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THE EFFECTS OF ENVIRONMENT SUBDIVISION ON MORPHOLOGICAL VARIATION IN THE "CAULIFLOWER" GALLS OF THE APHID *SLA VUM WERTHEIMAE* (HOMOPTERA, APHIDIDAE, FORDINAE)

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ABSTRACT

"Cauliflower" galls of the aphid, *Slavum wertheimae,* on *Pistacia atlantica*, are subdivided into branching tubular chambers. We set out to test for morphological differences among subgall means. Measuring 21 morphological characters on 8 aphids from each of 59 subgalls (12 galls from 5 trees), we discovered statistically significant differences among subgalls, but these amounted to only about *5%* of the total variation. The effect of microenvironmental differentiation on aphid morphology does not seem to be an important source of within-gall variation. We discuss the differences among gall means within trees, and among tree means, in comparison with previous studies, and comment on the possible sources of within- gall variation (Error).

KEY WORDS: Slavum wertheimae, gall aphids, morphological variation.

INTRODUCTION

The morphology of organisms is the final phenotypic expression of the genetic information stored in their cells, as modified by the environment during ontogeny. In most organisms it is not possible to measure the contribution of genetic and environ- mental determinants to the phenotype unless careful controlled breeding is carried out.

The unique biology of gall-forming aphids provides an exceptional case for study. Each gall is induced by a single female fundatrix. The growing plant tissues surround the aphid, which finally remains enclosed in the gall and in some cases the gall is completely sealed. The fundatrix reproduces parthenogenetically within the gall, so that all offspring carry identical copies of the fundatrix genome (cases in which this may not be true are mentioned in the discussion). Therefore, variation within a gall must be caused by developmental and environmental processes. Variation among gall means on the same tree represents genetic variation among fundatrix genotypes. Variation among tree means within localities, and variation among locality means, may be caused either directly by local environmental influences on the trees on which the galls are formed (in particular in characters related to aphid size), or indirectly by natural selection in different localities. It is the latter possibility which attracts the attention of biologists interested in geographic variation, adaptation and evolution. The methodology for the use of gall-aphid characters in studies of geographic variation has been

worked out by R.R. Sokal and his students, working on species of *Pemphigus* in the U.S.A. (e.g., Sokal, 1952, 1962; Sokal & Rinkel, 1963; Sokal & Thomas, 1965; Senner & Sokal, 1974; Sokal, Bird & Riska, 1980; Sokal & Riska, 1981).

Three species of aphids, forming galls on trees of the genus *Pistacia* (Anacardiaceae) in Israel, were analyzed using the same methods (Wool and Koach, 1976; Wool, 1977). Several interesting correlations were detected between morphometric measurements and environmental variables. In the present communication we refer to that part of the former results which concern the variation within galls.

We compared variance components of 17-19 morphological characters in 3 species: *Geoica utricularia* (Pass.), *Baizongia pistaciae* (L.) and *Slavum wertheimae* (H.R.L.). In all three species, the fundatrix is completely sealed in at a very early stage of gall formation, long before the onset of reproduction, so that immigration of alien aphids is ruled out. The final shapes of the three galls are strikingly different (Wool, 1977). *G. utricularia* forms globular galls about 1.5-2 cm in diameter. *B. pistaciae* forms elongate, very large galls (up to 30 cm in extreme cases), horn or banana shaped. *S. wertheimae* forms "Cauliflower" or coral-shaped galls, sometimes up to 15 cm across, made of branching interconnected tubes (Fig. 1).

Fig. 1. A typical gall of *Slavum wertheimae*, showing the branching tubes giving the "Cauliflower" appearance.

Comparing the variance components, within galls, of each of the morphological characters, we found that they were the smallest in *Geoica*, larger in *Baizongia* and the largest in *Slavum* (Wool, 1977, Table 3).

Since variation within galls is not genetic, it was suggested that the differences in magnitude of this variance component could be ascribed, in part, to differences in microenvironmental conditions within the gall. Inside the small, globular galls of *Geoica*, the environment may be more homogenous than in the branching tubes of the *Slavum* gall, with *Baizongia* intermediate. Environmental microdifferentiation would then affect the ontogeny of different individuals, resulting in larger within-gall variation.

The present study was designed to measure quantitatively the micro-environmental contribution to morphological within-gall variation in *Slavum wertheimae*.

MATERIALS AND METHODS

S. wertheimae is very common on *Pistacia atlantica* in many parts of Israel (Koach & Wool, 1976). In September, 1981, twelve large galls were collected from five trees – four in one locality at the northern foot of Mount Carmel, and the fifth about 100 km to the north, in the Hula Valley, Israel. At that time of the year all the aphids within the galls were alates.

Four to eight (usually five) "subgalls" were randomly sampled from each gall. A "subgall" is defined as a part of the gall beginning as a single branch at the base of the gall. Each "subgall" was separately cut open and all alates were preserved in 70% ethanol.

Eight randomly chosen alates of each of the 59 subgalls were mounted on microscope slides. Twenty-one morphological characters were measured on each alate. Measurements were taken on the screen of a Visopan projection microscope (Reichert, Austria). The list included the original 17 characters (measured in Wool, 1977) plus four new ones: WTL, MPTS, HWL and HWW (Table 1).

"Nested" analysis of variance (Sokal & Rohlf, 1969) was carried out on each character. Variation was partitioned into four components: aphids within subgalls; subgall means within galls; gall means within trees and among tree means. The four components were expressed as percentages of the total variation (Sokal & Rohlf, 1969).

In addition to the analysis of variance of each character, cluster and factor analyses were carried out among *characters*, to find patterns of association among them. These analyses were carried out using subgall medians as data. The use of the median, rather than the arithmetic mean, to represent the 8 subgall measurements considerably reduced computational load (since the median could be easily spotted without calculation) and is perhaps more desirable since the median is not affected by rare, extreme values.

Cluster analysis was done by the UPGMA method (Sokal and Sneath, 1963), beginning from the character correlation matrix. Factor analysis was done using the NTSYS program package written by F.J. Rohlf and his collaborators (now at the State University of New York, Stony Brook, Long Island). All computations were carried out at the Tel Aviv University Computation Center.

TABLE 1. MEANS AND STANDARD DEVIATIONS OF THE 21 CHARACTERS IN *SLAVUM*.
(Data were the 59 subgall medians for each character).
Measurements are in microns.

Code	*Description*	*Mean*	*S.D.*
WTL	Forewing total length	2532	170
WL	Length of forewing subcubitus (sc)	1733	108
WW	Forewing width	961	65
HWL	Hindwing length	1714	131
HWW	Hindwing width	542	45
HW	Head width	352	15
TW	Width of the large thoracic sternite	489	35
MPTS	Length of clypeus + rostrum	378	19
A1	Length of 3rd antennal segment	81	5
A2	Length of 4th antennal segment	55	5
A3	Length of 5th antennal segment	61	6
A4	Length of 6th (terminal) segment	108	7
F1	Foreleg femur	330	22
Ti1	Foreleg tibia	366	27
Tar1	Foreleg tarsus	120	6
F2	Midleg femur	273	2
Ti2	Midleg tibia	350	31
Tar 2	Midleg tarsus	123	6
F3	Hindleg femur	321	19
Ti3	Hindleg tibia	437	31
Tar3	Hindleg tarsus	142	7

RESULTS

Means and standard deviations of the 21 characters, calculated from the 59 subgall medians, are listed in Table 1. Since the 59 subgall medians represent only 12 independent genetic units (galls), one should divide the standard deviations by $\sqrt{12}$ rather than $\sqrt{59}$ to get standard errors for comparison with other data. The means in the Table are not significantly different from those reported in the previous study (Wool, 1977, Table 2).

Significant differences among galls within trees, and among subgalls within galls, were found in all 21 characters (Table 2). Most of these were highly significant ($P < 0.001$). In the leg characters (and two others) significant differences among tree means were also found ($P < 0.05$). This component was not studied previously in *Slavum*, since, while collecting galls of this species, no record was kept of the trees they were sampled from.

TABLE 2. PARTITIONING OF CHARACTER VARIANCE INTO COMPONENTS

	Variance components (%)			
Code	*Among trees*	*Galls within trees*	*Subgalls within galls*	*Individuals within subgalls (error)*
WTL	37.1 (ns)	45.1***	2.9***	14.9
WL	42.2 (ns)	36.4***	4.3***	17.1
WW	35.8 (ns)	43.2***	2.5***	17.1
HWL	40.0 (ns)	29.5***	6.1***	24.4
HWW	40.8*	20.4***	3.9*	34.7
HW	2.4 (ns)	43.4***	8.6***	45.7
TW	34.6 (ns)	43.2***	2.5***	19.7
MPTS	35.9*	12.1***	8.8***	43.2
A1	21.7 (ns)	35.5***	0.8(ns)	42.1
A2	12.3 (ns)	46.0***	6.1***	35.6
A3	20.0 (ns)	33.4***	3.5***	43.2
A4	3.7 (ns)	34.7***	15.8***	45.8
F1	57.2*	21.7***	3.6***	17.4
Ti1	53.6*	26.0***	2.1***	18.3
Tar1	39.0*	12.1***	3.0(ns)	45.9
F2	48.7*	31.5***	2.4***	17.5
Ti2	50.6*	26.6***	4.3***	18.5
Tar2	47.5*	8.6***	2.5*	41.4
F3	46.0*	32.0***	3.5***	18.5
Ti3	49.6*	28.2***	4.4***	17.8
Tar3	38.9*	20.0***	6.6***	34.5

ns = not significant, *P $<$0.05, **P $<$0.01, ***P $<$0.001.

Inspection of Table 2 indicates that the subgall added variance component comprised less than 5% in 15 of the characters and was more than 10% in only 1. (The mean square of subgalls was tested for significance over mean square error, which had about 350 degrees of freedom, so that small differences became significant).

The error variance component was quite large – more than 40% in 7 characters (more than 30% in 10). The most variable characters within subgalls were A 1-4, Tar 1-3, MPTS and HWW, which were the most difficult to measure accurately in this species of aphid: they seem to be easily deformed in the preparation of the slides. Preparation problems, causing measurement errors, seem to be the principal source of within-subgall variation. The error variance in the more stable characters, WL, F1-3 and Ti 1-3, was similar in magnitude to values reported previously (Wool, 1977).

Variation among gall means within trees – a measure of the genetic differences among fundatrices – was large in many characters (more than 30% in 11, more than 40% in 5). This was the case in all three species studied previously (Wool, 1977).

ASSOCIATIONS AMONG CHARACTERS

Factor analysis on the *Slavum* characters, using the 59 subgall medians as data, revealed three major axes, explaining respectively 86.6%, 4.4% and 3.7% of the variation. After rotation, the values changed only a little (to 90.0%, 4.1% and 3.2%, respectively).

The distribution of the characters in the field of the first two principal components is illustrated in Fig. 2. All characters have strong associations with factor I,

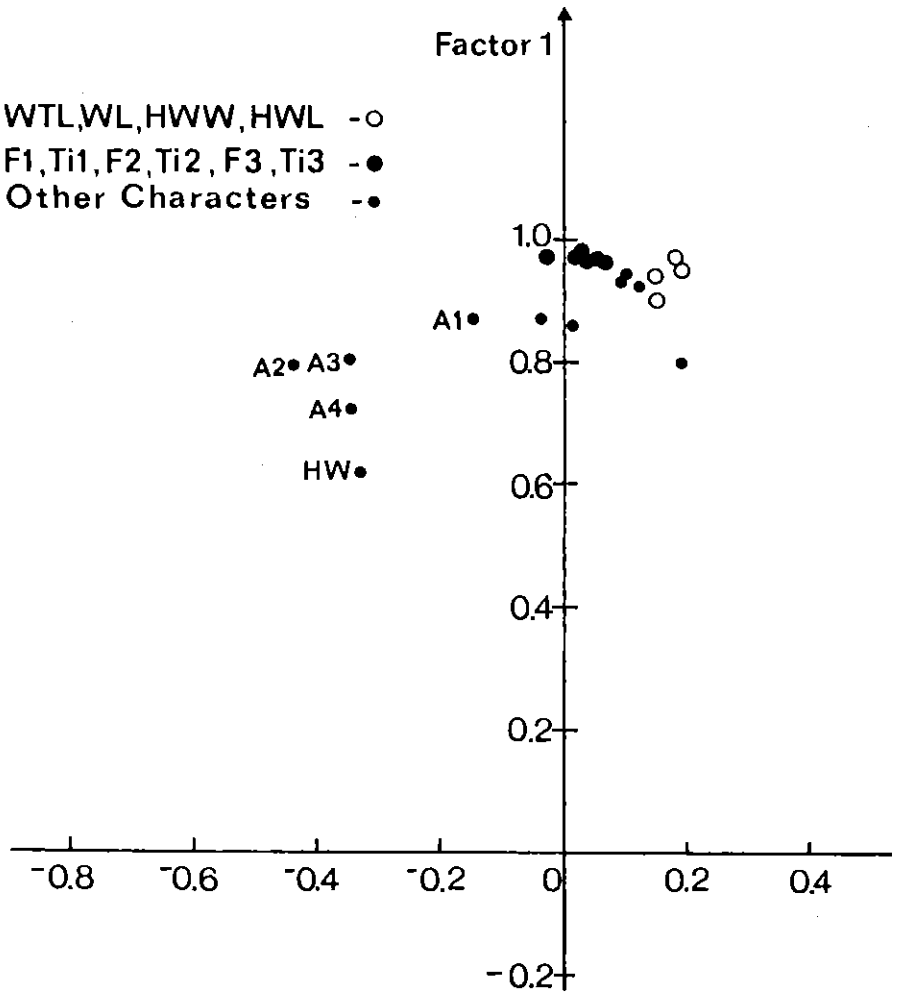

Fig. 2. Distribution of the 21 characters in the field of the first two principal components (before rotation). Antennal characters and HW are indicated by codes. Large dots = "leg" group. Circles = "wing" group. Small dots = all other characters.

which most probably is a "general size" factor. (Large aphids would have longer legs, wings, etc.). The antennal segments A2-4 and HW, are associated also with factor II. However, HW has negative loading on factor III, while A2-4 are positively associated with that factor.

When the scale in Fig. 2 is enlarged, there appears a more interesting grouping of characters, which is even better expressed in the results of the UPGMA cluster analysis (Fig. 3). Wing characters (with the exception of WW) form a distinct tight cluster, closely associated with the tight group of leg characters (not including tarsi). Antennal characters A2-4 form a less distinct group. Head, thorax and tarsal characters do not cluster clearly.

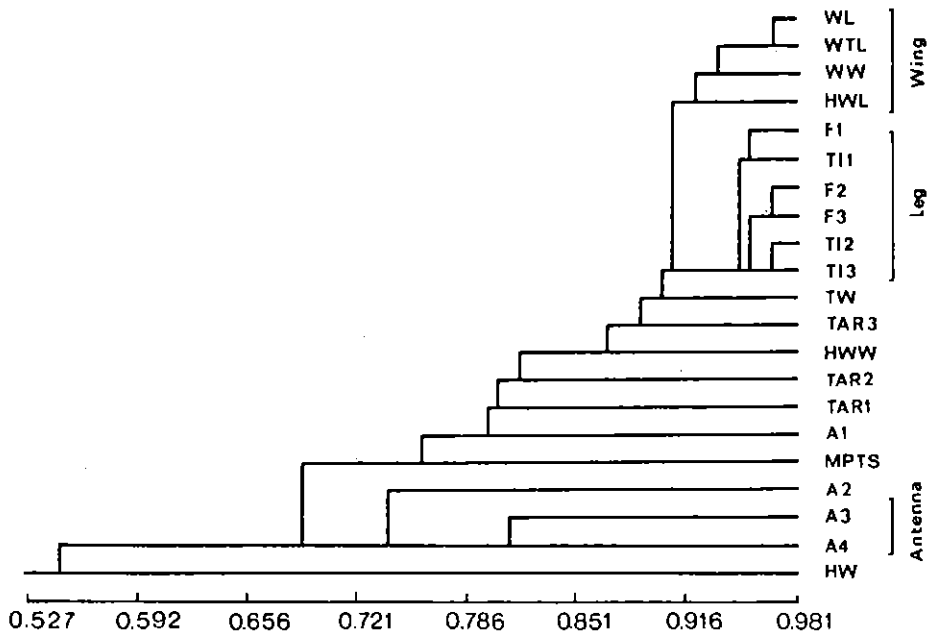

Fig. 3. Phenogram resulting from UPGMA cluster analysis of the 21 characters, using the 59 subgall medians as data. Note the tight clusters of the "wing" and "leg" groups.

This grouping shows that the 21 characters do not vary independently. In Table 3 we list the mean variance component for the four groups – wing, leg, antennae and other characters. We shall refer to this table in the discussion.

It is particularly reassuring that WL and WTL are very highly correlated. WTL is much more difficult to measure accurately on the microscope screen due to the transparency of the wings and, therefore, it was not measured in the 1977 work. The present study indicates that the correlation between WL and WTL is 0.97.

DISCUSSION

This study was designed to test for possible microenvironmental differentiation within a gall, which may affect aphid morphology. If aphids in different subsections of the same gall are exposed to different microenvironments during ontogeny, the same clonal genotypes may develop at different rates, increasing within-gall variation. We reasoned that in the branching tubular chambers which make up the *Slavum* gall, the environment may not be the same. This would represent the most micro-differentiated environment of the three species investigated by Wool (1977).

Within-gall variation may be due to several causes. One important cause is measurement error – in studies of the present kind, often because of distortions during preparation of the slides. The characters with the largest within-gall variation are often those most difficult to measure (Wool, 1977 and present study). The most "reliable" in this sense are the characters in the "leg" group, followed by the "wing" group (Table 3). Little can be done to avoid this source of variation, although improved technique may reduce it.

TABLE 3. MEANS (±STANDARD ERRORS) OF VARIANCE COMPONENTS FOR THE THREE CHARACTER CLUSTERS (Fig. 3) – WING, LEG AND ANTENNA – AND FOR THE FOURTH, "OTHER", GROUP

Character cluster	*#in cluster*	*Characters (Codes)*	*Among trees*	*Galls within trees*	*Subgalls within galls*	*Individuals within subgalls (error)*
Wing	4	WTL, WL, HWL, HWW	40.0±1.07	32.8±5.24	4.3±0.67	22.8±4.46
Leg	6	F1-3, Til-3	51.0±1.61	27.7±1.56	3.4±0.39	18.0±0.20
Antenna	3	A2-4	12.0±4.71	38.0±4.00	8.47±3.74	41.5±3.06
Others	8	Tar1-3, A1, HW,TW,WW, MPTS	32.0±4.92	27.3±5.51	4.41±1.10	36.2±4.07
All characters	21		36.1±15.6	30.0±11.2	4.7±3.20	29.2±12.2

Another possible source of within-gall variation may be immigration into the gall of individual nymphs originating in another gall (Setzer, 1980; and see Akimoto, 1981; Aoki & Makino, 1982). In *Slavum*, as well as in the other species investigated in 1977, this source can be safely ruled out since the gall is sealed around the fundatrix at a very early stage and no opening exists.

Microenvironmental subdivision comes next. Subgall environmental differences may arise, among other reasons, from an uneven distribution of nutrients in the gall tissues, effects of different amounts of sunlight or shade, or differences in atmospheric

composition in different chambers. The latter may perhaps be caused by respiration and metabolism of the different numbers of aphids. Our results show that significant differences among subgalls do exist in nearly all characters. However, the magnitude of these differences is very small, averaging only around 5% of the total variation (Table 3). The biological importance of this (statistically significant) portion of the variation may not be great. It stands to reason that in the more regular galls of *Geoica* and *Baizongia* (Wool, 1977), the importance of this level of environmental effect should be even less.

The variance component among trees is interesting. It could result from genetic differentiation of populations on each tree, due perhaps to random "founder effects". Genetically different groups of sexuparae may arrive at different trees in the summer. Another reason could be selection of particular aphid genotypes on different trees by some ecological property of the individual trees: it is very common to find adjacent trees, some of which are heavily infested with galls, while others carry none. However, it seems to us that the main source of the differences among tree means is environmental: the nutritional quality and the quantity of nutrients available to the tree must affect the growth of the gall, and, consequently, the nutrition of the aphids, which, in turn, should affect their size. It seems that the among-tree component measures in some way environmental heterogeneity within localities.

In morphological studies of geographic variation, natural selection and adaptation, one would like to use characters likely to be unaffected by direct local environmental conditions, which obscure the long term modifying effects of natural selection on the genetic composition of the populations. In gall-forming aphids, the best would be "leg" characters, which have low within-gall variance components and important genetic component (among galls). They seem to be also sensitive to environmental heterogeneity among trees. Similarly one should choose characters with low within-locality (among trees) variation and high inter-locality variation when searching for large-scale geographic patterns, such as in Wool (1977) and in the studies on *Pemphigus* listed above. Analyses of the type carried out here point out which characters should be used in future work and are essential for successful geographic variation analyses.

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