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(Callosobruchus maculatus)之繁殖行為與生活史策略(1/3)

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四紋豆象 (*Callosobruchus maculatus*) 之繁殖行為與生活史策略

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一、中文摘要

四紋豆象雌、雄蟲間交尾行為存在性別衝突 (sexual conflict)：雌蟲受傷且壽命減少，而雄蟲於此行為表現中可能有兩種獲利，假說一：增加雌蟲產卵速率假說；假說二：降低雌蟲的再交尾率假說。本研究根據上述假說進行檢驗並探討在性別衝突下，兩性的適應策略及其機制。結果發現，四紋豆象交尾過程可分成三個階段：安撫期 (Patting)、靜止期 (Stability)及拒斥期 (Kicking)；再針對每個階段行為之間的相關性以及與生殖力 (fecundity) 之關係進行探討。結果發現，經中斷拒斥行為的雌蟲，其再交尾的時間也較正常交尾且有拒斥行為的雌蟲提早，支持假說二；但其總產卵量則較正常交尾且有拒斥行為的雌蟲為高，並未支持假說一。根據試驗結果推論：就雌蟲而言，生殖孔道的傷害代價 (cost) 是很大的，但雄蟲可能可以經由生殖孔道傷害所造成的傷口傳送一些物質進入雌蟲體內刺激生殖組織；就雄蟲而言，利用交尾器的硬刺將雌蟲生殖孔道刺傷，延長了雌蟲再交尾的時間以降低精子競爭，雄蟲因此而獲利。

Abstract

The aedeagus of the male seed beetle, *Callosobruchus maculatus* bears sclerotized spines that damage the female genital tract during copulation. The female repeatedly kicks the male towards the end of copulation. These functionally diametric sexual traits were proposed to form the basis of sexual conflict. There are at least two non-exclusive ways in which genital wounding could increase the fitness of male *C. maculatus*. First, increased-oviposition hypothesis. Second, delayed re-mating hypothesis. This study was designed to test these two hypotheses. *C. maculatus* copulation behavior was observed in detail and divided into three distinct stages, patting, stability, and kicking. Females that performed a longer kicking duration, may have sustained more genital damage, did not laid significantly more eggs. This result thus does not support the increased-oviposition hypothesis. Moreover, when copulation was interrupted (so that kicking did not occur), interrupted females laid more eggs than did females that were not interrupted. These results suggest that male-induced genital-tract damage is costly for females. In addition, we found that females that copulated completely postponed re-mating for a longer time than did females whose kicking behavior was interrupted. Therefore, a male increased his fitness by extending the female re-mating interval, and the eggs that a female laid during that re-mating interval would be fertilized with his sperm. Higher fertilization success favor males that cause genital damages in females. Male-male competition therefore leads to sexual conflict in *C. maculatus*.

Keywords: genital damage, sexual conflict, copulating behavior, *Callosobruchus maculatus*.

二、緣由與目的

Ethologists used to view courtship rituals and mating as harmonious ventures in which males and females cooperated to propagate their respective genes. However, more emphasis is now placed on the idea that there are conflicts of interest between males and females in courtship and copulation (Krebs and Davis, 1993). Because the costs and benefits of polygamy differ between males and females, copulation is not always a cooperative venture between the sexes (Crudgington and Siva-Jothy, 2000). Chapman *et al.* (1995) demonstrated that seminal fluid products from accessory gland of the male *Drosophila melanogaster* increase female mortality rates, and therefore impose a cost of mating. Since these products affect the viability of sperm from other males and delay re-mating by females, this cost of mating to females may represent a side effect of male-male competition. In another well-known example, the bed bug, *Cimex lectularius*, has a unique mode of copulation termed “traumatic” insemination during which the male pierces the female’s abdominal wall with his external genitalia and inseminates her body cavity (Stutt and Siva-Jothy, 2001; Siva-Jothy and Stutt, 2003). It was concluded that traumatic insemination is a coercive male copulatory strategy that results in a sexual conflict of interests (Stutt and Siva-Jothy, 2001).

Here we investigate a potential sexual conflict in the seed beetle, *Callosobruchus maculatus*, the intromittent organ of the male bears strongly sclerotized spines which are everted in the central region of the female’s genital tract and therefore damage the female genital tract during copulation. The female repeatedly kicks the male towards the end of copulation, trying to reduce the extent of this damage. These functionally diametric sexual traits were proposed to form the basis of reproductive conflict (Crudgington and Siva-Jothy, 2000). Also, it was proposed there are at least two non-exclusive ways in which genital wounding could increase the fitness of male *C. maculatus*. First, increased-oviposition hypothesis: genital damage may increase immediate oviposition rates because females perceive genital damage as a threat to survival and invest more in current reproduction. Second, delayed re-mating hypothesis: it may reduce the risk of sperm competition if females postpone re-mating, and thus delay receiving male damage to their genitalia. Therefore an extended interval between female matings and/or increased oviposition rates benefits the copulated male, as he will achieve sperm precedence. These effects of genital damage are therefore in the male evolutionary interest. Benefits to the male are no doubt the main driving force to form the basis of male-male competition leading to sexual conflict. However, these two hypotheses have not been tested to date.

Therefore, this study was designed to test the hypotheses that genital damage increases immediate oviposition rates or reduces female receptivity to further copulation. Since references concerning *C. maculatus* copulation behaviours have not described its detailed patterns or possible functions. We first quantified copulation behavioural pattern of the seed beetle and determined the effects of copulation behavioural sequences on female fecundity. Hence possible functions of each copulation sequences can be clarified. To test these two hypotheses and to determine their effects on female fecundity, we artificially interrupted female kicking behavior during copulation and measured the oviposition rate and re-mating rate of these females. Based on these results, the hypothesis of male-male competition leading to sexual conflict between male and female seed beetles was validated.

三、結果與討論

Copulation Behavioral Sequences and Female Fecundity

Copulation duration was positively correlated with female lifetime fecundity (r = 0.317, N = 47, P = 0.0269). Copulation duration and female longevity were also positively correlated ($Y = 0.0081X + 8.8789$, r = 0.328, N = 47, P = 0.0392). However, kicking duration was not significantly correlated with female lifetime fecundity (r = 0.1762, N = 27, P = 0.3793, Fig. 1). A female that exhibited a longer kicking duration and may have sustained more genital damage, did not laid more eggs. This result thus did not support the increased-oviposition hypothesis.

The total number of eggs per female varied between 0 and 115, with an average of 63.5±6.4 (N = 47). From *C. maculatus* copulation observations, a few females did not present stability behavior during copulating. Females that did not exhibited stability behavior laid very few or almost no eggs (0.6±0.4 eggs, $N = 5$) compared with females that copulated completely. Thus spermatophore and sperm transfer may proceed during stability stage.

Interruption of Behavioral Sequences and Female Fecundity

Eggs laid by females whose kicking behaviour or stability behaviour were artificially interrupted and the control treatment were significantly different (ANOVA: $F_{2, 87} = 29.48$, $P<0.0001$). Females whose kicking behavior was artificially interrupted laid significantly more eggs over their lifetime than did the control treatment, i.e., females that had copulated completely ($P<0.05$) (Fig. 2). However, there was no significant difference between these two groups during each 12-h interval for the first 3 days (Fig. 3). Thus the female that copulated completely and possibly sustained higher genital-tract damage did not increase her immediate oviposition rate. Therefore, these results also did not support the increased-oviposition hypothesis. Females that were interrupted stability behavior laid very few eggs compared with control group ($P<0.01$). Therefore, the result confirmed that spermatophore and sperm transfer proceeded during this stage.

Interruption of Behavioral Sequences and Female Re-mating Rate

During each interval, if the female that had copulated once accepted a second copulation, she would copulate with a second virgin male within 5 min. But if the female rejected the second copulation, she would consistently run away from the second male that was courting her, or kicked at the male that was attempting to mount during a 15-min observation. Females that copulated completely, delayed re-mating for a longer period than did females whose kicking behavior was interrupted ($T = 2.29$, $N = 35$, $P = 0.0253$). Re-mating time of females whose kicking behavior had been interrupted ranged from 24 to 48 h with a mean of 35.0±1.2 h (Fig. 4). While the re-mating time of the control group of females who had copulated completely ranged from 32 to 56 h with a mean of 39.0±1.1 h (Fig. 4). Therefore, females of the control group delayed re-mating for 4 h than those of the manipulated treatment. Most females that were interrupted stability behavior remated after 8h (Fig. 4). Female longevity was not significantly different between treatment and control group ($P>0.05$).

四、計畫成果自評

Benefits to the male are the main driving force to form the basis of male-male competition leading to sexual conflict of the seed beetle. Such conflicts are key to understanding reproductive biology. By interrupting the specific copulation sequences and then assessing its impact on the female's fecundity and re-mating rate, we provide the evidence to support that males do acquire some benefits from damaging females' genital-tract. We were able not only to provide quantitative estimates of the effects of females' genital damage but also to clarify the possible function of each behavioral sequence.

五、參考文獻

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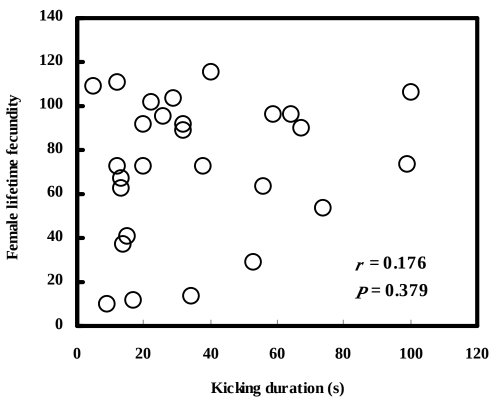

Fig. 1. Relationship between kicking duration and female fecundity of *C. maculatus*.

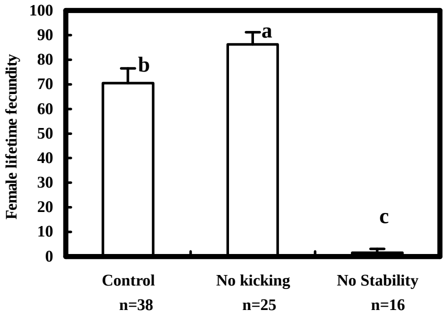

Fig. 2. Lifetime fecundity of female *C. maculatus* whose kicking or stability behaviour was interrupted and those had copulated completely.

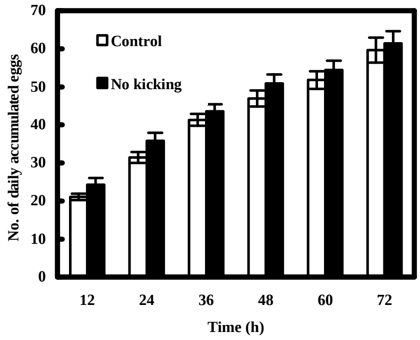

Fig. 3. Accumulated daily eggs of female *C. maculatus* whose kicking behaviour was interrupted and those had copulated completely.

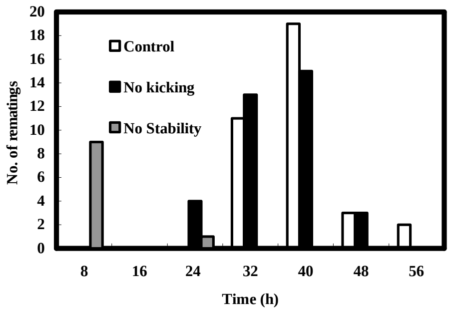

Fig. 4. Re-mating rate of female *C. maculatus* whose kicking or stability behaviour was interrupted and those had copulated completely. The re-mating time of manipulated treatment with no kicking (35.0±1.2 h) was significantly shorted than that of the control group (39.0±1.1 h) (T = 2.29, N = 35, P = 0.0253).