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Describing variations between reared and wild populations of the predatory earwig *Euborellia annulata*

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ABSTRACT

Since there was an increasing awareness on the environmental problems caused by the abuse of the use of insecticides in controlling insect pests, it was argued that other alternative measures be considered such as the use of biological control agents. The predatory earwig, Euborelliaannulata is considered effective in controllingan important pestof corn such as the cornborerOstriniafurnacalis thus was mass-reared. To be able to maintain the quality control of insect mass-rearing, many studies were focused on quantitative genetic studies of the life history and behavioral traits. However, quantitative analysis of phenotypic characters of the reared insects are limited. In this study, the different morphological body characters (head, pronotum, prosternum and elytra) of E. annulata between reared and wild populations were quantitatively analysed using landmark-based geometric morphometrics and correlation analysis based on distances (CORIANDIS). These tools were considered very useful in determining variations in characters of many organisms thus was applied in the examination of character shapes in the predatory earwigs in culture and in the wild. Results of the stacked bar graph analysis showed that reared male and female populations show similarities of characters (in the head, pronotum, prosternum and elytra). Disparity of characters were observed between sexes of the wild populations. These variations among characters can explain the differences between the reared and the wild populations of the insect. Inbreeding must have affected the insects in culture as the morphological characters have become similar between sexes for those that were in culture suggesting that in cultured insects genetic variance can be reduced and that it will lead to increased homozygosity among individuals resulting to phenotypic homogeneity.

Key words: inbreeding, homozygosity, *Euborelliaannulata*, CORIANDIS, geometric morphometric

INTRODUCTION

Corn borer is one of the important pests of corn causing yield loss up to 80-90% [1]. Host plant resistance has played an important role in the management of this pest but because of its limitations, insecticide use has been recommended [2]. Because of environmental problems brought about by the use of chemical pesticides, considerable efforts are made to reduce their use in controlling the pest. The predatory earwig, *Euborelliaannulata* is considered an option as a biological control agent being observed as one of the effective predators of *O. furnacalis* [3] including other corn pests such as the ear borer (*Helicoverpaarmigare*), aphids, and mites [4,5], leaf hopper, and caterpillar, larvae of beetles, centipede, and small insects. As a biological control agent, this insect is mass-reared for distribution and use by local farmers as this insect was found to efficiently prey on the larvae and eggs of *O. furnacalis* [6]. Because of this, a need for efficiency in producing the maximal number of reared insects with minimal man-hours and space, in as short a time and as inexpensively as possiblewas considered[7].

In maintaining the quality of insect mass-rearing programs [8],life history and behavioral traits are important. However, it is a common concern by those involved in rearing programs thatdeterioration in quality happens like those observed in the mass-reared melon flies [9,10,11,12,13,14]sweet potato weevils[15].Inbreeding depression changes important biological attributes by increasinghomozygositythus affecting the quality of reared insects especially its fecundity [16]. Common practice to avoid inbreeding depression is the introduction of wild or field collected individuals which is hypothesized to be a solution as it is believed it will lead to increase variability and fecundity of the reared population and to express traits that are desirable for pest control [17]. Wild populations are believed to be more diverse and more fecund than those reared populations. To be able to study diversity in populations, many tools in the past have been utilized – morphometric, cytological, biochemical, molecular, etc., however, with advances in biology, statistics, geometry and computer science, variation studies have become more quantitative [18]. An example of this is the application of geometric morphometric methods in many biological studies [19]. In this current study, phenotypic variations in reared and wild populations in*E. annulata* were investigated using the methods of landmark-based geometric morphometrics and correlation analysis based on distances (CORIANDIS). The morphological characters compared are the shapes of the head, pronotum, prosternum and elytra of *Euborelliaannulata*. The Geometric morphometrics (GM) and Correlation Analysis Based on Distances or CORIANDIS ver. 1.1 Beta [20] is used to determine the overall differences and/or similarities of the different body parts between populations. These methods combined with powerful and flexible tools of multivariate statistics make it possible to study morphological variation with direct reference to the anatomical context of the structure [21].

MATERIALS AND METHODS

Wild populationsof the predatory earwig (*Euborelliaannulata*) were obtained from Lanao del Norte (Inudaran, Lanao del Norte) and the reared population in the Entomology laboratory of the National Crop Protection Center at UPLB Los Baños, Laguna, Philippines. Fifty two males and 48 females were collected from the fields in Inudaran, Lanaodel Norte. Reared population consisting of 30 males and 30 females were obtained from the entomology research laboratory at UPLB, Los Baños Laguna, Philippines (Fig. 1). The following body parts were used in the evaluation: head (dorsal view), pronotum, prosternum and elytra. The images used in the evaluation were from those samples photographed by a digital camera. Digital images of four body parts were taken for each sample using a standardized procedure (Figure 2.). In landmark-based morphometric analyses, the morphology of an object is represented by coordinates of sets of landmark points [22]. Landmarks were chosen for their ease of identification, their homology and for the ability to capture the general shape of each morphological structure. The landmark data will provide some information such as the orientation, rotation and scale of the specimen [21]. Only (21) landmark points of the head, (18) landmark points for the pronotum, (10) landmark points for the prosternum and (12) landmark points for the elytra were chosen. Cartesian coordinates were digitized by TpsDig ver. 2 [23]. The analysis of superimposed specimens [24] describes shape variation by comparing individual specimen with a consensus configuration, representing the average Cartesian coordinates for each landmark across all specimens [21]. The raw coordinate data were aligned prior to analysis using tpsRelw (version 5.0) [24] to remove size and arbitrary positioning effects of the specimens relative to the reference axis [25]. Generalized Procrustes superimpositions make data standardize size of the landmark, thus removing differences due to translation and rotation [26]. The relative warps (RWs, which are the principal components of the covariance matrix of the partial warp scores) were computed using the unit centroid size as the alignment-scaling method. Histogram was generated using the PAST software [27] since it is a powerful display for comparing distributions. They also provide a compact view of where the data are centered and how they are distributed over the range of the variable [28]. Kruskal-Wallis test was also used to analyze whether or not the species differ significantly with regards to its shape [29].

The Correlation Analysis Based on Distances or CORIANDIS ver. 1.1 Beta [20] was used to determine the overall differences and/or similarities of the different body parts between reared and wild populations of *E. annulata*. The software implements most of the methods for a broad spectrum of data types, including 2-D landmark and distance data [19]. This was used to determine associations among multivariate datasets, projections on compromise space, trait variance or disparity, congruence and multivariate covariance measure on how similar the interspecific locations of the four body parts of *E. annulata*. It is interpreted as a decomposition of group distinctiveness for other groups in terms of specific traits or characters [30]. The squared distances of each group to the origin are computed for each of the shape data sets, and are plotted in a stacked bar graph to give an overall impression [31].

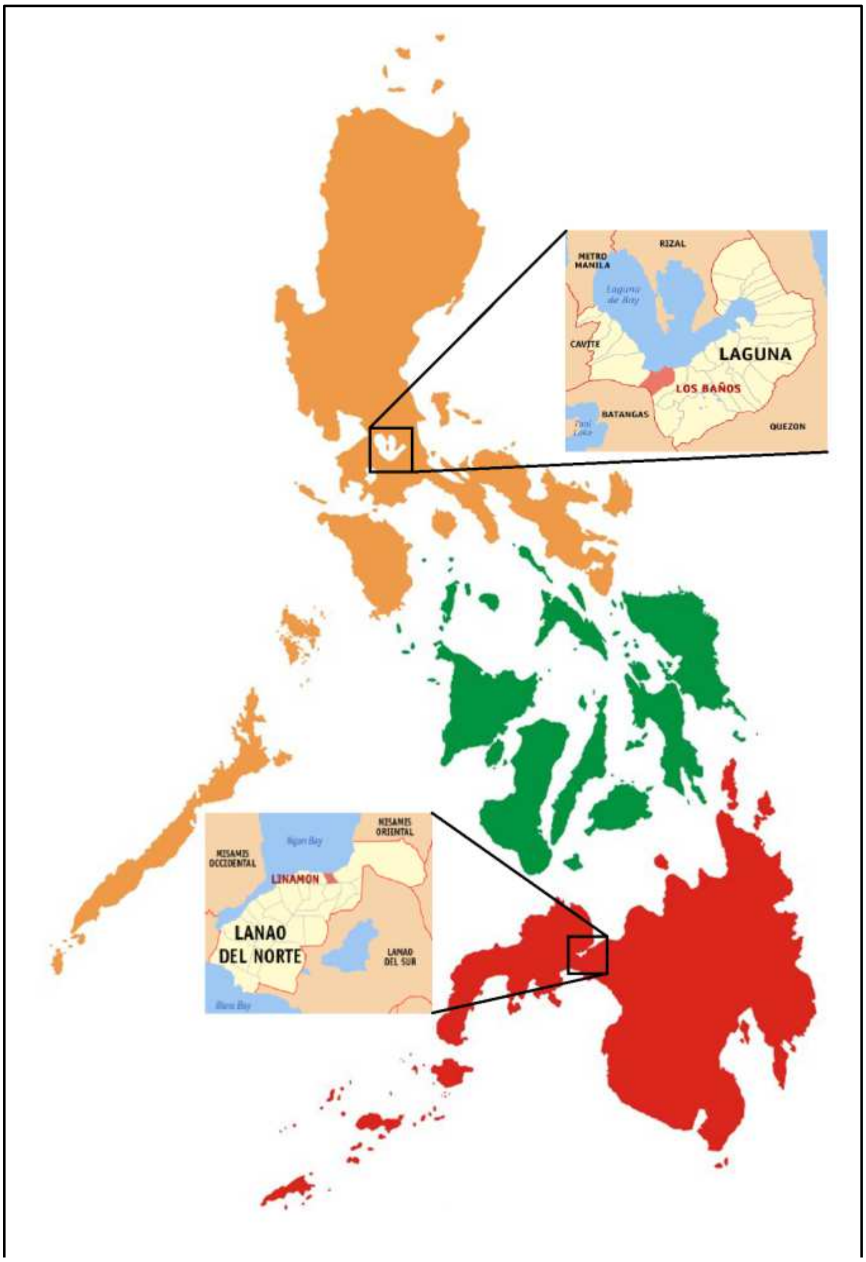

Figure 1. Map showing the study area

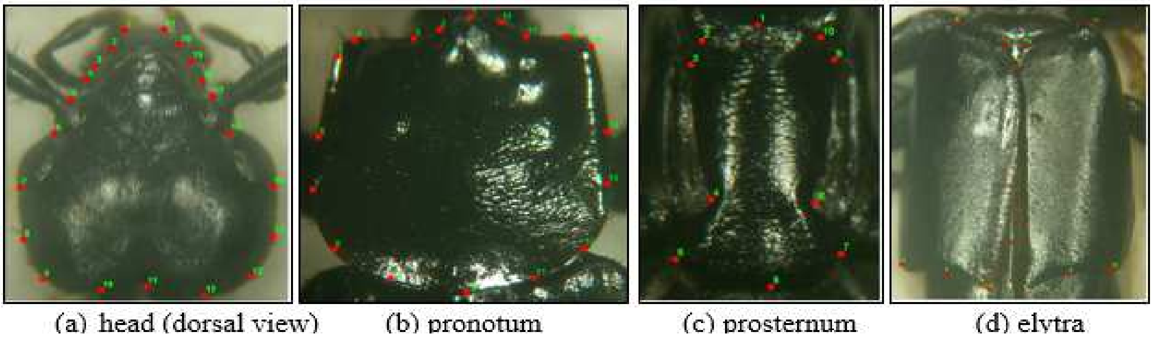

Figure 2. Photograph of four body parts of *Euborellia annulata* and the distribution of landmarks on thehead, pronotum, prosternum and elytra

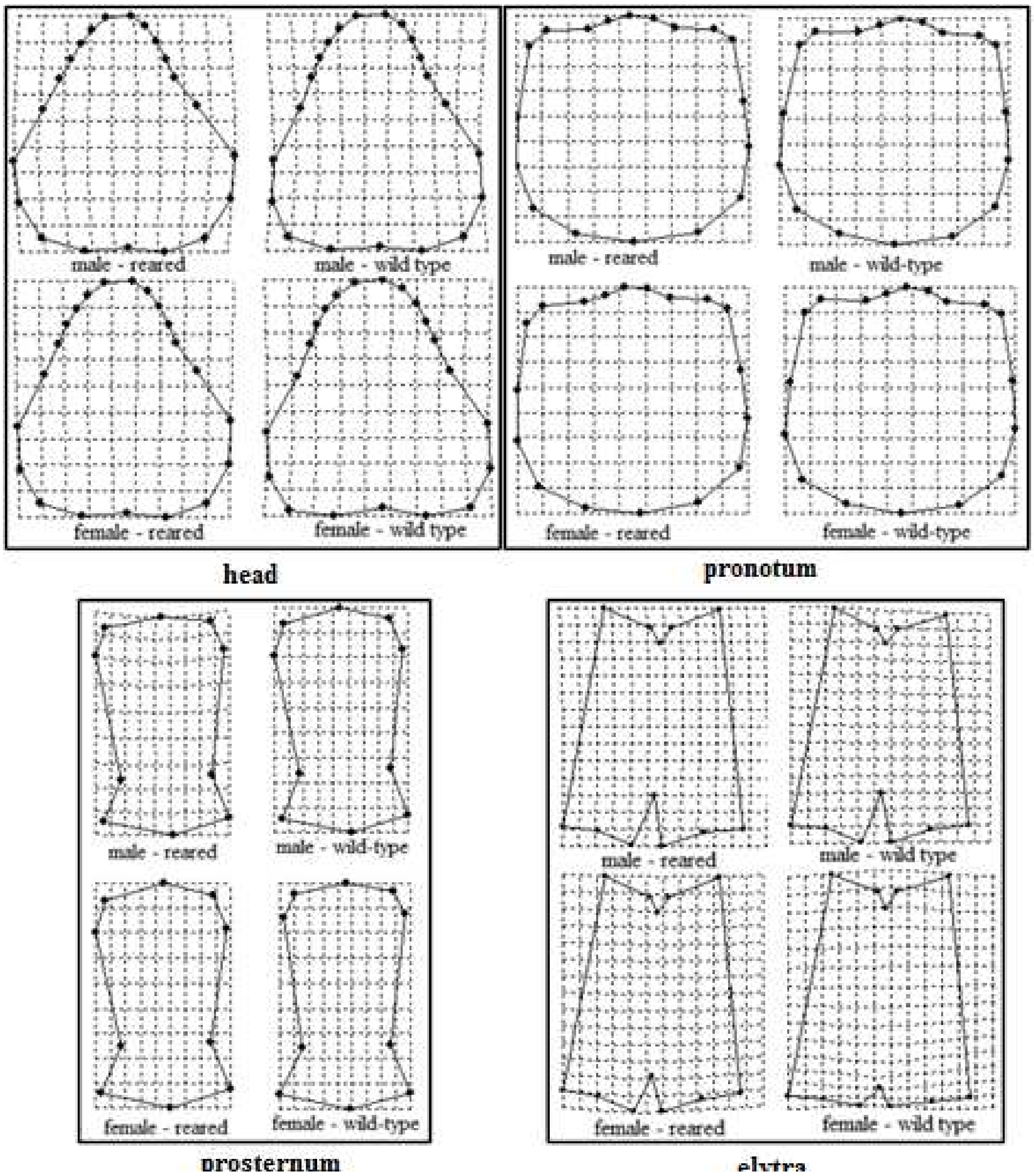

Fig. 3. Mean shapes of the head, pronotum, prosternum and elytra of *E. annulata*

RESULTS AND DISCUSSION

Thin-plate spline reconstructions of the shape of the head (dorsal view) of *E. annulata* are shown in Figure 3. A comparison of the mean shapes of the dorsal view of the head between sexes of the reared and wild populations of the insect show minor differences. Based on the observed bending of grids in the shape of the head show thereared populationhave more convexedhead shapes compared to the wild population. Males have long and thinner labrum compared to females from those collected in the field while those reared individuals. The shapes of the pronotum of *E. annulata* shows minor differences between sexes in reared and wild populations. Wild-type population of the males show more roundedposterolateral margin than females. Between sexes of the reared populationhave no remarkable differences except that the male is slightly convex in the pronotumshape. For the prosternum, reared and wild populations of *E. annulata* show minor differences. It was also observed that males have a narrow-shapedprosternum compared to females. For the elytra, differences were observed between the two populations although between sexes have shown only minor differences where the wild type population shows asymmetry in shape. Male has tilted apex while the female shows more declined apex. Regarding size, females in both populations has bigger elytra compared to males.

Table 1. Results of Kruskal –Wallis for significant differences in mean shape of the head of *E. annulata* between reared and wild populations

Relative Warp	Population		a	b	c	d
1	(a)	M-R		3.02E-11	3.02E-11	3.02E-11
	(b)	M-N			3.02E-11	3.02E-11
	(c)	F-R				3.02E-11
	(d)	F-N				
2	(a)	M-R		3.02E-11	3.02E-11	3.02E-11
	(b)	M-N			3.02E-11	3.02E-11
	(c)	F-R				3.02E-11
	(d)	F-N				
3	(a)	M-R		5.494E-11	3.02E-11	3.02E-11
	(b)	M-N			3.02E-11	3.02E-11
	(c)	F-R				3.02E-11
	(d)	F-N				
4	(a)	M-R		3.02E-11	3.02E-11	3.02E-11
	(b)	M-N			3.02E-11	0.4825
	(c)	F-R				3.02E-11
	(d)	F-N				

pronotum

Relative Warp	Species		a	b	c	d
1	(a)	M-R		3.02E-11	3.02E-11	3.02E-11
	(b)	M-N			3.02E-11	3.02E-11
	(c)	F-R				3.02E-11
	(d)	F-N				
2	(a)	M-R		3.02E-11	3.02E-11	3.02E-11
	(b)	M-N			3.02E-11	3.02E-11
	(c)	F-R				3.02E-11
	(d)	F-N				
3	(a)	M-R		4.504E-11	3.02E-11	3.02E-11
	(b)	M-N			3.02E-11	3.02E-11
	(c)	F-R				3.02E-11
	(d)	F-N				
4	(a)	M-R		3.02E-11	3.02E-11	3.02E-11
	(b)	M-N			3.02E-11	3.02E-11
	(c)	F-R				3.02E-11
	(d)	F-N				

Prosternum

Relative Warp	Species	a	b	c	d
1	(a) M-R		3.02E-11	3.02E-11	3.02E-11
	(b) M-N			3.02E-11	3.02E-11
	(c) F-R				3.02E-11
	(d) F-N				
2	(a) M-R		3.02E-11	3.02E-11	3.02E-11
	(b) M-N			3.02E-11	3.02E-11
	(c) F-R				3.02E-11
	(d) F-N				
3	(a) M-R		3.02E-11	3.02E-11	3.02E-11
	(b) M-N			3.02E-11	3.02E-11
	(c) F-R				3.02E-11
	(d) F-N				
4	(a) M-R		3.02E-11	3.02E-11	3.02E-11
	(b) M-N			3.02E-11	0.4825
	(c) F-R				3.02E-11
	(d) F-N				
		elytra			
Relative Warp	Species	a	b	c	d
1	(a) M-R		3.02E-11	3.02E-11	3.02E-11
	(b) M-N			3.02E-11	3.02E-11
	(c) F-R				3.02E-11
	(d) F-N				
2	(a) M-R		3.02E-11	3.02E-11	3.02E-11
	(b) M-N			3.02E-11	3.02E-11
	(c) F-R				3.02E-11
	(d) F-N				
3	(a) M-R		3.02E-11	3.02E-11	3.02E-11
	(b) M-N			3.02E-11	3.02E-11
	(c) F-R				3.02E-11
	(d) F-N				
4	(a) M-R		3.02E-11	3.02E-11	3.02E-11
	(b) M-N			3.02E-11	0.9941
	(c) F-R				3.02E-11
	(d) F-N				

Legend : (a) MR - Male –reared population , (b) MN – Male-wild population, (c) FR – Female –reared population, (d) FN – Female-wild population of E.annulata.

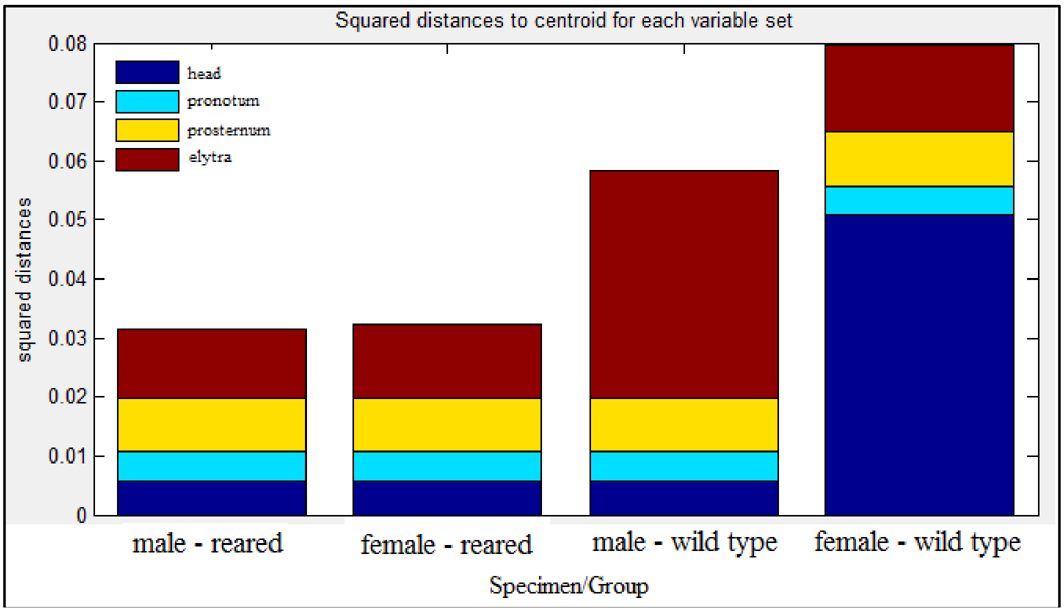

Figure 4. Stacked bar graph showing disparity between reared and wild populations of *E. annulata*

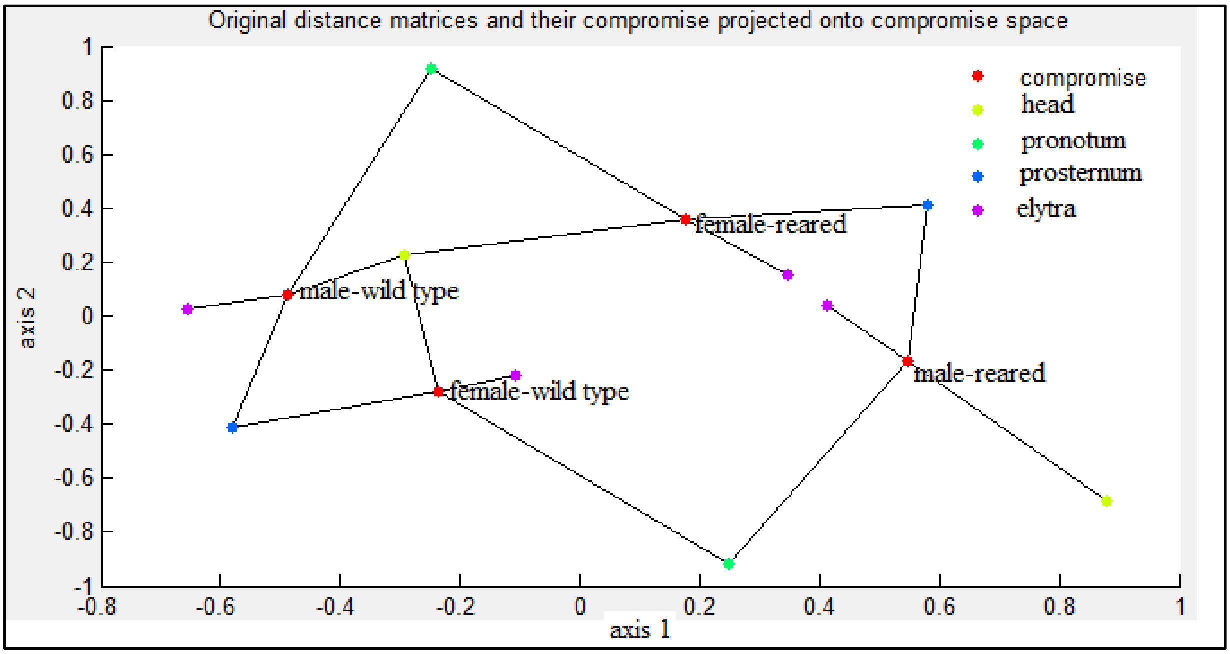

Figure 5. Plot of the principal components of "compromise" space axis of *E. annulata* between reared and wild populations

To have a graphical comparison of the variations between within and between sexes within and between populations of the insect, the association among all the traits between reared and wild populations were tested using the method of correlation analysis based on distances or CORIANDIS [20](Figures 4 and 5) usingthe landmark data analyzed to evaluate the variation in the different body parts. Kruskal-Wallis test showed significant differences between reared and natural populations (Table 1). The congruence and disparity of multivariate traits as shown in the stacked bar graph and compromise space(Figures 4 and 5) revealed that no differences are observable between sexes in reared *E. annulata*in all four characters. Disparity was observed in the head and elytra between sexes of the wild-types. Between populations, differences between reared and wild populations of *E. Annulata* was observed where differences in the elytra was pronounced between males and in the head between females (Figures 4 and 5).

Results of this study have shown that the reared population of the insect do not show between sexdifferences in all of the characters examined when compared to those collected from the wild. This indicates that inbreeding has resulted to homogeneity in the phenotypic attributes of the insect. When the four traits were collectively analyzed, both sexes of the reared population clustered together while those "wild" individuals have both sexes differ in the shapes of the head and elytra. This means that when reared in captivity, *E.annulata* exhibits morphologies different from those collected from the natural populations.Inbreeding could have reduced genetic variance in the reared population and lead to increased homozygosity among individuals[32]. Since the goal of many insect mass-rearing programs has been widely conducted to produce biological agents and sterile insects [33,34,35], [36,37,38,39]to control target pests on an area-wide level, this require quality control of the mass-reared insects [40] by not only understanding thegenetics of the life history [36,41,42,43,44] and behavioral traitsof the organism [45] but also changes in the phenotypes in culture. The problem of reduced quality in mass-produced insects due to inbreeding depression can be monitored, inferred and correlated with the insect phenotype and as shown by the current study where *E. annulata* were shown to be phenotypically homogeneous when in culture or mass-reared, it can be argued that studies of genetic coupled with phenotypic variability may help predict the potential of insect species to evolve in responseto farm management practices [46].

CONCLUSION

The use of geometric morphometrics and correlation analysis based on distances (CORIANDIS) could be useful in assessing population structures of insects. The shape and sizes of different morphological structures of the insect may reflect their function in nature. Organisms exhibit variability in adaptation to environment but when in culture, it is possible that genetic heterogeneity will be reduced and will affect the quality of the mass-reared insects.

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