行政院國家科學委員會補助專題研究計畫成果報告

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※　德國蜚蠊及雙紋姬蠊活動行為日週律動比較研究　※
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計畫類別：■個別型計畫　　□整合型計畫

計畫編號：NSC87－2313－B－002－045－
NSC88－2313－B－002－002－
NSC89－2313－B－002－015－

執行期間：86 年 8 月 1 日至 89 年 7 月 31 日

計畫主持人：李後晶

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- □赴國外出差或研習心得報告一份
- □赴大陸地區出差或研習心得報告一份
- ■出席國際學術會議心得報告及發表之論文各一份
- □國際合作研究計畫國外研究報告書一份

執行單位：國立台灣大學昆蟲學系

中　華　民　國八十九年　九　月　八　日

行政院國家科學委員會專題研究計畫成果報告

德國蜚蠊及雙紋姬蠊活動行為日週律動比較研究

Comparative study of locomotor circadian rhythm between *Blattella germanica* and *B. bisignata*

計畫編號：NSC87－2313－B－002－045－
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NSC89－2313－B－002－015－
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一、中文摘要

德國蜚蠊與雙紋姬蠊雖然親源相近、形態相似，但是兩者的生活環境卻有很大的差異：雙紋姬蠊是野外的種類，德國蜚蠊是居家害蟲。兩者在日週律動的表現有很大的差異，比較兩者日週律動的表現，是探討「日週律動在生態上的意義」很好的材料。因此本研究在建立雙紋姬蠊基本資料之後，藉雙紋姬蠊獨居或群居性試驗，探討其兩種蜚蠊生態上的差異；並針對雙紋姬蠊之生態特性，探討日週律動對雙紋姬蠊的配偶找尋行為的影響；另外由相反應曲線的研究，瞭解計時器如何因應環境的變化而調整其相位；藉由腦部顯微手術及組織切片的方法，證實了德國蜚蠊活動行為日週律動的調律器位於視葉；而聲音的干擾，對德國蜚蠊活動行為日週律動的表現，影響不大，對雙紋姬蠊影響則較明顯。

關鍵詞：德國蜚蠊、雙紋姬蠊、活動行為、日週律動、調律器、人類干擾

Abstract

Although German cockroach and *Blattella bisignata* are close-related species, their habitats are extremely different: .*B. bisignata* is a feral species, whereas *B. germanica* is a strictly domicile species. There are large differences between the expression of circadian rhythm of the two species. The comparative study of the expression of circadian rhythm, is a good theme in the investigation of ecological significance of circadian rhythm. Hence, after constructing the basic data of *Blattella bisignata*, by the solitary or aggregating experiments of *Blattella bisignata*, investigated the ecological differences between the two cockroaches. We also investigated the effect of the expression of circadian rhythm on mate searching behavior of *Blattella bisignata*, based on its ecological characteristics. In addition, by constructing of phase response curves of both species, we can understand how the pacemakers adjust their phase according to the environmental changes. Using brain microsurgery and tissue sectioning, we demonstrated the exact location of pacemaker of locomotion circadian rhythm of German cockroach is in its optic lobes. There is little effect on the expression of locomotor circadian rhythm of German cockroach caused by sound interference, but it seemed more effects on *Blattella bisignata*.

Keywords: German cockroach, *Blattella bisignata*, locomotor behavior, circadian rhythm, pacemaker, human interference

二、緣由與目的

德國蜚蠊是完全家居的種類，但是其親緣相近的雙紋姬蠊卻是野外的種類。兩者在日週律動的表現有很大的差異，比較

兩者日週律動的表現，是探討「日週律動在生態上的意義」很好的材料。近年來有報導雙紋姬蠊曾經於台北近郊的家居中被捕獲（吳與蕭，1994），因此探討雙紋姬蠊與德國蜚蠊的生活史特性、生殖策略與聚集性等差異，以及這些差異對兩者在居家環境互相競爭的影響，是本研究的另一個目的。

由相反應曲線(phase response curve)的研究中，可以瞭解計時器如何因應環境的變化而調整其相位(phase)(Brady, 1982；Moore-Ede *et al*., 1982)。而在日週律動的整個系統中，找出調律器(pacemaker)的位置及控制訊息的傳遞機制(Colwell and Page, 1990)是明白日週律動系統運作的基本要素，因此除了對相反應曲線進行探討外，亦利用腦部顯微手術及組織切片的方法，尋找調律器的精確位置。

人類活動的干擾可能是影響這二種蜚蠊在家居環境生存的主因，我們利開燈及聲的干擾，來探討德國蜚蠊及雙紋姬蠊活動行為日週律動的表現，藉此判定那種蜚蠊對人為環境的適應性較高。

三、結果與討論

(一)、雙紋姬蠊之壽命及生殖週期

雙紋姬蠊成蟲壽命以交尾雌蟲最長，處女雌蟲次之，而雄蟲最短。以一般昆蟲而言，交尾雌蟲在生殖上花費了許多能量與營養，所以交尾雌蟲的壽命明顯地比處女雌蟲短(Lessells, 1991)。但是雙紋姬蠊的生殖週期對雌成蟲的壽命卻有相反的效應。因為雙紋姬蠊不論處女或交尾雌蟲皆有週期性的排卵和懷孕，而且處女和交尾雌蟲一生平均都可產 9 個卵鞘。雌蟲在生殖方面的營養投資相當大，在相同的生殖週期中，處女雌蟲的活動量顯著地較交尾雌蟲活動量高，加上處女雌蟲的生殖週期較短，所以當處女雌蟲很快地產完 9 個卵鞘後，可能因養份與體力已消耗殆盡而死亡，因此處女雌蟲壽命顯著地較交尾雌蟲短。同樣地，德國蜚蠊也由於處女雌蟲生殖週期短，活動量高，養分及體力消耗較快而壽命比交尾雌蟲來得短(Lee and Wu, 1994)。而雄蟲的活動量又較處女雌蟲與交尾雌蟲高，所以體力消耗最快，壽命最短。

德國蜚蠊處女雌蟲的性接受程度是受到青春激素與交尾兩個因子所調控(Lin and Lee, 1998)。卵巢對性接受程度沒有直接的影響。至於雙紋姬蠊性接受程度的研究缺乏，其調控因子尚未瞭解清楚，僅知其性接受程度也具有週期性。

(二)、雙紋姬蠊與德國蜚蠊活動行為規律性之比較

1、亮暗週期下的晝夜律動

在 28°C，光週期 16L:8D 的環境條件下，雙紋姬蠊的雌雄成蟲都表現出明顯的晝夜律動，並且活動都侷限於夜晚。同樣地，德國蜚蠊的雌雄成蟲大致上也都表現出晝夜律動，大部份的活動也都侷限於夜晚(Dreisig and Nielsen, 1971; Sommer, 1975; Leppla *et al*.,1989; Lee and Wu, 1994)。而雌蟲在一般時期是有晝夜律動的，但是在性接受期及懷孕期則沒有晝夜律動(Sommer, 1975; Lee and Wu, 1994)。德國蜚蠊在性接受期間，不僅夜晚的活動量高，白天的活動量亦很高；而在懷孕期間，反而因為日夜的活動量都很低，沒有表現出明顯的晝夜律動 (Lee and Wu, 1994)。雙紋姬蠊雌蟲的活動模式則顯然與德國蜚蠊不同：雙紋姬蠊在性接受期間活動量也會增高，但大部份的活動仍侷限於夜晚；懷孕期間雖然活動量低，但亦僅侷限於夜晚活動。

在 28°C，光週期 16L:8D 的環境條件下，雙紋姬蠊與德國蜚蠊 1-4 齡若蟲皆因活動量太低，而沒有表現出晝夜律動。直到 5 齡以後活動量才漸增，呈現晝夜律動的現象。雙紋姬蠊有 67%的老熟若蟲可以表現出晝夜律動，但是德國蜚蠊卻只有 29%的老熟若蟲表現出晝夜律動。Sommer (1975) 也曾指出德國蜚蠊若蟲並沒有晝夜律動的表現。推測可能與生活環境穩定性有關，在較少天敵的居家環境，沒有晝夜律動的德國蜚蠊若蟲，對其生存不致造成太大的為害。

2、全暗環境下的日週律動

在 28°C，完全黑暗的環境條件下，雙紋姬蠊雄成蟲與處女雌蟲皆可以表現日週律動，並且活動都侷限於相對夜晚。但是在相同的環境條件下，德國蜚蠊卻只有雄成蟲表現出日週律動，活動也都侷限於相

對夜晚(Dreisig and Nielsen, 1971; Sommer, 1975; Leppla *et al*.,1989)，處女與懷孕雌蟲則不表現日週律動(Lin and Lee, 1996)。雖然雙紋姬蠊處女雌蟲可以表現日週律動，但是懷孕的雌蟲卻與德國蜚蠊懷孕雌蟲一樣，無法表現出日週律動。因為懷孕期間不需覓食及尋找配偶，所以活動量非常少，因此大部份雙紋姬蠊懷孕雌蟲(67%)無法表現活動行為的日週律動。這種只在懷孕期間日週律動無法表現的現象，就是掩蓋作用。根據掩蓋作用的定義(Page, 1989; Rietveld *et al*., 1993)，雌蟲攜帶卵鞘是一個負向的掩蓋因子，不但使懷孕雌蟲的活動量降低，也掩蓋住日週律動的表現。而德國蜚蠊處女雌蟲在性接受期間，不論相對夜晚或白天，活動量都很高，導致日週律動無法表現，這也是一種負向的掩蓋作用，是由卵巢發育引發性接受程度改變所造成的(Lin and Lee, 1996)。因此雙紋姬蠊雌蟲只具有懷孕的負向掩蓋因子，而德國蜚蠊雌蟲則具有懷孕和性接受期的雙重負向掩蓋因子。

在 28°C，完全黑暗的環境條件下，雙紋姬蠊與德國蜚蠊 1-4 齡的若蟲皆因為活動量低，而沒有表現出日週律動。直到 5 齡以後活動量漸增，才有日週律動的表現。雙紋姬蠊有 86%的老熟若蟲可以表現日週律動，但是德國蜚蠊卻只有 14%的老熟若蟲表現出日週律動。推測可能也與環境穩定性有關，雙紋姬蠊生活在野外，日夜變化明顯，天敵多，所以在適當時間出沒對其生存是很重要的。

(三)、雙紋姬蠊的配偶找尋行為

雙紋姬蠊與德國蜚蠊的若蟲不論在亮暗週期或是全暗的環境下，平均每日活動量隨著日齡增加而增高，而在末齡若蟲期達到最高峰。由於兩種蜚蠊的聚集性大不相同，所以末齡若蟲的日活動量增加所造成成蟲對空間分佈的結果，也有很大的差異。雙紋姬蠊並沒有聚集費洛蒙，聚集性低，末齡若蟲活動量增加將導致個體之間更為分散。若蟲羽化為成蟲之後，在找尋配偶方面，必須利用更有效率的方式，在時間的配合上需更為準確。而德國蜚蠊因為具有聚集費洛蒙，而且聚集性高，末齡若蟲活動量的增加，可以使近親個體分散開來，至其他聚落再聚集在一起。所以若蟲在羽化為成蟲之後，並不需要長距離的找尋配偶，雌、雄蟲很容易相遇而交尾(Brossut and Roth, 1977; Schal *et al*., 1983)。

雙紋姬蠊若蟲羽化為成蟲之後，因為雌雄蟲空間的隔離，相遇不易，所以雌蟲必須利用呼喚行為，釋放揮發性性費洛蒙，誘引雄蟲前來交尾，而這兩種行為（雌蟲的呼喚行為與雄蟲的活動行為）可由日週律動系統來控制，達到很緊密的時機配合性。這種配偶找尋策略與德國蜚蠊有所不同，因為德國蜚蠊雌蟲是利用聚集性與逢機相遇當作主要策略，並利用增加活動與呼喚行為當作次要策略(Lin and Lee, 1996; Tsai and Lee, 1997)，而雙紋姬蠊則是利用呼喚行為當作配偶找尋的主要策略。

(四)、雙紋姬蠊與德國蜚蠊相互競爭之可能性

在 28℃，光週期 16L：8D 的環境條件下，雙紋姬蠊的卵期約 20 天，而德國蜚蠊的卵期約 17 天（吳，1992）。在相同的環境條件下，雙紋姬蠊的卵期較長，使得雙紋姬蠊在競爭上稍處於劣勢。又雖然雙紋姬蠊的雌蟲壽命較長，一生所能產生的卵鞘數較德國蜚蠊為多，但是雙紋姬蠊交尾一次只能產生 2.7±1.8 個有效卵鞘，而德國蜚蠊交尾一次則能產生 3.4±1.6 個有效卵鞘（吳，1992），所以在穩定的環境條件下，由兩者生活史特性所推論出之生殖策略上，雙紋姬蠊是略遜一籌的。而將兩種蜚蠊各 10 對成蟲，在兩公升的透明塑膠桶內一起飼養，經過三個月的競爭結果，亦符合從兩者生殖策略上的差異所作的推論。

在穩定的環境條件下，雙紋姬蠊與德國蜚蠊兩者競爭的結果是德國蜚蠊會完全取代雙紋姬蠊。由於雙紋姬蠊與德國蜚蠊兩者的聚集性不同，所以在聚落密度很高的情形下，德國蜚蠊在攝食方面受到干擾的情況比雙紋姬蠊小，使德國蜚蠊在競爭上取得優勢。所以雙紋姬蠊與德國蜚蠊在競爭資源時，雙紋姬蠊因為攝食干擾與空間不足的雙重限制，而在環境穩定而空間狹小的環境中，處於不利的地位，而在短時間內（小於三個月）就被德國蜚蠊完全取代。

(五)、相反應曲線

在德國蜚蠊與雙紋姬蠊雄蟲對短暫光線刺激(2小時)的相反應曲線方面的研究發現，於16L：8D持續5天再改為DD的試驗環境下，不同的日週時刻曝光會造成不同的相移位：在相對白晝晚期至相對夜晚中期(CT 14至 CT 20)曝光，會造成相位延後(phase delay)；在相對夜晚末期至相對白晝早期(CT 21 至 CT 3)曝光，相位提前(phase advance)；其餘時間(CT 4至 CT 13)對相位的影響則較不明顯。與其他動物在12L：12D再改為DD的試驗環境下，呈現相同的趨勢(Brady, 1982； Moore-Ede *et al.*, 1982； Page, 1987； Cymborowski *et al.*, 1993)。而由於兩種蜚蠊相位提前的幅度皆小於相位延後的幅度，顯示相位提前的調整較相位延後困難。

在本試驗中發現，德國蜚蠊相位移的幅度遠小於雙紋姬蠊，顯示雙紋姬蠊生物時鐘的調整能力高於德國蜚蠊。此外，相位移與自由律動週期變化之間的關係並不顯著，其原因則有待進一步探討。

(六)、德國蜚蠊的調律器的精確位置

德國蜚蠊雄成蟲在二側視神經幹皆切斷後，不再表現活動行為的日週律動，而手術對照組的雄蟲活動行為仍可表現出日週律動。這個結果與前人的發現相同，可以證明德國蜚蠊控制活動行為日週律動表現的調律器是位於視葉，而且其訊息是透過前大腦視葉與中腦之間的神經聯結來傳遞。

在德國蜚蠊兩側視神經幹切割手術中，有26.9%在術後4週可重新表現日週律動，而存活超過6週的雄蟲中，表現出日週律動者，甚至可達 50%。此結果證明視神經幹在活動行為日週律動的表現上的重要性，也顯示了日週系統從切斷視神經幹的手術中，恢復活動行為的時間分布的控制的能力。

在德國蜚蠊活動行為日週律動回復試驗中，我們也發現有視神經幹切割手術再生後之自由律動週期較手術前週期長或短的情形都有(Stengl and Homberg, 1994)，我們的研究結果證實了德國蜚蠊活動行為日週律動的調律器位於視葉。

德國蜚蠊在切斷一側視神經幹後，仍能在另一側視神經幹完好的情形下，表現出活動行為的日週律動，顯示左右兩側的視葉皆擁有一個日週律動的調律器，而且任何一個都能獨力維持活動行為的週期。許多研究顯示，在成對的神經系統中，日週律動的調律器呈兩側對稱(Nishiitsutsuji-Uwo and Pittendrigh, 1968; Page *et al.*, 1977； Pittendrigh, 1981)，雖然在一隻蜚蠊體內具有二個調律器似屬多餘，但這可能是身體組織兩側對稱的演化結果。若兩個調律器的作用是緊密結合的，則它們的存在，並不違背天擇的作用。惟仍有待進一步的探討。

四、計畫成果自評

本計畫為三年期計畫，研究內容皆依原計畫期程分年執行，與原計畫大致相符。在計畫執行期間，我們達成了下列預期目標：

(一)、完成了雙紋姬蠊的生活史、生殖週期和性接受期等基本生態資料。

(二)、找出雙紋姬蠊若蟲、雌成蟲和雄成蟲在 16L:8D 及全暗的光週期下之活動模式。以及若蟲活動量與齡期之關係、成蟲活動量與生殖週期之關係。並將上述試驗所得到的雙紋姬蠊生態資料與已有的德國蜚蠊生態資料進行比較，探討兩者日週律動的作用與掩蓋機制之異同。

(三)、利用試驗確定雙紋姬蠊是獨居性種類，其配偶找尋的時機對其生殖成功率有決定性的影響，所以導致其日週律動模式與德國蜚蠊有相當大的差異。

(四)、德國蜚蠊與雙紋姬蠊雄蟲之活動行為相反應曲線皆有相同的變化趨勢。

(五)、利用破壞視葉特定部位及組織切片的方法，找出調律器的精確位置。

(六)、比較兩種蜚蠊對人類活動的敏感性，探究雙紋姬蠊侵入室內的可能性。

(七)、具掩蓋作用與不具此作用的個體所產的後代其掩蓋作用的比例並無明顯差異，表示有其他因子在影響掩蓋作用的表現。

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行政院國家科學委員會補助國內專家學者出席國際學術會議報告

89 年 4 月 日

<table>
<tr><td>報告人姓名</td><td>李後晶</td><td>服務機構及職稱</td><td>台大昆蟲系教授</td></tr>
<tr><td>會議 時間 地點</td><td>89年4月4至8日
突尼西亞</td><td>本會核定補助文號</td><td>德國蜚蠊及雙紋姬蠊活動行為日週律動比較研究(3/3)
NSC 89-2313-B-002-015</td></tr>
<tr><td>會議名稱</td><td colspan="3">(中文) 國際時間會議
(英文) International Symposium "Time"</td></tr>
<tr><td>發表論文題目</td><td colspan="3">(中文) 德國蜚蠊如何計時？
(英文) How the German cockroach tells time?</td></tr>
</table>

報告內容應包括下列各項：

一、參加會議經過

二、與會心得

三、考察參觀活動（無是項活動者省略）

四、建議

五、攜回資料名稱及內容

六、其他

此次時間國際會議是由突尼西亞國立科學博物館所舉辦，主要邀請法國及當地的學者參加，而我是唯一來自第三個國家的，也是唯一不懂法文的人。因此大會雇請了二位翻譯者，輪流做即時口譯給我聽，所以我可以全程參與會議而不致於鴨子聽雷。可見主辦單位相當用心，可以接受來自不同語系的人，對我國相當友好，絕無輕視或歧視之見。而主辦單位人員隨時會來與我交談，詢問事情，使我有賓至如歸之感。

會議首先由突尼西亞之高等教育部長主持開幕式，揭諸該國對此會議的重視，希望藉由學者的參與來提昇該國科學的水準。正式會議在部長與大家握手告別之後展開。

本次會議主要由各個領域，針對時間所造成的改變或現象，提出解釋。由於任何學術領域例如：地質、物理、天文、歷史、音樂、生物等皆受時間的影響，因此這個會議也變的格外有趣，從文史、宗教、音樂談到天體運行，及如何將時間測量。而與我領域相近的時間生物學也有兩位學者，討論人的生理時間及藥效作用之關聯性。由於這方面進展尚未達到全面應用的階段，因此他們也只能就一些原則性的現象，提出討論，無法深入探討生物時鐘在醫療體系中應考慮的角色功能。

我的演講排在第二天下午第一節，我用英文講演，所以有口譯者翻成法文。我談到德國蟑螂如何利用生物時鐘，來表現適時的活動行為。這是我多年來研究成果的綜合表現，由証明德國蟑螂生物時鐘的存在，再進一步談到這個生物時鐘如何將訊息透過傳導至胸部運動神經中樞，再控制 6 隻腳做出活動行為。由於德國蟑螂雌虫有類似懷孕階段來得保護胚胎發育，因此形成生殖週期的現象。此現象將每日活動行為日週律動的表現掩蓋住，使雌蟲無法表現出與雄虫一樣之活動行為日週律動現象。我們可以藉由手術將雌蟲之卵巢去除，則雌蟲表現出活動行為的日週律動現象。因此我們証實了德國蟑螂雌雄皆具有生物時鐘，可以控制活動行為表現出日週律動的現象。我們也藉由手術，証實德國蟑螂具有 2 個位於左右視葉的生物時鐘，並証實此 2 個左右生物時鐘是聯結一起，展現出一個同步的時鐘，然而聯結是有主從之分的，只是左右任一個都有可能成為主要的時鐘。

由於我的主題較為少見，因此引起不少人的注意，在演講後，休息時間，就有人來與我對談，希望多瞭解一些生物時鐘的研究與應用。其中主辦單位之館長 Dr. Tahar Gallali 是位地質學家，但也與我談了一些生物時鐘的問題。我也趁機做些國民外交，介紹國內一些科學博物館狀況，並且邀請他到國內訪問，藉由學術交流，達到外交上的一些成果，至少在提昇國際能見度上，有很大的助益。

第三天議程有位突尼斯國家劇院主任 Mohamed Driss 發表一篇有關藝術創作與時間的關聯演講，相當有趣。尤其他談到藝術工作者，希望抓住時間，而創造出不朽的作品。這篇論文，引發了我對藝術創作一些看法，是否藝術家的工作，就是希望從觀眾身上吸引到他們的時間？或者藝術創作的好壞，應該與它能抓住人們多少時間成正比？這些問題基本上與時間脫離不了關係，可能對藝術創作的評判也是相當切題的。

下午至加太基（Cathage）羅馬遺跡博物館去參觀。由於加太基深入地中海，可堅監控船隻進出地中海，是古代腓尼基據此建立起王國，而加太基將軍漢尼拔曾帶領軍隊，越過阿爾卑斯山，攻打羅馬帝國，雖然最後還是被羅馬軍打敗，但可見當時加太基的進步與文明。最後羅馬人佔領加太基，還將之建立起羅馬帝國非州省的一部分。此地雖然陸續遭拜占庭，阿拉伯人，及法國人佔領，但目前已將被整個埋在地下之羅馬遺跡挖掘出來。雖然只是些斷壁殘垣，但透過一些歷史，考古學者的解說及重畫，仍可瞭解昔日人民在羅馬帝國統治下的生活，只是在羅馬遺跡附近皆是阿拉子民，形成很奇特的對比，展現一種和義大利截然不同的風貌。

第四、五天大家乘坐遊覽車至南部參觀訪問一些地質、灌溉、歷史古蹟等與時間相關的地點。雖然突尼西亞國土不大，但是氣候多型，造成很多自然、人文景觀上相當大的變化。首先至 Kairouan 古城，參觀一座清真寺，建築相當雄偉，廣場內有日春（sun dial），可用來計時。一般遊客是無法進入清真寺參觀，但由於主辦單位是政府機關，因此特別帶我們這些非回教徒進入大廳參觀，內部的一些建築雕刻也特別介紹，對這座歷經數百年而仍在使用的清真寺，有較清楚的瞭解。隨後我們再往南走，到了已在沙漠中地質特殊的地點。由於地層的擠壓，造

成類似恐龍背脊上隆起的刺片，非常奇特。在地質學家的介紹下，看到表土上有些貝類的化石，証明此地在數百萬年前，是在海底。中餐就在沙漠上，由工作人員準備典型的北非烤肉，就著沙拉、麵包吃了一頓非常奇特的突尼西亞午餐。繼續向南行，來到一處沙漠中的湧泉小池塘，是個考古的地點。幾千年來，附近的人們，利用此水源過活。目前有個抽水馬達，人們除開車外，也有利用毛驢來載水，非常有趣。在到達晚上休息旅館之前，在一小鎮停留，參觀一下主辦單位在全國各地巡展的科學館。在鎮長及各位官員的歡迎下，我們領略了當地政府對活動科學館的歡迎。晚上到杜茲城，它是位於撒哈拉沙漠的北方，經由此地可進入舉世聞名的沙漠。到達旅館時天已暗，晚餐後就回房休息。第二天一早，我趁機花了一個鐘頭的時間，騎上駱駝往撒哈拉沙漠進入，去領略一下一望無際的沙漠及日出。生平第一次稍微親身體驗一下沙漠的情趣。景色非常壯麗，但對在那裡生活的人們，卻是艱苦萬分，使我對人在各種環境下皆能生存下去，感到相當的驚奇，也對我們生存環境，實在超越相當多的國家，應該惜福。在早餐後，就向北回程行去，首先在一望無際的鹽湖停靠，踩在烈日下厚實的鹽層上，已看不出這是一大片湖，只是仔細觀察，可看出表面結晶的鹽沙。而在湖邊仍有些許的水灘，由於結晶作用的關係，顯現不同色澤的水色，相當奇特。此地所生產之岩石結晶，有沙漠玫瑰之稱，相當漂亮。下一站到達一處綠洲，在古代當地政府就採取配給方式，讓人畜都有水喝。在目前仍有部分的渠道仍在使用。由於此綠洲的存在，使的當地的棕梠樹得以生產非常好吃的蜜棗（date)，是當地的特產。接下去的行程是參觀國營的磷礦，此磷礦年產一百萬噸，是全世界排名第二的磷礦，也是突尼西亞重要的進口外匯。由於此礦藏品質佳，採礦容易，因此銷路很好，但是該廠工程師評估，此礦藏可能只剩50年的壽命了，因此他們也極需新的開發技術，來延緩此礦藏的壽命。參觀完磷礦後，去餐廳聚餐，結束二天的學習參觀之旅。

首次至突尼西亞參與國際會議，讓許多該國人民認識了台灣，尤其有2位研究生，想至台灣來留學，希望我能替他們查詢申請學校的資訊及獎學金的支援。我想我國在第三世界國家裡，應扮演較積極的角色，尤其利用我們較為先進之科

技人文領域，訓練留學生，不但可以在國際社會中扮演輸出國的重要角色，這些留學生也是我們與該國之外交橋樑，對拓展我們國際空間有很大的助益，我們可以從美國大量吸收及訓練留學生，而得到重大的國家利益，我認為我國更應該積極往這個方向去做，建立起一套完整的留學生訓練計畫，積極吸收、援助、訓練外國知識分子，對雙方都有很大的助益。

How the German Cockroach Tells Time?

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1. Introduction

The German cockroach is a cosmopolitan species that lives closely with human. It's daily activities cause a threat to our health and environmental quality. Therefore, the German cockroach as a household pest is worth the attention of researchers to find a way of suppressing its population (Schal and Hamilton, 1990). If we know how the German cockroach tells time and avoid the contact with us, we should be able to design a control strategy against it.

The question "How the German cockroach tells time?" has two levels of aspects. One is at the molecular mechanism aspect to ask how the endogenous clocks of cockroach generate time signals and was sent out to control the expressing of overt rhythms. Another aspect is at the individual level to ask how the cockroach does a right thing on the right place at the right time (Enright, 1970). Although the molecular clock mechanism of the German cockroach is still unknown, we do have some information on the physiological and behavioral expression on the time (Lee and Wu, 1994； Lin and Lee, 1996； 1998； Tsai and Lee, 2000； Wen and Lee, 2000).

2. Why the existence of biological clocks?

The Earth's daily rotation along its axis provides organisms an important environmental cue on the control of behavioral state. The ubiquitous adaptation of organisms to daily alternating cycles of light and darkness and daily temperature fluctuations is the existence of an endogenous 24 h rhythmicity synchronizing life functions at the biochemical, physiological, and behavioral levels (Daan, 1982； Giebultowicz, 1999； Schwatz and Zlomanczuk, 1999). The circadian rhythms are self–sustained, persist in constant environmental conditions, and temperature compensated with close to a 24 h period. The endogenous period of many rhythms is not exactly 24 h, but they are entrained to the 24 h daily environmental fluctuations. This property of biological clock allows for more stable entrainment by environment cycles and organisms to adapt successfully to seasonal changes in photoperiod (Schwatz and Zlomanczuk, 1999).

Circadian clocks also provide organisms with a mechanism that allows body rhythms to be integrated for concerted action and phased to the local time of day, optimizing the economy of biological systems and allowing for a predictive homeostatic control (Brady, 1982 ; Schwatz and Zlomanczuk, 1999). Circadian clocks not only contribute to the regulation of reproductive rhythms, seasonal behaviors, and celestial navigation and migration, but also closely associate with internal physiological cycle and external behavioral rhythm.

3. Outline of molecular mechanism of biological clocks

Although no research has been done on the molecular mechanism of biological clocks in the German cockroach, a lot of progress have been down a fruitfly and some other model organisms. In each organism studied so far, the biological timekeeping mechanism is characterized by an intracellular feedback loop in which expression of a group of genes results in production of proteins that then switch off the expression of those genes (Hardin and Glossop, 1999). In order to be entrained, the feedback loop maintains synchrony with environmental light-dark cycles by shifting phase in responding to light. Light is speculated to destroy the clock protein at the rate that is proportional to the light intensity, at least up to a limit (Giebultowicz, 1999). This is concerted with the finding that PER levels is low under constant light (Zerr *et al.*, 1990). Our knowledge about molecules involved in circadian timekeeping comes mostly from the genetic researches on the fruit fly *Drosophila melanogaster*. The circadian feedback loops of *Drosophila* are controlled by a homologous set of transcriptional activator proteins (dCLK and CYC / dBMAL1), and inhibitors (PER-TIM heterodimer) that block these activators (Dunlap, 1999). Transcription of the *per* and *tim* genes is activated by dCLK-CYC protein dimers in the late night. These feedback loops are interlocked because the interaction of PER-TIM with dCLK-CYC inhibits *per* / *tim* transcription and releases *dClk* repression at the same time, thus enabling *per* / *tim* and *dClk* mRNA to cycle in opposite phases (Glossop *et al.*, 1999). Light applied early in the dark phase causes phase delays, whereas light applied late in the dark phase causes phase advances (Hardin and Glossop, 1999).

Light leads to the degradation of TIM protein (Hunter-Ensor *et al.*, 1996 ; Lee *et al.*, 1996 ; Myers *et al.*, 1996 ; Zeng *et al.*, 1996). Since it appears that the fly circadian clock is composed of two interlocked negative feedback loops, those loops are affected by light acting via CRY photoreceptors (Giebultowicz, 2000). The CRY protein interacts with TIM and activates phase shift responses when light stimulates on CRY photoreceptors (Stanewsky *et al.*, 1998 ; Emery *et al.*, 1998 ; Ceriani *et al.*, 1999).

Molecular characterization of the *per* gene in other fruit fly species rather than *D.*

melanogaster (Colot *et al.*, 1988), reveled conserved regions within this gene and led to isolation of the structural and functional *per* homologous from several species of moths and from a cockroach (Reppert *et a*l., 1994). Since homologues of the *period* gene were identified in humans and mice (Sun *et al.*, 1997, Tei *et al.*, 1997), these discoveries imply that molecular elements constructing circadian clocks have been conserved in evolutionary processes (Geibultowicz, 1999). Thus, probing circadian mechanism in insects should contribute to the understanding of the general principles of circadian timekeeping.

4. The clock study of the German cockroach

All the clock studies on the German cockroach are done in locomotor circadian rhythm. Although male German cockroach can express locomotor circadian rhythm under constant darkness conditions with a period less than 24 h, females are arrhythmic (Sommer, 1975 ; Leppla *et al.*, 1989 ; Lee and Wu, 1994 ; Lin and Lee, 1996). It has been found that the locomotor circadian rhythm of female German cockroach is masked by the development of ovaries. However, ovariectomized females show a robust free–running rhythm with a circadian period similar to the male's. Its locomotion occurs mainly during the subjective night as it does for males under constant darkness condition (Lin and Lee, 1996). Therefore, the locomotion of both male and female German cockroach is under the control of a circadian clock.

The circadian period of the German cockroach is temperature compensated. The free-running period appeared a range 22.73 to 23.67 h between 19 ℃ and 33 ℃ (Dreisig and Nielsen, 1971). These results meet the essential criteria of the circadian clock and enforce the finding that the locomotion of the German cockroach is under the control of circadian clock.

Since German cockroach is a nocturnal insect, the daily locomotory patterns show a diel rhythm, with most locomotion occurring in the scotophase. However, movement and behavioral patterns of the German cockroach are significantly depended on the sex, age and stage of reproductive cycle. Sommer (1975) used radiotrace techniques to monitor the activity of a single cockroach at a time. He found that the activity of males was highest, followed by non-pregnant females, pregnant females, old nymphs (4 to 6 instars), and the young nymphs (2 and 3 instars). Young nymphs (1 and 2 instars) were relatively immobile, and tended to aggregate (Bret *et al.*, 1983 ; Ross *et al.*, 1984 ; Rivault, 1989). After 3 instar stadia, nymphs became more active, especially when the resources (food and water) were scarce, they tended to leave the aggregated sites and dispersed (Ross *et al.*, 1984 ; Rivault, 1989). Denzer *et al.* (1987) pointed out that the adults displayed activity 4 times higher than that of the nymphs.

Although the majority of results showed that male adults are the most active of adult stages (Sommer, 1975 ; Owens and Bennett, 1983 ; Silverman, 1986). Demark and Bennett (1995) used computerized moving-image analysis to show that non-pregnant females were the most active of all adult stages. On the other hand, pregnant females are the most sessile, and spending most of their time in and around the shelter (Lee and Wu, 1994). Active locomotion of male adults occurs primarily during the night and females showed daily arrhythmic locomotion under light-dark cycles and DD conditions (Lee and Wu, 1994). However, the female will increase their locomotion so that they may even come up to walk during photophase against its nature of light avoidance (Lin and Lee, 1996 ; 1998). In addition, the female German cockroach shows a cyclic pattern of reproduction, its locomotive activity coincided with its reproductive cycle (Lee and Wu, 1994).

5. Mate-finding behavioral rhythm

The coordination of timing is crucial to the reproductive success of nocturnal insect in the wild (Loher, 1979). Therefore, both sexes should synchronize their temporal movements (Lin and Lee, 1996). Because the German cockroaches tend to aggregate, they usually do not need to travel far to find mates (Schal *et al.*, 1983). If they are alone and sexually receptive, both females and males increase their locomotor activity (Lin and Lee, 1996, 1998). Sexually receptive virgin females not only increase locomotion to search for mates, but also exhibit calling behavior during which volatile sex pheromone is released (Tsai and Lee, 1997). For adult female cockroaches, locomotion serves various functions: for example, searching for resources, finding mates, and escaping from natural enemies or harsh environment. Since locomotion of females was individually monitiored in a motion detector box with food and water supplied *ad libitum*, and no enemy was present in the studies (Lee and Wu, 1994 ; Lin and Lee, 1996 ; 1998 ; Tsai and Lee, 2000), we expected that the locomotion expressed by the females was primarily mate-finding. The significant reduction of activity after mating confirmed this expectation.

The existence of male odor to trigger female's locomotor rhythmicity indicates that the underlying mechanisms of both mate-finding locomotor activity and sexual receptivity are the same: 1). Juvenile hormone (JH) is an essential factor. 2). The ovaries have no direct effects on mate-finding locomotion and sexual receptivity, but could affect both of them through JH. 3). Mating decreases the frequency of mate-finding locomotion and inhibits sexual receptivity when sperm is transferred successfully (Lin and Lee, 1998). Although normal females' daily activities change cyclically and coincide with their own reproductive cycle (Lee and Wu, 1994), they do not show locomotor circadian rhythm (Lin and Lee, 1996). Since ovariectomized females do express circadian rhythm as males do, and the virgin females will regain their circadian rhythm when male's odor is present, the findings indicate that: 1). Mate-finding locomotory activity is under the control of a circadian oscillator that allows both sexes to synchronize their movements. 2). This locomotory circadian rhythm can be masked by internal factors. 3). Under certain social conditions, the masked circadian rhythm can be uncovered (Lin and Lee, 1996).

6. The coupling system of two locomotor pacemakers

By the bilateral severance between the optic lobes and midbrain, the locomotor circadian pacemaker is found to be located in the optic lobes and it controlled the locomotor circadian rhythm through neural pathways in the German cockroach (Wen and Lee, 2000). When unilaterally optic tract was severed, the cockroach still showed locomotor circadian rhythmicity without significantly changing the circadian period or level of locomotor activity. Therefore, both of right and left optic lobe contain a circadian pacemaker competent to drive the locomotor circadian rhythm (Wen and Lee, 2000). From only one locomotor circadian rhythm expressed either in entrainment or free-running conditions, the two functionally redundant pacemakers are mutually coupled. Regardless whether the optic nerve severed or not, the two pacemakers are still mutually coupled and only one component rhythm is expressed (Wen and Lee, 2000). The two pacemakers of the German cockroach are strongly coupled. Otherwise, there are two split rhythms observed in crickets under LL when the optic nerves were unilaterally severed (Wiedenmann, 1983； Tomioka, 1993).

Although one pacemaker is sufficient to drive a locomotor circadian rhythm, an unequal contribution from each pacemaker in determining the overt circadian period is found. There is a major-minor coupling relationship between these two pacemakers. No matter on which side of the optic lobe the major pacemaker was located; these two pacemakers are mutually coupled to produce an overt locomotor circadian rhythm, with a circadian period determined by the major one. Since the photic information is conveyed by the pacemaker to the contralateral pacemaker through a neural pathway (Page, 1978；1983), it is reasonable to assume that light intensity influences the coupling strength of the pacemaker (Wen and Lee, 2000). Influences of light intensity on the coupling strength have also been reported in mammals (Daan and Berde, 1978) and crickets (Tomioka, 1993).

7. Conclusion

At the present day, we can tell the German cockroach lives in a time orderly life. We have proven that an endogenous biological clock provides time signals for its locomotion. Although we just paint a partial picture of "how the German cockroach tells time", the outline or at least the frame of the picture is clearly visible. We hope that a complete story of "how the German cockroach tells time" can be unveiled in a near future and we can report to you once again in the City of Science in Tunis.

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