

## Phenotypic Plasticity in Morphological Characters of *Cinara* (*Schizolachnus* Mordvilko) (Aphididae: Lachninae) Species Collected From Different Localities in Turkey

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### Abstract

This study was performed in order to determine the effects of altitude and temperature on phenotypic plasticity in some morphological characters and character ratios of the monoecious species of the aphid *Cinara* (*Schizolachnus* Mordvilko) feeding on *Pinus* L. needles. *Cinara* (*Schizolachnus*) specimens were sampled from 242 different populations determined in Kütahya, Afyonkarahisar and Uşak in Inner Western Anatolia. Eleven morphological characters of 1 to 3 individuals were measured for each population to avoid clonal influences and the measurements were analyzed statistically to reveal any differences between mean values of the populations. Our results showed that, although the three sampling area were geographically close to each other, they significantly influenced some of the morphological characters of *Cinara* (*Schizolachnus*) species indicating presence of phenotypic plasticity. Multivariate analyses showed that Afyonkarahisar had a higher influence on the existing phenotypic plasticity, most probably due to its higher altitude and lower temperature than other two provinces. The effects of sampling area on taxonomically distinguishing characters such as ultimate rostral segment IV length (URS IV) and V (URS V) and hind tibia length (HT L) were found to be particularly significant. Aphid species like the members of *Cinara* (*Schizolachnus*) feed only on one type of host plant without host alternating and therefore the characteristics of sampling locality rather than host plant play a crucial role in diversity. Our results emphasized that the existing phenotypic plasticity as a result of selection pressures on the morphological characters based on altitude and temperature conditions of the sampling localities might play important roles in host utilization process of *Cinara* (*Schizolachnus*) populations in a long term period as they have a monoecious heteroecious life cycle.

**Keywords:** Locality, phenotypic plasticity, *Schizolachnus*.

## INTRODUCTION

Phenotypic plasticity which can be defined as a common response to environmental fluctuations can moderate an individual's morphology, fitness and behavior to cope with changes in environmental conditions. Studies on phenotypic plasticity in aphids have recently gained acceleration and it was shown that the capacity of a single genotype to display different phenotypes in different environments is a common phenomenon in aphids in response to changes both in the host plant and the locality conditions [1; 2; 3]. The degree of plasticity of herbivore insects can be defined as their capacity to make physiological, morphological and behavioral adjustments in response to the changes in nutritional, chemical and physical structures of the host plant environment [3; 4; 5; 6]. There are intraspecific differences among genotypes of conspecifics in their ability to phenotypically adjust themselves to environmental changes indicating genetic variation for phenotypic plasticity in aphids [4;7]. Genetically identical individuals living in different environments even on the same host plant species placed in the same genera may be different in form, physiology, morphology, performance or behavior. Aphids undergo morphological and physiological changes in response to environmental factors like seasonal changes in their host plants structures, food quality, microclimate conditions and short and/or long term environmental climatic changes that vary in space and time [8; 9].

Aphids are sup sucking, host plant specific, and taxonomically and ecologically diverse group of insects with over 5012 species described placed in 510 genera. Almost half of the species spend all or part of their lives

feeding on trees and about 250 species are regarded as important pests [10]. Aphids are directly influenced by host plant characteristics such as morphology, physiology and food quality and environmental conditions among which temperature, micro climatic areas, humidity, day length and altitude are of paramount importance. Some of the host plant and environment based conditions could act as a barrier against some aphid species in colonization, spread and utilization, whereas some others can be easily overcome [8]. Furthermore, particular life styles and genome structures (cyclical parthenogenetic reproduction, dispersal ability, host plant selection and utilization strategy, short generation time, telescoping generation, discovery of a functional DNA methylation system) of aphids lead to high amount of genetic and phenotypic diversity. These unique features made aphids a promising insect group to study phenotypic plasticity, ecological speciation and other ecological-evolutionary life history traits [1; 11;12]. Aphids were proposed as an important insect group that can be used in population differentiation and speciation studies through their host selection-utilization, dispersal ability and adaptive strategies to fluctuations in environmental conditions, behaviors related to their feeding style and the way of reproduction [13]. Recently, Moczek [14] reviewed the importance of phenotypic plasticity in insect diversity and clearly emphasized possible consequences of phenotypic plasticity in evolutionary processes and diversity. Schneider & Meyer [15] strongly emphasized the importance of phenotypic plasticity in diversification and divergence in various groups of organism.

*Schizolachnus* has been accepted as a separate genus but recent studies changed its taxonomic status and placed it as a subgenus in the genus *Cinara* by considering findings of nuclear and mitochondrial analyses [16; 17]. *Schizolachnus* subgenus has seven defined species, four in Nearctic and three in Palearctic, which are oval-bodied, hairy, wax-secreting and feeding on *Pinus* needles [10]. These species have monoecious holocyclic life cycles and therefore have close relationships with their host plants. No study has been conducted so far on possible phenotypic plasticity in *Schizolachnus* species related with host plant utilization and environmental interactions most probably due to their low economic importance. Recent climatic changes and molecular studies increased the importance of phenotypic plasticity studies in aphids, particularly in species with monoecious life cycles as in the case of members of the subgenus *Schizolachnus*. It has been proposed for members of *Schizolachnus* that since they do not show host alternation the role of environmental factors might play role on phenotypic plasticity in the group.

The aim of this study was to investigate morphological phenotypic plasticity among *Cinara* (*Schizolachnus*) populations sampled from 3 geographically close areas in inner western Anatolia, Turkey. The data obtained in this study can be considered to be important in evaluation of diversity and possible speciation routes in monoecious holocyclic aphid species.

## MATERIALS AND METHODS

The study material consisting of apterous viviparous females and nymphs of the *Cinara* (*Schizolachnus*) were sampled monthly on *Pinus* spp. needles from Afyonkarahisar, Kütahya and Uşak Provinces in Turkey from April to October between 2012 and 2014. The apterous viviparous females from each locality were slide-mounted following maceration procedure of Martin [18]. All samples were identified from permanent slides according to an online identification and information guide [10]. Voucher specimens are kept at the Biology Department of Niğde Ömer Halisdemir University. Although the three sampling localities are placed in the same geographical region, they differ from each other in terms of their altitudes and mean temperature values during samplings. The altitudes of the sampling localities in Afyonkarahisar, Uşak and Kütahya were 1021m, 906m

and 971m, respectively and the mean temperature values during the study period were 11.27°C, 12.01°C and 10.79°C, respectively. To detect morphological plasticity related with locality, eight morphological characters and three character ratios were measured for 316 apterous viviparous females from 242 populations sampled. Morphological characters (see below) were measured in mm scale on mounted permanent slides by using a stereo microscope (Olympus BX51) ocular-micrometer system. Measured characters and character ratios are;

- Body Length (BL)
- Antennal Segment Processus Terminalis (ANT PT)
- Length of the VI. Antennal Segment Base (ANT BASE)
- Length of the URS IV (URS IV)
- Length of the URS V (URS V)
- Diameter of Siphunculi (SIP BD)
- Hind Tibia Length (HT L)
- Basal Diameter of the Hind Tibia (HT BD)
- Ratio of the URS V and URS IV (URS V/URS IV)
- Ratio of the Body Length and Hind Tibia Length (BL/HT L)
- Ratio of the Antennal Processus Terminalis and Antennal Segment Base (ANT PT/ANT BASE)

Sets of one-way ANOVA were performed to verify significance of locality effects on measured morphological characters. The Post-Hoc Tukey test was performed for multiple analyses. All statistical analyses were conducted using the computer software package SPSS for Windows, version 16.0.

## RESULTS

The identifications of the sampled material showed that they belonged to *Cinara* (*Schizolachnus*) *obscura* (Börner, 1940), *Cinara* (*Schizolachnus*) *orientalis* (Takahashi, 1924) and *Cinara* (*Schizolachnus*) *pineti* (Fabricius, 1781). Morphological characters of only *C. orientalis* and *C. pineti* were considered for evaluation of possible phenotypic plasticity as population density of *C. obscura* was very (only 1 population sampled from Kütahya-Altıntaş on 08.X.2012) low in all localities. The initial descriptive morphological measurements for each population before evaluation of possible phenotypic plasticity revealed that some of the measured morphological characters and character ratios of *C. orientalis* population sampled in Uşak were higher than in Kütahya and Afyonkarahisar (Table 1).

**Table 1.** The measurement values for morphological characters and ratios (Mean±SE) of *C. orientalis* populations collected from the three localities. All measurements were conducted on 70 individuals on average for each locality.

Morphological characters and character ratios	Localities and sample size		
	KÜTAHYA (40 population)	AFYONKARAHİSAR (70 population)	UŞAK (64 population)
BL	1.80±0.047	1.80±0.037	1.86±0.102
ANT PT	0.02±0.000	0.020±0.000	0.02±0.000
ANT BASE	0.12±0.002	0.11±0.002	0.20±0.002
SIP BD	0.12±0.002	0.12±0.002	0.12±0.007
HT BD	0.09±0.002	0.08±0.002	0.09±0.002
HT L	1.09±0.028	1.02±0.029	1.24±0.030
URS IV	0.10±0.001	0.09±0.001	0.10±0.001
URS V	0.05±0.0001	0.04±0.0001	0.05±0.0001
URSV/URSIV	0.47±0.004	0.46±0.005	0.48±0.008
ANT PT/ANT BASE	0.17±0.002	0.18±0.003	0.17±0.003
BL/HTL	0.61±0.010	0.60±0.009	0.64±0.014

The results also showed that similar environmental influence existed for *C. pineti* populations although there were character specific effects. While some of the measured characters and characters ratios were higher for population collected from Kütahya, some of them were higher for the population collected from Afyonkarahisar (Table 2). The measurements of Uşak samples (only 2 population sampled on 19.VII. 2013 and 26.X.2013) were not included in the comparison due to the low number of individuals sampled here.

**Table 2.** The measurement values for morphological characters and character ratios (Mean±SE) of *C. pineti* populations collected from two localities. All measurements were conducted on 60 individuals on average for each locality.

Morphological characters and character ratios	Localities and sample size	
	KÜTAHYA (36 population)	AFYONKARAHİSAR (29 population)
BL	1.84±0.080	1.59±0.090
ANT PT	0.02±0.000	0.02±0.001
ANT BASE	0.11±0.004	0.11±0.004
SIP BD	0.12±0.003	0.11±0.005
HT BD	0.08±0.003	0.08±0.004
HT L	1.10±0.037	1.02±0.069
URS IV	0.09±0.002	0.09±0.003
URS V	0.04±0.001	0.04±0.002
URSV/URSIV	0.45±0.008	0.41±0.015
ANT PT/ANT BASE	0.18±0.005	0.20±0.008
BL/HTL	0.59±0.014	0.65±0.029

The measurement data revealed that the sampling locality and therefore environmental conditions had different effects on the selected morphological characters and character ratios for both *C. orientalis* and *C. pineti* populations. The one-way ANOVA test was run to reveal, if any, the possible phenotypic plasticity in *C. orientalis* populations collected from three different localities. The results of the analysis showed that the sampling locality had character specific effects as there were differences in measurements of some morphological characters and character ratios among the localities in contrast to non-significant differences for others (Table 3).

**Table 3.** Analyses of variance in *C. orientalis* populations morphological features sampled from 3 localities (One-Way ANOVA, Locality factor).

	Mean Square	Sum of squares	F	df	P
BL	0.074	42.86	0.190	2	0.827
ANT PT	0.000	0.003	0.399	2	0.671
ANT BASE	0.002	0.068	2.799	2	0.063
SIP BD	0.001	0.102	0.727	2	0.485
HT BD	0.002	0.108	2.531	2	0.082
HT L	0.934	16.422	6.283	2	<b>0.002</b>
URS IV	0.001	0.005	12.038	2	<b>0.000</b>
URS V	0.001	0.005	16.431	2	<b>0.000</b>
URSV/URSIV	0.032	0.429	8.312	2	<b>0.000</b>
ANT PT/ANT BASE	0.004	0.126	3.036	2	<b>0.050</b>
BL/HTL	0.036	1.741	2.119	2	0.123

The multiple comparison analysis with Post-Hoc Tukey test pointed out that the sampling localities as a factor acted on some morphological characters and character ratios in different ways and therefore caused significant differences in *C. orientalis* population. For instance, URS IV length of aphids sampled in Afyonkarahisar population differed from both Kütahya and Uşak populations ( $[(HSD)]_{(47,27)}=0.0038$ ,  $P<0.0001$  between Afyonkarahisar and Kütahya and  $[(HSD)]_{(47,27)}=0.0039$ ,  $P=0.002$  between Afyonkarahisar and Uşak). Differences in PT/BASE also caused by Afyonkarahisar population ( $[(HSD)]_{(46,97)}=0.008$ ,  $P=0.047$  between Afyonkarahisar and Kütahya,  $[(HSD)]_{(46,97)}=0.0005$ ,  $P=0.99$  between Kütahya and Uşak).

**Table 4.** Analyses of variance in *C. pineti* populations morphological features sampled from 2 localities (One-Way ANOVA, Locality factor).

	Mean Square	Sum of squares	F	df	P
BL	1.291	30.054	3.866	1	<b>0.052</b>
ANT PT	0.000	0.004	1.774	1	0.186
ANT BASE	0.000	0.079	0.003	1	0.954
SIP BD	0.001	0.055	1.702	1	0.195
HT BD	0.001	0.052	2.052	1	0.155
HT L	0.119	9.941	1.076	1	0.302
URS IV	0.000	0.024	1.058	1	0.306
URS V	0.001	0.007	7.669	1	<b>0.007</b>
URSV/URSIV	0.031	0.433	6.224	1	<b>0.014</b>
ANT PT/ANT BASE	0.008	0.135	5.182	1	<b>0.025</b>
BL/HTL	0.068	1.243	4.399	1	<b>0.039</b>

The sampling locality showed similar character specific effects on *C. pineti* populations collected from Afyonkarahisar and Kütahya. Considering the fact that the results of One-way ANOVA analyses showed that sampling localities had character specific effects on both *C. orientalis* and *C. pineti* populations and that same morphological characters and character ratios had been affected significantly for both species an analysis was performed to reveal, if any, influences of sampling localities on *Cinara (Schizolachnus)* irrespective of species specific data.

**Table 5.** The measurements for morphological characters and character ratios (Mean±SE) of *Cinara (Schizolachnus)* collected from three localities irrespective of the species sampled.

Morphological characters and character ratios	Localities		
	KÜTAHYA	AFYONKARAHİSAR	UŞAK
BL	1.82±0.041	1.75±0.037	1.86±0.102
ANT PT	0.020±0.0004	0.021±0.0004	0.021±0.0005
ANT BASE	0.11±0.001	0.11±0.002	0.12±0.002
SIP BD	0.12±0.002	0.11±0.002	0.13±0.007
HT BD	0.09±0.001	0.08±0.002	0.09±0.002
HT L	1.10±0.022	1.02±0.028	1.24±0.031
URS IV	0.10±0.0008	0.09±0.0009	0.098±0.0011
URS V	0.05±0.0005	0.04±0.0006	0.047±0.001
URSV/URSIV	0.47±0.004	0.45±0.006	0.48±0.008
ANT PT/ANT BASE	0.17±0.002	0.18±0.003	0.17±0.003
BL/HTL	0.61±0.008	0.61±0.011	0.64±0.014

The results showed that measurement values of morphological characters and character ratios were generally higher for samples in Uşak compared to the other two localities (Table 5) although there were no coherent changes for each character.

**Table 6.** Analyses of variance in *Cinara (Schizolachnus)* morphological features sampled from 3 localities (One-Way ANOVA, Locality factor) irrespective of sampled species.

	Mean Square	Sum of squares	F	df	P
BL	0.229	0.458	0.968	2	0.381
ANT PT	0.00002	0.00003	0.633	2	0.531
ANT BASE	0.001	0.002	1.931	2	0.147
SIP BD	0.001	0.001	1.447	2	0.237
HT BD	0.002	0.004	3.628	2	<b>0.028</b>
HT L	0.528	1.055	6.265	2	<b>0.002</b>
URS IV	0.0004	0.001	4.452	2	<b>0.012</b>
URS V	0.001	0.001	13.314	2	<b>0.000003</b>
URSV/URSIV	0.261	0.522	8.337	2	<b>0.0003</b>
ANT PT/ANT BASE	0.005	0.009	4.873	2	<b>0.008</b>
BL/HTL	0.013	0.025	1.171	2	0.312

The results clearly showed that sampling locality of *Cinara* (*Schizolachnus*) affected on at least some of the measured morphological characters and character ratios. URS V/ URS IV length, ANT PT/ANT BASE ratio and HT L were significantly influenced by the sampling locality and were evaluated as important distinguishing characters according to current identification keys applied. Post-Hoc Tukey analyses indicated that differences in measured morphological characters irrespective of sampled species caused by collection from Afyonkarahisar Province. For example, URS V length of Afyonkarahisar population differed from both Uşak and Kütahya ( $[HSD]_{(52,31)}=0.0037$ ,  $P<0.0001$  between Afyonkarahisar and Kütahya populations ( $[HSD]_{(52,31)}=0.0052$ ,  $P=0.002$  between Afyonkarahisar and Uşak)). Differences in PT/BASE also caused by Afyonkarahisar population ( $[HSD]_{(51,93)}=0.011$ ,  $P=0.013$ ). Statistical differences among 3 locations in URS IV length also induced by Afyonkarahisar ( $[HSD]_{(52,31)}=0.0029$ ,  $P=0.039$  between Afyonkarahisar and Kütahya).

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Aphids are phloem sap feeders showing specificity for their host plants and they need at least one plant species to survive, which in turn lead to formation of close relationships with their host plants. Most of the studies related with aphids were conducted on pest species and the remaining groups were paid less attention. Recent climatic changes and ecological fluctuations forced researchers to focus their studies on aphids irrespective of whether they have serious pest statuses or not. Aphids constitute a promising group to study ecological interactions and possible outcomes of these interactions including phenotypic plasticity due to their special way of reproduction, relatively shorter development time, telescoping generations, efficacy in host plant utilization, genome structure and discovery of a functional DNA methylation [19; 20;21].

Studies on aphids emphasized different types of phenotypic plasticity which can be defined as the capacity of a single genotype to display different phenotypes in different environments and this phenomena is common in aphid populations whether they are in serious pest group or not [3; 4;20; 22]. Madjdzadeh & Mehrparvar [23] studied on 21 characters of 11 *Macrosiphoniella sanborni* Richard (Hemiptera: Aphididae) populations collected on cultivated *Chrysanthemum* L. in different localities in Iran and indicated the presence of morphologically distinct groups as a result of the locality effects. In parallel with the findings of the Madjdzadeh & Mehrparvar [23], evaluation of *Cinara* (*Schizolachnus*) species sampled in the present study on the same host plant, *Pinus* sp., but from different localities revealed significant differences in some morphological characters and character ratios.

*Cinara* (*Schizolachnus*) species have monoecious holocyclic life cycles and therefore they have close relationships with their host plants. Host plants are considered one of the main ecological factors involved in the speciation process of phytophagous insects. Studies on various plant-feeding insects including aphids clearly demonstrated the existence of host races and suggested that host based selection plays important roles in speciation process in aphids [24]. However, the importance of locality-geography in aphid diversification has been given less importance most probably due to the fact that some aphid species are known to disperse over long distances and successfully find their host

plants during that type of selection pressures. It was thought that this characteristic could constrain the influence of geographic barriers on aphid diversification [25]. Host plants are not the only components of ecological niches of aphids, and temporal shifts in life cycles or changes in feeding habits due to changes in locality conditions can also play important roles in species diversity within a population [26]. Since the *Cinara* (*Schizolachnus*) populations included in the present study are monoecious holocyclic, they do not show host alternation and thus locality might have played an important role in phenotypic plasticity in the species evaluated. The present study is the first study related with phenotypic plasticity in *Cinara* (*Schizolachnus*). The evaluation of the findings on morphometric comparisons strongly indicated the importance of locality on phenotypic plasticity in the sampled *Cinara* (*Schizolachnus*) populations. The results showed that mean temperatures of the localities during the sampling period and their altitudes had a character specific influence on plasticity, but although the localities had trait specific effects, all localities influenced similar morphological characters which are important characters in identification key of the genus. The differences in temperature and altitudes of the sampling localities caused statistically significant differences particularly in some distinguishing characters of *Cinara* (*Schizolachnus*) species such as URS, ANT PT/ANT BASE and HT L. Mdellel & Kamel [9] indicated how strongly locality affected almost all morphological features of *Pterochlorides persicae* (Cholodkovsky) populations collected from different countries that had a higher amount of selection pressures on sampled aphid population due to ecological conditions, *P. persicae*, compared with *Cinara* (*Schizolachnus*) populations collection area conditions. Findings of both Mdellel & Kamel [9] and our study emphasized the importance of locality contribution to differentiation between different populations. The effects of sampling locality on *Schizolachnus* species in general can be evaluated as an indicator of existence of phenotypic plasticity as mean value of some of the morphological characters differed from each other significantly among the three localities such as basal diameter of the hind tibi (HT BD) and hind tibia length. Moreover, statistically significant influences of locality on particular distinguishing characters should be discussed for strong selection pressure on *Cinara* (*Schizolachnus*) group.

Possible shifts in locality conditions including temperature, precipitation, humidity, ecosystem disruption are likely to be as a result of the recent global ecological changes. Studies clearly indicated how differences in locality conditions, particularly temperature and altitude, caused reasonable differences in morphological features of aphid populations [23; 27]. These changes in habitat conditions directly influence aphid species and recent climatic changes reports indicate that Turkey is going to be one of the countries that is going to be dramatically affected from the recent climatic changes. As a result of these changes, higher amounts of plasticity in distinguishing characters and variation in plasticity between different traits play important role in aphid performance and future ecological processes, especially for species having monoecious holocyclic life cycle. Importance of phenotypic plasticity in aphid and host plant species in response to environmental changes were emphasized by different researchers [1; 20; 21; 28] and the phenotypic plasticity revealed in the studied *Cinara* (*Schizolachnus*) populations related with locality conditions needs to be supported with further studies.

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