IC-008(表 B)

國立陽明大學教師出席國際學術會議心得報告書

申請人姓名	曹譯友	服務單位及職稱	陽明大學微免所/博士後
出國時間	自 103 年 7 月 12 日 起 至 103 年 7 月 18 日 止	前往地點	荷蘭 阿姆斯特丹
會議名稱	中文：第七屆昆蟲分子科學國際研討會 英文：Seventh International Symposium on Molecular Insect Science		

心得報告

目的-

此次參與第七屆昆蟲分子科學國際研討會主要為藉由參與會議了解最新知識、研究設計邏輯及技術發展方向，並由張貼研究海報過程提升研究內容的國際能見度，透過介紹研究海報與內容的討論結識其他國家的昆蟲分子生物研究人員，增進國際間交流互動、擴增視野、激勵研究士氣與世界接軌。

過程-

此次的國際研討會.於 7/13 進行報到手續. 會議則於 7/14~7/16 舉行.議程主軸共分為九大區塊，依照三天的會議安排分別討論： Vector biology/Tropical diseases; i5K: 5000 Genomes Initiative; Molecular Insect Neurosciences; Evolution and development; Genomics and Proteomics; Agricultural insect molecular biology; Molecular regulation of insect behaviour; Evolutionary biology, systematics, molecular phylogeny; Symbiosis.為熱帶疾病/媒介生物,昆蟲基因組學,昆蟲分子神經科學,農業昆蟲分子生物學,昆蟲行為分子調控,進化生物學,分子系統發育,共生關係等重要議題。統計下來，大會出席人數近 350 人次,近 200 份海報張貼,有約 40 個不同國家的研究學者共同參與，為國際型的盛大會議，當然全程以英文舉行。

心得-

在此次會議中較受人矚目的是 i5K 這個議題，這個在未來 5 年內解序 5000 種昆蟲和節肢動物相關物種的基因組計劃自 2011 年提出以來,鼓勵昆蟲學者針對自己研究的昆蟲進行基因解序以及功能分析，並可藉由網路提交資訊到 GenBank, EMBL 或 DDBJ 加以註解。我認為，i5k 同時倡導閉門自居的科學家們一個重要的觀念，就是將節肢動物基因組研究的科學家利用國際合作關係的網絡串連，將基因資訊藉由該協會匯集資源，經濟且高效率地傳送及促進交流和分享將實驗結果,數據資料庫及標準命名；可以有系統的做連結並統一化，這些都有助於學者們未來利用於比較基因組學與系統發育學的相關研究； 以較寬廣的角度來看，龐大數目的昆蟲和節肢動物相關物種的基因組解序目的，除了在醫學面昆蟲可以媒介疾病，全球的農業面，食品安全，生態系統以至於未來的能源生產將會關係到人類，動物和植物健康的鏈鎖反應。不同國家的研究人員可以經由資料釋出及註解讓其他國家的研究人員所認識，這個協會的計畫不僅於資源共享與訊息串聯，不但研

究經費資源不會被重複浪費更可促成國際合作與鼓勵互動。而相較於哺乳類動物而言，多面向的系統生物性的昆蟲節肢動物資料庫一直是缺乏的，希望能夠有更多的研究人員與人才的參與能夠將日後龐大的數據資料整理成能夠輕易入手取得並利用資料的電腦介面。

另外，由於研究主題關係因此特別著重於蟲媒生物與熱帶疾病相關議題，其中，受到研究學者所困擾的昆蟲抗藥性,殺蟲劑的環境汙染與無有效疫苗控制，瘧疾的媒介岡比亞瘧蚊一直受人關注。 會議中提出岡比亞瘧蚊繁殖率高是為瘧疾媒介的主要組成部分之一，有學者提出利用減少傳播的新媒介為新的控制策略。 該學者認為，生物過程的繁殖力和生育率具有相關連，因此通過交配和交配栓形成的相互作用會引發雌蚊生理和行為的重大變化，包括：降低感受性進一步交配，產卵量增加，和產卵的誘導。 該學者研究發現，雌蚊生殖行為是經由交配時雄蚊的分泌物所進行監管並確定交配；此交配雄蚊的分泌物為雌蚊產卵，卵發育和生育能力的關鍵組成部分。 這些發現增加蚊子繁殖產量的重要生物過程的進一步了解，並揭示了岡比亞瘧蚊的交配生殖行為控制為可能的新的工具目標，未來可用於瘧疾媒介的自然種群控制。

不同於前述的議題，本實驗室在蟲媒傳播研究上則是以免疫相關基因調控的控制為目標，企期望於未來用於媒介昆蟲的的自然種群免疫能力控制。在會議期間張貼研究成果海報（ The dual roles of *Armigeres subalbatus* prophenoloxidase V in parasite melanization and egg chorion melanization in mosquito *Armigeres subalbatus*），酚氧化酵素被多數的研究學者認為是昆蟲天生免疫(黑化作用)中最具有潛力的基因，而長期以來人們認為酚氧化酵素在昆蟲各種生理功能中發揮關鍵作用，如:傷口癒合，卵殼硬化，骨骼形成和外來寄生蟲黑化等；蚊子較其他昆蟲，如:果蠅或天蠶蛾等(3 個)有較多數目(8-10 個)的酚氧化酵素存在。 酚氧化酵素的單一基因單一功能或單一基因多功能，更甚多基因單功能或多功能說眾說紛紜。 在本研究中發現，As-pro-PO V 的 mRNA 在蚊體內受到微絲蟲感染或吸食血液均顯著增加；以 As-pro-PO V dsRNA 沉寂基因表現後導致維絲蟲黑化程度顯著降低且卵殼黑化率和孵化率顯著降低；EMSA 分析證實 As-pro-PO V 基因是由 AP-1 免疫相關及 CdxA 發育調控相關調控因子正調控。這些結果顯示 As-pro-PO V 同時具有使寄生蟲黑化和卵殼黑化雙重角色。 由於前項種群控制議題的關係，此一研究成果受到其他國家研究人員關注與詢問，經以英文介紹實驗背景與研究目的與設計後進行實驗流程解說與結果解釋，經由解說與討論不同的研究學者給予我不同的意見以及對未來研究的期許。畢竟在國內做蟲媒昆蟲的人與單位不多，藉由會議參與提升台灣的國際能見度，以全英文思考及溝通，認識不同國國家與不同專業的昆蟲分子生物研究人員，了解最新知識技術發展與國際研究走向，增進國際交流與互動擴增視野是此次與會的最大收穫，而校方的出國補助亦是研究成果的最大鼓勵。

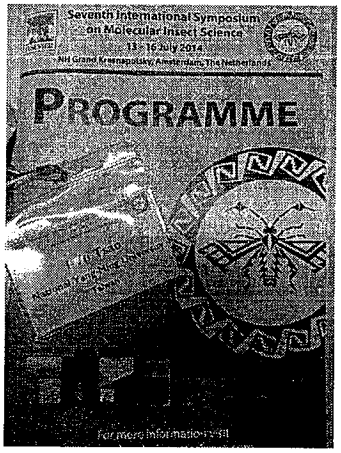
建議事項-

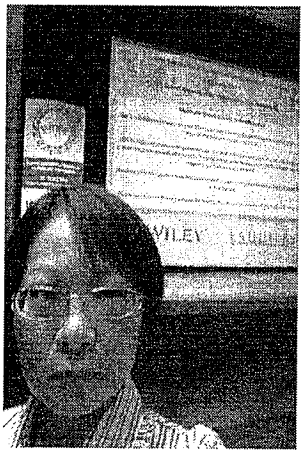
遠赴歐洲出國開會經費應是最大的問題，國際會議非常注重註冊這部分，且國際會議註冊費用也是高的嚇人，歐洲消費金額昂貴，在補助之外還需要自掏腰包，想找便宜一點的飛機，找便宜一點的青年旅店或民宿節省開支去開會貼海報； 若是能夠在註冊費上給予補助就也許能在歐洲就能多看到一些台灣的年輕面孔，打開台灣陽明大學的研究知名度。

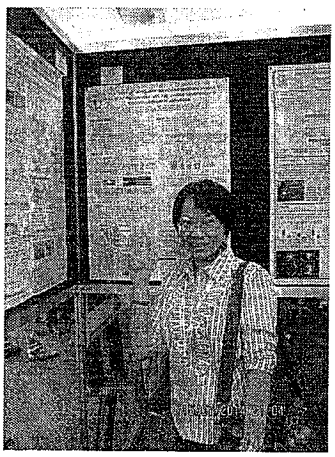
活動照片-

會議手冊及名牌/大會議場/張貼海報/海報內容

IC-008(表 B)







The dual roles of *Armigeres subalbatus* prophenoloxidase V in parasite melanization and egg chorion melanization in mosquito *Armigeres subalbatus*

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◎INTRODUCTION

Phenoloxidase (monophenol, L-Dopa:oxidoreductase; EC 1. 14. 18. 1; PO) has long been suggested to play significant roles in many vital physiological functions of insects, i. e. parasite melanization, cuticle sclerotization, wound healing, egg tanning, etc. Previously, we identified five pro-phenoloxidases (pro-POs), designated *As-pro-PO I* to *V*, from mosquito *Armigeres subalbatus* (Tsao et al., 2009) and found that the functions of As-pro-PO I and As-pro-PO III are associated with parasite melanization and cuticle formation, respectively (Shiao et al., 2001; Tsao et al., 2010). In this study, we delineated the function of As-pro-PO V in *Ar. subalbatus*.

◎RESULTS

(A) Expression profile analysis of *As-pro-PO V*

The level of *As-pro-PO V* was increased after mf challenge or blood feeding

Expressions of *As-pro-PO V* mRNA in mosquitoes were significantly increased after microfilariae (mf) challenge or blood feeding (Fig.1a). The expression of *As-pro-PO V* was decreased to normal level after oviposition in blood-fed mosquitoes (Fig.1b).

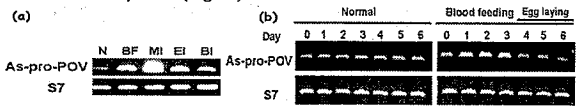


Fig 1. RT-PCR analysis of the expression levels of *As-pro-PO V* in *Ar. subalbatus*.
(a) As-pro- PO V transcription levels in mosquitoes at 48 hrs after blood feeding (BF); mf-inoculated (MI), *E. coli*-inoculated (EI), HBSS buffer-inoculated (BI), and normal (N). (b) *As-pro-PO V* transcription levels at various time intervals after BF. Mosquitoes were allowed to lay their eggs at the 4th day after blood feeding. S7 was used as a loading control.

(B) Promoter analysis of *As-pro-PO V* gene

As-pro-PO V is positively regulated by both AP-1 and CdxA.

Transient transfection analysis using C6/36 cells demonstrated that -730 to – 609 bp region contains putative AP-1 and CdxA regulatory elements (Fig. 2a,b). EMSA verified the binding of AP-1 motif with adult mosquito nuclear extracts was significantly enhanced after mosquitoes were challenged with mf (Fig. 2c). Whilst the binding of CdxA motif with mosquito nuclear extract was also significantly enhanced after mosquitoes were fed with blood (Fig.2d).

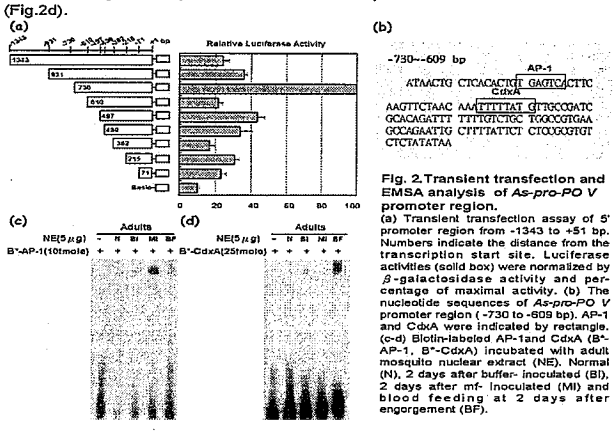


Fig. 2.Transient transfection and EMSA analysis of *As-pro-PO V* promoter region.
(a) Transient transfection assay of 5' promoter region from -1343 to +51 bp. Numbers indicate the distance from the transcription start site. Luciferase activities (solid box) were normalized by β-galactosidase activity and percentage of maximal activity. (b) The nucleotide sequences of *As-pro-PO V* promoter region (-730 to -609 bp). AP-1 and CdxA were indicated by rectangle. (c-d) Biotin-labeled AP-1and CdxA (B*-AP-1, B*-CdxA) incubated with adult mosquito nuclear extract (NE). Normal (N), 2 days after buffer- inoculated (BI), 2 days after mf- inoculated (MI) and blood feeding at 2 days after engorgement (BF).

(C) Functional analysis of As-pro-PO V

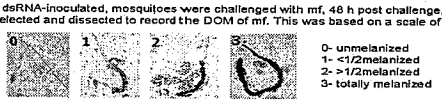
I. *As-pro-PO V* participates in parasite melanization

As compared with normal and GFP dsRNA-inoculated mosquitoes, knock-down of *As-pro-PO V* expression in mosquitoes by 450 bp dsRNA of *As-pro-PO V*, resulted in the significantly reduced in the degree of melanization (DOM) of mf (Table1).

Table 1.Inhibition of melanization of mf in *Ar. subalbatus* with dsRNA of *As-pro-POV*

	Normal		*GFP* dsRNA-inoculated		*As-pro-PO V* dsRNA-inoculated	
exp	DOM(0-3) Mean±S.D.	No. of mf recorded/ No. of mosquitoes dissected	DOM(0-3) Mean±S.D.	No. of mf recorded/ No. of mosquitoes dissected	DOM(0-3) Mean±S.D.	No. of mf recorded/ No. of mosquitoes dissected
1	2.80±0.40	534/30	2.82±0.39	568/30	1.82±0.38	578/30
2	2.83±0.32	575/30	2.75±0.43	572/30	1.66±0.47	545/30
3	2.75±0.43	562/30	2.80±0.40	553/30	1.75±0.43	567/30

Three days after dsRNA-inoculated, mosquitoes were challenged with mf, 48 h post challenge, Mosquitoes were randomly selected and dissected to record the DOM of mf. This was based on a scale of 0 to 3.



II. As-pro-PO V involves in Egg chronic melanization

The newly laid mosquito eggs are white and soft and the egg shells turn black and harden (tanning) about 2 h. As shown in Fig.3, knockdown of *As-pro-PO V* expression in blood-fed mosquitoes resulted in significantly reduced in both egg chorion melanization rate and egg hatching rate.

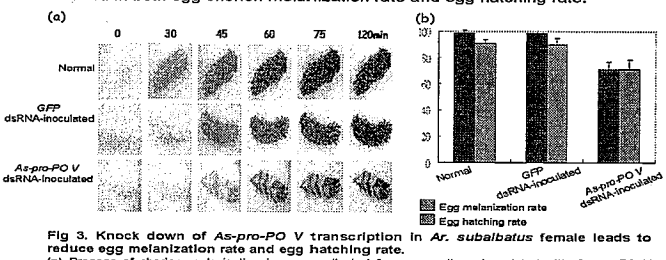


Fig 3. Knock down of *As-pro-PO V* transcription in *Ar. subalbatus* female leads to reduce egg melanization rate and egg hatching rate.
(a) Process of chorion melanization in eggs collected from mosquitoes inoculated with *As-pro-PO V* dsRNA,*GFP* dsRNA or were left untreated. (b) Egg melanization rate and egg hatching rate of normal, *GFP* dsRNA- inoculated and *As-pro-PO V* dsRNA-inoculated mosquitoes.

◎CONCLUSION

1. Knockdown of *As-pro-PO V* significantly reduced the degree of melanization of mf in *Ar. subalbatus*.
2. Knockdown of *As-pro-PO V* significantly reduced egg chorion melanization rate and egg hatching rate of *Ar. subalbatus*.
3. *As-pro-PO V* is positively regulated by both AP-1, a putative immune-related regulated element, and CdxA, a developmental regulatory element.
4. As-pro-PO V plays dual roles on parasite melanization and cuticle formation in *Ar. subalbatus*.

◎REFERANCES

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•Tsao, I.Y., Lin, U.S., Christensen, B.M. and Chen, C.C. 2009 *Armigeres subalbatus* prophenoloxidase III: Clong, characterization and potential role in morphogenesis. *Insect Biochem Mol Biol.* 39:96-104.
•Tsao, I.Y., Christensen, B.M. and Chen, C.C. 2010 *Armigeres subalbatus* (Diptera: Culicidae) prophenoloxidase III is required for mosquito cuticle formation: ultrastructural study on dsRNA-knockdown mosquitoes. *J Med Entomol.* 47:495-503.