Effects of Artificial Selection for Walking Movement on Reproductive Traits in the Red Flour Beetle, *Tribolium castaneum*

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Summary

This study investigated the effects of variation of walking movement in reproductive traits in the red flour beetle *Tribolium castaneum*. The results revealed that reproductive success of the beetle is differed between genetically higher movement strain and genetically lower movement strain. These strains make it possible to investigate whether track of walking is differed between the strains. Moreover, parameters derived from the two strains provide an opportunity for computational simulation of evolution of variation in movement activity.

***Abstract*—Animal movement is associated with their reproduction. Many studies have reported a trade-off between wing size and reproductive success of females in flight insects. However, there are few studies that focused on other ways of movement (e.g., walking). In the present study, we have established the strains of higher (LW) and lower (SW) walking activity by artificial selection in the red flour beetle, *Tribolium castaneum*. Moreover, we investigated and compared of reproductive traits of each sex between the selected strains. In males, individuals from LW strains showed significantly higher mating success than SW strain. While, in females, individuals from LW strains showed significantly smaller egg than SW strains. The present results suggest that walking activity is associated with reproductive traits in both sexes.**

Keywords—walking, reproductive trait, locomotor activity, *Tribolium castaneum*

1. INTRODUCTION

Many animals often move, and it is important to investigate exploration of foods or mating partner, dispersal, and migration [1]. However, in many animals, there are individual differences in movement activity within a population [2]. Therefore, this phenomenon suggests that each individual with higher or lower movement activity has benefits and costs [2].

Trade-off costs including movement trait had investigated by many previous studies that focused on flight movement. For example, in cricket that show polymorphism of wing size, females with larger wing showed significantly lower reproductive success than females with smaller wing size [3]. Similar results were also reported in other flight insect species [3]. This trade-off costs of flight are considered as a factor of maintain of wing polymorphism within a population. Although investigation of relationship between flight movement and reproductive traits were conducted by many previous studies, there are few investigations focused on other movement ways.

The red flour beetle *Tribolium castaneum* has wing for flight, but they often move by walking [4]. In the previous study, we carried out artificial selection for walking activity of *T. castaneum* and established genetically higher walking activity (LW) strain and lower walking activity (SW) strains, [5]. In the present study, we compared reproductive traits of both sex between LW and SW strains.

2. MATERIALS & METHODS

Insect

Base population of *T. castaneum* have been reared in laboratory for over 40 years. The beetles were reared with food (whole meal : brewer's yeast = 19 : 1) in a incubator maintained at 25□ with a 16h photoperiod.

Artificial selection for walking activity

We carried out two-way artificial selection for walking activity of the *T. castaneum* for 22 generations. Virgin males and females were randomly collected from the base population, and each beetle was measured walking activity by monochrome CCD camera (SEYE130SN, Science-eye, Japan) for 30 min. Walking activity was analyzed by software (2D-PTV Ver. 9.0, Digimo, Japan). As LW strain 10 males and 10 females with higher walking activity were selected. Similarly, 10 males and 10 females were selected as SW strains. The details of methods and results of this selection experiment are

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described in Matsumura & Miyatake [5]. We used virgin individuals (14-21days old) in this study.

Reproductive traits

To assess male's reproductive traits, we measured the mating success. In mating success, we pike upped males from each strain at 22nd generation, and a male is put on Petri dish (50×10mm) with five females from the base population. Then, we observed mating behavior of a male, and recorded number of mating as mating success during 15 min.

To assess female's reproductive traits, we measured egg size and number of eggs. Females were randomly collected from each strain and paired with a male from the base population. They were allowed mating at once, and the female was put on Petri dish (30×10mm) with enough food. Female was allowed oviposition for 50 days. Eggs were randomly collected from each Petri dish, and egg size and number of eggs were recorded. These data were recycled from Matsumura & Miyatake [5, 6].

Statistical analysis

Data of walking activity, mating success, egg size, and number of eggs were analyzed by generalized liner mixed model (GLMM) with gaussian distribution, which strain as fixed effect and replicate line as random effect. In GLMM for egg size, female's ID was included as random effect. All analyses were conducted by JMP Ver.12.0 (SAS 2015).

3. RESULTS

Figure 1 shows results of direct responses of artificial selection for walking activity at 22nd generation. LW strain showed significantly higher walking activity than SW strain (F = 584.32, P < 0.0001). Females showed significantly higher walking activity than males in both strains (F = 11.12, P = 0.0009). There were no effect of interaction between strain and sex (F = 0.10, P = 0.7574).

Males from LW strain showed significantly higher mating success than SW strain (F = 5.16, P = 0.0257). Figure 2 shows results of reproductive traits of female. Females from SW strain showed significantly larger size of eggs than LW strain (χ^2 = 12.73, P = 0.0004). Number of eggs was not shown significantly difference between LW and SW strains (F = 0.30, P = 0.5874).

4. DISCUSSION

The present results showed differences in reproductive traits between LW and SW strains in both sexes. These results suggest that walking movement is also associated with reproductive traits. Furthermore, variation of walking activity may be maintained within a population, because there are trade-off costs of higher walking activity in both sexes. In the results of mating success of male, individuals of LW strain increased mating success than SW strain. This result suggest

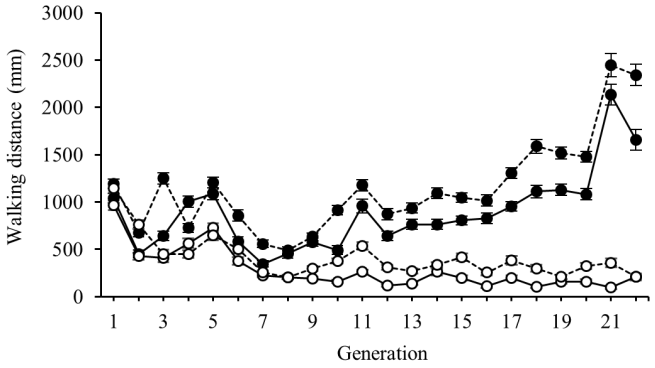

Fig. 1 Walking activity at 22nd generation of the beetles from LW (black circle) and SW (white circle) strains. Solid lines and dashed lines showed male and female, respectively. Error bars show SE.

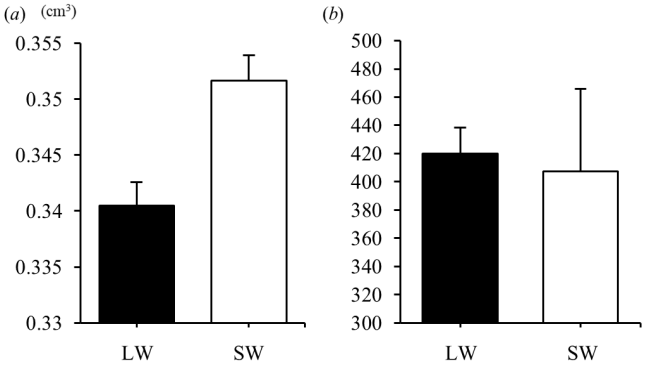

Fig. 2 Egg size (a) and number of eggs (b) of females. Black and white bars show LW and SW strains, respectively. Error bars show SE.

that male from LW strain may have higher encounter rate with the females due to their higher walking activity. Another factor that increase male's mating success is attractiveness [7, 8]. Males from LW strain may increase of investment to attractiveness than SW strain, not only encounter rate with females. On the other hand, males often show trade-off between mating success and fertilization success in polyandrous animals [9, 10]. Because resource is limited, males with more invest to mating success are often decreased investment for fertilization success. Therefore, males from SW strain may be increased investment for fertilization success than LW strain. We need additional studies that compare of fertilization success between LW and SW strains in the future.

In results of female's reproductive traits, females from LW strain had decreased egg size than SW strain, but not diffed in the number of eggs. That is, females from SW strain may invest more to reproduction than LW strain. In flight insects, females with larger wing size showed decreased investment to reproduction due to their use more energy for movement [3]. Females with higher walking activity may be decreased investment to reproduction due to higher activity, in *T. castaneum*.

Females showed significantly higher walking activity than males irrespective of strain used. The body size is larger in females than males of this beetle. Therefore, the moving speed may also higher in females than males. We need additional study that investigate evolutionary significance of sex difference in walking activity of this beetle.

The present study suggest that walking movement affects to reproductive traits in both sexes. There are few studies that focused on evolution of variation in walking movement. Therefore, this study reported fitness trade-off of higher or lower walking activity is important. We need additional studies that were investigated using animal species with different movement ways. Although we found trade-off costs of walking activity, we did not estimate evolution of variation for walking movement. We also need additional studies that conduct computer simulation of evolution of walking activity used by parameters from the selected strains.

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