Morphological genitalia variation in *Eudocima phalonia*

Catherine Terrell

Department of Entomology - Purdue University - West Lafayette, IN 47907, USA

Abstract An evaluation of morphological variation in geo-referenced *E. phalonia* specimens from the zoogeographical regions of Indomalaya and Australasia. Thirty specimens were dissected and measured, 14 female and 16 male. No overall variance was recorded but there was a significant difference between Indomalayan and Australasian specimens in clasper lengths and a marginally significant difference in antevaginal spine lengths. One specimen was identified post-dissection to be a different species, *E. homaena*. A non-morphometric difference that was observed was in corpus bursa thickness, potentially a result of copulation.

Introduction

Eudocima phalonia, also known as *Eudocima fullonia* (Clerck 1764), *Noctua dioscoreae* (Fabricius 1775), *Phalaena Noctua pomona* (Cramer 1776), and *Ophideres obliterans* (Walker 1858), is in the insect order *Lepidoptera* and in the family *Erebidae,* previously part of the family *Noctuidae* (Zilli, 2017).

What makes this insect important is its ability to pierce fruits with a sclerotized proboscis, allowing harmful bacteria and fungi to be introduced. There are two major types of fruit piercing categories: primary and secondary. Primary feeders are able to pierce through the skin of the fruit, while secondary feeders find puncture wounds caused by primary feeders (Banziger, 1982). These distinct feeders cause different types of damage: primary and secondary. Primary damage is the actual or potential ability to pierce intact skin, and secondary damage affects the pulp of an already punctured fruit (Banziger, 1982). It is thought that all primary feeders can also act as secondary feeders and cause the spread of pathogens by secondary feeding by picking up bacteria and fungi from inoculated fruit, leading to rot (Banziger, 1982).

Eudocima phalonia has a wide range of host plants, as both larvae and adults, that contains more than 100 plants over 34 families (Davis, 2005). Economically important host plant crops that *E. phalonia* feeds on include citrus, apples, pears, melons, tomatoes, and strawberries. The larvae tend to feed primarily on the foliage of wild hosts mainly within the Menispermaceae and Fabaceae families, but have also been shown to feed and develop on red apple under laboratory conditions (Davis, 2005). *Eudocima phalonia* was also shown to have a preference for sweet aromatic fruits compared to those with low sugar content (reference). An adult food preference index was developed for this moth, and the highest rated fruits were bananas and brinjal (aka, eggplants) in 1998, followed by guava and bananas in 1999 (Bhumannavar, 2012).

Eudocima phalonia is known to be present in temperate broadleaf and mixed forests, tropical and subtropical grasslands, savannas and shrubs, and tropical and subtropical forests (Davis, 2005). With these known biomes and previously mentioned host plants, there is a chance that *E. phalonia* could become a pest across about 30% of the continental US (Davis, 2005). However, the host plants for larval stages are not commonly found in the US and entry potential is considered low, as only 5.8% of all interceptions of previously identified Noctuidae moths to the USA originated in a

country known to have *E. phalonia* (Davis, 2005). *E. phalonia* has also been identified as"particularly mobile" and "being a powerful flyer known to undergo migrations" (Fay, 1999; Zilli, 2017). This behavior could aid in becoming an invasive pest in the Americas.

Eudocima phalonia is commonly confused with other moths in the genus *Eudocima*, including *E. materna* (which has occurred in Florida and Texas), *E. homaena,* and *E. jordani* (neither of which have occurred in the US) (Davis, 2005). They possess similar body color and patterns, and the only certain way to identify one of these moths is through genitalic dissection by a qualified taxonomist (Davis, 2005).

The goal of this study was to examine variation among geo-referenced specimens collected for Vernon A. Brou Jr. Increased understanding of variation in *E. phalonia* would aid quarantine scientists engaged in the process of identification. Being able to identify this moth if it reaches the US will be crucial to inhibit its spread, and knowing any potential variation will only benefit this prevention.

Materials and Methods

The specimens used for these dissections were collected in the Indomalayan and Australasian zoogeographic areas, (see fig. A). They were collected from as early as 1988 and by various collectors acting for Vernon Antoine Brou Jr. They were stored in a freezer between handling, to avoid mold forming on any of the specimens and prevent its spread on specimens that had been affected by mold previously.

The materials used for dissections were glass vials, 10% KOH, a 250 mL beaker, acetic acid, camel hair paint brushes, iris scissors, petroleum jelly, watch glasses, and a M165C Leica imaging microscope.

Dissections started by boiling water in a 250 mL beaker, removing it from the hot plate, and placing vials with the moth abdomen covered by 10% KOH. They were kept in the hot water for 5 to 10 minutes so the fat body would break apart and the exoskeleton would become relaxed. Scales were brushed off the body with a paint brush and then an incision was made on the right side following the spiracles to avoid cutting any reproductive organs. The fat body was brushed away with the paint brushes to give a clear view of the genitalia. The genitalia was then carefully removed and more debris were removed in a fresh dissecting tray. Petroleum jelly was placed onto a dry dissecting tray, covered in 90% ethanol, and the genitalia placed on top. A watch glass was placed over the genitalia to flatten it in an attempt to keep measurements uniform during imaging.

The reproductive parts measured were the claspers, juxta, and uncus in the male and for the females the antevaginal spine and corpus bursa were measured looking at the length and width for all. All images and measurements were taken on a M165M Leica microscope for uniformity.

Results

Principal Component Analyses (PCA), using JMP SAS ver. 14.2.0, were calculated on the male and female specimens, looking at all genital parts measured, for males (M): clasper length, clasper width, juxta length, juxta width, and uncus length; for females (F): antevaginal spine length, antevaginal spine width, corpus bursa length, and corpus bursa width; and comparing Indomalayan specimens to Australasian specimens. The levels of determining significance were: significant p-value = <0.05 and marginal significant p-value = <0.10. There were no subgroupings found in either males or

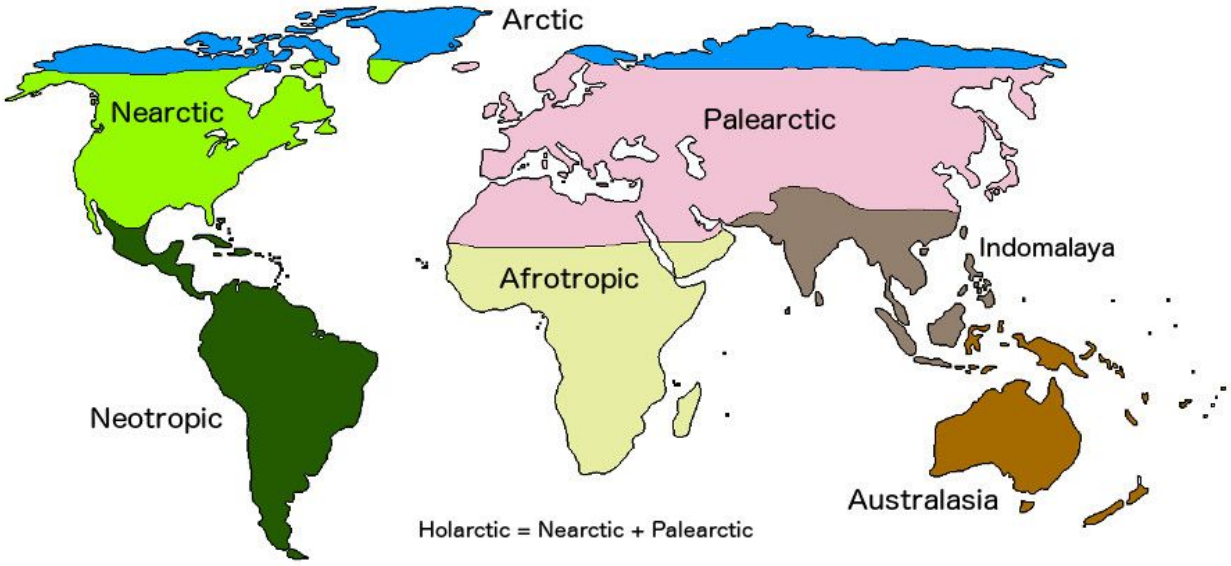
females, (see fig. B and C). For the male PCA results, there was one clear outlier so a test was run to locate any additional outliers (see fig. D). Once those outliers were removed (three in total), a second PCA was run on the male moths again and component 2 (composed of 0.736 eigenvector as clasper length) gave a marginally significant p-value of 0.0896 (see fig. E). This prompted further investigation into the data to find if any individual parts were significantly different between Indomalayan and Australasian specimens. To accomplish this, T-tests using Microsoft Excel 2016 were performed. From these tests there was one marginally significant different part: antevaginal spine length ($t(5) = -1.681$, $p = 0.07$) (see fig. F and fig. G). Additionally, there were differences in corpus bursa shape, but these were not quantified as it is a complex, non-linear shape (see fig. H and fig. I). Finally, one male moth stood out from the other male moths. Further investigation showed that it was misidentified as *E. phalonia*, (see fig. J) but after dissection this specimen was found to be *E. homaena* (see fig. K).

Discussion

There were no major overall variances found in the genitalia of *E. phalonia*, from across the zoogeographic regions of Indomalaya and Australasia. Previous studies have found several biotypes within *E. phalonia* that need evolutionary value assessments, such as between Afrotropic and Indoaustralian specimens (Zilli, 2017). Our current study would imply that all moths across this area (Indomalaya plus Australasia) have been selected for the same sets of traits across these areas. Further investigation of Afrotropical specimens is needed for this lack of variation across the entire species to be confirmed.

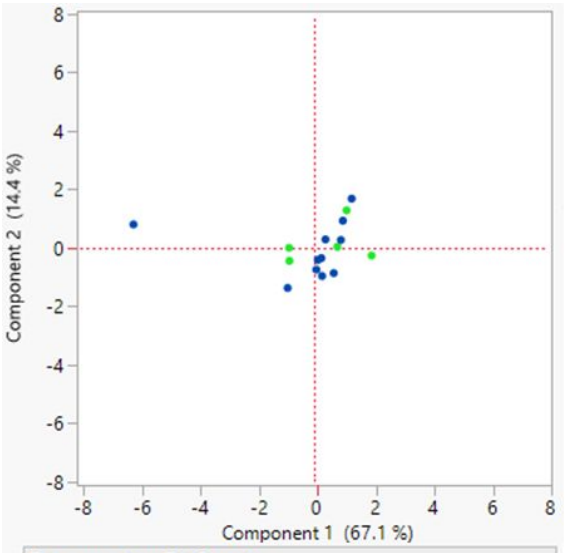
Small variation is visible, however, when looking at individual genital parts. Looking more beyond the qualitative results at the corpus bursae shape in female moths, some had thicker mid sections than others, (see fig. H and I. fig. H). Thicker corpus bursae show that there are cornuti present in the bursa, which could indicate that there are physiological differences between mated and unmated female moths. Further investigation is also needed for this to be confirmed.

fig. A



Map of commonly used zoogeographic regions where individuals are more similar to each other within regions rather than outside.

fig. B



Parameter Estimates

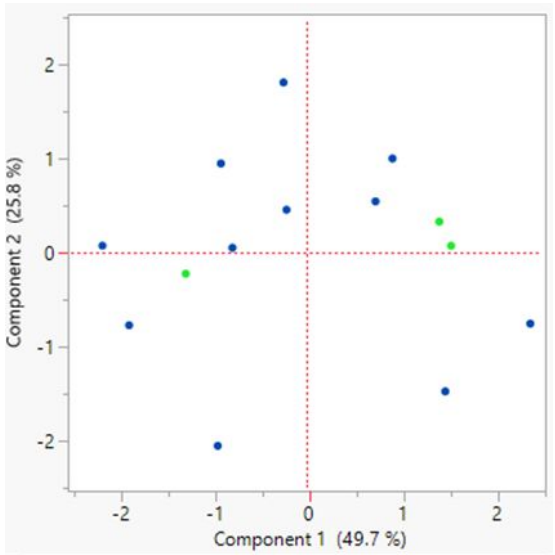
Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.1176472	0.504312	0.23	0.8189
Column 1[A]	0.3137259	0.504312	0.62	0.5439

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.0353724	0.235594	0.15	0.8828
Column 1[A]	0.0943264	0.235594	0.40	0.6949

Principal component analysis of male moth genitalia comparing different reproductive parts for Indomalayan and Australasian specimens. The first parameter estimates is for component 1 and the second is for component 2.

fig. C



Parameter Estimates

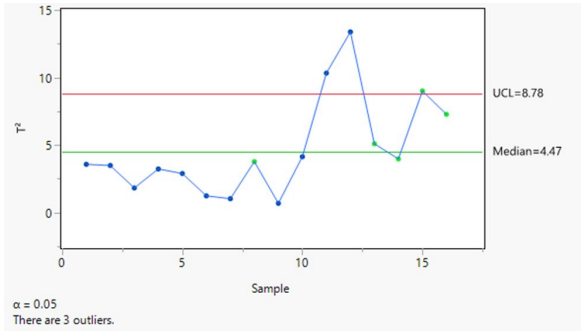
Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.2012098	0.46695	0.43	0.6742
Column 1[A]	0.3521172	0.46695	0.75	0.4653

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.0215047	0.343965	0.06	0.9512
Column 1[A]	0.0376333	0.343965	0.11	0.9147

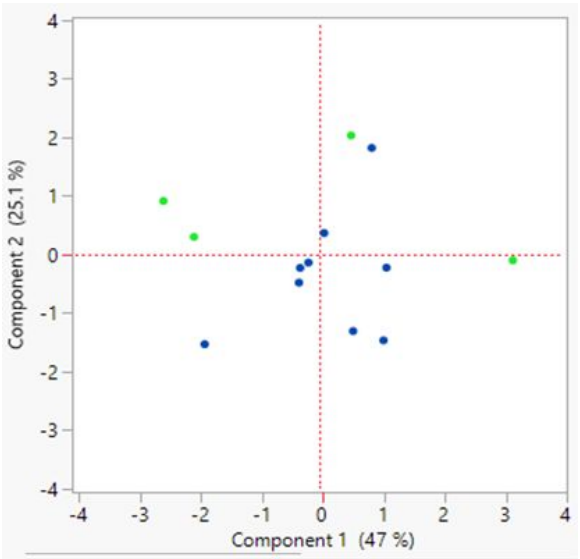
Principal component analysis of female moth genitalia comparing different reproductive parts for Indomalayan and Australasian specimens. The first parameter estimates is for component 1 and the second is for component 2.

fig. D



Outliers found using JMP SAS.

fig. E



Parameter Estimates

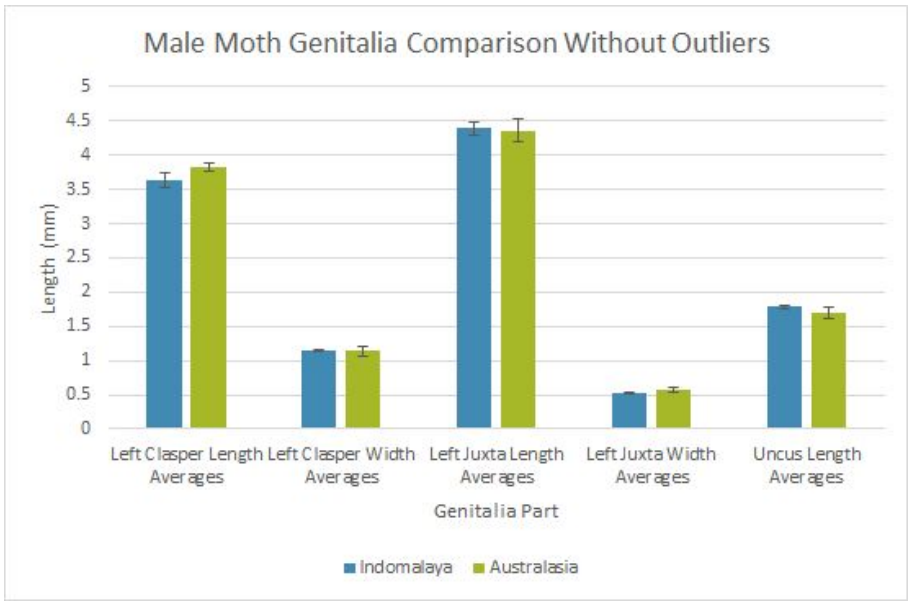
Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-0.064193	0.478616	-0.13	0.8957
Column 1[A]	-0.166901	0.478616	-0.35	0.7339

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.2195611	0.306727	0.72	0.4890
Column 1[A]	0.570859	0.306727	1.86	0.0896

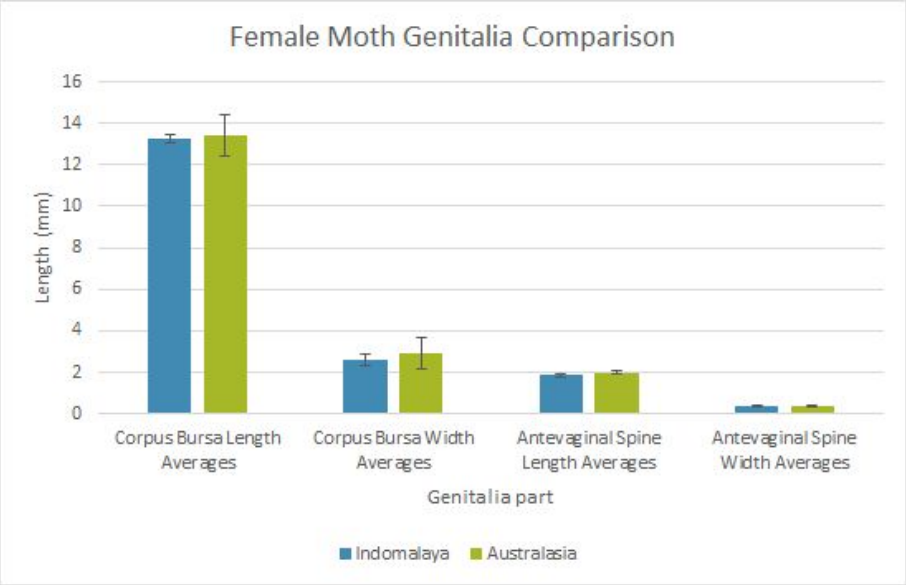
Principal component analysis of male moth genitalia comparing different reproductive parts for Indomalayan and Australasian specimens after outliers were taken out. The first parameter estimates is for component 1 and the second is for component 2

fig. F



Bar graph showing standard error means for male reproductive parts for zoogeographical regions.

fig. G



Bar graph showing standard error means for female reproductive parts for zoogeographical regions.

fig. H

Female reproductive organs (corpus bursa and antevaginal spine present) showing qualitative difference. E. phalonia.

fig. I

Female reproductive organs (corpus bursa and antevaginal spine present) showing qualitative difference. E. phalonia.

fig. J

Male reproductive organs (claspers, juxta, and uncus present) E. phalonia.

fig. K

Male reproductive organs (claspers, juxta, and uncus present) E. homaena.

Acknowledgements

Dr. Stephen Cameron
Crystal Klem
Taylor Nelson
Dr. Laura Ingwell
Dr. John Couture

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