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海外特別研究員最終報告書

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海外特別研究員としての派遣期間を終了しましたので、下記のとおり報告いたします。

なお、下記及び別紙記載の内容については相違ありません。

記

1. 用務地（派遣先国名）用務地： Halifax （国名： Canada ）
2. 研究課題名（和文）※研究課題名は申請時のものと変わらないように記載すること。
免疫・摂食・求愛から紐解く、動物の適応戦略の包括的理解
3. 派遣期間： 平成 29 年 4 月 1 日 ～ 平成 31 年 3 月 31 日
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Department of Psychology and Neuroscience, Dalhousie University
5. 所期の目的の遂行状況及び成果…書式任意 **書式任意 (A4 判相当 3 ページ以上、英語で記入も可)**
(研究・調査実施状況及びその成果の発表・関係学会への参加状況等)
(注)「6. 研究発表」以降については様式 10—別紙 1～4 に記入の上、併せて提出すること。

During the two years of this fellowship, I have successfully completed a planned research project, where I have published a book chapter and preparing two original research articles for publication. The latest results will also be presented at an international conference in August 2019. Below I will describe the detailed explanation of the research done in this project with a brief summary of background information.

In behavioural ecology, we often observe plasticity in physiological configurations and behavioural traits in a range of species. For example, animals often feed and explore less when infected than they do in normal conditions, which is called sickness behaviours. The sickness behavior may be adaptive since reducing resource demands from physiological compartments other than immunity may contribute to increase their chance of recovery, by preventing resources from being sequestered to those non-urgent systems. However, exceptions do exist, such that organisms can increase investment towards certain traits under infection. A good example for this is that animals can increase reproductive investments under infected condition, which is often referred to as ‘terminal investment’. This function (i.e. terminal investment) is often interpreted as a fecundity compensation. In other words, under a severe infection where life expectancy is low, it may be adaptive to abandon immunity and reproduce anyways since it may maximize the fitness. When we planned this study, it was unclear what factor(s) determines whether an organism increase or decrease reproductive output when infected.

To answer this question, we chose an insect species (the Texas field cricket, *Gryllus texensis*) as a model species. Insects have multiple advantages to answer this type of questions: (1) they are easy to handle, (2) their physiological system is simpler than higher organisms (e.g. mammals), and (3) they can circumvent ethical issues that we may confront when using mammalian animals. Among insects, crickets are advantageous because their relatively large body size allows us to collect sufficient amount of blood sample to measure multiple aspects of their immune system (e.g. phenoloxidase and lysozyme-like activities, key insect immune molecules (Cerenius, Lee, & Söderhäll, 2008; Kanost & Gorman, 2008). It is important to measure multiple aspect of immune function on each animal, because the immune system is a complex multifactorial system and is capable of shifting its emphasis on pathways over time (Armitage & Boomsma, 2010; Piñera, Charles, Dinh, & Killian, 2013; Srygley & Jaronski, 2018). Another advantage of using insect species is that female reproductive output can be quantified by counting the number and quality of eggs (Z. R. Stahlschmidt, Rollinson, Acker, & Adamo, 2013). Although we primarily focus on the effect of immune challenge on reproduction, we also take other intrinsic factors, such as age and dispersion capability, into account in the study design. Such multifactorial approach is particularly important, because the dynamic regulation of reproduction likely involves multiple factors (Duffield et al., 2018). There is evidence that age affects the effect of immune challenge on reproduction (Duffield, Bowers, Sakaluk, & Sadd, 2017). Also, especially in crickets, dispersion capability is associated with plasticity of reproduction (A. J. Zera, 2003, 2005). Thus we take the age and the dispersion capability into account for our study design. Also, our lack of knowledge of the physiological mechanisms mediating interactions between immune responses and reproduction (Schwenke, Lazzaro, & Wolfner, 2016) hampers our ability to precisely choose which molecular marker(s) to measure as a proxy for immune activity. Given the multifactorial, complex nature of the insect immunity, it should be necessary to measure multiple, at least two, immune-related molecules (Duffield et al., 2018).

Also, many cricket species consist of two discrete variants within a population: long-wing and short-wing variants, which show distinct reproductive outputs (Roff & Gelinas, 2003). The long-wing variant has a fully functional flight apparatus with long wings and functional flight muscles. The other variant, short-wing, has a reduced flight apparatus with shortened wings and histolyzed flight muscles (A. J. Zera, 2003, 2005; Anthony J. Zera & Denno, 1997). To minimize the variability due to the polymorphism from experimental designs, only one morph is often chosen in the analyses (Z. Stahlschmidt, O’Leary, & Adamo, 2014; Z. R. Stahlschmidt et al., 2013). In *G. firmus*, newly-emerged long-winged adults have pink (functional) flight muscles but they transform into white (histolysed) muscles, and the histolysis is associated with entering a reproductively active phase (Anthony J. Zera, Sall, & Grudzinski, 1997). In *G. texensis*, a similar phenomenon has been observed (AM, unpublished data). Although we provide females with a food limited diet in this study to uncover resource conflicts between immunity and reproduction, this characteristic of crickets may give rise to a potential experimental

difficulty, as the additional resources released by flight muscle abandoning can mask the physiological trade-offs (van Noordwijk & de Jong, 1986)(Anthony J. Zera & Denno, 1997; Anthony J. Zera & Harshman, 2001). In this study we note whether our long-winged females have white or pink muscles at the end of the experiment (i.e. day 36).

Given these backgrounds, we first conducted a literature survey, and then conducted an experimental study. The literature survey was a part of our attempt to put our study in a wide context of insect stress responses. The literature survey was published in a form of book chapter (Miyashita and Adamo, 2019). In the experiments, we have found that (1) females of *G. texensis* do not alter their reproductive output in response to immune challenges, (2) flight muscle histolysis has a strong link with reproductive output, (3) one of their immune factors may be a dual-functional molecule, being also involved in reproduction, and (4) the females may form a reproductive cluster with at least three classes that show distinct temporal reproductive strategies. Results will be published in at least two separate original articles. I am also planning to present these results in an international conference that will be held in August 2019.

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