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Differentiation in wing shape in the *Bactrocera tau* (Walker) complex on a single fruit species of Thailand

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ABSTRACT: The *Bactrocera tau* complex contains fruit fly pests which damage several species of cucurbit crops. Two cryptic species, A and C, of the *B. tau* complex both occur on the host fruit *Momordica cochinchinensis*. In this study, wing geometric analysis was used to differentiate the *B. tau* complex on *M. cochinchinensis*. A total of 586 wings (297 males, 289 females) were discriminated into two groups corresponding to the reference species A and C of the *B. tau* complex. *B. tau* A and C were reclassified at higher than 96% and 99% accuracy, respectively. *B. tau* C had larger wings than *B. tau* A. Interspecific differentiation was significant due to wing size, indicating that wing shape can be used to separate the species. The *B. tau* C was found to be the dominant species on *M. cochinchinensis*. A classification tree based on Mahalanobis distances suggested that the variation within *B. tau* C is related to seasonal variation. The variation among seasonal populations was similar in males and females. Wing shape analysis, which can differentiate between species within cryptic complexes helping to detect variation within species, may have important applications in pest control programs.

KEYWORDS: wing geometrics, environmental factors, species distinction

INTRODUCTION

Bactrocera (Zeugodacus) tau (Walker) is a primary pest that damages fruits and vegetables of the family Cucurbitaceae throughout South and Southeast Asia, including Thailand [1]. One difficult problem in fruit fly control is to accurately identify the pest species. *B. tau* in Thailand now consists of eight forms: A, B, C, D, E, F, G, and I, which are common in different host plant species and habitats [2,3]. Species identification in the *B. tau* complex is difficult, and therefore they have been classified within the single taxon *B. tau*. Attempts to verify the species of the *B. tau* complex have been made using several methods.

The original identification of *B. tau* was based on morphological description [4,5]. In Thailand, the *B. tau* complex was proven by cytotaxonomy to contain seven closely related species tentatively designated as *B. tau* A, B, C, D, E, F, and G [2]. The cryptic species were also supported by allozyme electrophoresis [6] and by molecular analysis of the COI gene [3], and an eighth species (*B. tau* I) was added. These forms have never been separated morphologically. However, the wings of *B. tau* A and C were successfully distinguished by geometric morphometric analysis [7]. *B. tau* C is associated with a specific host plant species, *Momordica cochinchinensis* (gac fruit) while *B. tau* A is a generalist infesting several cucurbit species and occasionally *M. cochinchinensis* [2,7]. Intraspecific variation within *B. tau* A was found to be associated with host plant species in the Cucurbitaceae [7]. Therefore, wing geometric analysis may be useful for identification of the *B. tau* complex and may aid our understanding of species differentiation.

Although some evidence suggests that development and growth have effects on phenotypic variation [8,9], several investigators have suggested that environmental factors also affect phenotypic variation [10–12]. As *B. tau* C has been historically associated with *M. cochinchinensis* [2,7], this host may provide the natural habitat for morphometric variation of *B. tau* C, since it produces fruit over several months of the year. If so, variation in wing shape could be associated with environmental conditions as a consequence of differential developmental responses. In this paper, we present data on the differentiation of the species complex (i.e., *B. tau* A, C, etc.) and investigate environmental variation affecting wing shape of the dominant species on the host plant.

MATERIALS AND METHODS

Insect collections

Fruit flies of the *B. tau* complex were collected from wild "gac fruit" (*M. cochinchinensis*) in Ratchaburi province for 10 months (March–December 2009).

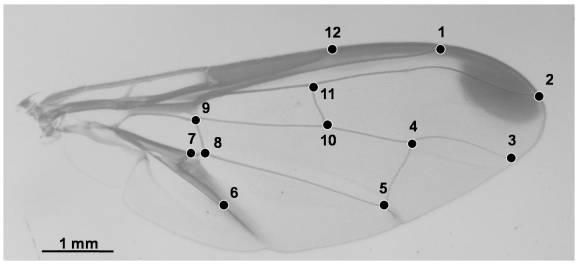

Fig. 1 Twelve landmarks on the *Bactrocera tau* wing used in wing geometric analysis.

The plants were growing in undisturbed areas under various environmental conditions (temperature, rainfall, humidity, etc.) according to time of collection. Fruit collections infested with larval *B. tau* were taken to the laboratory once each month. After adult emergence, all *B. tau* were maintained in plastic cages at $25 \pm 2\,^{\circ}$C and $70 \pm 5\%$ relative humidity. They were provided with a mixture of sugar and yeast hydrolysate (3:1) and 10% honey in distilled water as food for approximately 10–14 days, or until development of wing patterns suitable for processing.

Specimen processing and data collecting

All wings were processed and mounted on glass slides with Hoyer mounting solution. The wing images were captured using a digital camera on a stereomicroscope (40×). The study used 12 landmarks (Fig. 1) following type I (venation intersections) design [13]. The coordinates of landmarks were digitized by the COO module of software (see "Software"). The connections between 12 landmarks provided polygons used for further analysis, including comparison of wing size and shape.

The coordinates of landmarks were superimposed (translation, scaling, rotation) using the MOG module (see "Software") which computed procrustes superimposition, centroid sizes, and partial warps (as shape variables).

Interspecific analysis

Size: Centroid size, an isometric estimator of wing size, was calculated from the square root of the sum of the squared distances between the centre of the polygon and each landmark. Then all centroid size values and their variances were compared by non-parametric analysis based on permutations (1000 runs) [14].

Shape: The shape variables of the wings were computed with discriminant analysis. The reference data of *B. tau* A from KN(CG)26 (12 males, 14 females) and *B. tau* C from NA(MC)16 (21 males, 21 females) in Kitthawee and Dujardin [7], were pooled with observed data and investigated in the analysis. The specimens were distinguished and classified based on the reference data. A re-classification of individual specimens was computed using Mahalanobis distances under discriminant analysis in the PAD module. Wing shape variation was presented by the factorial map of the first two discriminant factors, DF1 and DF2. The relationship between wing shape and size differences was examined by regression analysis.

Intraspecific analysis

Only the dominant species was used in the analysis. The shape variables of *B. tau* C from ten months were analysed with discriminant analysis and then classification trees were constructed based on Mahalanobis distances among their collection times. Stepwise regression analysis [15] was performed for the relationships between environmental factors (i.e., temperature, rainfall, humidity) as independent factors and wing shape variables from the first principal components of shape [13].

Software

Collecting landmarks made use of the COO module. Centroid size and partial warp scores were obtained from the MOG module. All discriminant analyses were performed by the PAD module (software components are available at http://www.mpl.ird.fr/morphometrics). Classification trees were constructed with the unweighted pair group method with arithmetic mean (UPGMA) of the PHYLIP computed by external software at http://www.mpl.ird.fr/morphometrics. STATISTIX 8 [15] was used to perform the multiple regression analysis.

RESULTS

Interspecific analysis

A total of 586 wings (297 males and 289 females) of the *B. tau* complex infesting *M. cochinchinensis* were processed (Table 1). All wing shape variables analysed with discriminant analysis gave two separate groups for each sex (Fig. 2). The scatter plots showed the total first and second discriminant factors 97% (DF1 = 87%, DF2 = 10%) and 96% (DF1 = 87%, DF2 = 9%) for male and female wings, respectively. The interspecific differences were greater in males than in females. When reference groups of *B. tau* A and C from Kitthawee and Dujardin [7], supported by genetic differences, were added into the analysis, they were still supported by wing geometry. All specimens were reclassified in discriminant analysis based on geometry of the wing. The percent correct

Table 1 *Bactrocera tau* complex infesting *Momordica cochinchinensis* collected from Ratchaburi province for ten months (March–December 2009). *Bactrocera tau* A and C are groups according to Fig. 2.

Month	Males			Females		
	Tested	*B. tau* A	*B. tau* C	Tested	*B. tau* A	*B. tau* C
Mar	47	8	39	45	10	35
Apr	9		9	17		17
May	16		16	12		12
Jun	15	15		15	15	
Jul	45		45	45		45
Aug	45		45	45		45
Sep	6		6	4		4
Oct	46	6	40	44	2	42
Nov	49		49	49		49
Dec	19		19	13		13
Total	297	29	268	289	27	262

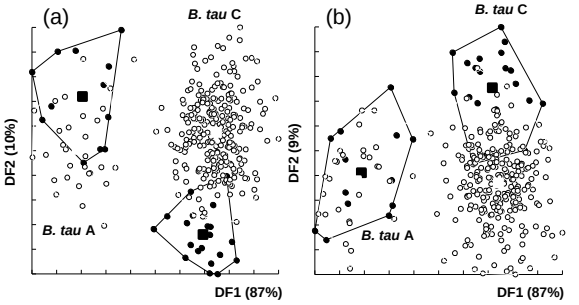

Fig. 2 Scatter plots of the discriminant analyses performed on *B. tau* complex (open circles) distinguished based on reference *B. tau* A from KN(CG)26 and *B. tau* C from NA(MC)16 in Kitthawee and Dujardin[7] (the polygon-enclosed dark circles). (a) males, (b) females. The horizontal axis (DF1) is the first discriminant factor derived from shape variables, representing 87% (both male and female) of the total variation.

classification was almost perfect in *B. tau* A (100% males and 96% females) and in *B. tau* C (99% males and 99% females).

The *B. tau* A and C groups were analysed by the month of collection (Table 1). *B. tau* A was found in small numbers in three months (March, June, and October 2009) and almost all *B. tau* A was found mixed with *B. tau* C. *B. tau* C was found in nine monthly collections and was the dominant group.

Size: Wing size of *B. tau* C was significantly larger than that of *B. tau* A ($P < 0.01$). The variance of wing size did not differ significantly between *B. tau* A and C overall, but within each species, female wing size was significantly larger than male wing size ($P < 0.01$), and there were no overlap in size ranges between the sexes in *B. tau* A. However, the ranges of wing size in *B. tau* C males and females overlapped broadly (Fig. 3).

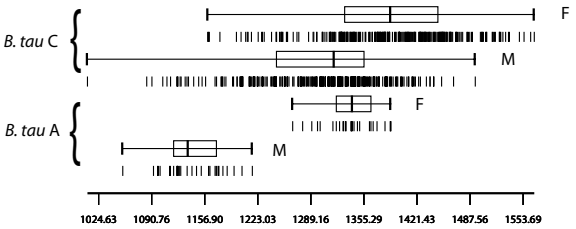

Fig. 3 Quantile plots showing range of size variation in each species and each sex. Each box presents the median as a line across the middle and the quartiles (25th and 75th percentiles) as its ends. Units are pixels.

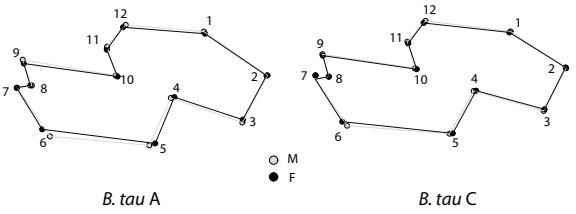

Fig. 4 Mean coordinates of landmarks in males (grey) and females (black). Landmarks correspond to those shown in Fig. 1. Landmark no. 6 was observed to be different between males and females in both species.

Shape: The superimposition of coordinates showed that there were shape differences in wing venation between sexes. The landmark no. 6 clearly distinguishes males and females in both species (Fig. 4).

The interspecific allometry was computed by linear regression analysis of the wing shape differences (CV1) of *B. tau* A and C on their centroid sizes. The allometry was significantly different for males ($R^2 = 28\%$; $P < 0.01$) but not for females ($R^2 = 2\%$; $P > 0.01$) (Fig. 5).

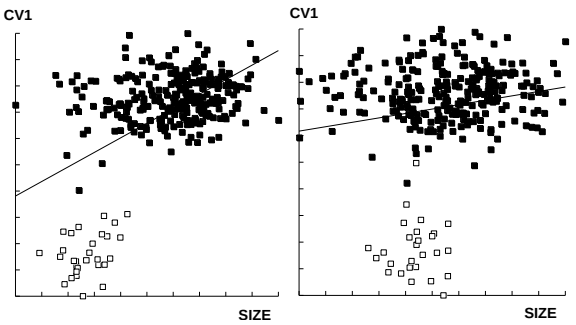

Fig. 5 Plots of linear regression of the CV1 as dependent variable on centroid size as independent variable of *B. tau* A (open squares) and *B. tau* C (dark squares). Left: $R^2 = 28\%$ in males; right: $R^2 = 2\%$ in females.

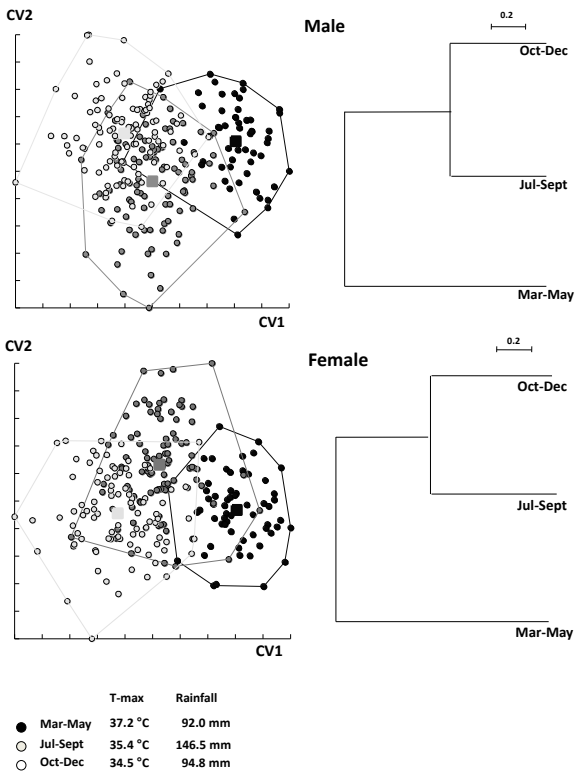

Fig. 6 Left: Discriminant analyses of three populations analysed from three-month pooled samples (black circles, March–May; grey circles, July–September; white circles, October–December). Right: The UPGMA based on Mahalanobis distances of three populations grouped by seasons. T-max is maximum temperature.

Intraspecific analysis

B. tau C is common and has become the dominant species on the host plant *M. cochinchinensis* (Table 1), in agreement with previous work[2,7]. In this study, *B. tau* C specimens were pooled into 3-month groups for the discriminant analysis (Fig. 6, left). Mahalanobis distances were calculated from partial warp scores and a classification tree was constructed from these distances. The classification tree showed almost the same pattern in both sexes. There were two separate clusters: one containing the March–May collections and another containing the July–September and October–December collections (Fig. 6, right). Significant distances were found between these two clusters by permutation checks (1000 runs).

Environmental factors

Regression analysis of canonical variable on female wing size was not significant. The results suggested that wing shape changes were independent of wing size (allometry free) which is in line with previous studies[16,17]. Therefore, stepwise regression analysis was performed for the relationships between wing shape changes and environmental factors (temperature, humidity, rainfall) observed at each month. The analysis indicated that male wing shape changes related to maximum temperature, rainfall, and humidity ($R^2 = 0.40$, $F = 56.32$, df $= 3, 246$, $P < 0.01$) (Table 2). A correlation analysis of environmental factors showed that average temperature and minimum temperature were not directly important in the model, but were correlated with other directly important factors ($P < 0.01$). Stepwise analysis also suggested that female wing shape changes related to maximum temperature and rainfall ($R^2 = 0.53$, $F = 136.04$, df $= 2, 241$, $P < 0.01$) (Table 3).

Table 2 Results of stepwise linear regression predicting wing shape changes in *B. tau* C males from a variety of environmental measures. Model: $R^2 = 0.3999$; $F = 56.32$; df $= 3, 246$; $P < 0.01$. Factors without regression coefficients did not meet 0.05 significance levels for entry into the model.

Variable	Coefficient	T	P
Intercept	−0.11868	−2.36	0.0000
T-max[a]	0.00642	8.03	0.0000
Rainfall	1.221×10^{-4}	6.33	0.0000
Humidity	−0.00158	−4.04	0.0000
T-min[b]	–	–	–
T-average[c]	–	–	–

[a] maximum temperature
[b] minimum temperature
[c] average temperature

Table 3 Results of stepwise linear regression predicting wing shape changes in *B. tau* C females from a variety of environmental measures. Model: $R^2 = 0.5264$; $F = 136.04$; df $= 2, 241$; $P < 0.01$. Factors without regression coefficients did not meet 0.05 significance levels for entry into the model.

Variable	Coefficient	T	P
Intercept	−0.36747	−16.36	0.0000
T-max[a]	0.01007	16.27	0.0000
Rainfall	7.681×10^{-5}	8.47	0.0000
Humidity	–	–	–
T-min[b]	–	–	–
T-average[c]	–	–	–

[a] maximum temperature
[b] minimum temperature
[c] average temperature

Based on the 3-month groups, the average maximum temperature and rainfall were: 37.2 °C and 92.0 mm during March–May; 35.4 °C and 146.5 mm during July–September; 34.5 °C and 94.8 mm during October–December. The data indicate that seasonal differences in wing shape changes occurred and were similar in males and females (Fig. 6).

DISCUSSION

Interspecific analysis

Geometric morphometric analysis of wing shape revealed that the host fruit *M. cochinchinensis* contains at least two groups or two species: *B. tau* A and C. All wing specimens of *B. tau* A and C were reclassified using discriminant analysis. The percent of correct classification was almost perfect with more than 96% and 99% accuracy for *B. tau* A and C, respectively. Only one specimen of each species was incorrectly assigned. This warrants recognition of *B. tau* A and C as separate species. The degree of interspecific divergence exhibited by molecular genetic analysis supports this conclusion [3].

In general, females are larger than males in tephritid flies [18]. As expected, centroid wing sizes of females were larger than those of males in the *B. tau* complex. The differences in landmarks 6 and 7 permit sexual identification in both *B. tau* A and C (Fig. 4). Although it is clear that sex organs are responsible for sex determination, this result is useful in identifying incomplete specimens from wings.

The centroid wing size differences between *B. tau* A and C are important in species recognition on the same host fruit species. Males showed greater differences than females in wing size and shape between species. Absence of female size differences in wing shapes between species A and C may indicate complex environmental relationships. It is possible that female larvae of both species had their own favourable microhabitat on the same fruit species. The wing shape differences between *B. tau* A and C could also result from larval use of different host species [7] and genetic differences [3]. These two species have been shown to be sympatric differentiation in the microhabitats of the same fruit species.

Intraspecific analysis

B. tau C is specific and very commonly associated with the fruit *M. cochinchinensis* while *B. tau* A is only occasionally present (Table 1). Here, we studied environmental factors affecting variation within *B. tau* C natural populations. Wing sizes of *B. tau* C females were not related to wing shape. The changes appeared to be related to environmental conditions (temperature, rainfall, and humidity) during the times of collection. All *B. tau* C were collected from the same province (Ratchaburi) at different times of the year, which indicates that most wing shape variation was likely to be a response to environmental variation (Tables 2 and 3). Maximum temperature appeared to be the most relevant factor for both male and female wing shape variation, and may have affected wing shape change in the March–May populations either directly through physiology and the developmental process or indirectly by altering the quantity and quality of food in the habitat. In *Drosophila* populations, variation in wing shape may result from temperature change and is associated with survival [19, 20]. Similarly, the shape change of *B. tau* C may be adaptive and related to temperature. However, insect wing development may be sensitive to a variety of factors [10, 12, 21]. Low rainfall was also associated with wing shape change in both sexes and was apparently responsible for the seasonal variation (Tables 2 and 3, Fig. 6). Wing variation tended to be greater in the dry season (March–May) than in the rainy season. Humidity affected wing shape variation in males but not in females. No explanation can be offered for this finding. However, humidity was correlated with rainfall and was an indirect factor for females. Similarly, minimum temperature and average temperature may not directly affect wing shape, and may be correlated indirect factors. The results indicate that wing shape of *B. tau* C undergoes complex changes in response to environmental conditions. It is clear that the environment can greatly influence wing shape changes which may permit better environmental tolerance [22] and/or a greater capacity to endure environmental stress [23]. Indeed, wing shape changes may be an adaptive response to the environment. For instance, such changes may relate to individual variations in flight capacity, development, survival, reproductive capacity, etc., and allow selection to act when specific changes are adaptive. Further research on these phenomena could lead to a better pest-management strategy. For example, the changes associated with critical periods of survival or preceding optimal conditions for reproduction could be targeted for control efforts.

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