Physiological Parameters Reflecting Longevity of a Parasitoid Wasp (Hymenoptera: Ichneumonidae)

Kouhei Matsushita, Kanako Ueno, and Takatoshi Ueno

Abstract*—Itoplectis naranyae* is a common parasitoid wasp inhabiting rice paddies in most part of Asia and is a candidate of biological indicators to assess biodiversity and eco-friendly agricultural practice in rice paddies. Here, we examined physiological factors affecting the longevity of *I. naranyae* to investigate its basic biology. For this purpose, life expectancy of the male and female was assessed without food (=carbohydrate) provision. As expected, the parasitoid had a much shorter lifetime under food deprivation. Curiously, both forewing length and body weight were not significant indices reflecting the longevity of male and female *I. naranyae*. Therefore, individual differences in life expectancy under starvation are not simply predicted with body size. Body weight loss during the lifetime instead correlated positively with the longevity, regardless of the sexes. Thus, weight loss, rather than body size, reflects the longevity probably because it reflects nutritional storage available for sustaining the life of *I. naranyae*.

***Keywords*—**natural enemies, rice fields, bioindicator, pest management, fitness.

I. Introduction

PARASITOID WASPS are well known as natural enemies of insects, and many of them play an important role in suppressing agricultural pests [1—3]. Because of their importance in crop production, many researchers have focused on ecology, life history, behavior, taxonomy and physiology of parasitoids [2, 4]. Biological control, i.e., use of natural enemies in pest management or control, has been a central subject in the field of applied entomology [3, 4].

Itoplectis naranyae Ashmead (Hymenoptera: Ichneumonidae) is a parasitoid wasp attacking a variety of lepidopterans [5—7]. It is a solitary species in which only one larva can reach maturity on each host even when more than one egg are deposited in one host [8, 9]. *Itoplectis naranyae* usually parasitizes host pupae and prepupae [7]. Importantly, this parasitoid is widely common and abundant in rice paddies, and attacks serious pests of rice, such as rice leaf folder [7, 10, 11, 12]. *Itoplectis naranyae* is thus a significant natural enemy of rice pests, contributing to natural control in the ecosystem of rice paddies.

Recently, we have highlighted the usefulness of natural enemies, including *I. naranyae*, as biological indicators in rice paddies where eco-friendly agricultural practices have been made [12, 13]. Eco-friendly practice is one of the main key features in sustainable agriculture. There are strong demands to practice eco-friendly agriculture or eco-agriculture with conservation and rural development strategies, which focus on an integrated approach to conserving biodiversity to enhance sustainable agricultural production and improve livelihood [14]. However, it is often difficult to evaluate in what degree a given agricultural practice is environmentally friendly. Use of biological indicators is ideal for assessing of agro-biodiversity and eco-friendliness of farming practices [13, 15, 16].

The parasitoid wasp *I. naranyae* is one of the candidates of biological indicators in rice paddies because its abundance reflects both biodiversity and farming practice [12, 13]. For example, the density of the parasitoid is negatively correlated with how much insecticides have been applied while it is positively correlated with diversity of the arthropod fauna in a given rice field.

However, many aspects of the biology of *I. naranyae* remain unknown though laboratory studies have been conducted to reveal sex allocation, host discrimination, super- and multi-parasitism behavior, etc. [8, 9, 17—19]. In particular, it is largely unknown in what environmental conditions the parasitoid can become abundant in the field. Our recent study has revealed that host density is a significant factor influencing parasitoid density (Ueno, unpublished).

Another factor may be the availability of food resources for adult *I. naranyae*. Because adults of many parasitoid wasps generally require food (e.g., sugar source) to sustain their life and activity [20, 21], lack of food resources in agricultural environment can result in low density of parasitoid wasps [22, 23]. Dependence on food may differ among parasitoid wasp species, however. It is not well understood in what degree *I. naranyae* rely on food resources during the adult stage. Reliance may differ depending on the size, sexes and age but few studies have addressed the importance of, or dependence on, food in *I. naranyae*.

Longevity or life expectancy is a good measure to evaluate such reliance on food in parasitoid wasps but, again, little information is available for what factors could affect the longevity of *I. naranyae*; it is assumed that a variety of physiological and environmental factors could affect the longevity.

The purpose of the present study was to reveal the relation of physiological parameters to longevity of *I. naranyae*. For this purpose, analysis was made to examine the relationship between body size and longevity or mortality of *I. naranyae* when no food was given. Under food deprivation, parasitoid life expectancy would depend on nutritional resources that

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parasitoids at emergence carried over from the larval development. The results would be useful as basic knowledge on factors affecting life expectancy of *I. naranyae*.

II. Materials and Methods

A. Parasitoid and host

Basic rearing procedure of *Itoplectis naranyae* was reported in Ueno and Tanaka [7] and Ueno and Ueno [16]. A parasitoid laboratory colony was established with adult parasitoids collected from rice paddies in Fukuoka City, Japan. Wild-caught female *I. naranyae* were individually placed in plastic cups (10 cm in diameter, 4.5 cm in height), together with tissue paper saturated with a 20% honey solution. The tissue paper was replaced twice a week thereafter. The plastic cups were kept at 20 ± 1°C, 60—70% RH under a photoperiod of 16L:8D.

The parasitoid colony was maintained on pupae of a laboratory host, *Galleria mellonella*. Host cocoons containing pupae were presented to female parasitoids in the cups. After parasitization, hosts were removed from the cups and were held at 25 ± 1°C under a photoperiod of 16L:8D, until adult parasitoid emergence. Newly emerging females were placed individually in the plastic cups, and were paired with a male. Then, they were maintained as mentioned above.

B. Laboratory assays

Longevity of adult *I. naranyae* was investigated under laboratory conditions at 25 ± 1°C, 60—70% RH under a photoperiod of 16L:8D. Newly emerged adult parasitoids were collected from rearing cups, sexed and then weighed with a minimum accuracy of 0.1mg using an electronic balance, in order to measure fresh body weight at the time of wasp emergence. The parasitoids were then placed individually in plastic cups for assay.

The parasitoids were provided with water only during their lifetime. Tissue paper saturated with water was attached on the inside of the plastic cups. Tissue paper was replaced every two days. Parasitoid mortality was recorded every day until all parasitoids died. When died, the weight of dead parasitoids was measured with an electronic microblance. Forewing length of the parasitoids also was measured under a stereomicroscope. In all, 28 females and 23 males were used in this experiment.

Statistical analyses were made with the aid of JMP version 9.0 [24]. Survival analyses assuming Weibull distribution were applied to examine the effects of biological parameters on wasp longevity. Regression analyses and ANOVA, where appropriate, were also made to analyze the relationships between two parameters.

III. Results and Discussion

Male *I. naranyae* lived on average 5.93 ± 0.62 (with SE) days while female *I. naranyae* had a mean longevity of 5.65 ± 0.72 days (Fig. 1). No significant difference was detected for the longevity between the sexes (Log-rank test; df = 1, x^2 = 0.039, P = 0.84).

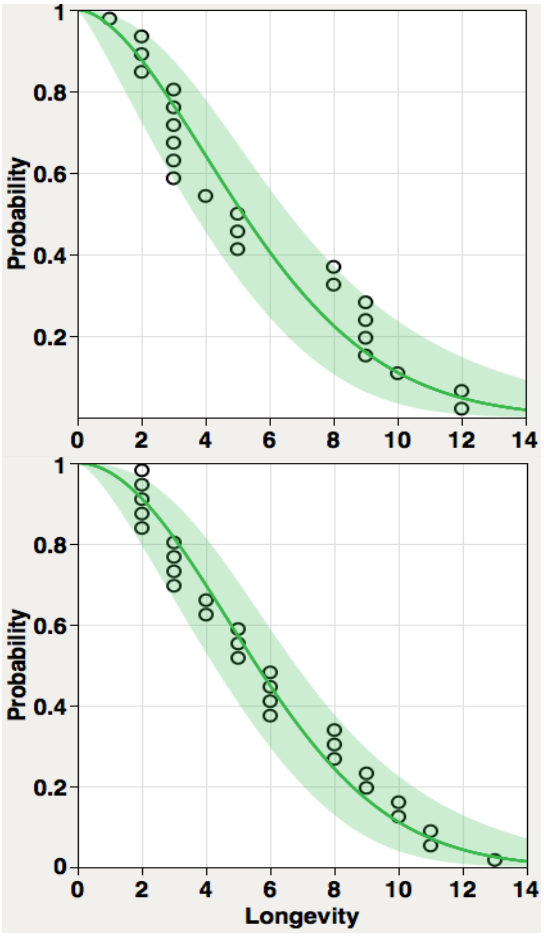

Fig. 1 Survival curves of male (above) and female (below) *Itoplectis naranyae*. Longevity in days is shown. The curves are estimated with Weibull fitting, and green areas show 95% confidence intervals

Body size indexes did not affect the longevity of the parasitoids though the average body weights differed between the sexes; females (21.1 ± 0.7mg) were heavier than males (17.3 ± 0.7mg) (ANOVA; df = 1, F = 14.3, P = 0.0004). Forewing length of *I. naranyae* had no significant relations with longevity of the males (Regression analysis; t = 0.72, P = 0.48) and females (t = -0.44, P = 0.67) (Fig. 2). Likewise, body weight at the time of parasitoid emergence had no significant influence on parasitoid longevity (Regression analysis; t = 0.24, P = 0.81 for males; t = -1.05, P = 0.30 for females) (Fig. 3). Both indexes similarly reflected the size of parasitoids, and correlated significantly each other (Regression analysis; r^2 = 0.64, t = 6.14, P < 0.0001 for males; r^2 = 0.73, t = 8.34, P < 0.0001 for females) (Fig. 4).

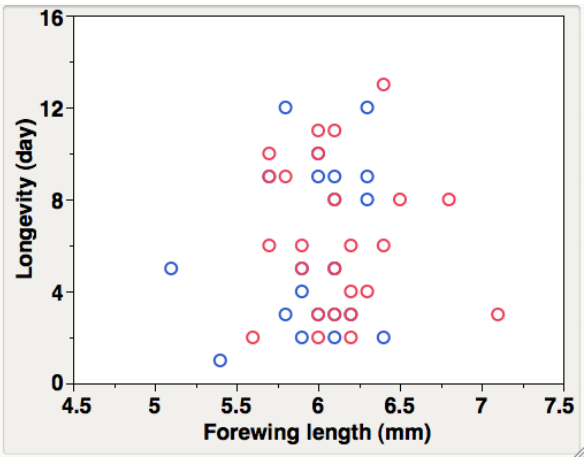

Fig. 2 Relationships between forewing length and longevity of male (blue) and female (red) *Itoplectis naranyae*. No significant relations were detected with survival analyses ($P > 0.05$)

On the basis of body weight at the time of emergence and death, the loss of body weight during the lifetime was estimated (subtraction of body weight of the dead from that of newly emerged wasp) and was used as an index of consumed nutritional stock during the lifetime. Curiously, regression analyses detected significantly positive relationships between weight loss and longevity for both male and female *I. naranyae* ($r^2 = 0.34$, $t = 3.28$, $P = 0.0035$ for males; $r^2 = 0.25$, $t = 2.93$, $P = 0.0071$ for females) (Fig. 5). Weight loss did not correlate with the two body size measures, however ($P > 0.05$).

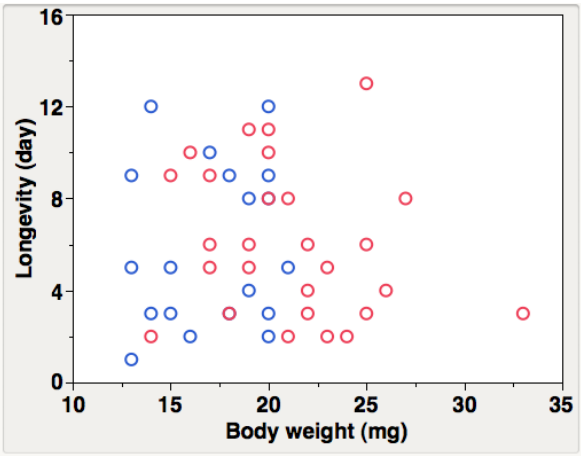

Fig. 3 Relationships between body weight at the time of wasp emergence and longevity of male (blue) and female (red) *I. naranyae*. No significant relations were detected with survival analyses ($P > 0.05$)

The average longevity of *I. naranyae* in the present study was much shorter than that of the previous study under food provision; adult *I. naranyae* can live more than 3 weeks at 20°C and more than 2 weeks at 25°C when sugar source is provided [7, 25]. It is well established that provision of carbohydrates can greatly extend parasitoid longevity [21, 23]. This fact suggests that adult parasitoids upon emergence from their host carry a limited resource available for sustaining their life. Thus, many species of parasitoids, including *I. naranyae*, require, during the adult stage, food to maintain their life.

In animals, it is often evident that higher body weight or body mass is linked to shorter life span within a species level though larger animal species live longer [26]. This is not the case for most of insects; larger individuals generally have higher life expectancy [27—29]. Our current study allowed analysis of the relationship between body size and longevity or mortality in *I. naranyae*, and the finding indicated that body weight or size itself has no influence on the longevity for both males and females (Figs. 2 & 3). This finding contrasts with general observations for insects.

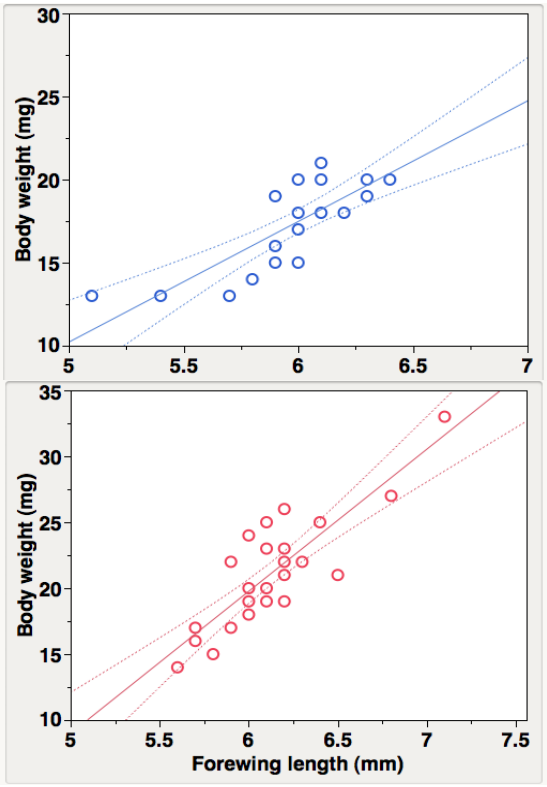

Fig. 4 Relationships between body weight at the time of wasp emergence and forewing length of male (blue) and female (red) I. naranyae. Significantly positive relations were detected with regression analyses ($P < 0.001$). Dot lines show 95% confidence intervals

Studies with parasitoid wasps have generally shown positive relationships between body size and fitness parameters like fecundity and longevity [2, 28, 29]. Similarly, larger female *I. naranyae* can produce more eggs at any one time and during their lifetime and have longer lifetime [25]. The present results therefore contrast with previous observations for the same parasitoid species. We used body weight at wasp emergence as an index of body size, which might reflect the amount of resources that each individual could store during the larval development. Contrary to expectation, however, no evidence was obtained that individuals with higher body weight were more likely to have higher life expectancy for both sexes (Fig. 3). The key factor causing the difference should then be whether or not carbohydrate food is given for the parasitoids.

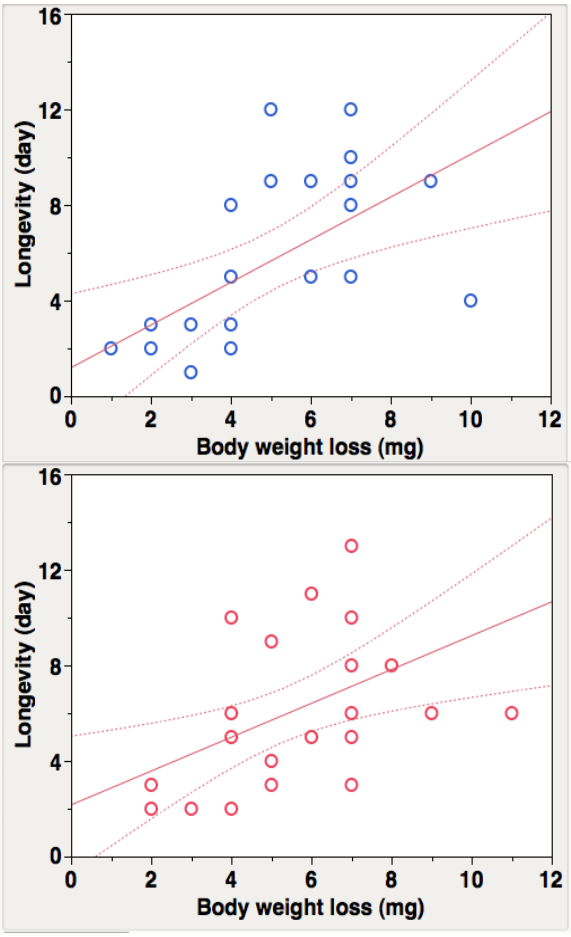

Fig. 5 Relationships between weight loss during lifetime and longevity of male (above) and female (below) *I. naranyae*. Significantly positive relations were detected with regression analyses ($P < 0.005$). Dot lines show 95% confidence intervals

We also found no gender differences in longevity despite a distinct difference in body size. The difference in body size between male and female *I. naranyae* arises because female *I. naranyae* are more likely to lay female eggs on larger hosts, which causes females being larger than males [7, 25]. A previous study has shown longer life expectancy for female *I. naranyae* under food provision [7]. Again, under food deprivation, biological factors affecting longevity appear to be different, and sex difference in potential life expectancy disappears in *I. naranyae*.

Although it is known body size is associated with longevity and slow aging, such an association may arise only when organisms can attain physiological potential for longevity. In our laboratory assays, no carbohydrate sources were provided for test parasitoids. Under this condition, parasitoids may sustain their life using nutritional stocks obtained during the larval development, as possible as they can, but the stocks may not be enough or may be limited for maintaining their activity. Larger individuals may stock more nutrients usable for reproduction such as egg production but such nutrients may not be usable for sustaining their life. As a consequence, they die shortly, regardless of the body size.

Another finding in the present study was that the loss of body weight during lifetime positively related to longevity of both male and female *I. naranyae* (Fig. 5). This suggests that body weight loss reflects the amount of resources that each individual has used during its lifetime. It would be hence reasonable to assume such resources are used for maintaining the life under starved conditions. However, the amount of consumed resources was not associated with body size in our study. It is thus unclear what factors are involved in individual variation of the consumption of resources for life maintenance.

Parasitoid wasps are among one of the most diversified groups of insects with a variety of life-history traits, fascinating many researchers in evolutionary and behavioral ecology [2, 4]. They are also of particular importance in biological control and integrated pest management because they play an essential role in controlling insect pest populations [1, 3]. Additionally, the present study demonstrates that parasitoid wasps can be valuable model organisms to investigate physiological aspects of life expectancy and aging.

IV. Conclusion

Unlike many other cases known for parasitoid wasps, the longevity of the solitary pupal parasitoid *Itoplectis naranyae* is not influenced by their forewing length and body weight at the time of wasp emergence from host when no sugar sources as food are given though body size (wing length and body weight) differs considerably between males and females and among individuals. Instead, body weight loss during the lifetime is positively associated with longevity of the parasitoids. This means that the weight loss can reflect the amount of resources usable for maintaining their life. However, the amount of resources should not relate to the body size of *I. naranyae*. Thus, individual differences in life expectancy under starvation are not simply predicted with body size, and physiological parameters affecting life expectancy under food deprivation remain to be examined in future studies.

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