行政院國家科學委員會專題研究計畫　成果報告

粉蝨科昆蟲形態及分子分類之研究

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計畫主持人：柯俊成

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行政院國家科學委員會專題研究計畫成果報告

計畫名稱：粉蝨科昆蟲形態及分子分類之研究

Morphological and molecular studies on the taxonomy of whiteflies (Hemiptera: Aleyrodidae)

計畫編號：NSC 91-2313-B-002-370
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中文摘要

本研究以傳統形態及生物技術等方法進行台灣粉蝨科 (半翅目) 裸粉蝨屬昆蟲 5種之形態以及分子分類學研究，其中包括 1 新種以及成蟲特徵的利用。本年度計劃已完成 1 篇研究報告之初稿，目前發表中。如果長期累積資料及經驗，定可逐步深入探討其分類體系、類緣關係及進化過程。往後將將粉蝨分類朝向多元的方向發展，尋找更多分類特徵的資料來分析粉蝨的類緣關係，以期重建合理完整的粉蝨科分類系統。以發表國際期刊之工作為首務，增加國際上之曝光度。

關鍵詞：昆蟲綱，粉蝨科，粉蝨，形態分類，分子標記。

英文摘要

Dialeurodes Cockerell of Taiwan were studied and one new species are described: *D. citri* (Ashmead), *D. daphniphylli* Takahashi, *D. kirkaldyi* (Kotinsky), and *D. swidi* Ko, n. sp. *D. agalmae* Takahashi and *D. citri* are synomynous. A key to Taiwanese species of this genus based on pupal cases and adults are provided The long-term will apply more techniques such as molecular markers, acoustic signal analysis, and mating behavior. To gather more data in understanding the diversity and evolution of whitefly species, for assessing and reconstructing the phylogenetic tree of whiteflies. A higher priority for the project in this year is to publish with the combination of morphological and molecular traits.

Keywords: Insecta, Aleyrodidae, whiteflies, morphological taxonomy, molecular marker.

I. 前言

Dialeurodes is by far the largest whitefly genus, with over 130 described species. It is an Old World genus, with a large majority of species having been described from the Oriental and Austro-Oriental Regions. A few species have been described form the Ethiopian, Palaearctic and Neotropical Regions, and fewer still from other parts of the world.

Dialeurodes, is the largest whitefly genus, but is probably not a single evolutionary group. *Dialeurolonga*, which appears to replace *Dialeurodes* in the Malagasy/Mascarene region, is almost certainly a figment of the taxonomic imagination. These two genera need to be considered together, their species re-examined and then re-sorted into species-groups on carefully considered structural characters.

The practical definition of *Dialeurodes* in use today encompasses a great variety of species, the primary shared characters of which are some sort of distinct marginal tracheal pores, a vasiform orifice with the lingula included and with the operculum nearly filling the orifice and usually covering the lingula, and a lack of many specialized characters found in related genera.

Dialeurodes Cockerell has historically been the most speciose genus of whiteflies, with more than 130 species worldwide. In preliminary work, Jensen (1999, 2001) discussed the history and characters of the pupal case of *Dialeurodes*, and he formally proposed alternative generic names and groupings, indicating that the genus was paraphyletic.

II. 研究目的

成蟲的形態分類是一項挑戰性的工作。因成蟲標本不易採集，雖透過飼養之方法並不能確保成蟲之順利羽化。又因其個体柔軟，標本容易變形而難以觀察；目前已有完善的標本製作及飼育技術。成蟲利用的分類特徵包括生殖器、複眼、管狀孔、黏腺以及觸角之感覺器等，至於翅脈及質地則尚未充份利用。如何尋找更多有用的分類形質以建立完整的成蟲分類系統是往後研究的重點工作。另外對於形態特徵不穩定的種類，除成蟲特徵的利用之外，如果能進行操作生物技術，以輔助種類確定以及類緣關係的推斷，對於研究內容深度的提升，將是往後研究的一大課題。

III. 文獻探討

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3. Ko, C. C. 2003. *Dialeurodes* (Hemiptera: Aleyrodidae) of Taiwan (draft)

IV. 研究方法

Leaves containing late instar nymphs of the species were collected in the field, and colonies were established in the laboratory. Descriptions and terminology of external and interior morphological structures are based on Bink-Moenen (1983), Martin (1985), Gill (1990), and Guimarães (1996). In the text, the following abbreviations are used for the depositories of material: (ANIC) Australian National Insect Collection, Canberra; (CDFA) California Department of Food and Agriculture, Sacramento; (HDA) Department of Agriculture, Honolulu, Hawaii; (NHM) Natural History Museum, London; (NMNS) National Museum of Natural Science, Taichung; (NTU) National Taiwan University, Taipei; (SIE) Shanghai Institute of Entomology, Shanghai; (TARI) Taiwan Agricultural Research Institute, Taichung; (USNM) United States National Museum of Natural History, Washington DC.

DNA Extraction

DNA was extracted from single, whole specimens, in the manner described by De Barro and Driver (1997). All samples were washed briefly in sterile distilled water to remove alcohol prior to homogenization. Individual whiteflies were homogenized in 10 µL of the lysis buffer (50 mM KCl, 10 mM Tris pH8.4, 0.45% Tween 20, 0.2% genatin, 0.45% NP40, 60 µg/mL proteinase K). Homogenisation was carried out using a 1.5 mL microcentriguge tube and a disposable microtube pestles. After homogenization, a further 15 µL of lysis buffer was added. The homogenate was then incubated at 65℃ for 30 mins. After incubation the sample was then boiled for 10 mins to inactivate the proteinase K. Sterile distilled water 25 µL was then added to yield a final homogenate volume of 50 µL. Samples were then stored at -20℃.

RAPD assays

RAPD analyses were carried out according to Willams *et al*. (1990) with some modifications. Total reaction volumes of 25 µL were used with final concentration of 1 unit Tag polymerase (Promega), 1× reaction buffer, 0.1 mM dNTPs, 1.5 mM $MgCl_2$, 0.2 µM primer and 20 ng template DNA (about 2 µL). Amplification was using a AG-9600 Thermal Station using the following parameters: one cycle of 2 min at 94℃ (denature), followed by 1 min at 94℃ (denature), 1 min at 36℃ (primer annealing) and 1 min at 72℃ (amplification) for 45 cycles, and 5 min at 72℃. PCR products were separated electrophoretically using 1.6% agarose gels in 1× TAE buffer and the DNA fragments were stained with ethidium bromide. The DNA fragments were visualized on a UV transilluminator and photographed.

Cytochrome oxidase I gene (COI) sequencing

PCR primer pair LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') was subsequently used to amplify a fragment about 700 bp of the COI gene (Folmer *et al*., 1994). PCR assays were conducted using 2 µL of each template DNA in a total reaction volume 25 µL. The PCR reaction mix contained 0.15 µM of dinucleotide triphosphates (dNTPs), 2.5 mM of $MgCl_2$, 0.75 units of Taq DNA polymerase and 0.6 µM of each primer. Amplification was using a AG-9600 Thermal Station using the following parameters: one cycle of 1 min at 94℃; five cycles of 1 min at 94℃, 1.5 min at 45℃ and 1.5 min at 72℃; 35 cicles of 1 min at 94℃, 1.5 min at 50℃ and 1.5 min at 72℃ and a final cycle of 5 min at 72℃. PCR products were subsequently gel purified using the PCR Clean Up-M (Viogene, Taiwan) and sequenced in one direction on an ABI 3730 DNA Analyzer (Applied Biosystems, CA, USA) using ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit, V3.1 (Applied Biosystems, CA, USA)

Phylogenetic analysis

A total of 5 *Dialeurodes* were included in the analysis. One outgroup was selected: *Aleurodicus disperses*. All whiteflies were identified morphologically using the 4th instar. A total of 15 sequences representing one to three individuals from each population were used in

the analysis (Table 1). Phylogenetic analyses were performed using PAUP 4.0b10 (Swofford, 1998). The aligned sequences were analysed by applying the Kimura two-parameters genetic distance model (Kimura, 1980) and the phylogeny represented with a bootstrap tree.

Data analysis

PCR amplification products were scored as presence (1) or absence (0) of bands. The data matrix was used to calculate Jaccard's similarity coefficient which does not consider the joint absence of a markers as an indication of similarity. A dendrogram was constructed using the unweighted pair-group method analysis (UPGMA). These analysis were performed using NTSYS-pc version 2.01b (Rohlf, 1997).

V. 結果與討論

未來粉蝨科分類之首務應透過飼育幼蟲期及生物學之記錄，若能廣泛的利用成蟲特徵，將有助解決幼蟲期外部形態變異的困擾。往昔利用蛹殼形態無法解決的問題，特別是對族群或種間判別或近緣種間的鑑別仍需開發新的技術尋求有用的分類形質以輔助分類系統或類緣關係的推斷。

粉蝨的形態分類已累積十年以上的基礎與經驗，文獻與材料相當豐富，咸信“臺灣粉蝨誌”專論之完成指日可待。雖然目前以傳統的蛹殼分類為主，輔以生物技術之操作，但是如果長期累積資料及經驗，定可逐步深入探討其分類體系、類緣關係及進化過程。往後將以發表報告為為首務；世界性粉蝨屬級之專論及探討類緣關係為中程目標；長程目標將以支序學方法，納入生物學、成蟲形態、成蟲求偶訊息 (acoustic signal) 及分子分類等形質，將粉蝨分類朝向多元的方向發展，尋找更多分類特徵的資料來分析粉蝨的類緣關係，以期重建合理完整的粉蝨科分類系統。