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The *Diachasmimorpha longicaudata* complex: Reproductive isolation and geometric patterns of the wing

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ABSTRACT

Diachasmimorpha longicaudata is an endoparasitoid of Tephritid fruit fly larvae and is regarded as an important biocontrol agent. However, it is likely that under this specific name several biological species may be contained, the correct identification of which is essential for effective use in control programs. In this paper, three populations (DLA, DLB and DLBB) of *D. longicaudata* designated according to geography and/or natural hosts were reared in the same laboratory. They were tested for reproductive compatibility and characterized by morphometric analyzes. Forced-contact mating technique showed either complete lack of inter-population reproductive compatibility or the production of rare, sterile female offspring. The three populations, indistinguishable on the basis of morphological characters alone, were readily identified by the geometry of the wing. Results strongly suggest that the DLA, DLB and DLBB are distinct biological species, and highlight the usefulness of wing geometry to distinguish them.

1. Introduction

Braconid fruit fly parasitoids (Opiinae) are typically solitary endoparasitoids. Current interest in their systematics comes from their use as biological control agents (Ovruski et al., 2000; Wharton and Gilstrap, 1983). Although there has been some controversy, most opiine genera have now been revised and redescribed (Wharton, 1997). However, this revision did not include the species level, especially for *Diachasmimorpha longicaudata* (Wharton and Gilstrap, 1983). There is suspicion that recognized species may in fact be cryptic species complexes (Kitthawee, 2008; Wharton and Gilstrap, 1983). The biological control user, lacking a reliable means of identification, may find it difficult to determine exactly which species has been released.

The parasitoid, *D. longicaudata*, is a common fruit fly parasitoid and is native to many countries of Southeast Asia where it has been reported infesting a wide variety of host flies in the genus *Bactrocera* (Bess et al., 1961; Clausen et al., 1965; Wharton and Gilstrap, 1983). It has been introduced and established in several other countries for biological control (Clausen, 1978; Ovruski et al., 2000; Sivinski and Webb, 1989; Vargas et al., 1993; Wong and Ramadan, 1987). However, its taxonomic status remains unclear. Wharton and Marsh (1978) observed morphological variations in specimens from different geographical localities, and Wharton and Gilstrap (1983) listed a number of subspecies (*compensan, formosanus*) and varieties (*chocki, malaiaensis, novocaledonicus* and *taiensis*). In these earlier descriptions and keys, color was used as a major character but different populations of *D. longicaudata* are often proved indistinguishable on the basis of morphology alone (Kitthawee, 2008). Because of this, *D. longicaudata* has been treated as a single taxon in most research and biological control programs. Recently, Kitthawee (2008) reported that the subspecific subdivision was actually more than local differentiation, suggesting that in Thailand *D. longicaudata* is a complex of cryptic species.

Correct taxonomic identification of biological control agents such as *D. longicaudata* is essential to a successful biological control program (DeBach and Rosen, 1991). Therefore, we examined this group of fruit fly parasitoids in Thailand by using reproductive compatibility tests and morphometric techniques for tentative group identification. Reciprocal cross-breeding experiments allowed for the detection of reproductive barriers among three populations. The venation of the wings was then tested as an alternative and low-cost identification technique to accurately differentiate the populations.

2. Materials and methods

2.1. Parasitoid cultures

Parasitoid populations of *D. longicaudata* were obtained from ripe fruits collected from different types of trees and from various

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Table 1
Number specimens of *D. longicaudata* complex (DLA, DLB and DLBB), males (M) and females (F) used for the morphometric study. DL, *D. longicaudata*; *N*, number of measured specimens; F_1, offspring of DLB females × DLA males; Host fly, genus *Bactrocera*; Host plant, plant on which *Bactrocera* was reared; M. s., *Musa sapientum* (banana); lab, laboratory; nat, nature.

DL	*N*		Host fly		Host plant	
Code	F	M	Lab	Nat	Lab	Nat
DLA	53	51	*correcta*	*correcta*	M. s.	*Psidium guajava*
DLB	49	49	*dorsalis*	*dorsalis*	M. s.	*Terminalia catappa*
DLBB	52	49	*dorsalis*	*carambolae*	M. s.	*Averrhoa carambola*
F_1	11	0	*dorsalis*	–	M. s.	

geographic locations. They were identified as *D. longicaudata* using the key of Wharton and Gilstrap (1983) and confirmed by Dr. Robert Wharton, Texas A&M University, USA, in 1997. They were tentatively labeled as DLA, DLB and DLBB[1] according to the time and location of collection, the host plant and the host fly (Table 1).

The DLA and DLB samples were collected in Thailand from Nakhon Pathom Province in the central part of the country (Fig. 1). These colonies were initiated with approximately 15–20 pairs in 1997 and 2000 respectively. The DLBB sample came from Phatthalung Province in the more southern area (Fig. 1) and the colony started from approximately 10 pairs in 2001. All these colonies were maintained at the Department of Biology (Mahidol University) for more than 100 consecutive generations before the present experiments were conducted.

2.2. Cross-breeding

Combinations of reciprocal pair-matings (Table 2) among the different populations (DLA, DLB and DLBB) were performed by the forced-contact mating technique (Kitthawee, 2008). Each cross pair-mating consisted of reciprocal crosses and controls. In preparation for the cross pair-mating, parasitized pupal hosts from colonies to be crossed were isolated and kept individually in vials in order to obtain virgin males and females. Emerging parasitoids were grouped by population and sex.

The crossing process was conducted as in Kitthawee (2008): in short, an immobilized, virgin female was placed in a plastic vial and arranged in the flight position; an active winged male was then released into the same vial and the vial was slowly moved until the male touched the immobilized female. After successful copulation, females were transferred in groups of 5–10 mated females to a new cage provided with 10% honey in distilled water. They were allowed to lay eggs for 10 days into the same fruit fly species used in parasitoid cultures (Table 1). In each cross pair-mating 3–4 replicates (a total of 25–30) were prepared.

Parasitoid progeny were counted and sexed to determine successful fertilization (genetic compatibility). Due to the haplodiploid mode of reproduction in *D. longicaudata*, incompatibility was attested by the absence of female offspring. Only the presence of female progeny indicated that mating and egg fertilization were both successful. Genetic compatibility was estimated by the percentage of F_1 progeny relative to mating within-population controls, and these frequencies were compared using the χ^2 test with Yates' correction (Sokal and Rohlf, 1995).

2.3. Sample processing for morphometric analyzes

A total of 104 DLA (53 females, 51 males), 98 DLB (49 females, 49 males) and 101 DLBB (52 females, 49 males) were studied (Table 1). Specimens from each colony of DLA, DLB and DLBB were dissected. Both left and right fore wings of female and male parasitoids were mounted on the glass slides. Right fore wings only were photographed using a digital camera connected to a stereo microscope at 40× magnification.

[1] The voucher specimens of each population were kept at Mahidol University (Bangkok, Thailand).

Fig. 1. Geographic origin (see black stars) of the parasitoids: DLA and DLB from central Thailand, province of Nakhon-Pathom, and DLBB from southern Thailand, province of Phatthalung.

2.4. Data collection and analyzes

Wings were digitized at 10 landmarks (Fig. 2), all of them of "type I" (venation intersections) (Bookstein, 1991). To avoid

Table 2
Crossing combinations among the DLA, DLB and DLBB populations of *D. longicaudata*. Between brackets, the number of F_1 females.

Crosses Female × Male	Pairs tested	Total progeny	% (F_1 female)
DLA × DLA*	30	548	51 (280)
DLA × DLB	30	380	None
DLB × DLA	30	522	3 (17)
DLB × DLB*	30	614	53 (330)
DLB × DLBB	25	470	None
DLBB × DLB	30	411	None
DLBB × DLBB*	30	408	35 (144)
DLBB × DLA	25	285	None
DLA × DLBB	25	320	None

* Control crosses.