# 行政院國家科學委員會專題研究計畫 期中進度報告

## 四紋豆象生活史策略之演化生態學：多次交尾、棄卵與學習行為(2/3)

## 期中進度報告(精簡版)

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計 畫 主 持 人 ：李後晶

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# 行政院國家科學委員會專題研究計畫期中進度報告

## 四紋豆象生活史策略之演化生態學：多次交尾、棄卵與學習行為(2/3)

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### 一、中文摘要

許多昆蟲都有棄卵行為的發生，即產卵在非寄主上。過去研究都認為棄卵是個體因為生理限制（例如：卵成熟能力差異、載卵壓力太大）而產生的不適應行為；然而近來也有學者提出適應性假說，認為棄卵是個體在缺乏適當寄主時維持卵持續成熟的機制，以便在寄主再度出現時能儘速將卵產出。為檢驗豆象棄卵行為的適應性假說，本試驗透過操作成功於 24 小時內將族群區分成棄卵與不棄卵個體，以便進行後續產卵行為及生活史特質比較。結果發現，棄卵與不棄卵個體在豆象族群中比例約為 3 比 1，透過解剖發現，棄卵雌蟲與非棄卵雌蟲卵成熟能力並沒有顯著差異。而曾經歷較好寄主經驗的棄卵雌蟲在面臨沒有寄主時，明顯較對照組延後棄卵的現象，因此否證棄卵主要乃受生理限制的假說。另提出棄卵為豆象面臨寄主缺乏時產卵決策的適應性假說，棄卵與不棄卵雌蟲對寄主接受度不同，棄卵雌蟲降低其寄主接受閾值，將有利於豆象族群開發新寄主。

**關鍵字**：四紋豆象、棄卵行為、產卵決策、寄主隔離、生理限制假說

### Abstract

It is puzzling that some insect species dump their eggs on unsuitable places in the absence of a host. This egg-dumping behavior, in previous studies, was generally considered to be maladaptive behavior under physiological constraints (i.e., female weight, egg maturation, and egg-storage ability). In this study, a physiological-constraint hypothesis of egg-dumping behavior in *Callosobruchus maculatus* was tested. It was first revealed that when deprived of hosts, the egg-number distribution was bimodal, and females within population could be differentiated into two groups by the number of eggs dumped, i.e., dumpers and non-dumpers. By dissecting the ovaries and comparing the life history traits of the two groups, we falsified the physiological-constraint hypothesis. Herein we integrated to form a new hypothesis from an egg-laying decision viewpoint. By changing the host acceptance threshold, the dumpers could be redefined as a conditional adaptation which can exploit the novel host.

**Keywords:** *Callosobruchus maculatus*, egg dumping, egg-laying decision, host deprivation, physiological-constraint hypothesis.

### 二、緣由與目的

Egg-laying decisions on hosts have

been well described in *Callosobruchus maculatus*. Mated females can readily discriminate host quality (Mitchell 1975; 1990; Messina 1991; Fox & Mousseau 1995). Also, female *C. maculatus* make egg-laying decisions by comparing the present host and the previous one (Mitchell 1975; Mark 1982). They even adjust their oviposition rate to cope with variation in host availability (Horng 1997; Horng *et al.* 1999). When host resources are deficient, they either lay fewer eggs or leave the inferior patch to search for a better one (Charnov 1976; Mitchell 1990). These oviposition behaviors are usually considered egg-laying decisions responding to cues from the host.

However, even without a host, most mated females of *C. maculatus* will still deposit several eggs on unsuitable substrates (Wilson & Hill 1989; Messina & Slade 1999). This is the so-called egg-dumping behavior. These dumped eggs will hatch, but quickly die from lack of nutrients (Wang & Horng 2004). Furthermore, because the *C. maculatus* female is a capital breeder in storage, trade-offs between traits, such as longevity and reproduction (Messina & Slade 1999), present and future reproduction, and reproduction and survival (Huang *et al.* 2005), are easily found in the population (Ellers & van Alphen 1997). Accordingly, egg-dumping behavior not only wastes fertile eggs but also reduces the longevity or future fecundity, and hence, in previous studies, it was generally considered to be a maladaptive behavior (Wilson & Hill 1989; Wang & Horng 2004).

Most behaviors affected by host absence are usually considered in terms of physiology, mainly concerning the egg-load pressure (Terkanian, 1993; Fletcher *et al* 1994; Rivero-lynch & Godfray 1997; Sadeghi & Gilbert 2000). Although the concept of being overloaded with eggs is widely accepted, the overwhelming state of egg-load pressure on the egg-laying behavior of *C. maculatus* has been challenged. In fact, virgin *C. maculatus* females are able to retain eggs in their bodies even if suffering from high egg-load pressure (Wang & Horng 2004), and mated females with high egg-load pressure can still disperse their eggs uniformly on hosts (Cope & Fox 2003). In addition, the physiological-constraint hypothesis not only confounds the egg-maturation ability and egg-storing ability but also ignores the body weight effect on fecundity, and thus cannot sufficiently explain individual variation in the number of dumped eggs (Messina & Slade 1999). Therefore, we argue that the so-called egg-dumping behavior is not merely the result of physiological constraints, but a life history strategy for exploiting novel hosts.

## 三、結果與討論

**Early detection of dumper and non-dumpers**

During the observation period,

dumpers did not hold eggs for over 24 h. The eggs of dumpers were mainly dumped in the early days of the oviposition period and hence, we provide the first operational protocol for judging if a female is a dumper in 24 h (Fig. 1). This suggests that the differentiation of egg-dumping behaviour did not occur when the females were approaching the end of oviposition, but happened in 24hrs after emergence, since the females were deprived of hosts. The total number of eggs dumped (*Y*) could be predicted very well by the number of eggs dumped in the first day (*X*). ($Y = 5.41 + 1.92X$, $R^2 = 0.863$, $P < 0.001$)(Fig. 2)

**Life history traits of dumpers and non-dumpers**

The average body weight of dumpers and non-dumpers was not significantly different (Table 1), and thus the trade-off between fecundity and longevity was evident. Non-dumpers lived about 8 days more than dumpers (*t* test: $t = -4.58$, $P = 0.0005$). Considering eggs in the ovaries, non-dumpers were able to mature sufficient eggs (28.8 ± 0.4) , but they retained these eggs in body rather than depositing them on the Petri dish (1.9 ± 0.7). Also, they laid eggs (80.5 ± 5.3) as well as dumper did when we provided them with abundant azuki beans(74.6 ± 3.5)($t = -0.95$, $P = 0.3555$) (Table 1).

**The host-abundant experience retarded the egg-dumping rate**

Dumpers that had experienced 5 beans took more time to dump the first egg (36.0 ± 5.5 h) in an empty Petri dish than those which had experienced only one bean (19.6 ± 2.1 h) or an empty Petri dish (15.7 ± 1.6 h) (ANOVA: $F_{2,14} = 9.01$, $P = 0.0006$) (Fig. 3). This result suggests that the egg load was not the only cause for egg dumping. The experience of relative host abundance may also be involved.

In this study, dumpers and non-dumpers had similar body weight but dumped distinctly different numbers of eggs (Table 1). This result suggests that differences between them did not result from different amount of energy accumulated in the larval stage but from distinct allocations of energy during ovipositing. In addition, the anatomic description of their ovaries proved that non-dumpers were able to produce eggs as well as the dumpers (Table 1). Also, they have ability to lay eggs when host was available (Table 1). Accordingly, the difference between them was mainly because non-dumpers did not deposit eggs as dumpers did under host absence.

But why the dumpers dumped eggs? According to the egg-loading hypothesis (Wilson & Hill 1989), it should be attributed to the inability of retaining eggs in the body when egg-load pressure is too high. However, the overloading with eggs cannot explain how the dumpers, suffered almost the same egg-load pressure, can hold eggs for 20 more hrs merely because of experiencing high-quality hosts (Fig. 3). Apparently, even though the egg loading could be one of the forces to drive egg deposition, it was not the only one and even not the crucial one driving the differentiation of oviposition behavior, when host was deprived. Based on our results, the mechanism controlling egg dumping is more complex than merely being overloaded with eggs.

However, the phenomenon about influence of host-experience can be more logically explained by a decision-making aspect. Reasonably, if egg-dumping behavior is an egg-laying decision from the dumper, it would be expected that the decision was influenced by dumper's previous experience of abundant host (Fig. 3) (Mitchell 1975; Mark 1982). In addition, the mated females' responses to a host-deprived environment were differentiated immediately (in 24 hrs), not at the late period of their lives (Fig. 1) and the eggs remain in dumpers' ovaries were significantly less than those in non-dumpers' (Table 1) (Wilson & Hill 1989; Wang & Horng 2004). Both implied that the egg-dumping behavior result from active egg-laying decision but not merely passive overloading with eggs.

Why did dumpers employ such an energy-consuming strategy? Besides the hypothesis proposed by Wang and Horng (2004), a view from life history strategy was proposed here. Dumpers may change their host acceptance threshold and thus offer the offspring a chance to survive on the novel host.

## 四、計畫成果自評

We successfully divided seed beetles with or without dumping behavior within 24 h and then could test the relative hypothesis. It was clarified that the mechanism of the differentiation was not because of physiological constraints but crucially resulted from different egg-laying decisions to non-host substrate. In addition, we further proposed an adaptive hypothesis of egg-dumping behavior as female beetle's life history strategy while facing novel hosts.

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**Table 1.** Life history traits of dumpers

and non-dumpers in *Callosobruchus maculatus*

| Trait | Dumper | Non-dumper | df | t | p |
|---|---|---|---|---|---|
| Weight (mg) | 6.8 ± 0.1 | 7.2 ± 0.3 | 15 | -1.28 | 0.2216 |
| No. of eggs dumped | 24.4 ± 3.1 | 1.9 ± 0.7 | 7 | 7.12 | 0.0002 |
| No. of eggs in the ovaries | 10.1 ± 1.3 | 28.8 ± 0.4 | 16 | -13.8 | < 0.0001 |
| Longevity (days) | 16.3 ± 1.6 | 24.8 ± 0.8 | 13 | -4.58 | 0.0005 |
| No. of eggs on hosts† | 74.6 ± 5.5 | 80.5 ± 5.3 | 15 | -0.95 | 0.3555 |

†Except for number of eggs on hosts, all traits in the table were measured during host deprivation.

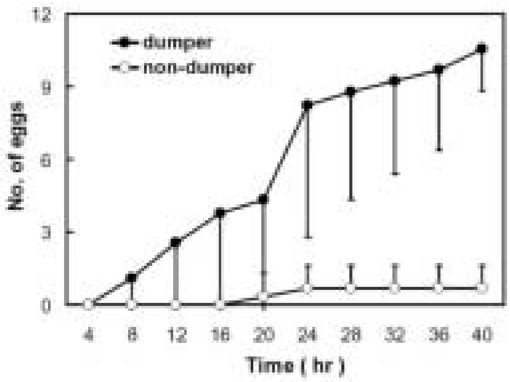


Fig. 1. Discrimination between dumpers and non-dumpers. The egg-dumping behaviour (measured by numbers of eggs dumped ± 2SD) of dumpers and non-dumpers could be distinguished within 24 h.

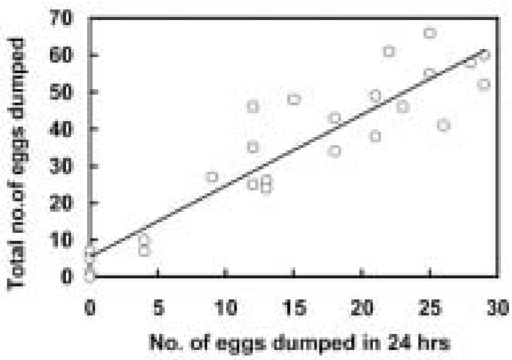


Fig. 2. Regression for the initial and total numbers of eggs dumped. The high correlation not only confirmed the reliability of the early detection of the two groups, but also allowed us to estimate the ultimate number of eggs dumped ($Y = 5.41 + 1.92X$, $R^2 = 0.863$, $P < 0 001$).

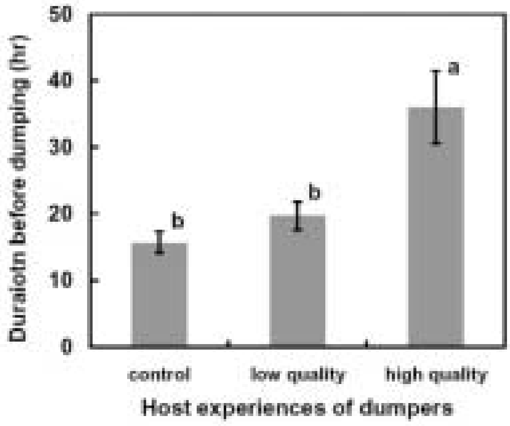


Fig. 3. Host-experience effect on the egg-dumping behaviour. Dumpers that had experienced 5 clean beans (high quality) took more time to dump an egg in an empty Petri dish than those which experienced a single bean (low quality) and those without host experience (control). This suggests that the egg load is not the only cause of egg dumping. The relative quality of the host experienced may also be involved. (ANOVA: $F_{2,14}=9.01$, $P = 0.0006$)

# 出國開會心得報告

台大昆蟲系李後晶教授

此次高登時間生物學會議在法國 Aussois 舉行，由於位處歐洲之 Alps，因此交通非常不便，我從瑞士日內瓦搭火車，須 6 小時的車程，其中 2 小時是在一小站等換車，那想到該路段每星期日修路，因此必須用計程車將客人載至 40 公里外的車站再接續旅程，由於不懂法文，一陣比手畫腳之後也無法溝通，幸好當時也有其他乘客需要轉載，其中一位女士可以說英文，如此才替我解決了所有問題，我也如願抵達 Aussois,展開一週的討論與開會的活動。

整個會議的主題是依據不同生物，例如微生物、植物、動物、人等為主題，每個小主題安排了 3 至 4 位演講者，大家針對每一主題之最新趨勢提出探討，雖然所用的研究材料不同，但基本的時鐘運作模式，或調整方法皆相當類似，顯示這個特徵經過長時期的演化，仍是生命所必須的，因此這些跨物種的特性成為聯繫所有與會者興趣的關鍵，當然每一類物種皆有其獨特性，這些特性是研究演化生態學的焦點，可惜在這次領域的研究者非常稀少，也沒有在出席會議者間引起大家的注意，這也是我對高登時間生物會議較為不滿的地方。因為每一參與者都需經過申請而獲得主席的同意，因此非主流研究者，很容易就會被排除在外，因此討論主題廣度較不足。

此次會議，很高興我的博士班研究生李琦玫也被接受參與會議，她首次與這個領域的出名學者齊聚一堂，聽講及參與討論相關的研究主題，尤其是她的研究成果也已正著手在寫，希望在一個月內就可以開始投稿專業期刊。此次會議帶給她很好的機會，可以將研究結果與國際同儕討論，不但可以發現自我缺點，也可吸收他人意見，使得成果討論會更有意義，增加被期刊接受發表的機會。這次的參與會議，對琦玫的幫助是很大的。

本次會議的最大收獲是知道我的研究方向與主流趨勢相符，尤其是琦玫的研究主題 PDF 在時鐘運作的角色，可說是目前相當熱門的題目，只是我們的材料是以德國蟑螂而不是果蠅這種模式昆蟲，不過非模式動物的研究可提供較多的比較資訊，對整體研究主題有絕對的必要，然而大部分學者還是較易忽視這類非主流的研究。

高登研究會議（Gordon Research Conference）是個自然科學界財團法人所支持的會議，每年都有上千個小領域的小型會議再舉行。它是一種非正式的研討會，將全世界該領域的佼佼者齊聚一堂，以自由交談及腦力激盪的方式針對共同的領域，希望激出推進研究方向的火花，在沒有保留的情境下，每位參與者都能有所收穫，只是所有參與者，都需經過主席的認可才能取得出席權，因此顯然主席們本身是這領域的頂尖科學家，然而太大的權利使得有些人會成為剛愎自用，所找的人就會形成幾個小團體，將大多數的科學家排斥在會議之外。然而我們在這領域研究的人員很少，因此也沒有太多的瓜葛，在參與這個會議時雖然無法得到太多的關注，也不會被排擠，因此我覺得我們應繼續參與這類的會議，一邊提升自我實力，一邊也可藉著認識一些出名學者，建立起交流管道，可增加我國在這領域研究成果被接受的機會。

在本次會議中，大家恭喜來自美國 Prof. Michael Yang 成美國科學院院士。算算在最近 4 年內，就有 5 位從事時間生物學的學者成為美國科學院的院士，顯示這個領域漸漸受到重視。因為從低等的單細胞生物至高等的人類皆具有生物時鐘，因此這個領域的發展就顯得非常的有意義，尤其是一些人類健康維持及疾病診治與生物時鐘的調整與規範有很密切的關係。而藥物的投給是與生物時鐘的相位有密切關聯，這一切基礎與應用的研究是和人類醫藥發展息息相關，因此學界開始給與這個領域應有的關注，於是才有很多這方面領域的學者一再被提升。我們鄰國日本也有很充足的研究人力在這個領域中，他們甚至有日本時間生物學學會的組織，每年聚會發表學術成果，因此在高登時間生物學會會議中也佔有很高的地位。我國目前真正從事這個領域研究的學者屈指可數，因此要結合成一個學會是非常困難的。然而許多睡眠醫學的研究者，可能參與這個領域的研究，如果我們能藉助台灣睡眠醫學會，可能可以將我國在相關領域的研究學者整合在一起。讓我們的研究成果可以更加充實精進，可能在不久的將來，我們也可以有台灣時間生物學會的產生，在這個研究領域也發揮我們的引響力。

本次會議中，很多來自歐洲的學者都在最近一、二年內加入了 EU-clock 的整合型計畫，這是歐盟近年來整合歐洲各國時間生物學者，希望讓各國的研究人才與資源能從基礎的生物時鐘運作至其在人類健康上的運用的研究整合在一起。不但可以在這個領域上與美國競爭主導地位，也加強歐盟成員國之間的科技合作。這個 EU-clock 的總召集人就是本次高登時間生物學的主席 Prof.Till Roenneberg，他是德國慕尼黑大學的生理學教授。他的一位研究生鄧資新博士目前正在中興大學農藝系任教，因此對台灣也深具好感，我也私下邀請他來台灣訪問，希望藉他的到訪，可以讓我國的時間生物學者齊聚一起討論一些合作議題，最好也能和 EU-clock 計畫掛勾，不僅可以提升我們這領域的研究水準，也可增加與國際學界交流的機會，尤其與歐盟各國做更緊密的合作，Prof. Roenneberg 初步答應明年初來台訪問一至二星期，屆時希望國科會能大力支持。

最後附上此次參加會議發表之 Poster，請參考。

# The knockdown effects of *pdf* gene expression on locomotor circadian rhythm of the German cockroach, *Blattella germanica* (L.)

Chi-Mei Lee and How-Jing Lee

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## Introduction

Pigment dispersing factor (PDF), a neuropeptide, is expressed specifically and constantly in the locomotor circadian clock cells of many insects. The function of PDF has not been determined. However, it is suggested that PDF might sever as the output and coupling signal of locomotor circadian clocks. In this experiment, we use RNA interference (RNAi) technique to study the function of PDF in the German cockroach. The effects of double-strand RNA (dsRNA) injection has been analyzed in molecular and behavioral levels.

## Results

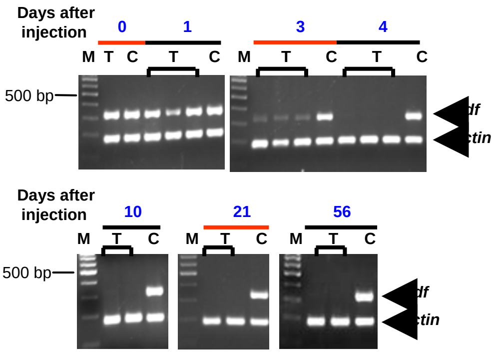


Figure 1. The RNA expression pattern in the heads of male German cockroaches. The total RNA is extracted and analyzed by RT-PCR. M: DNA marker, T: *pdf* dsRNA injected male cockroaches, C: depc. $H_2O$ injected male cockroaches.

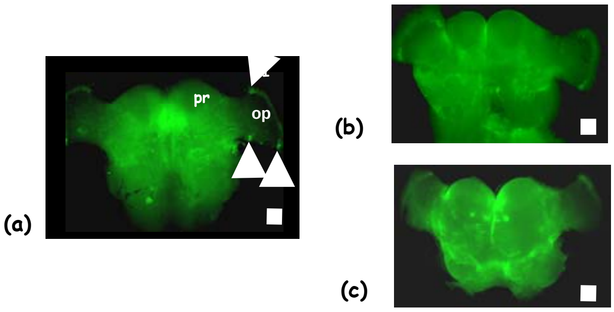


Figure 2. The immunostaining results of the brains of male German cockroaches. (a) The brain of a depc-treated $H_2O$-injected male cockroach. Three groups of PDF-immunoreactive (PDFir) cells were in the optic lobe (arrow). (b) and (c) The brains of a *pdf* dsRNA-injected male cockroaches. Group I and II PDFir cells can be weakly stained, but group III PDFir cells can not be stained. pr: protocerebrum; op: optic lobe; I: group I PDFir cells; II: group II PDFir cells; III: group III PDFir cells. Scale bar =100 μm.

Table 1. The effects of *pdf* gene knockdown on the expression of circadian rhythm in the male German cockroach. There is a significant difference between depc. $H_2O$ injection and *pdf* dsRNA injection (Chi-square test, $p<0.005$).

| | before injection | after injection |
|---|---|---|
| Injected with depc. $H_2O$ | 100% (n= 10) | 90% |
| Injected with dsRNA | 100% (n= 20) | 35% |

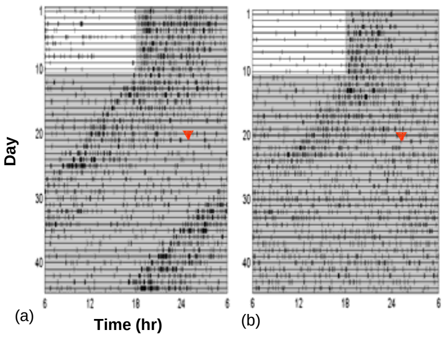


Figure 3. The actograms of (a) depc. $H_2O$ injected male cockroach and (b) *pdf* dsRNA injected male cockroach. From first to tenth days, the cockroaches are entrained in a light:dark = 12:12h schedule. After 10 days of entrainment, the cockroaches freerun in the constant darkness environment. The red triangles indicates the time of injection.

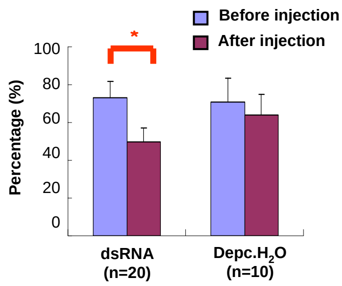


Figure 4. The percentage of daily locomotor activity in the subjective night period. The *pdf* dsRNA injection signi-ficantly decreases the activity ratio between subjective night and day (Student's t-test, $p<0.05$).

## Conclusion

1. *pdf* dsRNA injection can effectively block the *pdf* mRNA synthesis.
2. The knockdown of *pdf* gene expression causes the male cockroaches to become arrhythmic in locomotion.
3. PDF involves in the control of locomotor circadian rhythm.