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RESEARCH ARTICLE

Phenotypic plasticity in morphological features of *Thelaxes suberi* (Del Guercio, 1911) (Hemiptera: Aphidoidea) feed on *Quercus* (L.) species

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Abstract: Phenotypic plasticity is a common and important phenomenon in aphids, particularly in host plant utilization. It has been shown that *Thelaxes suberi* species fed on three closely related *Quercus* species showed high amount of phenotypic plasticity in morphological characters. *Thelaxes suberi* population has the largest morphological features on *Quercus infectoria* and the smallest morphological features on the *Quercus coccifera*. It was thought that these differences related with the total protein content and level of amino acid in acorns of the host plant where *Thelaxes* individuals were collected during this study. Findings of the study indicated how high is phenotypic plasticity capacity of aphid species and interactions with host plant properties. Another important implication of the result is that as aphid has a close relationship with host plant and even small amount of changes in the same *Quercus* species caused that amount of plasticity in *Thelaxes* species. Recent ecological changes including climatic fluctuations directly influences host plants morphological/nutritional composition and might also impacts the diversity and distribution of aphid species. Findings were also discussed for possible changes in host plant range of the *T. suberi* as a result of the current phenotypic plasticity and possible interactions between different types of phenotypic plasticities.

Key words: Aphid, host utilization, phenotypic plasticity, *Thelaxes*.

Introduction

The known world aphid fauna recently reached about 5000 species, placed in 510 currently identified genera and about half of them maintain all or part of their life feeding on tree species (Blackman & Eastop 2016). Aphids are sap sucking insect group and various properties of host plant such as morphology, physiology and food quality directly influence them even when aphids feed on host plant species placed in same genera. Plants also vary in both external and internal structure such as nutritious component, secondary chemicals, hair, hook, waxy layer and texture. Some of these plant structures could act as a barrier against some aphid species in colonization and utilization, whereas others might easily overcome them (Agarwala 2007).

With a special way of reproduction, short development time, telescoping generations, efficacy in host plant utilization, genome structure and discovery of a functional DNA methylation system, aphids are promising group to study phenotypic plasticity. Phenotypic plasticity, which can be defined as the capacity of a single genotype to display different phenotypes in different environments, is common in aphids host plant selection and utilization and can be expressed either in short term or long term (Görür 2005; Srinivasan & Brisson 2012; Mukherjee & Baudach 2016). The degree of plasticity of herbivores can be defined, in an ecological context, by their capacity for making physiological, morphological, and behavioral adjustments in response to the nutritional, chemical, and physical structure of the host plant environment. Recent findings support the importance of DNA methylation in phenotypic plasticity in aphids. (Görür *et al*. 2005; Moraiti *et al*. 2014; Ogawa & Miura 2014; Mukherjee & Baudach 2016). There commonly are differences among genotypes within a species in their ability to phenotypically adjust to environmental changes, in other words there is often genetic variation for phenotypic plasticity (Görür *et al*. 2005, 2007). Genetically identical individuals living in different environments even in the same host plant species placed in same genera may be different in form, physiology, morphology or behavior. These individuals display phenotypic plasticity in response to environmental factors like seasonal changes in their host plants structures, food quality and environmental climate that vary in space and time (Agarwala 2007).

It has been proposed that aphids are an important group that can be used in population differentiation and speciation studies through host selection-utilization behavior due to their feeding style and the way of reproduction (Powell *et al*. 2006). As aphids have close relationships with their host plants, host race formation through phenotypic plasticity in aphids has been considered one of the important initiator of sympatric speciation (Görür 2000, 2005; Görür *et al*. 2005). Recently, Moczek (2010) published a review about importance of phenotypic plasticity in insect diversity and clearly emphasized results of possible consequences of phenotypic plasticities in evolutionary processes and finaly diversity.

Thelaxes species are defined as oak-feeding species and *Thelaxes suberi* mainly occurs on oak species in southern Europe and in UK, but usually not on English oak (*Quercus robur*) (Blackman & Eastop 2016). There has been no study conducted related with host plant utilization of oak-feeding species, *Thelaxes* Westwood 1840, up to date. Although various types of phenotypic plasticity in different species of aphids have been clearly demonstrated and discussed (*Uroleucon* by Moran (1986), *Cryptomyzus* species by Guldemond (1990), *Acyrthosiphon pisum* by Via (1999), *Brevicoryne brassicae* by Görür (2003), *Aphis fabae* by Görür *et al*. (2005), *Myzus persicae* by Nikolakakis *et al*. (2003) and so on.), there has been no study carried out so far for oak-feeding species possibly due to their unimportant pest status. Due to the less interest in host plant utilization of *Thelaxes* species, there was also no study published concerned with phenotypic plasticity in this particular aphid genera in contrast to vast amount of study published in phenotypic plasticity in other group of aphids. Four oak-feeding species have been identified around the world so far, three in Europe and the Mediterranean region, and one in North America. All determined oak-feeding species have been recorded from Turkey including one North American species recently indicating that they might invade new areas (Görür *et al*. 2011; http://www.aphidsonworldsplants.info). *Thelaxes* species do not host alternate and is found on many species of oak (*Quercus* spp.). Colonise at the tips of the shoots, on stems, leaf petioles and along mid ribs on the undersides of the leaves. They are also found on the developing acorns basically on cup (Dransfield & Brightwell 2016). In these aspects, the objective of this study was to verify the amount of phenotypic plasticity in morphological properties of *Thelaxes suberi* population sampled on 3 *Quercus* species placed in botanically different sections.

Material and methods

Features of Host Plants

Thelaxes suberi population sampled on acorn of 3 *Quercus* (*coccifera*, *ithaburensis, infectoria*) species which generally grown mixed. These host plants are placed in different sections botanically. General characteristics of the host plants are as follows (Yılmaz & Akkemik 2014).

Quercus coccifera (in *Ilex* section) is usually a shrub less 2 meters tall, rarely a small tree and it is evergreen, with spiny-serrated coriaceous leaves. The acorns are 2–3 cm long and 1.5–2 cm diameter when mature about 18 months after pollination. Acorns are held in a cup covered in dense, elongated, reflexed scales. *Quercus ithaburensis* (in *Cerris* section) is a small to medium sized semi-evergreen to tardily deciduous tree growing to a maximum height of around 15 metres with a rounded crown and often with a gnarled trunk and branches. The leaves are 4-9 cm long and 2-5 cm wide, oval in shape. The acorns are generally oval, up to 5 cm long and 3 cm wide with a cup covering roughly 1/3 of the acorn. The cup is covered in long stiff loose scales which are rolled backwards or involutes especially along the edges of the cap.*Quercus infectoria* (in *Quercus* section) is a deciduous, semi-evergreen, small tree or shrub that grows up to the height of 2 to 20m. The stem is crooked. The leaves are ovate-oblong. The hemisphere shaped cup covered the one third of the acorn. The cup is strictly covered with short hairy scales.

Sampling, Preparation and Identification of *Thelaxes suberi*

Thelaxes suberi samples were collected on the cup-acorn of each *Quercus* species from different localities in Turkey including Afyonkarahisar, Kütahya, Niğde and Uşak Provinces. Totally about 70 colonies sampled from 3 host plants, 20 adult aptera individuals were sampled from each collected colony and preserved in 70% alcohol. All samples were identified according to an online identification and information guide (Blackmand & Eastop 2016) from permanent slide. Voucher specimens are kept at the Biology Department of Ömer Halisdemir University.

Morphological Measurement and Evaluations

In total, at least 50 individuals of *T. suberi* morphological characters measured for each host plant. In order to avoid clonally effects on measured properties, randomly maximum 3 adult individuals chosen from permenant slides for each sampled colony. Determined morphological characters were measured on mounted permenant slides by using Olympus BX51 stereo microscope ocular-micrometer system. Measured morphological characters were; Body Length (**BL**), Length of the Processus terminalis of Antennal segment VI (**PT**), Length of the basal part of Antennal segment VI (**BS**), Length of the Rostrum IV+V

(**URS**), Hind tarsus I Length (**HTI**), Hind Tarsus II Length (**HTII**), Length of the knob of cauda (**CL**), Maximum width of the knob of cauda (**CW**), Ratio of cauda length to the cauda diameter (**CL/CW**), Ratio of the length of the last antennal segment processus terminalis to base of last antennal segment length (**PT/ BS**) and Ratio of the length of the Rostrum IV+V to length of the last antennal segment processus terminalis (**URS / PT**).

Sets of one-way ANOVAs were performed to verify significance of host plant effects on measured morphological characters. The post-hoc analyses were performed to determine which host plant influences resulted in significant differences according to ANOVA results. The estimates of correlations among measured characters were estimated by the Pearson correlation coefficient. All statistical analyses were performed using the computer software package SPSS for Windows, version 15.0.

Results

As a result of the evaluation of the 8 measured morphological features, it has been clearly showed that host plant had strong influences on the morphology of the *Thelaxes suberi* population. Almost all measured morphological traits of *T.suberi* population were larger on *Quercus infectoria* than on other two *Quercus* species (Fig. 1).

Whether host plants effects on the measured traits were significantly different from each other or not, One Way Anova analyses performed. Analyses revealed that host plants had significant influences on most of the measured characters including Body Length, Basal Part Length of the VI antennal segment, and Proximal Part Length of the VI antennal segment except length of the ultimate rostral segment (Table 1) and all measured characters were positively correlated with BL. (e.g. r_{cm}=0,648, P<0.001 between BL and BS, r_{cm}=0,687, P<0.001 between BL and CW).

It is important to have attention to significant effects on both the cauda length and maximum width of the examined species knob of cauda which are one of the important distinguishing characters among *Thelaxes* species. In order to find out which host plant resulted in that amount of differences among measured characters in *T. suberi* population, Post Hoc-Tukey test performed. Multiple comparison analyses clearly indicated that most of the differences among measured morphological characters caused by the effects of the *Q. ithaburensis*. For example even there was a overall significant difference in body length (BL) measurement among populations collected from 3 *Quercus* species, Post Hoct analyses indicated that body length of the *T. suberi* population on *Q. ithaburensis* significantly differed from other two *Quercus* feed populations (i.e. Tukey HSD $_{[64.25]}$=0.105, *P*=0.00001 between *Q.coccifera* and *Q.ithaburensis* and Tukey HSD $_{[64.25]}$=0.063, *P*=0.0066) while there was no difference between *Q. coccifera* and *Q. infectoria* fed population body length (Tukey HSD $_{[64.25]}$=0.041, *P*=0.1469). The only exception was the host plant effects on the length of the last antennal segment proximal part (PT). There was a clear host plant induced changes on PT but Post-Hoc analyses pointed out that main factor resulted in this difference was *Q. coccifera*, not *Q. infectoria* (i.e. Tukey HSD $_{[65.02]}$ =0.0042, *P*=0.000006 between *Q. coccifera* and *Q. ithaburensis*, Tukey HSD $_{[65.02]}$ =0.0044, *P*=0.0000008 between *Q. coccifera* and *Q. infectoria*, Tukey HSD $_{[65.02]}$ =0.0003, *P*=0.9309 between *Q. ithaburensis* and *Q. infectoria*). This different interaction can be explained with possible character specific interaction between traits and driving factors, host plant.

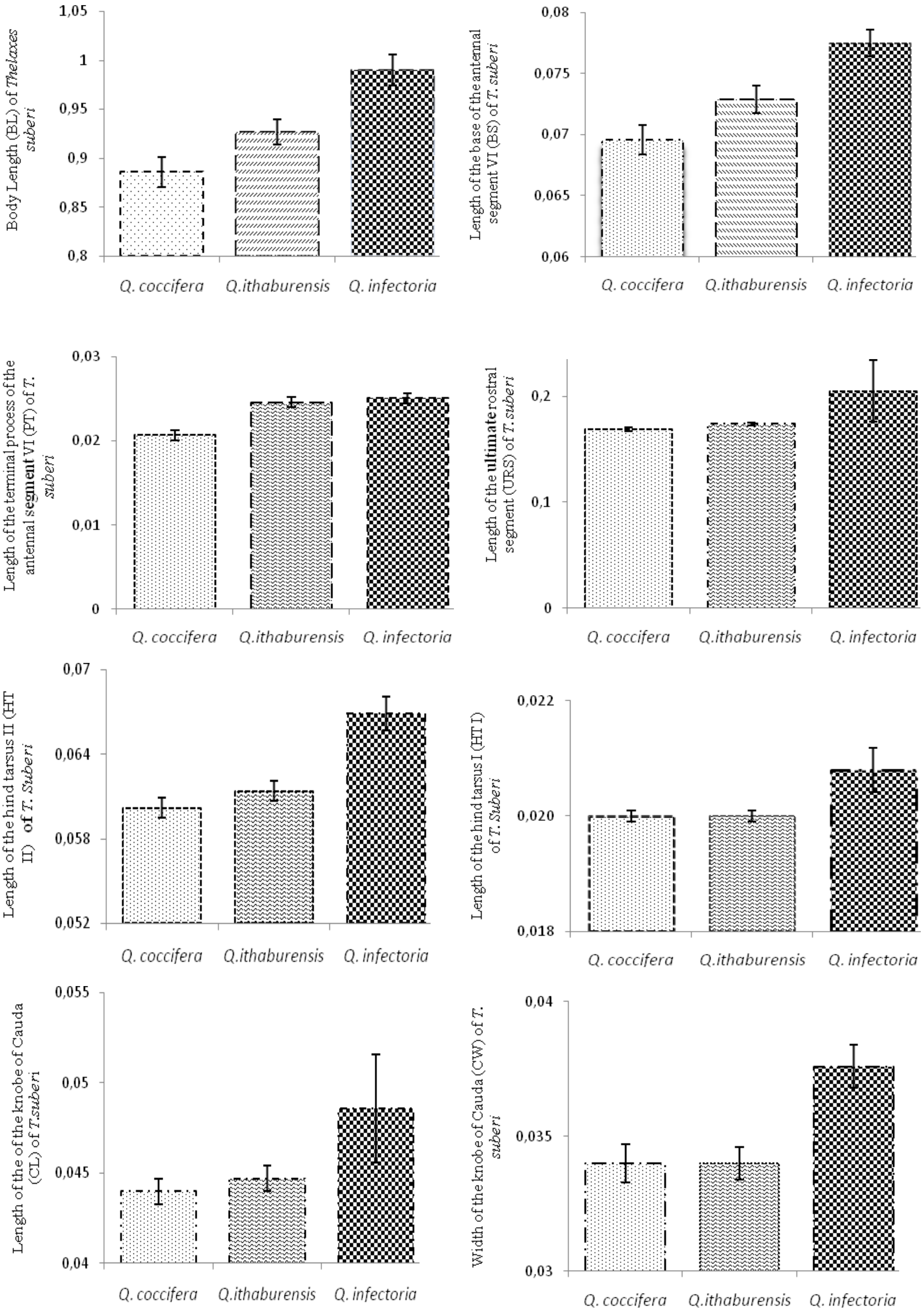

Figure 1. Expression (mean±standard error) of the measured morphological characters of *T. suberi* on *Quercus* species.

Table 1. Analyses of variance of the measured morphological characters of *Thelaxes suberi* populations feed on 3 *Quercus* species (One Way Anova).

		Sum of Squares	d.f	Mean Square	F	Sig. (P)
BL	Between Groups	,353	2	,177	11,872	**,000014**
	Within Groups	2,870	193	,015		
	Total	3,224	195			
BS	Between Groups	,002	2	,001	13,264	**,000004**
	Within Groups	,015	193	,000		
	Total	,017	195			
PT	Between Groups	,001	2	,000	17,069	**,0000001**
	Within Groups	,004	194	,000		
	Total	,005	196			
URS	Between Groups	,051	2	,025	1,494	,227118
	Within Groups	3,294	193	,017		
	Total	3,345	195			
HT I	Between Groups	,000	2	,000	5,681	**,004006**
	Within Groups	,001	193	,000		
	Total	,001	195			
HT II	Between Groups	,001	2	,001	15,166	**,0000008**
	Within Groups	,009	190	,000		
	Total	,011	192			
CL	Between Groups	,001	2	,000	12,736	**,000006**
	Within Groups	,006	196	,000		
	Total	,007	198			
CW	Between Groups	,001	2	,000	9,935	**,000078**
	Within Groups	,006	196	,000		
	Total	,007	198			
PT/BS	Between Groups	,066	2	,033	9,055	**,000174**
	Within Groups	,705	193	,004		
	Total	,771	195			
URS / PT	Between Groups	71,621	2	35,811	1,670	,191
	Within Groups	4161,215	194	21,450		
	Total	4232,836	196			
CL / CW	Between Groups	,011	2	,005	,317	,729
	Within Groups	3,268	196	,017		
	Total	3,278	198			

Discussion

Almost all organisms show some degree of plasticity related with changes in environmental conditions and various types and mechanisms of phenotypic plasticity has been well documented in different aphid species (Görür *et al*. 2005; Whitman & Agrawal 2009; Mehrparvar *et al*. 2012; Thieme *et al*. 2014; Brisson *et al*. 2016; Mukherjee & Baudach 2016; Tagu *et al*. 2016; Yuan *et al*. 2016). Phenotypic plasticity has been considered to be a facilitator of phenotypic diversification and also host race formation which might end with sympatric speciation in aphid (Görür 2005). Features of host plant utilized by aphids including quality, morphology, physiology and various defense mechanisms are the main initiator of the phenotypic plasticity and may cause expression of reasonable differences in aphid morphological properties and behavior. This was the first study conducted on *Thelaxes* population host utilization and existed phenotypic plasticity related with characteristics of the host plant traits. Statistical evaluation of the findings strongly emhazised plausible influences of host plant features on morphological features of the *T. suberi* population, namely, phenotypic plasticity. *Thelaxes suberi* population sampled from the acorn of the *Quercus coccifera*, *Q. ithaburensis* and *Q. infectoria* species. Özcan (2006) conducted study on *Quercus* species existed in Turkey and examined the total protein and amino acid compositions in the acorn of these species. *Quercus infectoria* had the highest amount of total protein and essential amino acid concentrations, *Q. ithaburensis* followed *Q. infectoria* and *Q. coccifera* had the lowest among these three host plants. *Thelaxes suberi* population expressed the largest morphological traits on *Q. infectoria* and became smaller on *Q. ithaburensis* and *Q. coccifera*, respectively. Total protein and amino acid contents reflect host plant quality for aphid species and *T. suberi* populations existed larger traits on good quality host plant in terms of morphological plasticity. These findings consistent with the idea that host plant quality directly influence aphid morphology. Thieme *et al*. (2014) reported higher amount of phenotypic plasticity in *Macrosiphum albifrons* in response to host plant quality, namely, alkaloid content which act as a defense mechanism of plant species. Amount of morphological phenotypic plasticity in response to food quality of the host plant placed in same genera might have important ecological implications. Bauerfeind & Fischer (2013) highlighted the significant effects of temperature induced changes in host plant quality (both increased CO_2 and temperature resulted in changes in host plant quality in different amount and ways such as plant nitrogen content, C/N ratio, water content, terpenoids, carbohydrates.) and emphasized that these changes may increase the negative effects of global warming on herbivore host- plant quality. *Thelaxes* population existed high amount of phenotypic plasticity in almost all measured morphological characters in response to changes in protein content of particular part of (acorn) the *Quercus* species which have grown mixed. Recently Yuan *et al*. (2016) indicated how growth of the plant species are affected from ecological factors namely light level in terms of phenotypic plasticity. These changes in plant species properties as a result of the phenotypic plasticity in plants directly influences aphid species as they have a close relationship with their host plants and they quickly respond to any changes in their host plant quality by expressing different amount of phenotypic plasticity and even get benefit from these changes. Aphid species including *T. suberi* might have ability to show different types of phenotypic plasticity in response to phenotypic plasticity in host plants. Sigro *et al*. (2016) pointed out importance of different types of phenotypic plasticity and interactions between this phenotypic plasticity. Interactions between different types of plasticity could facilitate herbivorous insect host shift. Kindlmann *et al*. (2010) gave detail about how aphid might behave under environmental changes mainly mediated by global warming. If environmental changes cause new niches (changes in host plant features…) and resulted in favorable host plant quality, *T. suberi* population might also start to utilize *Q. robur*, yet *T. suberi* species has not been recorded on.

In addition, as *T. suberi* species expressed statistically important amount of morphological phenotypic plasticity even three host plants of the aphid species grown mixed, in a long term there might be host race formation of *T. suberi* on particularly *Quercus infectoria*. Importance of phenotypic plasticity in host race formation in aphid population and other organisms strongly emphasized by different researchers (West-Eberhard 2003; Görür

2005; Pigliucci 2005) and statistically supported phenotypic plasticity obtained from *T. suberi* population fed on *Q. infectoria* might coincide with general approaches.

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