan *Cyclophora Puppilaria* (Lepidoptera)'nın Doğu Akdeniz Popülasyonlarındaki Allozim Lokuslardaki Genetik Varyasyon ve Mikroevrim

Research Article

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ABSTRACT

Genetic variation at 7 allozyme loci were determined in 4 Meditarranean populations of the moth *Cyclophora puppillaria*. Polymorphism levels, and gene diversities as Hardy-Weinberg heterozygosities are mostly substantial for the loci except for those in one of the samples. Pairwise genetic distances between the samples are expressed as F_{ST} values, and a high degree of differentiation exists for almost all of the pairwise comparisons. A test of natural selection, Ewens-Watterson test of neutrality, shows no sign of selective difference among electrophoretic allelic states over loci in all samples. Our results presents the the first picture of population genetic variation in this species and in accordance with the result of a previous DNA barcoding analysis pointing to low level of genetic variation.

Key Words
Allozyme polymorphism, *Cyclophora puppillaria*, Geometrid moths, natural selection.

ÖZET

Cylophora puppillaria'nın Akdeniz Bölgesi'nden örneklenen 4 popülasyonundaki 7 allozim lokusuna dayalı genetik varyasyonu ele almıştır. Hesaplanan polimorfizm düzeyleri, Hardy-Weinberg heterozigotluğu şeklindeki gen çeşitlilikleri popülasyonların biri dışında önemli büyüklüktedir. Popülasyonlar arası genetik uzaklıklar FST şeklinde ifade edilmiştir ve hemen tüm ikili karşılaştırmalar yüksek bir farklılaşma düzeyi olduğuna işaret etmektedir. Aynı zamanda, Ewens-Watterson nötralite testi, doğal seçilimin varlığını bulgulamak için kullanılmıştır ve test sonuçları bütün popülasyonlardaki tüm elektroforetik lokusların alelik durumları için seçilim önemli olmadığını göstermektedir. Elde ettiğimiz sonuçlar bu türe ilişkin ilk poülasyon genetik varyasyon profilini sunmaktadır ve daha önce gerçekleştirilen ve düşük düzeyli bir genetik varyasyon bulunduğunu ifade eden DNA barkodlama analizin sonucuyla da uyuşmaktadır

Anahtar Kelimeler
Allozim polimorfizmi, *Cyclophora puppillaria*, Geometrid güveler, doğal seçilim.

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INTRODUCTION

Detecting the amount of genetic variation in a species is one of the necessary steps to focus on its evolutionary genetics history [1,2]. Determining the electrophoretic variation at allozyme loci is a quicker method of genetic variation detection in which electrical charge and conformational changes from the mutations that result in amino acid replacements are revealed as different alleles of genes coding numerous enzymes [3]. It is limited in extent in reflecting the total amount of genetic variation of a given species [1,4]. But it is still a powerful tool that gives a correct picture of the amount of variation at loci with moderate polymorphisms [4].

Here in this article, we present results of the analysis of genetic variation of allozyme loci in natural populations of the geometrid moth *Cyclophora* pupilliaria (Hübner 1799). *C. puppillaria* (Hübner, [1799]), together with *Rhodometra saccraria* (Linnaeus, 1767) and *Orthonoma obstipata* (Fabricius, 1794) is known as "emigrant species" in Geometridae. In undertaking flights over one thousand kilometers from southern Europe to central Europe, in the North, it sometimes reaches up to southern Scandinavia and Ireland [5]. *C. puppillaria* is reported from eastern Alps at: 3200 m [5,6]. It is widespread in Mediterranean and common southern Europe where it flies from 0 up to 1000 m above sea-level [5]. In Turkey, from where our population samples were taken, it is found up to 1500 m of sea level [7]. *C. puppillaria* adults are nocturnal and readily attracted by light. The species is plurivoltine from mid March to late October or early November, its larva is polyphagous and possibly preferring evergreen oaks for feeding [7].

In our study we performed population genetic analyses of the allozyme variation data from *C. puppillaria* population samples taken in various localities in Eastern Mediterranean part of Turkey. We screened the populations for seven allozyme loci and estimated the basic parameters of level of the genetic variation and natural selection. Our study provides first results of population genetic analyzes in this relatively less studied Geometrid moth, *C. puppillaria* and we discuss our findings with respect to possible evolutionary mechanisms that may have underlined this species' genetic evolution.

MATERIAL and METHODS

Populations

Field collections were performed from 25 May to 6 July in 2007. Specimens were caught by light trap after the sunset in four localities. The exact GPS coordinates of sampling sites is given in Table 1, and Figure 1 shows a map of those sites. After collection, specimens were placed individually in small boxes with food and shipped alive to the laboratory where they were put immediately into a deep-freeze (at -80°C).

Allozyme Electrophoresis

Starch electrophoresis was performed for each population for the 7 loci chosen. Thorax homogenates were obtained and starch gel electrophoresis was performed, using thorax homogenates, as described in Manchenko [8] in which, after each run, gel slices were taken and and each slice was stained for a particular allozyme locus. Genotype determination and scoring were visual. The seven loci were phosphoglucomutase (Pgm)-PGM (E.C.2.7.5.1), phosphoglucose isomerase (*Pgi*)-PGI (E.C.5.3.1.9), glucose-6-phosphate dehydrogenase (Gd)-GD (E.C.1.1.1.49), isocitrate dehydrogenase (Idh)-IDH (E.C.1.1.1.42), hexokinase (Hk)-HK (E.C.2.7.1.1),phosphogluconate dehydrogenase (Pgd)-PGD (E.C.1.1.1.44) and malate dehydrogenase 1 (Mdh1)-MDH (E.C.1.1.1.37).

Statistical Analysis

Allozyme allele frequencies, gene diversities as expected (Hardy-Weinberg) heterozygosities were estimated. Intensities of selection at individual loci were determined by testing the departure of allele frequency distribution from neutrality [9,10]. An analogous test of selection [11] was also performed. Allele frequency and gene diversity estimations and tests for the presence of selection at allozyme loci were performed using the software Arlequin version 3.1 [12]. Genetic distances between the population pairs were estimated as FST values using the same software. Effective number of alleles per population was estimated as in Weir [13].

Figure 1. Collection sites.

Table 1. Population collection sites and their basic geographical data.

POPULATION	N	LOCATION^a	ALTITUDE (meter)
BATIAYAZ	16	36 09 N ; 35 59 E	454
DÖRTYOL	23	36 54 N ; 36 13 E	178
OSMANİYE	10	37 09 N ; 36 26 E	540
ALANYA	20	36 34 N ; 31 55 E	146

N; number of individuals.
[a] as coordinates from GPS measurement.

RESULTS

Genetic Variation and Allele Frequencies

Table 2 shows allele number and frequency data for the 7 loci studied. For almost all of the samples, the loci are mostly polymorphic, except for those of Osmaniye in which only two loci have two or more alleles satisfying one or other (i.e 95 or 99 percent for the allele with highest frequency) conventional polymorphism criterion. A notable part of the allele number data is that one locus, *Pgi*, has more than two alleles (i.e. 3 alleles) almost in all populations except for Alanya. Most of the loci are in Hardy-Weinberg equilibrium; two loci, Hk and *Pgi* deviate significantly from the equilibrium but this is not common for all the populations (Table 3, loci marked with a star). For the *Pgi*, all the "extra" (i.e third) alleles are slower variants with different mobilities from the common slow,S, hence their different notations as S_1 or S_0. Overall, the major feature of allele frequency data is that for almost all the loci with two or more allele numbers, the common allele frequency exceeds the conventional 95 percent polymorphism treshold making them to be regarded as practically monomorphic.

We estimated the gene diversities per locus per population as expected (Hardy-Weinberg) heterozygosities from the allele frequencies. Table 3 shows the individual gene diversities and their means over the 7 loci. Overall, the population most polymorphic and with the polymorphism criteria most conservatively satisfied, BATIAYAZ, has the highest mean diversity (0.19067) whereas the population mostly monomorphic, OSMANİYE, has the least (0.06466). We also estimated the effective allele numbers (Ae) per population over

Table 2. Allele frequencies of the loci. Number of the allele per locus and the frequency of a third allele is also given if present.

Population	Locus	Number of Allele	Frequency[a]	Frequency Other (third) Allele
BATIAYAZ	GD	2	0.969	
	PGD	2	0.938	
	PGM	2	0.969	
	IDH	2	0.969	
	MDH	1	1.000	
	HK	2	0.625	
	PGI	3	0.594	0.039 (S0)
ALANYA	GD	2	0.900	
	PGD	2	0.900	
	PGM	2	0.850	
	IDH	2	0.900	
	MDH	1	1.000	
	HK	2	0.975	
	PGI	2	0.975	
DÖRTYOL	GD	2	0.957	
	PGD	2	0.957	
	PGM	2	0.913	
	IDH	2	0.957	
	MDH	1	1.000	
	HK	1	1.000	
	PGI	3	0.652	0.022 (S1)
OSMANİYE	GD	1	1.000	
	PGD	1	1.000	
	PGM	2	0.950	
	IDH	1	1.000	
	MDH	1	1.000	
	HK	1	1.000	
	PGI	3	0.8	0.050 (S0)

[a] The most common allele.

all loci. In all the populations, effective allele number does not exceed two, population with the lowest gene diversity (i.e. Osmaniye) having the lowest effective number of around 1 (Table 3, Ae). Mean gene diversity over all loci and populations can serve as a species level genetic diversity and this value is 0.129 (Table 3).

Genetic Distances

Genetic distances between pairs of populations were estimated as overall (over all loci) FST values. Table 4 shows the FST distances and their associated P values. The table also gives the putative number of migrants per generation, Nm, estimated as function of FST values. Genetic

Table 3. Expected heterozygosities for the loci with their Means (average gene diversities) and effective allele numbers (A_e).

Locus	BATIAYAZ	ALANYA	DÖRTYOL	OSMANİYE	Mean	s.d.
GD	0.063	0.185	0.085	0.000	0.083	0.077
PGD	0.121	0.185	0.085	0.000	0.098	0.077
PGM	0.063	0.262	0.162	0.100	0.147	0.087
IDH	0.063	0.185	0.085	0.000	0.083	0.077
MDH	0.000	0.000	0.000	0.000	0.000	0.000
HK	0.484	0.050	0.000	0.000	0.133	0.235
PGI	0.542	0.050	0.478	0.353	0.356	0.219
Mean	0.191	0.131	0.128	0.065	0.129	0.051
s.d.	0.224	0.097	0.164	0.132	0.154	0.054
A_e	1.352	1.163	1.198	1.094		

distances between pairs of samples are all highly significant ($P<0.001$) except for the pair DÖRTYOL-OSMANİYE.

Natural Selection

To determine if natural selection could shape the genetic variation at the loci studied, we performed the Ewens-Watterson test of neutrality for the polymorphic loci. We found no indication of selection for these 7 allozyme loci, as Table 5 demonstrates. The close match between observed and expected F values, and the high P values associated unequivocally point to the absence of selection at the level of electrophoretic variation for the 7 loci. An exact version of the Ewens-Watterson test, Slatkin's (1994), was also estimated and confirms our finding of no selection (Slatkin's exact p value, Table 5).

DISCUSSION

Level of genetic variation in a species and its partition among the populations comprising it are indicative of the evolutionary history of a species. Here in this study we present results of genetic variation and its possible distribution among some of the populations of the Geometrid moth *Cyclophora puppillaria* sampled from Eastern Mediterranean. Although well studied taxonomically [5,7,14], to date, no study has been conducted on the population genetics of *C. puppillaria* in general terms and with respect to its population evolutionary history. In our study, we used allozyme electrophoresis to detect the amount of genetic polymorphism in the sampled populations of this nocturnal moth species. Although we have analyzed relatively smaller number of loci (i.e. 7 allozyme loci coding enzymes of various functions), our results show remarkable points. First of all, almost all the allozyme loci have their common allele frequency over the conventional polymorphism treshold, making them practically monomorphic (Table 2). Accordingly, average gene diversity as expected (Hardy-Weinberg) heterozygosity estimated over all the loci is rather low in each population and the mean (species) gene diversity estimated over all loci and populations is just about 13 percent (Table 3). This gene diversity profile, togetherwith the effective allele number data, may indicate that the level of genetic variation in *C. puppillaria* is rather low. Indeed, this inference of ours is quite in line with the finding from recent molecular work on the comprehensive DNA barcoding profiles of the Geometrid moths of Europe by Hausmann et al. [14]. Their study, alongwith the calibration and identification of the species statuses, also provides intrapopulation genetic variation data for the *Cyclophora* genus and its shows that *C. puppilaria* has a very low level of intraspecies genetic variation, as evidenced by an only one base pair variation it shares with the genus' other widely distributed species *C. porata* and *C. linearia*. We also checked the presence of an effect of natural selection operating on them using appropriate tests [9-11] and found no

Table 4. Genetic distances as F_{ST} values between population pairs and the putative number of migrants (N_m) per generation.

Population pair	FST	Nm
BATIAYAZ-ALANYA	0.508***	0.194
BATIAYAZ-DÖRTYOL	0.100***	2.239
BATIAYAZ-OSMANİYE	0.128***	1.707
DÖRTYOL-ALANYA	0.520***	0.231
DÖRTYOL-OSMANİYE	0.010NS	25.312
OSMANİYE-ALANYA	0.627***	0.149

*** P<0.001 NS: Not significant

Table 5. Ewens-Watterson neutrality tests and the associated p- values. Analogous Slatkin test values are also given.

	BATIAYAZ	ALANYA	DÖRTYOL	OSMANİYE
No. Of loci	7	7	5	4
Observed F value	0.356	0.540	0.408	0.655
Expected F value	0.287	0.309	0.430	0.432
Watterson F p-value	0.814	0.963	0.503	0.960
Slatkin's exact p-value	0.860	0.919	0.574	0.960

significant selection (Table 5). Putative gene flow amounts are very low almost for all population pairs reflecting the high genetic differentiation among them (Table 4). There is only one population pair (i.e. DÖRTYOL-OSMANİYE) with a trivial, nonsignificant genetic differentiation and consequently high putative number of migrants between them. This population pair has the smallest geographical distance between them and may reflect the general conclusion that gene flow is operational for considerably shorter distances within any set of demes [15]. In lights of these findings, our results seem to be well concerted with the isolation by distance model of genetic variation in which the shaping process is essentially the genetic drift of the selectively neutral alleles. This inference would be supported by very low level of heterozygosity we found in the populations, which may indicate that the populations may have been established by a small number of migrants to their current locations. Although *C. puppillaria* is a highly migrant species, this high migration rate may entail small number of dispersing individuals ensuing in the colonization of the new places [5,7]. However, the number of the populations sampled were low in our study and therefore the inference of any definite population structure at this moment is limited. Population structure inference could be made after extensive sampling from the distribution of *C. puppillaria*.

In conclusion, our study provides the first results of population genetic and microevolutianary analysis in a less studied geometrid moth *C. puppillaria*. We also show that the level of genetic variation is quite low in this species. A DNA work focusing on the level of genetic variation and estimation of the respective molecular population genetic parameters could also be supplementary.

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DISCLOSURE

The authors declare that they do not have any conflict of interest.

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