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MORPHOMETRICS OF FOUR GENITAL TRAITS IN SOME PHILIPPINE POPULATIONS OF THE ASIAN CORN BORER, *Ostrinia furnacalis* (Guenée) (Lepidoptera: Crambidae)[1]

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ABSTRACT

The morphometrics of four genital traits were examined among the 27 local populations of Asian corn borer (ACB), *Ostrinia furnacalis* (Guenée). Comparison in measurements of genital traits, namely: aedeagus, sacculus and valva lengths and clasper width, showed significant differences among and within the local populations compared. Cluster analysis based on percent correct classification revealed four groups within ACB populations with 96% accuracy. However, the more sensitive jackknife correct classification analysis revealed that the groups formed were 85% accurate. The results also showed that groups were formed regardless of geographical distance. The variations in genital traits of the different ACB populations can be attributed possibly to their adaptive mechanisms to their respective localities.

Key words: Asian corn borer, *Ostrinia furnacalis* (Guenée), genital traits, aedeagus length, sacculus length, valva length, clasper width

INTRODUCTION

The Asian corn borer (ACB), *Ostrinia furnacalis* (Guenée), remains the most important insect pest of corn in the Philippines. This insect can cause yield loss ranging from 20% to 80% or even result in zero profitability of the crop at severe infestation (Sanchez, 1971). Preliminary studies showed that variations occur among the local ACB populations. Barrion et al. (1981a) reported that the ACB in Los Baños was chromosomally polymorphic with very large genetic versatility but with very little genetic stability. Their results showed that ACB is one of the most progressive species since it is better adapted to the present environment and more likely to survive temporal changes. In another study, abdominal chaetotaxy indicated that ACB population from Laguna was significantly different from that in Musuan, Bukidnon (Barrion et al.1981b). Mendoza et al. (1994) concluded that wide variations exist among and within the three local populations of ACB based on the significant difference in the esterase isozyme patterns of ACB from Laguna compared with ACB from Leyte and South Cotabato. However, there was no significant difference observed between the two latter populations. They noted that the Laguna population is subjected to higher selection pressure since this population thrives in the area considered as the center of experimental activities in corn breeding. Recently, Caasi-Lit and Sapin (2012) noted that larval and pupal stage durations of the different ACB populations were significantly different. Based on the data gathered, the ACB populations from Leyte and Laguna had some larvae that underwent six, and others five, larval instars.

Morphometric data can be useful as a tool in determining the variations within the species. Currently, there are no documented local studies on the genital traits of the different populations of adult male ACB. Generally, genital characteristics are very useful in providing distinction of a species. Researchers working on species with similar external morphology typically use male genitalia for species diagnostic (Mutanen et al. 2007, Song 2009) since the male genitalia seems to be more variable and more elaborate than the female genitalia, to a larger extent, easier to measure because of identifiable morphological structures, and the female genitalia are less often described in literatures (Eberhard 1985 as cited by Arnqvist, 1997).

Eberhard et al. (1998) noted that according to the good viability genes model of male genitalic evolution, females might use male genitalia to evaluate overall male size, and choose sires with superior viability genes by favoring males with larger genitalia. Thus, larger male size may be associated with superior abilities to survive and accumulate resources (Andersson, 1995).

The objectives of the study were: 1) to determine the variations in the four genital traits of male ACB adults collected from the different localities; 2) to determine extent of variability of the four genital traits; and, 3) to classify the 27 different ACB populations using the combined genital traits.

MATERIALS AND METHODS

Insect Collection

Geographic ACB populations used in this study were collected from 27 localities in the Philippines (Figure 1)* from November 2010 to August 2011. All developmental stages of ACB randomly found in each site were considered in the collections.

In Luzon area, ACB populations were collected from Ilocos Norte (1)* towns of Bangui, Banna, Batac, Dingras, and Paoay; Isabela (8) towns of Aurora, Cauayan, Echague, Ilagan, and Tumauini; Pangasinan (13) town of Sta. Maria; Pampanga (18)

*Designated code number for the province in the map of the Philippines (Fig. 1) town of Magalang; Laguna (24) towns of Bay, Calamba, Calauan and Los Baños; and Camarines Sur (28) towns of Buhi, Iriga, Naga, and Ocampo. ACB populations from Mindanao were collected from Bukidnon (61) towns of Don Carlos, Malaybalay, Musuan, and Valencia; Sultan Kudarat (67) town of Bagumbayan and South Cotabato (68) towns of Banga and Koronadal.

Figure 1. Collection sites (encircled) in the Philippines of Asian corn borer, *Ostrinia furnacalis* (Guenée), used in morphometric study of four genital traits of the insect pest.

Laboratory Rearing of ACB

Asian corn borer larvae were reared on Multispecies Diet (Southland Products Incorporated, Arkansas, USA). Proper laboratory handling of the different ACB populations was practiced to prevent mixture of the populations. The containers were labeled according to the collection sites. Rearing was done under the laboratory temperature range of 25°C to 27°C. A room with screen door and windows was used to house the oviposition cages to prevent escape of adult ACB from the laboratory.

Uniformity in rearing conditions was ensured by providing a uniform amount of artificial diet to a standard larval density. Pupae were collected from the folded tissue paper and transferred to a Petri dish. Six days after collection, the pupae were transferred to an oviposition cage. Cotton moistened with honey solution was provided to the adult moths. A piece of wax paper was taped on top of the oviposition cage, which served as an oviposition medium for the ACB.

Preservation of the Test Insects

The newly emerged male F_1 adults were anaesthetized by freezing for 40 minutes. Once immobilized, the adults were transferred individually to a 1.5 ml microcentrifuge tube with 75% ethanol.

Measurement of the Four Genital Traits

Thirty male adults were characterized for each population. Four genital traits, namely, lengths of valva, sacculus, aedeagus, and the width of the clasper (Figure 2) were measured based on Ohno *et al.* (2003).

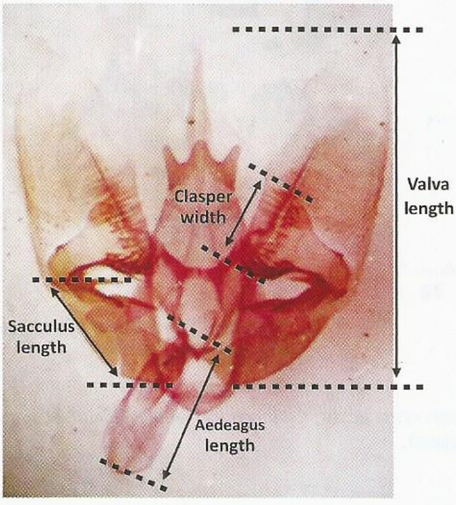

Figure 2. The four genital traits of the male Asian corn borer, *Ostrinia furnacalis* (Guenée), considered in determining variabilities among the populations collected from different localities in nine provinces in the Philippines.

Dissecting, Slide Mounting and Measuring the Genital Traits

Slide mounts of male genitalia were prepared based on the procedure of Franclemont (1983). The tip of male abdomen was removed and placed in 10% potassium hydroxide (KOH) for 24 hours, then removed from the KOH solution, rinsed with distilled water and placed on a dissecting pan. The cuticle was carefully removed using a fine needle, and the genital part cleaned by careful brushing. The genital parts were stained in 5% acid fuchsin for 20 minutes, rinsed with distilled water, and finally, placed in 95% ethanol for 30 minutes. The genitalia were then mounted on microscope slide using Hoyer's medium. Measurements of male genitalia were determined using ImageJ 1.33 (NIH, USA).

Data Analysis

Data were analyzed using SAS 9 (Cary, North Carolina, USA) and GraphPad Prism Version 6 softwares. One-way analysis of variance (ANOVA) and Bonferroni's multiple comparison tests (single pooled variance) at 0.05% family-wise significance and 95% confidence interval were performed for all the traits measured. However, for the South Cotabato populations where only two populations were compared, an unpaired t-test was used at 95% confidence level. Multivariate statistics were performed to determine the relationships among the different traits. Hierarchical cluster analysis and discriminant analysis were performed using SYSTAT 9 (San Jose, California, USA).

RESULTS AND DISCUSSION

Variations in Four Genital Traits of ACB Collected from Nine Different Provinces

Table 1 shows comparison of measurements of the four genital traits of ACB collected from nine different provinces. Consistently, in all of the measured genital traits, the ACB from Bukidnon had the highest mean length while the ACB from Pampanga had the lowest. One way analysis of variance (ANOVA) revealed significant difference in the lengths of all genital traits measured.

The longest aedeagus mean length was observed in ACB from Bukidnon (0.0107 mm) while the shortest was recorded in Pampanga population (0.0093 mm). Same mean aedegus length (0.0097 mm) was observed in ACB populations from Pangasinan and South Cotabato while ACB populations from Laguna and Sultan Kudarat had 0.0094 mm. The mean aedeagus lengths of ACB in decreasing order were Bukidnon>Ilocos Norte > Camarines Sur > Isabela > Pangasinan = South Cotabato > Laguna = Sultan Kudarat > Pampanga. Bonferonni test revealed that Bukidnon population was significantly different from all of the populations except those from Camarines Sur and Ilocos Norte. On the contrary, the mean aedeagus length of ACB from Isabela was similar to those of all other the populations except those from Bukidnon and Pampanga.

Table 1. Mean measurements of genital traits, namely, aedeagus length, clasper width, sacculus length, and valva length (mm) of Asian corn borer, *Ostrinia furnacalis* (Guenée), collected from nine provinces in the Philippines

PROVINCE	SAMPLE SIZE	MEAN[1] ± SD (RANGE)			
		Aedeagus Length (AL)	Clasper Width (CW)	Sacculus Length (SL)	Valva Length (VL)
Bukidnon	120	0.0107 [a] ± 0.0010 (0.0105- 0.0108)	0.0057[a] ± 0.0007 (0.0056- 0.0058)	0.0096 [a] ± 0.0008 (0.0095- 0.0097)	0.0249[a] ± 0.0018 (0.0246- 0.0252)
Camarines Sur	120	0.0102[abc] ± 0.0009 (0.0100- 0.0104)	0.0054[b] ± 0.0006 (0.0053- 0.0055)	0.0095 [a] ± 0.0009 (0.0094- 0.0097)	0.0236 [b] ± 0.0021 (0.0232- 0.0240)
Ilocos Norte	150	0.0103[ab] ± 0.0009 (0.0102- 0.0105)	0.0053[bc] ± 0.0005 (0.0052- 0.0054)	0.0094[ab] ± 0.0007 (0.0093- 0.0096)	0.0235[bc] ± 0.0015 (0.0233- 0.0238)
Isabela	150	0.0099[bcd] ± 0.0008 (0.0098- 0.0100)	0.0051[bcd] ± 0.0005 (0.0050- 0.0052)	0.0090[c] ± 0.0007 (0.0088- 0.0091)	0.0230[bcd] ± 0.0014 (0.0227- 0.0231)
Laguna	120	0.0094[de] ± 0.0010 (0.0092- 0.0095)	0.0050[cd] ± 0.0005 (0.0049- 0.0051)	0.0086[cd] ± 0.0009 (0.0085- 0.0088)	0.0226[cd] ± 0.0017 (0.0223- 0.0229)
Pampanga	30	0.0093[e] ± 0.0006 (0.0091- 0.0096)	0.0049[d] ± 0.0002 (0.0049- 0.0050)	0.0083[d] ± 0.0004 (0.0082- 0.0084)	0.0212[e] ± 0.0008 (0.0209- 0.0215)
Pangasinan	30	0.0097[cde] ± 0.0006 (0.0095- 0.0100)	0.0050[cd] ± 0.0004 (0.0049- 0.0051)	0.0087[cd] ± 0.0006 (0.0085-0.0089)	0.0226[cd] ± 0.0011 (0.0222- 0.0230)
South Cotabato	60	0.0097[cde] ± 0.0009 (0.0095- 0.0099)	0.0052[bcd] ± 0.0005 (0.0051- 0.0053)	0.0087 [cd] ± 0.0006 (0.0086- 0.0088)	0.0228[bcd] ± 0.0017 (0.0223- 0.0232)
Sultan Kudarat	30	0.0094[de] ± 0.0009 (0.0092-0.0097)	0.0051[bcd] ± 0.0005 (0.0049- 0.0053)	0.0090[bc] ± 0.0005 (0.0088-0.0092)	0.0222[de] ± 0.0014 (0.0217- 0.0227)

[1]Means under each column with a common letter are not significantly different at α = 0.05 using Bonferroni (Dunn) t Tests

The biggest mean clasper width was observed in Bukidnon population (0.0057 mm) while the smallest was recorded in Pampanga population (0.0049 mm). The same mean clasper width was recorded for ACB populations of Laguna and Pangasinan (0.0050 mm). The Sultan Kudarat and Isabela populations also had the same mean clasper width of 0.0051 mm. The mean clasper widths of ACB population in decreasing order were Bukidnon > Camarines Sur > Ilocos Norte > South Cotabato > Isabela = Sultan Kudarat > Laguna = Pangasinan > Pampanga. Clasper width of

Bukidnon population was significantly greater than those of all the other populations. On the other hand, the Pampanga population, which had the smallest mean width, was not significantly different from those of Isabela, Laguna, Pangasinan, South Cotabato, and Sultan Kudarat populations.

The biggest mean for sacculus length was recorded in Bukidnon population (0.0096 mm), while the smallest was observed in Pampanga population (0.0083mm). Same mean length (0.0090 mm) was recorded from Isabela and Sultan Kudarat populations, so with those from Pangasinan and South Cotabato (0.0087 mm). The mean sacculus lengths of the different ACB populations in decreasing order were Bukidnon > Camarines Sur > Ilocos Norte > Isabela = Sultan Kudarat > Pangasinan = South Cotabato > Laguna > Pampanga.

The biggest mean for valva length was recorded from Bukidnon (0.0249 mm) while the smallest was from Pampanga population (0.0212 mm).The decreasing order for the mean valva lengths of the different ACB population was Bukidnon > Camarines Sur > Ilocos Norte > Isabela > South Cotabato > Pangasinan = Laguna > Sultan Kudarat > Pampanga. Again, Bukidnon population showed significantly longer valva than those of the other populations.

The results also revealed that regardless of geographical distance, genital trait measurements may or may not differ significantly. For instance, male ACB from Bukidnon was not significantly different in aedeagus length from their counterpart from Ilocos Norte despite their geographical distance. On the contrary, since South Cotabato and Sultan Kudarat are not that distant apart, the mean aedeagus lengths of ACB from the two provinces were not significantly different.

Variations in Four Genital Traits Within ACB Provincial Populations

Ilocos Norte population. Table 2 shows the measurements of four genital traits of the ACB collected from five different municipalities of Ilocos Norte. The biggest mean lengths for all the genital traits were observed in Dingras population. One-way ANOVA revealed that mean measurements for all genital traits of the ACB collected from five different localities were statistically different.

The biggest mean for aedeagus length was recorded in Dingras (0.0111mm) while the smallest was observed from Batac population (0.0098 mm). Bonferroni test revealed that Dingras population was significantly different from the rest of the populations.

Valva length was the most variable trait among the ACB populations collected from five different municipalities, some differences being significant as revealed by Bonferroni test.

Table 2. Mean measurements (mm) of four genital traits of Asian corn borer, *Ostrinia furnacalis* (Guenée), collected from five municipalities of Ilocos Norte

MUNICIPALITIES	GENITAL TRAITS MEASUREMENTS (MEAN[1]± SD RANGE)			
	Aedeagus Length (AL)	Clasper Width (CW)	Sacculus Length (SL)	Valva Length (VL)
Bangui	0.0101^{bc} ± 0.0006 (0.009-0.011)	0.0052^{b}± 0.0005 (0.004-0.006)	0.0096^{a}± 0.0006 (0.008-0.010)	0.0231^{c}± 0.0010 (0.021-0.025)
Banna	0.0105^{b}±0.0006 (0.009-0.012)	0.0055^{a}±0.0005 (0.004-0.006)	0.0098^{a}±0.0005 (0.009-0.011)	0.0243^{b}±0.0010 (0.023-0.026)
Batac	0.0098^{c}±0.0007 (0.008-0.012)	0.0051^{b}±0.0003 (0.005-0.006)	0.0088^{b}±0.0005 (0.008-0.010)	0.0221^{d}±0.0010 (0.020-0.025)
Dingras	0.0111^{a}±0.0011 (0.009-0.014)	0.0058^{a}±0.0006 (0.005-0.007)	0.0099^{a}±0.0007 (0.009-0.011)	0.0252^{a}± 0.0013 (0.023-0.027)
Paoay	0.0101^{bc}±0.0005 (0.009-0.011)	0.0050^{b}±0.0004 (0.004-0.006)	0.0091^{b}±0.0005 (0.008-0.010)	0.0229^{c}±0.0008 (0.021-0.025)

[1]Means under each column with a common letter are not significantly different at $\alpha = 0.05$ using Bonferroni (Dunn) t Tests

Isabela population. Table 3 shows the measurements of the genital traits of the ACB collected from five municipalities of Isabela. Aurora population had the biggest mean for aedeagus length while Echague population had the biggest means for clasper, sacculus, and valva measurements. One-way ANOVA revealed that mean measurements for all genital traits of the ACB collected from five different localities were statistically different.

The difference being significant as shown in the table, aedeagus of ACB population from Tumauini is significantly shorter than those of the ACB populations from Aurora, Echague, and Ilagan. Meanwhile, claspers of ACB populations from Aurora and Cauayan are significantly narrower than those of other populations. In terms of sacculus length, that of Cauayan population is significantly shorter than those of the others except for Aurora population. Again, Tumauini population is significantly different in valva length from the rest of the populations.

Table 3. Mean measurements (mm) of four genital traits of Asian corn borer, *Ostrinia furnacalis* (Guenée), collected from five municipalities of Isabela

MUNICIPALITIES	GENITAL TRAITS MEASUREMENTS (MEAN[1]± SD RANGE)			
	Aedeagus Length (AL)	Clasper Width (CW)	Sacculus Length (SL)	Valva Length (VL)
Aurora	0.0103^{a}±0.0006 (0.009-0.012)	0.0048^{b}±0.0004 (0.004-0.005)	0.0087^{bc}±0.0005 (0.008-0.010)	0.0220^{c}±0.0013 (0.019-0.025)
Cauayan	0.0096^{bc}±0.0006 (0.008-0.011)	0.0049^{b}±0.0004 (0.004-0.006)	0.0083^{c}±0.0005 (0.007-0.009)	0.0220^{c}±0.0007 (0.021-0.023)
Echague	0.0100^{ab}±0.0010 (0.008-0.012)	0.0054^{a}±0.0005 (0.005-0.006)	0.0094^{a}±0.0007 (0.008-0.011)	0.0239^{a}±0.0014 (0.021-0.026)
Ilagan	0.0101^{ab}±0.0006 (0.009-0.011)	0.0052^{a}±0.0004 (0.005-0.006)	0.0093^{a}±0.0005 (0.008-0.010)	0.0238^{a}±0.0010 (0.022-0.026)
Tumuaini	0.0095^{c}±0.0009 (0.008-0.011)	0.0053^{a}±0.0005 (0.005-0.006)	0.0090^{ab}±0.0007 (0.008-0.011)	0.0228^{b}±0.0012 (0.020-0.025)

[1]Means under each column with a common letter are not significantly different at α = 0.05 using Bonferroni (Dunn) t Tests

Laguna Population. Table 4 shows the mean measurements of genital traits of the ACB collected from four different localities of Laguna. The biggest means for aedeagus length, clasper width, and sacculus length were recorded from Calauan population while for valva length it was from Bay population. On the other hand, the smallest mean measurements for all the genital traits were recorded for Calamba population.

Table 4. Mean measurements (mm) of four genital traits of Asian corn borer, *Ostrinia furnacalis* (Guenée), collected from four municipalities of Laguna

MUNICIPALITIES	GENITAL TRAITS MEASUREMENTS (MEAN[1]± SD RANGE)			
	Aedeagus Length (AL)	Clasper Width (CW)	Sacculus Length (SL)	Valva Length (VL)
Bay	0.0096^{ab} ± 0.0011 (0.007-0.011)	0.0051^{a} ± 0.0006 (0.004-0.006)	0.0090^{ab} ±0.0011 (0.007-0.011)	0.0231^{a} ± 0.0023 (0.019-0.029)
Calamba	0.0087^{c}± 0.0007 (0.007-0.010)	0.0048^{b} ± 0.0005 (0.004-0.006)	0.0079^{c}±0.0006 (0.007-0.009)	0.0216^{b} ± 0.0013 (0.019-0.024)
Calauan	0.0099^{a}± 0.0009 (0.008-0.011)	0.0051^{a}± 0.0003 (0.005-0.006)	0.0092^{a} ±0.0006 (0.008-0.010)	0.0229^{a} ± 0.0013 (0.019-0.025)
Los Baños	0.0093^{bc} ± 0.0009 (0.008-0.012)	0.0050^{ab} ± 0.0004 (0.004-0.006)	0.0085^{b} ±0.0006 (0.007-0.010)	0.0228^{a} ± 0.0013 (0.020-0.025)

[1]Means under each column with a common letter are not significantly different at α = 0.05 using Bonferroni (Dunn) t Tests

Camarines Sur population. Table 5 shows the measurements of the four genital traits of the different ACB populations in the province. The biggest means for all genital traits were recorded in Buhi population, while smallest mean lengths were recorded in Naga except for aedeagus length, which was recorded in Ocampo population.

In all genital traits, Buhi population revealed to be significantly bigger than the rest of the populations. The Naga population is significantly smaller in valva length than the other populations except for population in Iriga.

Table 5. Mean measurements (mm) of four genital traits of Asian corn borer, *Ostrinia furnacalis* (Guenée), collected from four municipalities of Camarines Sur

MUNICIPALITIES	GENITAL TRAITS MEASUREMENTS (MEAN[1]± SD RANGE)			
	Aedeagus Length (AL)	Clasper Width (CW)	Sacculus Length (SL)	Valva Length (VL)
Buhi	0.0110^{a} ±0.0010 (0.009-0.013)	0.0060^{a}±0.0006 (0.005-0.007)	0.0107^{a} ± 0.0006 (0.010-0.012)	0.0260^{a}± 0.0019 (0.022-0.030)
Iriga	0.0100^{b}±0.0008 (0.009-0.012)	0.0053^{b}±0.0004 (0.005-0.006)	0.0092^{b} ±0.0006 (0.007-0.0100	0.0225^{bc}± 0.00133 (0.020-0.025)
Naga	0.0100^{b} ±0.0008 (0.009-0.011)	0.0050^{b}±0.0002 (0.005-0.006)	0.0091^{b}±0.0004 (0.008-0.010)	0.0223^{c}± 0.0008 (0.020-0.024)
Ocampo	0.0098^{b} ±0.0007 (0.009-0.011)	0.0053^{b}±0.0006 (0.004-0:006)	0.0091^{b}±0.0007 (0.008-0.010)	0.0234^{b}± 0.0017 (0.020-0.027)

[1]Means with the same letter (per column) are not significantly different at α = 0.05 using Bonferroni (Dunn) t Tests

Bukidnon population. Table 6 shows the mean measurements of the genital traits of the ACB that were collected from the four different localities. The biggest means for clasper width and valva length were recorded from Musuan population while the smallest were in ACB populations from Malaybalay for clasper width and from Don Carlos, for valva length. For aedeagus length, biggest mean was recorded in Valencia population while the shortest was recorded again in Don Carlos. For sacculus length, highest mean was recorded for both Musuan and Valencia populations while the lowest was for Don Carlos population.

In terms of significant difference, the populations from Don Carlos and Malaybalay consistently differed in all genital traits from the ACB populations from Musuan and Valencia.

Table 6. Mean measurements (mm) of four genital traits of Asian corn borer, *Ostrinia furnacalis* (Guenée), collected from four four municipalities of Bukidnon.

MUNICIPALITIES	GENITAL TRAITS MEASUREMENTS (MEAN[1]± SD RANGE)			
	Aedeagus Length (AL)	Clasper Width (CW)	Sacculus Length (SL)	Valva Length (VL)
Don Carlos	0.0098[b] ± 0.0008 (0.009_0.012)	0.0055[b]± 0.0005 (0.005_0.006)	0.0089[b] ± 0.0007 (0.008_0.0010)	0.0236[b] ± 0.0016 (0.021_0.026)
Malaybalay	0.0102[b]± 0.0007 (0.008-0.012)	0.0051[b] ± 0.0005 (0.004-0.006)	0.0091[b] ± 0.0006 (0.008-0.010)	0.0237[b] ± 0.0012 (0.021-0.026)
Musuan	0.0111[a]± 0.0010 (0.009-0.012)	0.0063[a] ± 0.0006 (0.005-0.007)	0.0102[a]± 0.0005 (0.009-0.011)	0.0262[a] ± 0.0014 (0.021-0.029)
Valencia	0.0114[a]± 0.0009 (0.010-0.013)	0.0060[a] ± 0.0004 (0.005-0.007)	0.0102[a]± 0.0004 (0.010-0.011)	0.0261[a] ± 0.0008 (0.025-0.027)

[1]Means with the same letter (per column) are not significantly different at α = 0.05 using Bonferroni (Dunn) t Tests

Table 7. Mean measurements (mm) of four genital traits of Asian corn borer, *Ostrinia furnacalis* (Guenée), collected from two municipalities of South Cotabato

TRAITS	MUNICIPALITY AND MEAN ± SD (RANGE)		T-VALUE
	BANGA	KORONADAL	
Aedeagus Length	0.009 ± 0.0008 (0.008-0.011)	0.0010 ± 0.0007 (0.009-0.012)	**4.754**[****]
Clasper Width	0.005 ± 0.0006 (0.004-0.006)	0.004 ± 0.0005 (0.004-0.006)	0.0[ns]
Sacculus Length	0.009 ± 0.0005 (0.008-0.009)	0.009 ± 0.0006 (0.008-0.010)	1.780[ns]
Valva Length	0.022 ± 0.0009 (0.020-0.024)	0.023 ± 0.0020 (0.020-0.027)	**3.646**[***]

Level of significance: ** p<0.01, ***p<0.001; ****p<0.000

Cluster and Discriminate Analyses

The measurement data for genital traits of the 27 geographic ACB populations were analyzed using the Ward's method or Ward's minimum variance criterion and a Mahalanobis distance to generate hierarchical clusters. The Ward's method minimizes the total variance within-cluster variance, wherein merging is done in each step of cluster pairing having the minimum cluster distance. On the other hand, the

Mahalanobis distance is based on correlations of data set and scale-invariant. Figure 3 shows the clusters generated using the combined measurements of respective genital traits and four clusters were generated.

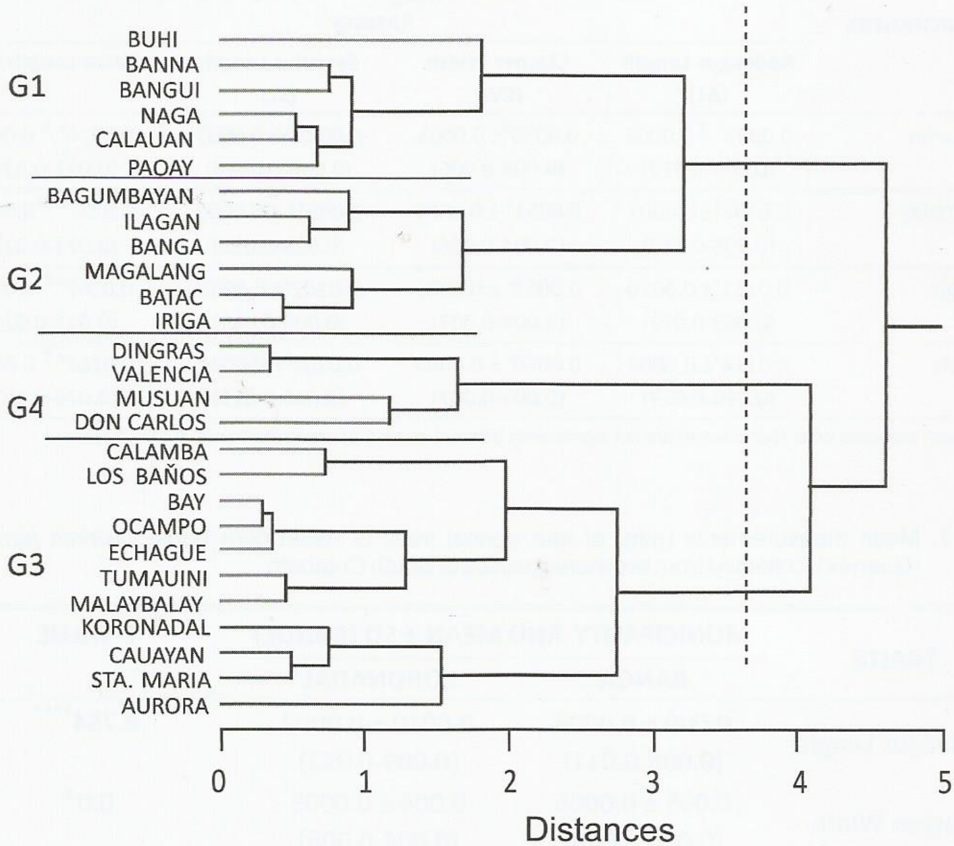

Figure 3. Hierarchical cluster analysis based on the combined measurements of the genital traits; namely: aedeagus length, clasper width, sacculus length, and valva length of the Asian corn borer, *Ostrinia furnacalis* (Guenée) collected from 27 different municipalities in the Philippines.

The frequencies of the four clusters (G1 to G4) were 6, 6, 11, and 4, respectively. G1 included two ACB populations from Camarines Sur: Buhi and Naga; three populations from Ilocos Norte: Banna, Bangui, and Paoay; and lastly, one population from Laguna: Calauan. Meanwhile, G2 was composed of ACB populations from Bagumbayan (Sultan Kudarat), Ilagan (Isabela),. Banga (South Cotabato), Magalang (Pampanga), Batac (Ilocos Norte), Iriga (Camarines Sur). On the other hand, G3, the, largest group, was composed of three populations from Laguna: Bay, Calamba, and Los Baños; four populations from Isabela: Aurora, Echague, Cauayan, and Tumauini; one population from Camarines Sur: Ocampo; one population from Bukidnon: Malaybalay; a population from Koronadal, South Cotabato, and a population from Sta. Maria, Pangasinan. G4 on the other hand, consisted of only three populations from Bukidnon: Don Carlos, Musuan, and Valencia; and one population from Ilocos Norte: Dingras.

The results revealed that clusters were generated regardless of the geographic locations. The G1 was the only group, which consisted of ACB from Luzon area. Some ACB populations collected under the same province were grouped together. For instance, the G4 was predominantly composed of ACB collected from Bukidnon. However, only the Malaybalay population was included in the other group (G3). The ACB from different municipalities of Isabela was present in all the groups except in G4.

The percentages of correct classifications were 83%, 100%, 100%, and 100% for G1 to G4, respectively. This resulted in 96% as an overall accuracy of classification. However, these values decreased when a more sensitive test, the jackknife classification, was used for analysis. Jackknife classification revealed 83%, 100%, 82%, and 75%, correct classifications for G1, G2, G3, and G4, respectively, with an overall classification accuracy of 85%.

Based on cluster analysis, four groups were formed (G1 to G4) using the measurements of four genital traits of the 27 different ACB populations and these resulted in three canonical discriminant functions. Thus, the number of discriminant functions is determined by subtracting one from the number of groups formed.

Table 8 shows the values of the canonical discriminant functions for the four genital traits. The values of discriminant functions show the importance of each predictor or variable. The first important predictor had the highest value while the second important has the lowest value and those predictors are known to be good predictors. In the first canonical discriminate function, valva length and sacculus length were identified as the good predictors. For second and third discriminate functions, the sacculus length and clasper width were also identified as good predictors.

Table 8. The canonical discriminant functions* (standardized by within variances) of the four genital traits of the 27 different populations of Asian corn borer, *Ostrinia furnacalis* (Guneeé).

PREDICTORS OR VARIABLES	DISCRIMINANT FUNCTIONS		
	1	2	3
ValvaLength (VL)	**4.518**	1.646	0.049
SacculusLength (SL)	**-2.887**	**1.781**	**-1.575**
Clasper Width (CW)	-2.000	**-3.037**	**0.795**
AedeagusLength (AL)	0.554	-1.070	-0.120

*This determines the importance of each predictor, the highest value is the first important predictor while the lowest is the second important predictor

Variations in Four Genital Traits

Results revealed that the measurements of four genital traits were varied in different ACB populations. There are several factors that could have influenced the final dimensions of any morphological traits, namely: patterns of gene expression, patterns of cell growth and division, actions of hormones, and growth of other tissues (Sterns and Emlen 1999). Emlen and Nijhout (2000) also noted that larval environments affect the final body size of an adult insect. Fluctuations in nutrient availability, temperature, relative humidity, larval competition, and larval population density generally affect larval growth and development. They also concluded that the growth of body parts may permit to be sensitive in the environmental changes but this characteristic does not mean to be non-genetic. Different individuals within a population may vary genetically in how they respond to changes in the growth environment. In the study, uniformity in rearing the ACB larvae was maintained to eliminate variation due to external factors. However, the feeding behavior of the ACB larvae cannot be controlled.

Size of any morphological trait is influenced by many factors and can have variable results. However, according to Mutanen *et al.* (2007), the importance of genital size should not be underestimated since in general, genital size is less variable compared to body size. The variations in genital traits of the different ACB populations perhaps could be attributed to their adaptive mechanisms to their respective localities. Local environments differ greatly and insect pests have the capability to evolve rapidly under their stressed environments (Kim and McPheron, 1993).

As mentioned earlier, the male genitalia can be used by the female to evaluate the overall male size, choosing superior sires with superior viability genes by favoring males with larger genitalia (Eberhard *et al.* 1998). Thus, larger male size may be associated with superior abilities to survive and accumulate resources (Andersson, 1995). In the results, those populations with bigger measurements for genital traits might have superior abilities in terms of survival, and therefore, might have selective advantage over those populations with smaller measurements. On the other hand, perhaps the size of the male genitalia only implies that different ACB populations might have established their own genital sizes, in which those respective sizes are just fitted to the their female counterparts in their respective populations, or those populations belonging to the same group in the cluster analysis probably can mate exclusively. Interestingly, this can be verified with further studies.

Rapid differentiation within species has posed serious problems in conventional strategies for managing arthropod pests and vectors (Kim and McPheron 1993). Differentiation within ACB populations might also affect the stability of any Insect Resistance Management (IRM) strategy designed for Bt corn. Perhaps, more studies should be conducted to investigate further the variations in local ACB populations.

SUMMARY AND CONCLUSION

The variations of some local Asian corn borer (ACB), *Ostrinia furnacalis* (Guenée), populations were determined using four genital traits. ACB populations were collected from nine different provinces comprising of 27 different municipalities namely, Bukidnon (Don Carlos, Malaybalay, Musuan, and Valencia); Camarines Sur (Buhi, Iriga, Naga, and Ocampo); Ilocos Norte (Banna, Bangui, Batac, Dingras and Paoay); Isabela (Aurora, Echague, Ilagan, and Tumauini); Laguna (Bay, Calamba, Calauan, and Los Baños); Pampanga (Magalang); Pangasinan (Sta. Maria); South Cotabato (Banga and Koronadal); and Sultan Kudarat (Bagumbayan).The genital traits measured were aedeagus length, clasper width, sacculus length, and valva length.

The decreasing orders for morphometric values obtained from the different ACB populations were as follows: for aedeagus length: Bukidnon>Ilocos Norte>Camarines Sur >Isabela>Pangasinan = South Cotabato> Laguna = Sultan Kudarat> Pampanga; for clasper width: Bukidnon>Camarines Sur >Ilocos Norte > South Cotabato>Isabela = Sultan Kudarat> Laguna = Pangasinan> Pampanga; for sacculus length: Bukidnon>Camarines Sur >Ilocos Norte >Isabela = Sultan Kudarat>Pangasinan = South Cotabato> Laguna > Pampanga; and lastly, for valva length: Bukidnon>Camarines Sur>Ilocos Norte >Isabela> South Cotabato>Pangasinan = Laguna > Sultan Kudarat> Pampanga.

All of the four genital traits were significantly different among and within the different ACB populations collected from the nine different provinces. Results also revealed that four groups were formed using the cluster analysis and groups were formed regardless of the geographic distances.

The percent correct classification of each group was assessed using the discriminant analysis. The percent correct classification were 83%, 100%, 100%, and 100% for G1 to G4, respectively, with overall percent correct classification of 96%. Using jackknife classification, the percent correct classifications obtained for G1, G2, G3, and G4, were 83%, 100%, 82%, and 75%, respectively, with overall percent correct classification of 85%. Results also revealed that the valva length, sacculus length, and clasper width were good predictors.

Generally, insect genital characters are used in taxonomy at species level and, very often, genitalia provides the way to distinguish species (Mutanen et. al, 2007). The variations in genital traits of the different ACB populations perhaps can be attributed to their adaptive mechanisms in their respective localities. Perhaps, different ACB populations might have established their own genital size, which corresponds with the female genitalia in their respective populations. Maybe, the size of the male genitalia can be attributed to population fitness. Those populations with larger male genitalia size may be associated with superior abilities to survive and accumulate resources (Andersson, 1995) and therefore, have higher fitness as compared to those with smaller male genitalia size.

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