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Quantitative Descriptions of head shapes of three different instar-larvae of the Asian corn borer *Ostrinia furnacalis*

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ABSTRACT

Ostrinia furnacalis or the Asian corn borer (ACB) is a well-known agricultural pest of corn. In the Philippines a yield loss of 20 to 90% or even a total crop loss was reported. It is being argued that phenotypic variation can be found in this pest because it differs in population structure and mating barrier. Likewise the extent of damage on the corn by the larvae also varies and this may be due to variations in the capability of the larvae to scrape and bore to the plant. Since the head of the larvae has the feeding structures that will damage the plant, we examined this by quantitatively describing the head shape of the larvae at three different developmental instars using geometric morphometric (GM) tools specifically outline-based analysis. Results revealed that among the three instars, the third instar obtained highest total variability (92.88%). CVA/MANOVA indicated head shape differences among the three instars between the two generations. DFA also confirmed correct classification of the different developmental stages of the insect.

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INTRODUCTION

Ostrinia furnacalis or the Asian corn borer (ACB) was first recorded in the Philippines by Banks in 1906 and two decades later its biology was first studied (Buligan, 1929) followed by Calora *et al.* (1965) who investigated egg laying, larval habits, and control strategies. Camarao (1976) conducted a comprehensive study of the population dynamics of the species which was considered a milestone in the history of corn borer research in the Philippines. The insect is a well-known agricultural pest on several crops such as bell pepper, cotton, hops, millet, pearl millet, foxtail millet, sugarcane, sorghum, ginger but it prefers corn. The larva feeds on almost any part of the plant but when it bores into the ear to feed on the silk and kernels, considerable damage to the plant happens thus it is considered one of the worst corn pests in Japan and China (Huang *et al.*, 1998; Zhou and He, 1995), Guam and the Northern Mariana Islands, Java, Sulawesi, the Philippines, Borneo, New Guinea, the Solomon Islands, and Micronesia including the western Pacific region of Asia (Huang *et al.*, 1998). It is also considered one of the worst pests overall, second only to maize downy mildew (Nafus and Schreiner, 1991). In the Philippines, losses of 20 to 90% have been reported (Sanchez, 1971; Rejesus, 1983; Morallo-Rejesus and Punzalan, 2002; Gerpacio *et al.*, 2004) or even a total crop loss. In Taiwan it has reached 95%, and in the Marianas, 100% (Nafus and Schreiner, 1991).

Several control measures were developed against the pest. Up to the early 1970s, the use of pesticides was the most popular but because of the hazards including their high costs that are brought by their applications, other measures were explored such as host plant resistance (HPR). In the Philippines, the establishment of the Institute of Breeding (IPB) in 1975 helped advance the growing awareness of host plant resistance as an approach to pest population regulation and management (Lit *et al.*, 1987). However, the presence of ecotypes of the borer in the different corn growing areas in the Philippines poses a problem in the usefulness of a resistant cultivar especially if the cultivar was exposed to a different ecotype and bred in a different region. The work of Barrion (1981) showed morphological variations in ACB populations mainly based on setal patterns of the last-instar larvae. Likewise, the observations of diffused centromeres of the chromosomes and the extraordinary wide scattering of the frequency and haploid chromosome numbers ranging from 20 to 169 with 27 as predominating number (Barrion *et al.*, 1982) may indicate that the species is chromosomally polymorphic with large genetic variability but with little genetic stability. This was further supported by the results of a study on

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isozymes where genetic variability in geographical populations of the pest was also observed (Mendoza *et al.*, 1994). It can therefore be argued that this insect pest differ in population structure and mating barriers may have existed affecting rates of gene flow within the species thus phenotypic variations can be found (Mutebi *et al.*, 2002). This is evident in the closely related species of ACB- the North American *O. nubilalis*. Population of this corn pest shows phenotypic variation in pheromone production and perception, and the number of generations per year (voltinism) (Showers, 1993; Mason *et al.* 1996). To date however, no phenotypic variation was detected between sympatric voltinism ecotypes of *O. furnacalis*. Only the unpublished chaetotaxic study by Barrion (1981) was always referred to. Thus, it is the objective of this study to further examine phenotypic variations in the species by quantitatively describing differences in the phenotype of the larvae of the insect. The head where the mandible is attached is also a significant morphological feature in the feeding behavior of the larvae since it facilitates the entry of larvae to the host (Albutra *et al.*, 2014). Since differences in the head capsule is correlated with differences in mandibular power, it is argued that this morphological feature have a direct effect on the ability of the insects to ingest hard foods rapidly (Bernays, 1986) thus was examined in this study. The first and second generations of *O. furnacalis* larvae were examined using the tools of geometric morphometrics (GM). GM is based on landmark coordinates which preserves the geometry of the landmark configurations throughout the analysis and thus permits to represent statistical results as actual shapes or forms (Bookstein, 1991). GM is applied not only in taxonomy and other fields but as a complementary tool to understand the origin of phenotypic differences (Klingenberg, 2011). Two common methods in describing the form of an organism through GM are landmark-based which uses a set of landmarks to describe the object or specimen; and outline-based which extracts the margin around the specimen (Klingenberg, 2011, Albutra *et al.*, 2012). GM includes the Procrustes superimposition-based techniques which offer an advantage over traditional analyses of linear morphometrics in that geometric shape space presents all various shape configurations of designated landmarks (Bookstein, 1996; Rohlf, 1998). The shape variables (partial and relative warp scores) focused on functional adaptation and evolutionary change in extant and extinct taxa (Singleton, 2002; Nicholson & Harvati, 2006) was used in the quantitative description of the shapes of the head of the larvae. Three instars (3rd, 4th, and 5th) of two generations of *O. furnacalis* were used in the current study since these were the most damaging stage in the life of the insect pest.

Methodology:

Two generations of *Ostrinia furnacalis* (Guenée) larvae reared at Tranca Bay, Laguna were obtained from Entomology laboratory of the National Crop Protection Center at UPLB Los Baños, Laguna, Philippines. A total of 51 larvae samples from first generation (6 third instar, 22 fourth instar, 23 fifth instar) and 52 larvae samples from second generation (15 third instar, 20 fourth instar, 17 fifth instar) were examined. The head of the larvae samples were removed using dissecting needles and forceps. The dissected heads were then mounted on white clay in a clean glass slide. The slides were labelled properly for imaging. Images of the head shape of *O. furnacalis* viewed under a dissecting stereomicroscope were taken using 14.1 megapixel Sony digital camera with uniform magnification.

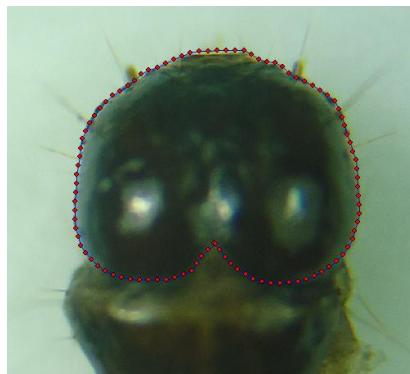


Fig. 1: Digitized image of *O. furnacalis* head showing the 100 outline points.

Analyses of the head shapes of the third, fourth and fifth instars from the two generations were first done separately. Then a combination analysis of the two generations was carried out to establish morphological variations between the larval stages. A total of 100 closely connected points were made along the outline of the head shape (Fig. 1) to capture the general shape of the head using tpsDig version 2.12 (Rohlf, 2008a). The tps curve was converted into landmark points (XY coordinates) that would serve as raw data for outline analysis. The raw coordinate data were then subjected to generalized Procrustes analysis (GPA) to superimpose the landmark configurations using least-squares estimates for translation and rotation parameters (Adams *et al.*,

2004). The coordinates of superimposed configurations in all aligned specimens were used for the thin-plate splines relative warp analysis (Bookstein, 1991) in order to analyze and display the direction of shape differences among species.

The relative warps analysis and computation of partial-warp scores were done using tpsRelw ver. 1.46 (Rohlf, 2008b). The relative warp scores were then subjected to MANOVA/CVA and DFA using PAST software version 1.91 (Hammer *et al.*, 2001) to determine whether the head shape of Asian corn borer differ significantly between the two generations.

RESULTS AND DISCUSSION

Relative warp analysis shows the degree of phenotypic variation in the head shapes between the two generations of the three different instar larvae of *O. furnacalis*. The pattern of these variations from the mean shape is summarized in Figure 2.

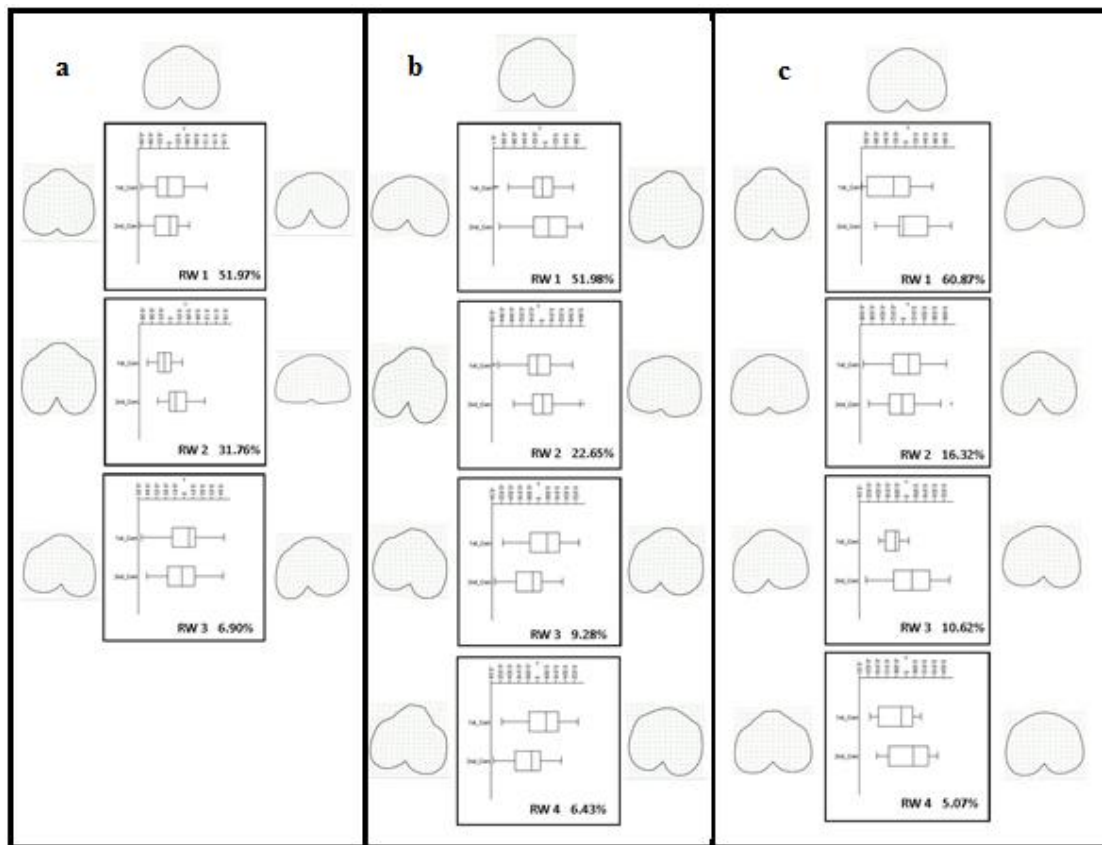


Fig. 2: Summary of head shape geometric morphometric analysis of the three instars (5th, 4th, and 3rd) of *O. furnacalis* showing its relative warps and boxplots comparing the first generation from the second. A. Head shape variations of fifth instar having three significant relative warps (RW1= 51.97%; RW2= 31.76%; RW3= 6.90%). B. Head shape variations of fourth instar having four significant relative warps (RW1= 51.98%; RW2= 22.65%; RW3= 9.28%; RW4= 6.43%). C. Head shape variations of third instar having four significant relative warps (RW1= 60.87%; RW2= 16.32%; RW3= 10.62%; RW4= 5.07%).

Among the three instars studied, the third instar larvae showed more variant forms than those of the 4th and 5th instars. Relative warp analysis showed 92.88% total variability from the four significant relative warps was obtained by the third instar larvae. This is followed by fourth instar with a total variability of 90.43% from four significant relative warps. Fifth instar obtained 90.63% total variability from three significant relative warps. Description of head shape variations between the two generations on the three instars were listed in Table 1.

Table 1: Description of head shapes between first and second generations of 3rd, 4th, and 5th instar larvae of *O. furnacalis*.

RW	% Variance	
5 th Instar		
1	51.97%	Variations observed at the anterior and posterior ends of the head. Both generations approached the mean shape but the first generation tends to have more defined anterior end than the second generation. The second generation however has more defined curvature at the base of the vertex.
2	31.76%	Variations observed at the anterior and posterior ends of the head. The first generation has more defined anterior region and defined curvature at the basal region than the second generation. The second generation however has broader head.
3	6.90%	Variations found at the curvature at the base of the head shape. Both generations tend to approach the mean shape.
4 th Instar		
1	51.98%	Variation observed at the anterior end of the head. The first generation has blunt anterior end while the second generation has more defined region.
2	22.65	Both generations tend to approach the mean shape but the heads of the second generation appeared to be blunt.
3	9.28%	Variations found at the curvature of the basal region of the head. More defined curvature at the right portion of the basal of the head was evident on the first generation while the second generation tends to have more defined curvature at the left portion.
4	6.43%	Variations found at the anterior region of the head. First generation has broader head shape and appeared to be blunt while the second generation has more defined anterior region.
3 rd Instar		
1	60.87	Variation was observed at the anterior and posterior regions of the head. First generation showed more defined anterior end and curvature at the basal end. Second generation on the other hand has blunt anterior end and less defined curvature at the basal end.
2	16.32	The first generation tend to have more defined anterior region and curvature at the basal end while the second generation has blunt anterior region and broader head shape.
3	10.62%	Variation was observed at the anterior region of the head. The first generation has more defined anterior end while the second generation has broader head shape.
4	5.07%	The first generation tends to have more defined anterior region while the second generation has blunt anterior region and broader head shape.

Phenotypic variations were further analyzed using canonical variate analysis (CVA). The scatter plots showed the distribution of head shapes of the first and second generations of the three instars larvae (Fig. 3). While there are some overlaps, the values obtained using multivariate analysis of variance (MANOVA) showed that between generations, larval differences were observed (Table 2). Discriminant function analysis (DFA) also showed significant variations in the frequency distribution of the relative warp scores between the first and second generations of the three larval instars. Analysis shows that higher than 70% of the pooled population of *O. furnacalis* is correctly classified (Table 3).

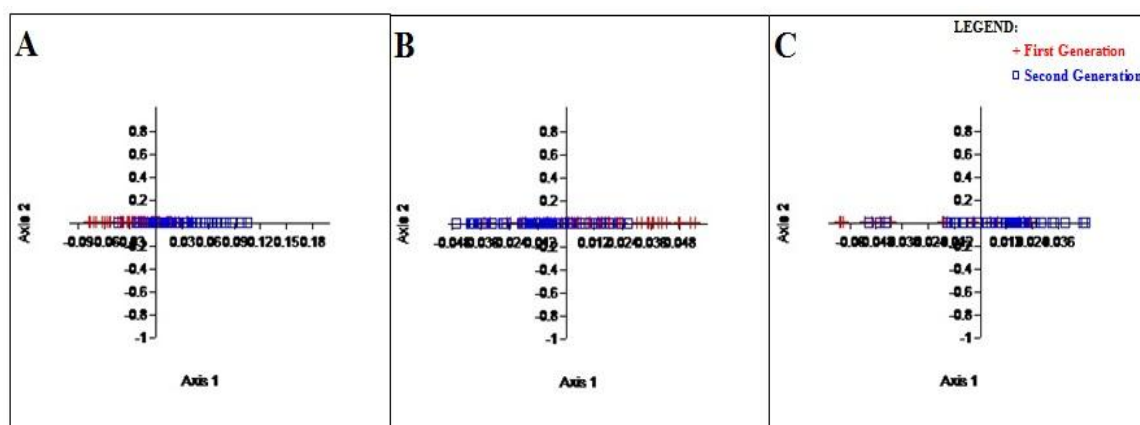


Fig. 3: CVA scatter plots showing the head shape distribution of the first and second generation on the three different instars of *O. furnacalis* population. A. Fifth Instar. B. Fourth Instar. C. Third Instar

Table 2: Results of MANOVA test for significant variation in the head shape between different populations of white stem borer.

	Wilks' lambda	Pillai trace	Eigenvalue1	Eigenvalue2	P(same)
Fifth Instar	0.6393	0.3607	0.5643	1.072E-16	2.829E-11
Fourth Instar	0.7451	0.2549	0.3422	3.387E-16	3.04E-07
Third Instar	0.6376	0.3624	0.5683	3.612E-16	2.474E-05

Table 3: Summary of DFA results of the three instars of *O. furnacalis* population.

Instars	Correctly Classified %	Generation	Classification	
			First	Second
Fifth	75.00%	First	53	16
		Second	14	37
Fourth	70.63%	First	45	21
		Second	16	44
Third	82.54%	First	12	6
		Second	40	5

Data obtained from the different analyses of outline based geometric morphometric revealed that there exist a significant variation on the head shape between the first and second generation among the three instars of *O. furnacalis* population being studied. Presently, no known factor that accounts for the variations found between the first and second generation of Asian corn borer. However, Zhou and He (1995) reported that the first and second generation of the Asian corn borer attack at different developmental stage of the maize plant. Thus, this indicates that the two generations might have adapted different feeding behavior. This is supported by the findings of Kaliontzopoulou *et al.* (1991) which indicated that bite performance is determined importantly by the dimension and shape of the head. Furthermore, it was argued that there is a direct effect on the ability of the insects to ingest hard foods rapidly from differences in the head capsules, with the correlated differences in mandibular power (Bernays, 1986). Albutra *et al* (2014) pointed out the significance of the head since it facilitates the entry of larvae to the host.

Conclusion:

In conclusion, Geometric morphometric analysis based on outline approach is a valuable tool for entomological problems. Significant phenotypic variation was observed on the head shape between the first and second generation on the three instars of reared *O. furnacalis* population. Head shape variation between the two generations was accounted from its feeding behaviour on the different developmental stage of it's the host plant.

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