Nutrient Acquisition, Allocation and Utilization in the Pupal Parasitoid Wasp Itoplectis naranyae Ashmead (Hymenoptera: Ichneumonidae) in Relation to its Reproductive Strategy

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Chapter 1. General Introduction

Nutrition is one of the most important factor influencing reproduction and survival in all organisms, but the strategies of acquisition and allocation employed are different and affected by a lot of factors. The primary aim of evolutionary ecology has been to explain the divergent patterns in life-history among and within animal species. Insect parasitoids are one of the most diverse groups of insects with various life-history strategies, and have been shown as an excellent model organism in evolutionary ecology. The patterns of nutrient allocation, utilization, and acquisition in parasitoids are important in understanding their reproductive strategies (Zhang *et al.*, 2004; Bernstein and Jervis, 2007; Jervis *et al.*, 2008). Parasitoids can show species-specific differences in reproductive strategies, which have attracted numerous biologists and evolutionary ecologists, and it is an essential constituent of parasitoids in biological control (Zhang *et al.*, 2004). Moreover, the reproductive strategies of natural enemies, including parasitoids, are fundamental in the evaluation of a potential biological control agent (Jervis and Kidd, 1986).

Parasitoids acquire nutrients during both larval and adult stages. The development of parasitoids before emergence is completed inside the host (Sequeira and Mackauer, 1992, 1993; Harvey and Strand, 2002; Harvey *et al.*, 2004). As a result, host resources for parasitoid development are often highly limited (Slansky, 1986). Immature parasitoids use host nutrients to support their development, and a part of these resources is also allocated to egg production in the adult stage (Godfray, 1994; Harvey, 2005). After emergence from host, adult parasitoids feed on sources of carbohydrates such as honey, honeydew and nectar to support their daily maintenance and reproduction (Boggs, 1981). Many species exhibit a behavior called host-feeding, which is the consumption of the host body fluids and tissues, to acquire the crucial nutrients (mainly protein and lipid) for egg production (Jervis and Kidd, 1986; Heimpel and Collier, 1996).

Nutrient acquisition and allocation strategies of parasitoids are critically correlated to life-history traits, i.e., the modes of egg production and parasitism. Flanders (1950) divided parasitoids into two types in terms of egg production: (1) Pro-ovigenic species that emerge with a full egg complement and do not mature additional eggs during adult life, which means the nutrient stored in larval stage should mainly be allocated to reproduction; and (2) Synovigenic species that emerge with no or few mature eggs and continue to mature during the adult stage, so the nutrient stored in larval stage should represent only a part of resources to be used for reproduction.

On the other side, Askew and Shaw (1986), according to the host performance after parasitism, divided parasitoids into idiobionts and koinobionts. Usually, strictly pro-ovigenic species are koinobionts, which produce small, yolk-deficient eggs. In those species, the fecundity is very high in their early adult stage, but decreases fast with age, and the adult diet is utilized for their activities and maintenance. In contrast, extremely synovigenic species are mostly idiobionts, which produce large, yolk-rich eggs in compensation of the low fecundity; adult nutrient would partly invest to reproduction.

Besides those external factors, internal factors such as body size also affect parasitoids' fitness. When considering reproductive resource allocation, female body size is a crucial trait in life history (Jervis *et al.*, 2008). Body size, which directly reflects the resource acquiring and/or storing capability of an individual, is one of the key traits because it is correlated with life-history parameters such as mating efficiency, dispersal capability and most importantly, reproductive success (Ueno, 1998, 1999b; Ellers *et al.*, 1998). Theoretically, larger body size enables parasitoids to store more energy and to produce more eggs, resulting in longer life span and higher reproductive success (Ueno, 1999b; Rivero and West, 2002; Ueno and Ueno, 2005). Numerous laboratory studies have reported the positively correlation between body size and life-history traits such as longevity or fecundity in parasitoids (Nakamura, 1995; Bezemer *et al.*, 2005; Harvey, 2008).

The evolution of life history models assume that trade-offs will arise when two traits are limited by the same resource, such as between early and late reproduction, between body size and fecundity and between reproduction and longevity (Sterns, 1989; Roff, 1992). Generally, trade-off emerges when a change in one trait leads to an increase in some fitness while another trait also changes at the same time, which leads to a decrease in other fitness. Under the constraint of some conditions, a trade-off is a correlation between traits -- a positive change in one by itself is opposed by a negative effect in another (Jervis and Kidd, 1986; Ellers *et al.*, 1998; Roff and Fairbairn, 2007).

For example, an investment of more internal resources towards egg production during adult life is at the cost of a reduced life-span (Harvey, 2008). Many factors can constrain resource availability including ecological conditions and the structure of an organism's life cycle, especially in natural field. Thus, the developmental, physiological and morphological traits exhibit a species specialization.

Study organism

Itoplectis naranyae Ashmead (Hymenoptera: Ichneumonidae) (Fig. 1-1) is a solitary endoparasitoid wasp that attacks a variety of lepidopterous pupae (Ueno and Tanaka, 1994). This parasitoid is widespread and abundant in rice paddies of Japan and other East Asian countries, where it is an important natural enemy of lepidopterous pests such as rice leaf folder (Ueno and Tanaka, 1994).

This species produces relatively large yolk-rich eggs and has a relatively long lifetime (Ueno, 1998). Ueno and Ueno (2007) have demonstrated that it is typically synovigenic, and host-feeding positively affects its egg production. The fecundity is relatively low and only a small fraction of eggs is stored in the ovary throughout lifetime. Female *I. naranyae* carries no mature eggs at eclosion, but fecundity quickly increases even when hosts are unavailable. They can produce about 30 eggs without host-feeding using nutrients stored during the larval stage. Host feeding allows females to produce additional eggs, but there is a three days' delay between the host-feeding behavior and egg maturation (Ueno and Ueno, 2007). *I. naranyae* is a destructive host-feeder, so the hosts that are used for host-feeding would be not appropriate for oviposition (Ueno, 1998; Ueno and Ueno, 2005). Thus, when encountered a host, female has to make a decision between ovipositing and feeding as food (Ueno, 1998).

Wild *Itoplectis naranyae* females were collected from grassy fields on the shore of the Umigawa River, Higashi-Ku, Fukuoka City, and then placed individually in plastic containers (7.5 cm in diameter, 4.5 cm in height). Absorbent cotton saturated with 20% honey solution was offered as food source, and the cotton was replaced twice a week. Parasitoids were incubated under a controlled condition (20 ± 1℃, 60 – 80 % RH, 16:8 h L:D regime). The pupae of greater wax moth *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae) were used as hosts. Host cocoons with fresh pupae were offered to female *I. naranyae* in the container. After parasitization, the hosts were removed from the container and were incubated at 25 ± 1℃ until parasitoid emergence. The voucher specimens were placed in the Laboratory of Insect Natural Enemies, Kyushu University.

Host

Galleria mellonella is a moth of the family Pyralidae and the only member of the genus *Galleria*. It is widespread in most of the world as a pest of honeybee. They were mass-reared in plastic cases (30 cm in length, 20 cm in width, 5 cm in height), and artificial food – a mixture of wheat bran, honey, bee wax, glycerin and yeast, was presented in the cases. The cases were placed in a climatic incubator at 30 ± 1℃ and 60 – 80 % RH. After pupation, the fresh cocoons were collected and used in the experiments. The rest part of hosts was stored in a refrigerator to prevent them from further development.

Fig. 1-1. Females of the Ichneumonid parasitoid *Itoplectis naranyae* Ashmead (Hymenoptera: Ichneumonidae). (A) visiting a flower to feed on nectar in the field; (B) parasitizing the pupa of greater wax moth *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae).

Thesis outline

The present thesis was performed to examine the effects of nutrients on the life-history reproductive strategies in the pupae parasitoid *Itoplectis naranyae*. The objectives of this study were:

Chapter 2 examined the sugar source availability on the longevity and fecundity of female *I. naranyae*. The body size was employed as a parameter to evaluate the effect on life-span.

In Chapter 3, the benefit of host-feeding and the cost of reproduction of female *I. naranyae* were discussed. The impact of host availability and body size on female survival was tested, and the interaction of these two traits was analyzed.

In Chapter 4, in response to a fixed amount of different food sources, the strategy of nutrients allocation on the egg production and maturation in the later life stage of *I. naranyae* was examined. The effect of body size on fecundity was also evaluated.

Chapter 5 addressed the investment to eggs under variable conditions of adult nutrient types and body size in adult stage of *I. naranyae*.

In Chapter 6, the effect of egg size on the fitness traits of larval *I. naranyae*, including hatching time, developmental time, survival, and sex ratio was assessed.

Based on the results above, I summarized life time strategies of *I. naranyae*, and emphasized the flexibility and variation of reproductive strategies under the constraints of both internal and external factors. Hence, the benefit of such flexible strategies was discussed, and the potential prospect as a successful biological control agent in the field was evaluated.

Chapter 2. The importance of food for female reproduction of *I. naranyae*

2.1 Introduction

All of organisms need a variety of nutrients, such as carbohydrates, proteins, and lipids, to support their development, reproduction and daily maintenance. Classical life history models assume that trade-offs will arise between reproduction and survival when resources are limited (Sterns, 1989; Roff, 1992). Likewise, trade-offs have commonly been detected between variable life history traits (Jervis and Kidd, 1986; Ellers *et al.*, 1998; Roff and Fairbairn, 2007; Harvey, 2008). For example, an investment of more internal resources towards egg production during adult stage is at the cost of a reduced life-span, which is a trade-off between reproduction and survival (Harvey, 2008). Many factors can constrain resource availability including ecological conditions and the structure of an organism's life cycle.

Parasitoids lay their eggs on or in the body of other insects, and the larvae develop by feeding on the host and eventually kill it. Nutrient acquisition and allocation can critically impact the fitness of parasitoids (Rivero and Casas, 1999; Jervis *et al.*, 2008). Food acquisition by adult parasitoids was described by numerous researches in parasitoid biology and biological control, and information regarding the effect of feeding on the parasitoids' key life-history variables, such as fecundity and longevity. Nutrient limitation or stress might affect fitness of parasitoids in many aspects, such as host-finding and dispersal efficiency (Nakamatsu and Tanaka, 2003). Moreover, parasitoids should be under selection to optimize utilization of limited resources to competing fitness functions such as reproduction and survival. Carbohydrates, for example, obtained by adults from host or non-host resources enhance the reproduction and survival of parasitoids (Boggs, 1981; Fadamiro and Chen, 2005; Lee *et al.*, 2006; Ho and Ueno, 2011). Most of daily maintenances are also from sugar sources. Carbohydrates are the only nutrient used for flight by Hymenoptera and this nutrient class is rapidly depleted if adults are starved (Harrison and Fewell, 2002). In the absence of carbohydrates, fat body reserves may be metabolized for maintenance instead (Ellers *et al.*, 1998, 2000). Proteins and lipids obtained through host feeding or other sources increase the production of yolk-rich eggs by allowing females to invest less reservation in maintenance functions. Consequently, the optimal phenotype is likely to be determined by a trade-off between life-history characters when extrinsic sources of nutrition are in short supply (Rivero and West, 2002).

Body size, which directly reflects the resource acquiring and/or storing capability of an individual, is one of the key traits determining parasitoid fitness (Ueno, 1998, 1999b; Ellers *et al.*, 1998; Rivero and West, 2002; Ueno and Ueno, 2005). Numerous laboratory studies have reported the positively correlation between body size and longevity or fecundity in parasitoids (Nakamura, 1995; Bezemer *et al.*, 2005; Harvey, 2008).

The present study examined: longevity with or without food source; the relationship between female body size and longevity; the egg production with or without food source. The reproductive strategies of synovigenic parasitoids are discussed here.

2.2 Materials and Methods

2.2.1 Insect culturing

All experiments in this study were carried out under controlled conditions in the laboratory (20 ± 1℃, 16:8 h L:D regime). Newly emerged females for testing were collected from rearing containers (10 cm in diameter, 4.5 cm in height) and were anesthetized with CO_2 to weigh on a microbalance. The females were then individually placed in plastic containers (10 cm in diameter, 4.5 cm in height) together with a male. The male was removed after 24 hours.

2.2.2 Longevity

This experiment was conducted to estimate life expectancy of *I. naranyae* under different food conditions, which is 20% honey solution and water as a control treatment. The food source was saturated in cotton, and the cotton was replaced twice a week. Forewing length of females was also recorded as a parameter of body size.

2.2.3 Fecundity

In this experiment, I designed an experimental tool to estimate egg production by female *I. naranyae* in the absence of host meal (host-feeding). Plastic tubes (8 mm in diameter) each with 40-50 little holes (0.3 mm in diameter) on the surface were prepared. A host pupa was put into each tube when given to test females. Female *I. naranyae* were able to recognize the host pupae inside the tubes and attack them. Oviposition into host pupae was possible through the holes made on the surface of plastic tubes but feeding on host materials was prevented.

From the 3rd day after emergence, each female was offered two plastic tubes containing a fresh host pupa for 2 hours every day, and this procedure was continued in the next 9 days. Then, the saturated cotton was removed from the containers, and test females were starved for 24 hours. Twenty-four hours later, test females were orally fed 3μL different food sources (i.e., 20% sucrose solution, or water as a control) with a micro-injector. Thereafter, the females were offered with water only. On the 3rd day after the treatment above, test females were dissected under a microscope at 25× magnification. The mature eggs have a smooth and clear surface and relatively straight which can be easily separated from immature eggs with a rough surface (Fig. 2-1). The numbers of total eggs, immature and mature eggs were counted and recorded under a microscope.

2.2.4 Statistical analyses

Statistical analyses were made with the aid of JMP (SAS Institute 2005).

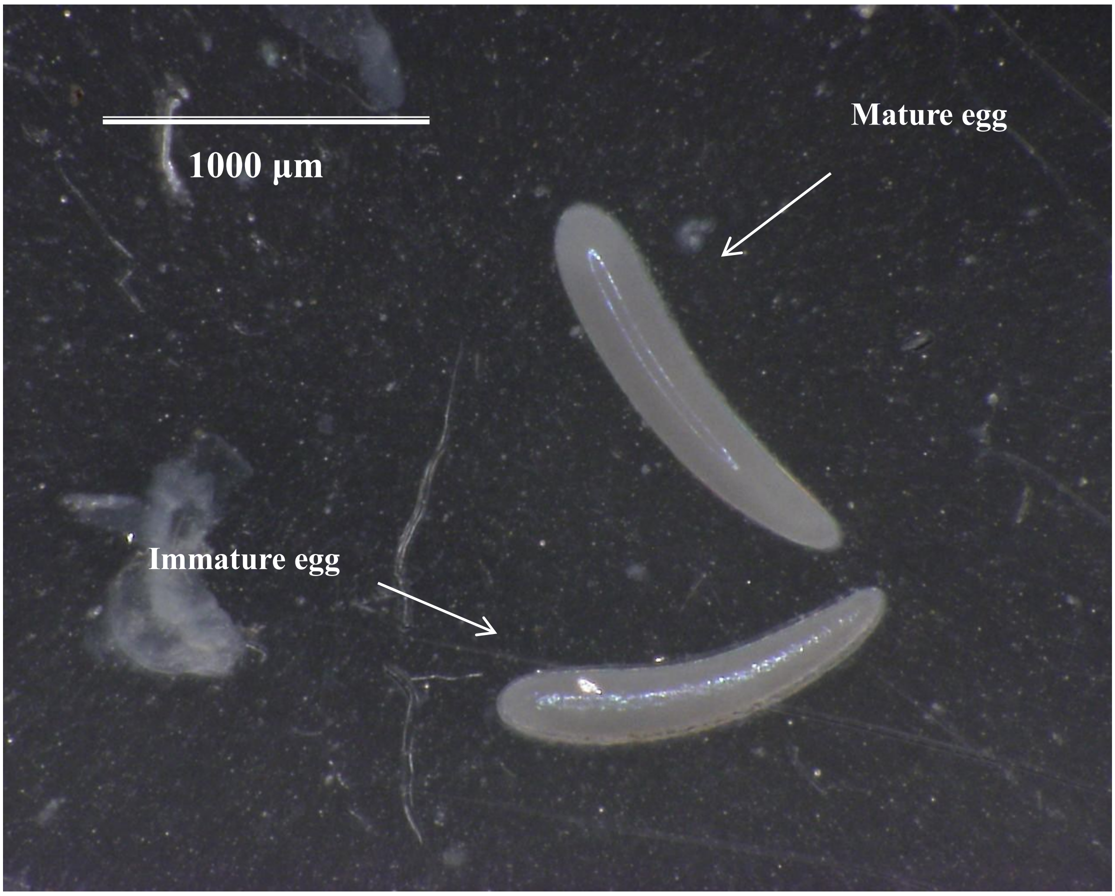

Fig. 2-1. Comparison of mature egg and immature egg of *I. naranyae* at 100× magnification.

2.3 Results

2.3.1 Longevity

A comparison of survival curves indicated that the longevity of females sharply decreased when food source removed females (survival analysis; x^2 = 28.23, $P < 0.0001$, Fig. 2-2). The mean longevity (with ±SE) was 52.33 ± 2.34 days when honey solution provided while that with water was 11.56 ± 0.81 days. I applied proportional hazard fits for analyzing the longevity data. Because I had two indexes for body size, i.e., body weight at parasitoid emergence and forewing length, and because I did not know which index was better, two models were constructed (see Table. 2-1). Whole models obtained were highly significant (model 1; N = 33, df = 3, x^2 = 42.11, $P < 0.0001$, model 2; N = 33, df = 3, x^2 = 42.15, $P < 0.0001$).

Simple regression analyses were made to examine the relationships between longevity and body weight or forewing length. Forewing length was significantly and positively correlated to female longevity regardless of food presence (r^2 = 0.94, F = 139.84, $P < 0.0001$; Fig. 2-3b), and the same with body weight (r^2 = 0.91, F = 101.69, P < 0.0001; Fig. 2-3a). Although body weight at emergence and forewing length were strongly correlated with each other (Fig. 2.4; N = 33, r^2 = 0.74, F = 87.24 P = 0.0001), However, in the effect tests, comparing with body weight, the forewing length may be a better predictor of body size of female *I. naranyae*.

Table 2-1. Survival analysis for accessing factors affecting the longevity of *I. naranyea* in the food availability. Proportional hazard fits are applied.

Factors	Chi-square	P
Model 1		
Forewing Length (mm)	2.05	0.15
Food Availability	34.16	< 0.0001
Interaction	1.20	0.27
Model 2		
Body Weight (g)	0.32	0.57
Food Availability	39.13	< 0.0001
Interaction	1.18	0.28

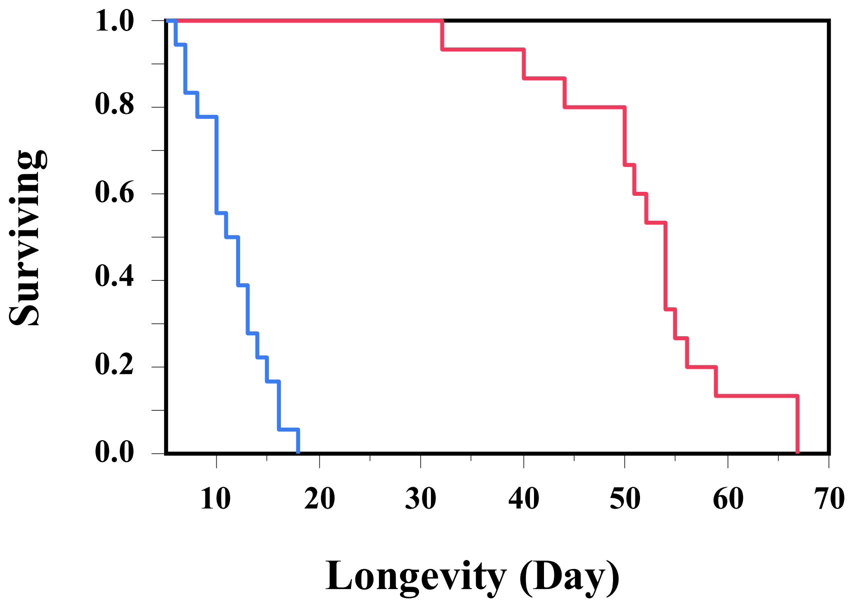

Fig. 2-2. The comparison of survival curves of *I. naranyea* between the females with honey solution (—) and the ones with water only (—). The longevity of females sharply decreased when food source removed females (p < 0.0001).

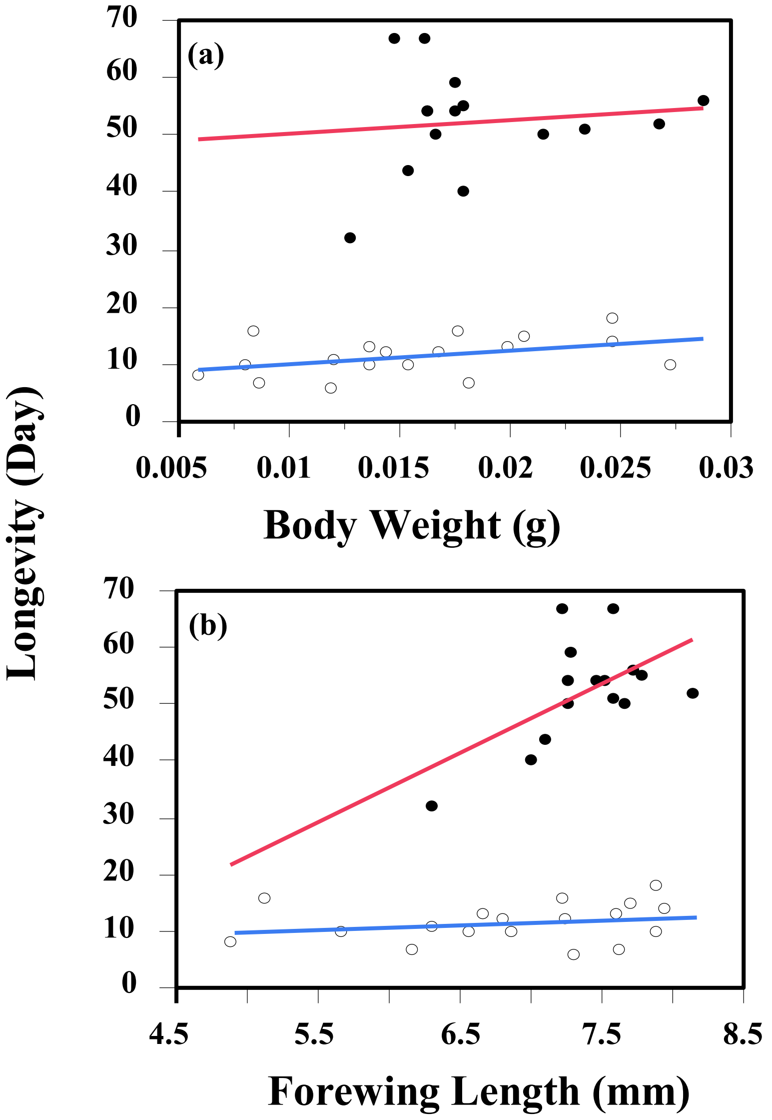

Fig. 2-3. Simple regression analyses between longevity and body weight (a) or forewing length (b) of *I. naranyea*. Both of them significantly affected the longevity no matter the honey group (—●—) or water group (—○—).

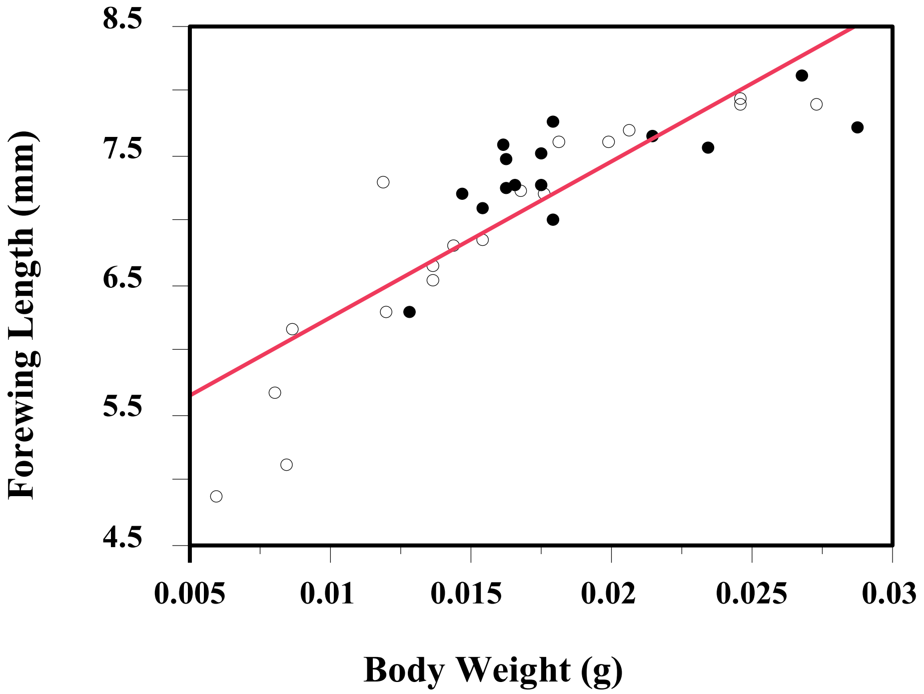

Fig. 2-4. The regression analyses between body weight at emergence and forewing length of *I. naranyea*. The two parameters are significantly correlated with each other (P < 0.0001).

2.3.2 Fecundity

One way ANCOVAs were performed to access the effect of food availability on the fecundity. The number of total eggs that female *I. naranyae* carried was significantly different between the two groups (Fig. 2-5a; F = 7.59, P = 0.0115). Females orally fed with sucrose solution had a greater number of eggs (mean ± SE: 31.1 ± 2.66) in their ovary, those fed with water (21.3 ± 1.96) intermediate. Also, the similar phenomena were detected for immature eggs (Fig. 2-5b; F = 5.14, P = 0.0336; for sucrose treatment: mean ± SE = 25.3 ± 2.15; for water treatment: mean ± SE = 18.7 ± 1.63). The most significant correlation has been found in mature eggs (Fig. 2-5c; F = 7.09, P = 0.014; for sucrose treatment: mean ± SE = 5.86 ± 0.99; for water treatment: mean ± SE = 1.74 ± 0.37). Without food source, female fecundity decreased critically in no matter egg production or egg maturation.

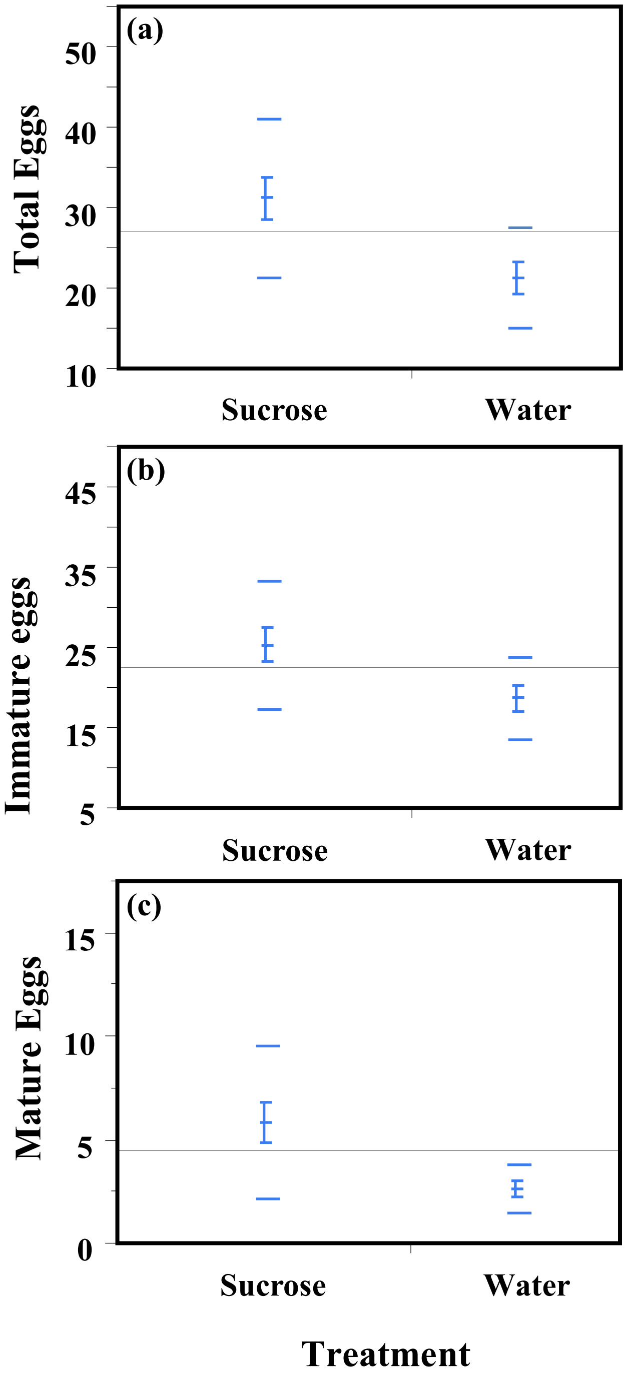

Fig. 2-5. The ANOVAs performed to assess the effects of food on the total eggs (a), immature eggs (b) and mature eggs (c) of female *I. naranyea*. The differences were significant in both total eggs and immature eggs, the most in mature eggs.

2.4 Discussion

Sugar sources can have a dramatic impact on the activity, longevity and lifetime fecundity of parasitoids (Lee *et al.*, 2006). In the wild field, it is normal for parasitoids to face a situation of food shortage temporally and spatially. Thus, the availability of food can be very important to their efficiency in biological control (van Rijn and Tanigoshi, 1999; Winkler *et al.*, 2006). Typically, the food availability has a strong impact on survival and reproductive of the adults (van Rijn *et al.*, 2006; Winkler *et al.*, 2006).

Many parasitoids mainly rely on carbohydrates as a source of energy to fuel activity, maintenance, metabolic expend and reproduction (Jervis *et al.*, 2008). For example, arthropods such as Hymenoptera and Diptera show high frequency wing muscle contractions where primarily contain oxidize carbohydrates. The fact that the mobilization of energy from carbohydrates can be very rapidly makes sugars especially suit to supply the large requirement in energy during flight (Hoferer *et al.*, 2000). Carbohydrates obtained by adults from host or non-host resources enhance longevity and survival (Jervis *et al.*, 2008).

In the present study, female *I. naranyae* were allowed unlimited access to a carbohydrate source, i.e., honey. In this experimental condition, their mean longevity is beyond 1.5 months, the value of which demonstrates *I. naranyae* has a considerably long lifetime among parasitoid wasps. The sharp decrease of lifetime in the absence of carbohydrate sources indicates that internal storage in *I. naranyae* is rapidly depleted when resources are allocated to both physical and metabolic activities.

Many parasitic wasps can reabsorb eggs as a temporal nutrient source under food limitation or in the absence of hosts, which was known as ‘egg resorption’ (Jervis *et al.*, 2001). Egg resorption is frequently associated with parasitoid species that produce large, yolk-rich eggs and show host-feeding (Flanders, 1950; Bell and Bohm, 1975). Also, it is believed that such parasitoids convert nutrients taken from their eggs into energy for maintenance or activity in order to increase the future reproductive opportunity. Egg resorption may particularly be advantageous for long-lived species because flexible re-allocation of resources allows them to endure temporary severe conditions. In the present study, fecundity sharply decreased in the control group when compared with the female group fed with sucrose. This result indicates that egg resorption also takes place in *I. naranyae* under food limitation. As we have shown, *I. naranyae* has a long life span, when sugar source is lacking, *I. naranyae* show ‘egg resorption’, which implies re-allocation of resources they carry and big size egg production.

Most insects exhibit a variation of adult body size led by the environmental and genetic effects. The size-dependency of reproduction and morality rates is induced by the differences in growth rate and the duration of important periods (Roff, 2002; Nijhout *et al.*, 2006). Numerous studies have reported that body size is correlated with life-history traits such as mating efficiency, dispersal capability, and longevity (Visser, 1994; Rasa *et al.*, 2000; Skow and Jakob, 2003; Bezemer *et al.*, 2005; Jervis *et al.*, 2008). Larger wasps are presumably better able to storage metabolic resources for maintenance than small wasps. This may explain why body size always positively and significantly affects longevity of female *I. naranyae*, no matter with or without food source.

If food limitation occurs, the degree of the cost may be much higher depending on the size of females. Future studies will then focus on the interaction between food and cost of reproduction.

Chapter 3. The cost of reproduction of *I. naranyae*

3.1 Introduction

Life-history models suggest trade-offs among traits, which may play an important role in shaping the structure of ecological communities (Sterns, 1989; Roff, 1992). Generally, under the constraint of some conditions, a trade-off is a correlation between traits -- a positive change in one by itself is opposed by a negative effect in another (Jervis and Kidd, 1986; Ellers *et al.*, 1998; Roff and Fairbairn, 2007). For example, an investment of more internal resources towards egg production during adult life is at the cost of a reduced life-span (Desouhant *et al.*, 2005). Parasitoids have long been considered as model organisms in testing many aspects of evolutionary theory (Godfray, 1994) but the cost of reproduction has seldom been examined in parasitoids.

Nutrient acquisition and allocation critically impact the fitness of all organisms (Rivero and Casas, 1999; Jervis *et al.*, 2008). During the larval stage, parasitoids acquire nutrients by feeding on hosts mainly for their development but part of the nutrients is stored for future egg production in adult life (Godfray, 1994; Harvey, 2005). Adult parasitoids, particularly the females, feed on sources of carbohydrates such as nectar and honeydew to support their daily maintenance and reproduction (Boggs, 1981). In the other side, many species exhibit a behavior called host-feeding, which is the consumption of the host body fluids and tissues, to acquire the crucial nutrients (mainly proteins and lipids) for egg production (Sandlan, 1979; Jervis and Kidd, 1986; Heimpel and Collier, 1996; Ferracini *et al.*, 2006). Synovigenic parasitoids are typically born with no or a limited number of mature eggs and continue to mature eggs during the adult stage (Jervis *et al.*, 2001). Thus, their fitness is strongly dependent on the number of further eggs that they can produce during their adult life (Rosenheim, 1996).

Itoplectis naranyae Ashmead, (Hymenoptera: Ichneumonidae) is typically synovigenic and is a host-feeding species; female *I. naranyae* carries no mature eggs at eclosion, but fecundity quickly increases even when hosts are unavailable. They can produce about 30 eggs without host-feeding using nutrients stored during the larval stage. Host feeding allows females to produce additional eggs, but there is a three days' delay between the host-feeding behavior and egg maturation (Ueno and Ueno, 2007). This species is destructive, which means hosts that had been fed on are heavily damaged and not suit for oviposition.

Although carbohydrate food for the adults and host meal obtained via host-feeding are known to affect the fitness of pupal parasitoids like *I. naranyae*, it is unknown how these two nutrient sources interactively affect the reproductive success of pupal parasitoids. The present study compared the female longevity when different food sources were given and when host availability was changed.

3.2 Materials and Methods

3.2.1 Insect culturing

Experimental hosts and parasitoids were prepared and cultured as described in Chapter 2.

3.2.2 Experimental procedure

This experiment was conducted to estimate life expectancy of *I. naranyae* under different host and food conditions. Test females were first divided into two groups. Females of the first group were reared in the absence of host throughout their lifetime whereas, in the other group, each female was provided with 3 hosts for 2 hours every day until the death of them. Both of the two groups were further divided into three groups, and each of the groups was subject to different food type treatments: 20% sucrose solution, 20% honey solution and water as a control. The food source was saturated in cotton, and the cotton was replaced twice a week. Thus, in all, 6 female groups were set up and 90 individuals in all were tested. Forewing length of females was also recorded as a parameter of female size.

3.3 Results

The mean longevity (with ± SE) of female wasps was 54.35 ± 1.96 days when no hosts had been provided while that with hosts was 45.56 ± 0.60 days. We applied proportional hazard fits for analyzing the longevity data. Because we had two indexes for body size, i.e., body weight at parasitoid emergence and forewing length, and because we did not know which index was better, two models were constructed (see Table. 3-1). Whole models obtained were highly significant (model 1; N = 27, df = 3, $x^2 = 17.82$, P = 0.0005, model 2; N = 27, df = 3, $x^2 = 15.20$, P = 0.0017). For both models, host provision strongly affected the life expectancy of females, and a comparison of survival curves indicated that females that had given hosts had shorter lifetime than those without hosts (Fig. 3-1).

Simple regression analyses were made to examine the relationships between longevity and body weight or forewing length. Forewing length was significantly and positively correlated to female longevity regardless of host presence (Fig. 3-2a; N = 12, df = 1, F = 13.69, P = 0.0041 for host provided group; N = 15, df = 1, F = 6.22, P = 0.027 for host deprived group). However, body weight had a significant effect only for host provision group (Fig. 3-2b; N = 12, df = 1, $r^2 = 0.60$, F = 14.93, P = 0.0031), but not for host deprived group (N = 15, df = 1, $r^2 = 0.014$, F = 0.18, P = 0.68). Although body weight at emergence and forewing length were strongly correlated each other (Fig. 3-3; N = 27, $r^2 = 0.74$, F = 71.64, $P < 0.0001$), forewing length may be a better predictor of body size of female *I. naranyae*.

On the other side, the lifetime of females feed on sucrose solution (number = 30;

mean ± SE: 84.5 ± 2.44; $p < 0.0001$; Fig. 3-4a) were apparently improved than ones with honey solution (number = 27; mean ± SE: 49.1 ± 6.22; $p < 0.0001$; Fig. 3-4b), while longevity sharply decreased in the absence of food (number = 33; mean ± SE: 11.3 ± 1.44; $p < 0.0001$; Fig. 3-4c). The body size had a positive effect on the longevity among food types, but only in sucrose treatment ($r^2 = 0.95$; $p < 0.0001$; Fig. 3-4a) the positive interacttion between body size and longevity was significant, while not in honey ($r^2 = 0.26$; $p < 0.0001$; Fig. 3-4b) and water treatment ($r^2 = 0.17$; $p < 0.0001$; Fig. 3-4c).

Table. 3-1. Survival analysis for accessing factors affecting the longevity of *I. naranyea* in the absence and presence of host. Proportional hazard fits are applied.

Factors	Chi-square	P
Model 1		
Forewing Length (mm)	4.84	0.028
Host Provision	16.13	0.0001
Interaction	0.52	0.47
Model 2		
Body Weight (g)	1.00	0.32
Host Provision	14.58	0.0001
Interaction	1.63	0.19

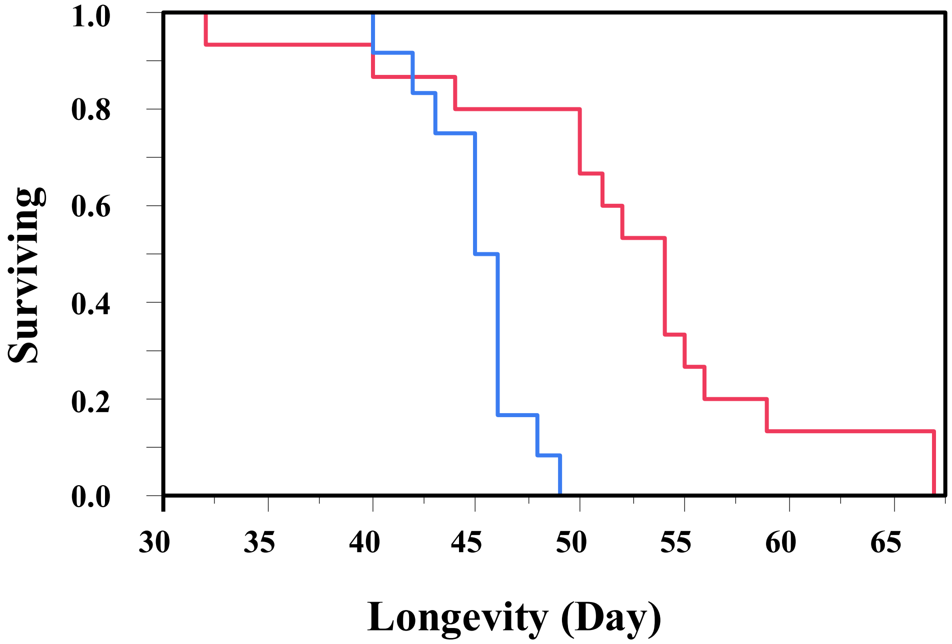

Fig. 3-1. The comparison of survival curves of *I. naranyea* between the females with hosts (——) and the ones without hosts (——). Females that had given hosts had shorter lifetime than those without hosts

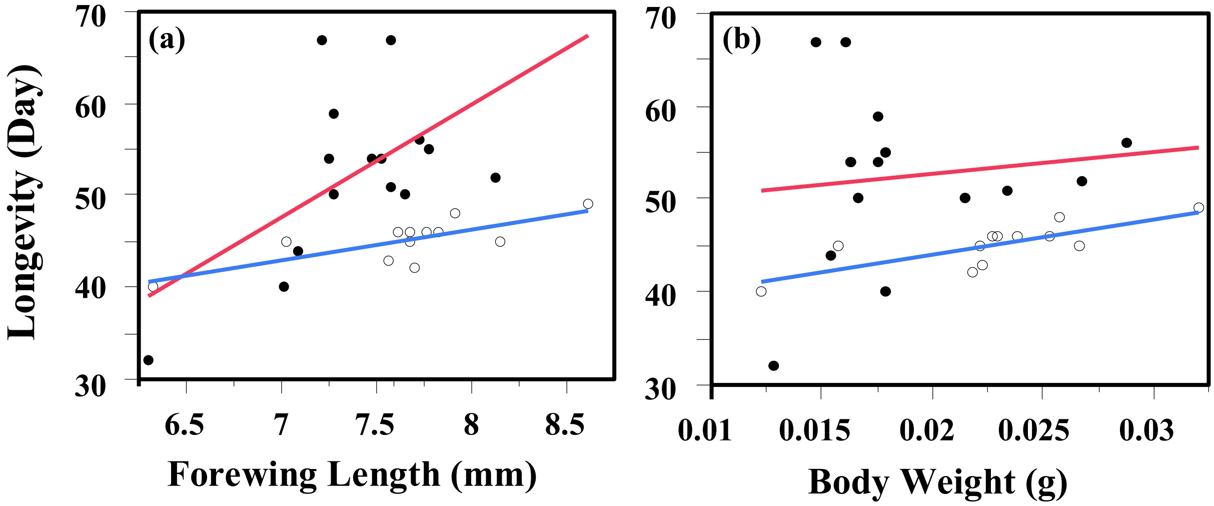

Fig. 3-2. Simple regression analyses between longevity and forewing length (a) or body weight (b) of *I. naranyea*. Both of them significantly affected the longevity no matter the host presence (—○—) or host absence (—●—).

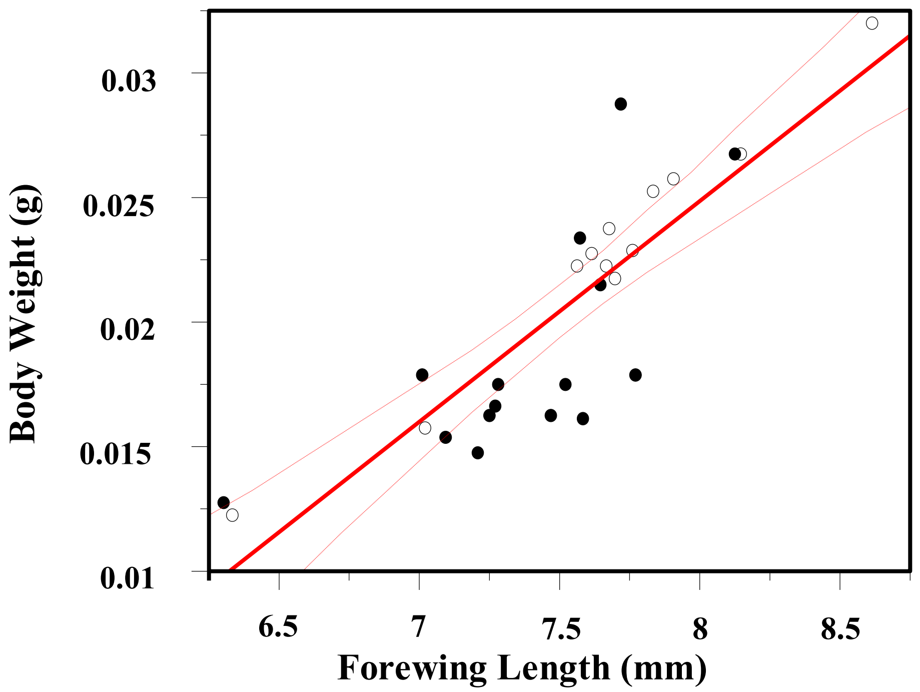

Fig. 3-3. The regression analyses between body weight at emergence and forewing length of *I. naranyea*. The two parameters are significantly correlated with each other (P < 0.0001).

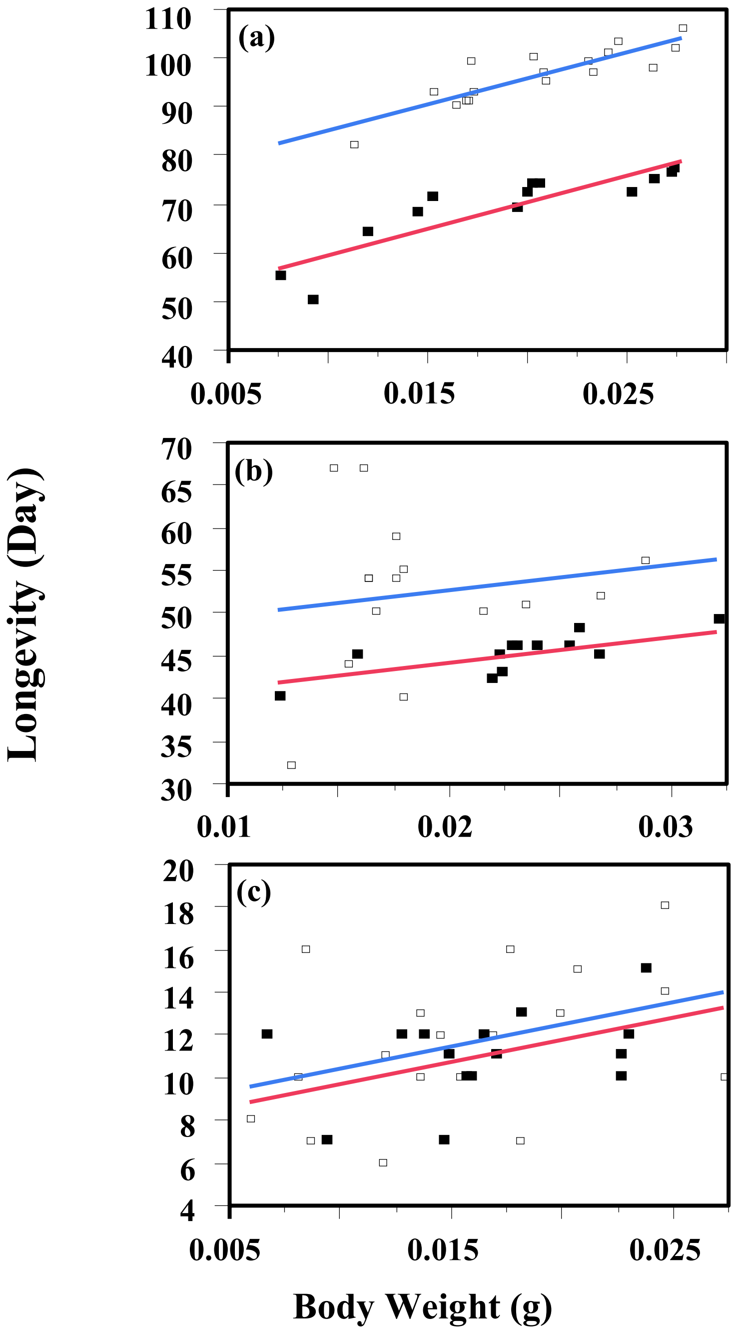

Fig. 3-4. The interaction of host availability and body weitht on the longevity of *I. naranyea* with different food treatment: Sucrose (a), Honey (b), Water (c). With host (—□—); Without host (—■—).

3.4 Discussion

As a host-feeding species, upon encountering a host, a female should face a dilemma whether to oviposit or feed on that host (Briggs *et al.*, 1995). She can obtain an immediate reproduction success by using the host for oviposition while oviposition will decrease her life expectancy due to the cost of reproduction; she can gain nutrients for future reproduction by host feeding but loses the current reproductive chance, i.e., oviposition, because host feeding causes the direct mortality of hosts making them unsuitable for oviposition (Rivero and Casas, 1999; Giron and Casas, 2003; Burger *et al.*, 2005). Traditionally, a potential reduction for future reproduction is at the cost of a current reproduction, this trade-off is essential for researches of the evolution of reproductive investment (Desouhant *et al.*, 2005). Thus, how much energy an organism invests to the eggs is supposed to depend on its reproductive chances in the future, which will be affected by both of physiological state (e.g. age, nutrition) and environmental factors (e.g. resource availability), but eventually aim at the optimal reproductive success.

The differing effects of host access on longevity may be based on the strategies parasitoids employ to obtain resources for maintenance versus reproduction (Harvey, 2005; Jervis *et al.*, 2008). In the present study, the longevity of female *I. naranyae* was lower when provided with hosts than those deprived of hosts. This finding indicates the presence of a trade-off between reproduction and life expectancy, i.e., cost of reproduction. Such a trade-off is the basis of the hypothesis of evolutionary theory of life-history (Roff and Fairbairn, 2007).

Adult parasitoid species with host-feeding behavior are highly dependent on resource acquisition from both the environment (carbohydrates and proteins) and host (proteins and lipids), because of the rapid depletion when resources are allocated to both metabolic activity and reproduction (Jervis *et al.*, 2008). The suitability of a particular food source is not only a function of food source characteristics but also depends on how well consumers are adept to its exploitation. Insects have the behavioral, morphological and physiological adaptations that enhance their ability to locate and digest specific food sources. They are supposed to develop adaptation to the food sources within their habitat.

Parasitoids have to balance between the searches for food and hosts. The search for food resources mainly depends on the temporal and spatial relationship between hosts and food resources (Bernstein and Jervis, 2008; Downes and Dahlem, 1987; Sirot and Bernstein, 1996). Carbohydrates obtained by adults from host or non-host resources can enhance longevity and survival (Jervis *et al.*, 2008). In the natural field, sugar sources for parasitoids are various, such as honeydew, nectar and so on. Wäckers *et al.* (2008) suggested that such sugar sources may not always be suitable as food because of the complex contents. As known, the contents of those sources are varied not only in carbohydrates and also proteins or other nutrients. Some of these may be harmful or bring a trouble to metabolite them, such as some harmful oligosaccharides, which negatively affect the survival and reproduction as a result (Jervis and Kidd, 1986).

In the present study, female *I. naranyae* were allowed unlimited access to a carbohydrate source, i.e., honey or sucrose. In this experimental condition, their mean longevity was beyond 1.5 months, the value of which demonstrates *I. naranyae* has a considerably long lifetime among parasitoid wasps. Moreover, individuals fed with sucrose solution exhibited a relative longer lifetime than those with honey solution. Comparing with honey, the content of sucrose solution is much simpler and more easily to metabolize and convert to energy they need. Even under such a favorable condition, I have detected the cost of reproduction; if food limitation occurs, the degree of the cost may be much higher depending on the size of females.

Some studies in parasitoids have found evidence of a trade-off between reproduction and survival (Hohmann *et al.*, 1989; Harvey, 2005), whereas others have not (Bai and Smith, 1993). Thus, the cost of reproduction is likely to differ among parasitoid species (Ferracini *et al.*, 2005). Harvey (2008) found that even between related species – *Lysibia nana* and *Gelis agilis*, there is a strong divergence in reproductive biology because of the difference in life-history strategies they employ. The trade-offs among traits are constrained by different factors (Roff, 2000). In solitary parasitoid species, which often grow only marginally smaller than their host, host size should limit their body size and resources they can stock (Ellers *et al.*, 1998; Ueno, 1998, 1999b; Rivero and West, 2002; Harvey, 2005). Because of an absence of lipogenesis during the adult stage, constraints on lipid reserves gained during the larval stage can be a major factor influencing the reproductive strategies of parasitoids.

In the present study, I have shown that female *I. naranyae* in the absence of host are able to prolong their lifetime to acquire a future reproductive opportunity, but with sufficient numbers of host, they invest to egg production at the cost of longevity. As I have shown, *I. naranyae* has a long life span, and resource re-allocation may be advantageous for species with long lifetime because environments (host and food availability) should change temporarily during their lifetime. The next step should be focused on the effects of food source on adult reproduction of *I. naranyae*.

Chapter 4. Food and egg production of *I. naranyae*

4.1 Introduction

The diet of adult female parasitoids can have important effects on their lifetime reproductive success (e.g. Jervis *et al.*, 1996; Heimpel *et al.*, 1997). Adult nutrients generally support the mating, foraging, physical activities and also reproduction (Jervis and Kidd, 1986; Heimpel and Collier, 1996; Jervis *et al.*, 1996; Thompson, 1999). The mode of egg production is one of life-history traits, which strongly link to the nutrient acquisition and allocation strategies of parasitoids (Iwasa *et al.*, 1984; Roff, 1992). Egg production and deposition by female parasitoids have received considerable attention from researchers (Price, 1975; Rosenheim 1996; Sevenster *et al.*, 1998). The pattern of allocation of nutritional resources to reproduction can have important influences to the fitness of parasitoids and is fundamental to numerous aspects in behavioural and evolutionary ecology (Rivero *et al.*, 2001). A female's egg production includes the total number of oocytes, the number of mature eggs she can store, the rate of egg maturation, and the capacity of egg resorption (Price, 1975; Jervis and Kidd, 1986). Thus in studies of parasitoids' reproductive biology, patterns of nutrient allocation, utilization, and acquisition are important in understanding their reproductive strategies (Bernstein and Jervis, 2007; Jervis *et al.*, 2008).

Parasitoids show species-specific differences in reproductive strategies, which have attracted numerous biologists and evolutionary ecologists, and their reproductive strategies are an essential constituent of parasitoids in biological control (McGinley *et al.*, 1987).

Solitary species rely on resources from a single host individual during their entire developmental stage, so at least a part of these resources must be allocated to reproduction and survival in adult stage. According to the egg production strategies, Flanders (1950) divided parasitoids into two types: (1) pro-ovigenic species that emerge with a full egg complement and do not mature additional eggs during adult life; and (2) synovigenic species that emerge with no or only a fraction of their potential complement of mature eggs and continue to mature eggs during the adult stage. To characterize this, Jervis *et al.* (2001) developed the 'ovigeny index' (OI), which characterizes the proportion of an adult female's eggs that are ready for oviposition at adult emergence. It is the ratio of the initial egg load (mature eggs) to the lifetime potential fecundity (Jervis *et al.*, 2001). An OI of 1 (strict pro-ovigeny) means that all oöcytes are mature at emergence, while an OI of 0 (extreme synovigeny) means the absence of mature oöcytes at emergence. If OI is one, larval resources are the only sources for egg maturation, which is known as 'capital resource' (Boggs, 1992, 1997a; Stearns, 1992; Tammaru and Haukioja, 1996). In contrast, if OI is zero, no or only a small part of larval resources would be allocated to egg maturation, and females mature most of their eggs in the adult stage using the nutrients acquired in later adult life (Jervis *et al.*, 2001). The fitness of synovigenic parasitoids is largely dependent on the rate at which eggs can be matured after eclosion, thus the role of adult diet in egg maturation and reproductive success should be more important for syonovigenic than pro-ovigenic species

No-prey food, such as floral and extrafloral nectars, pollens, honeydew etc., is a main carbohydrate source, but is low in quality in terms of proteins (Boggs, 1997b). Host-feeding parasitoid species feed on host fluids or tissues as a part of adult nutrient sources that are directly used for egg production (Briggs *et al.*, 1995), and are mostly synovigenic species that produce large, yolk-rich eggs (Jervis *et al.*, 2001). As a result, females require large amount of resources for egg production, but nutrients stored during larval stage can not cover the requirement for egg production and maturation. Therefore, adult food, both host-feeding and non-prey sources, is important in those species.

Ueno and Ueno (2007) have done the research on life history traits of *I. naranyae*; the adult females emerge with no mature eggs, and start to produce eggs after eclosion, meaning that this parasitoid is typically synovigenic with the synovigeny index OI of zero. The fecundity is low in the earliest stage of adult life, but subsequently increases with female age. Host-feeding plays an important role in its egg production, and, in the early stage of adult life, *I. naranyae* use a part of hosts for feeding. However, it is still not known the effect of nutrients on their later stage of life. In addition, females can produce about 30 eggs without host-feeding, indicating that this parasitoid can allocate the nutrients stored during larval stage for egg production (Ueno and Ueno, 2007). In this chapter, I examined the effects of a fixed amount of food on the egg production and egg maturation in the later life stage of *I. naranyae*. The importance of food on fecundity will also be discussed.

4.2 Materials and methods

4.2.1 Insect culturing

Experimental hosts and parasitoids were prepared and cultured as described in Chapter 2.

4.2.2 Experimental Procedure

In this experiment, I designed an experimental tool to estimate egg production by female *I. naranyae* in the absence of host-feeding. 5 cm in length plastic tubes (8 mm in diameter) were prepared and each with 40-50 little holes (0.3 mm in diameter) on the surface. The tube was stuck on a plastic board (20mm * 50mm) to prevent tubes rolling inside container (Fig. 4-1). A host pupa was put into each tube when given to test females. Female *I. naranyae* were able to recognize the host pupae inside the tubes and attacked them. Oviposition into host pupae was possible through the holes made on the surface of plastic tubes but feeding on host materials was prevented.

From the 3rd day after emergence, each female was offered two plastic tubes containing a fresh host pupa for 2 hours every day, and this procedure was continued in the next 9 days. As a result, females used the nutrients stored during larval stage and carbohydrates in adult life to support their reproduction and maintenances.

Then, the saturated cotton was removed from the containers, and test females were starved for 24 hours. Twenty-four hours later, test females were orally fed with 3μL different food sources, i.e., 20% sucrose solution, host blood or water as a control, with a micro-injector. Thereafter, the females were offered with water only. On the 3rd day after the treatment above, test females were dissected. The numbers of total eggs, immature and mature eggs were counted and recorded under a microscope.

4.2.3 Host fluid extraction

Newly pupated hosts were collected and the cocoons outside pupae were removed. Then, the pupae were mashed in an Eppendorf tube (4cm in length). The tube containing host tissues and fluids was heated in 60 - 65 ℃ water for 5 minutes, and then centrifuged for 1 minute in 10000 rpm. Only host fluids (= hemolymph) were collected from the tube and used for the following experiments.

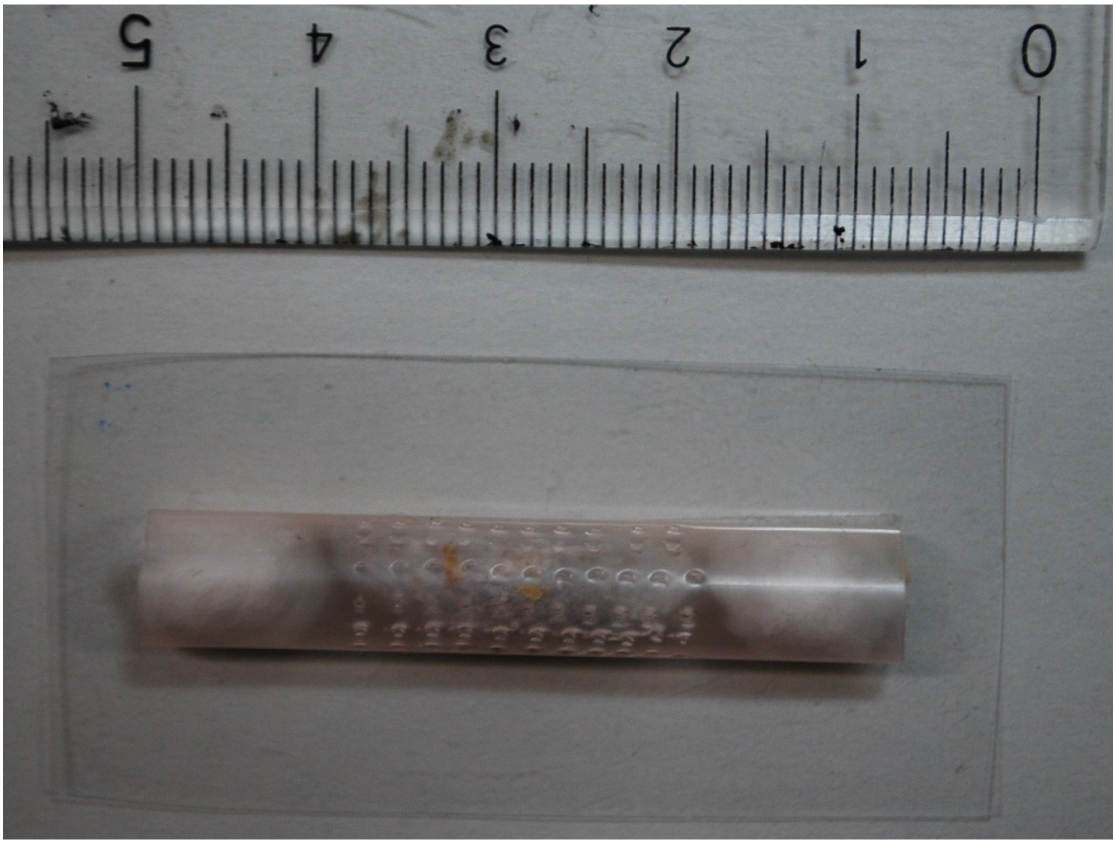

Fig. 4-1. The experimental tool to estimate egg production by female *I. naranyae* in the absence of host-feeding

4.3 Results

4.3.1 The effect of food type

The egg production and maturation of *I. naranyae* differ with food types. One way ANCOVAs were performed to access the effect of different food sources on the fecundity. The number of total eggs that female *I. naranyae* carried was significantly different among the groups (Fig. 4-2a; Table. 4-1; n = 58, $r^2 = 0.137$). Females orally fed with host blood had the greatest number of eggs (mean ± SE: 30.4 ± 2.27), those fed with sucrose (25.5 ± 1.50) intermediate, and the control group carried the least number of eggs (20.6 ± 2.45). The difference was the most significant in mature eggs of *I. naranyae* (Fig. 4-2b; Table. 4-1; n = 58, $r^2 = 0.415$). Females orally fed with host blood had the greatest number of mature eggs (mean ± SE: 9.29 ± 1.05), those fed with sucrose (4.59 ± 0.51) intermediate, and the control group carried the least number of eggs (2.41 ± 0.34). However, the number of immature eggs in *I. naranyae* was 21.2 ± 2.19 for feed with host blood, 21.1 ± 1.23 for feed with sucrose solution and 18.2 ± 1.59 for feed with water only. Although the immature eggs in control treatment (water) was lower than the rest two, it was not significant different (Fig. 4-2c; Table. 4-1; n = 58, $r^2 = 0.030$). Without food source, female fecundity decrease critically in no matter egg production or egg maturation.

Table. 4-1. One way ANCOVAs were performed to access the effect of different food sources on the fecundity of *I. naranyae*.

Factors	F	P
Total Eggs	4.35	0.0176
Mature Eggs	19.47	< 0.0001
Immature Eggs	0.84	0.4374

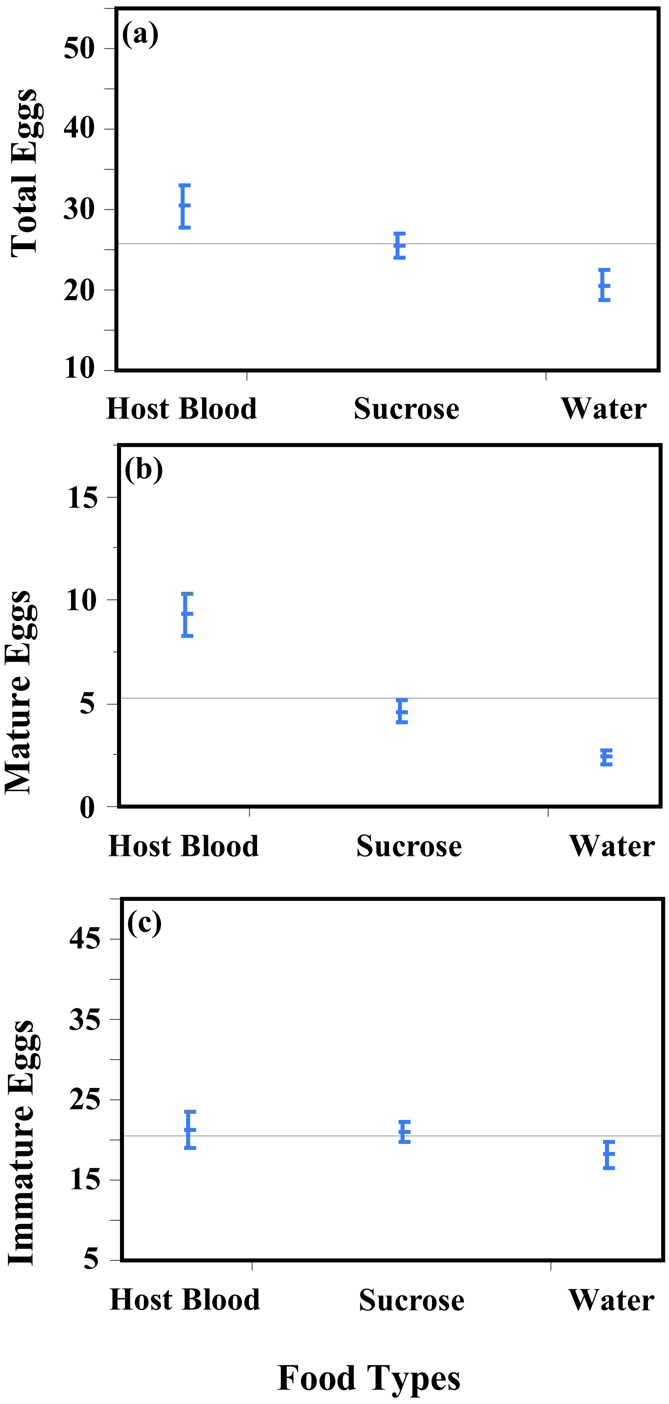

Fig. 4-2. One way ANCOVAs were performed to access the effect of different food sources on the fecundity of *I. naranyae*. The differences were significant in the number of total eggs (P = 0.0176) and mature eggs (P < 0.0001), but not for immature eggs (P = 0.4374).

4.3.2 The effect of body size and interaction with food type

Two indexes for female body size, i.e., body weight at parasitoid emergence and forewing length were strongly correlated with each other (Fig. 4-3; N = 36, r^2 = 0.84, F = 184.51, P < .0001). Thus, I analyzed the effect of food type with each body size parameter respectively, and simple regression analyses were made.

4.3.2.1 Forewing length

The forewing length was significantly and positively correlated to female fecundity regardless of food type. The effects of both forewing length and food types on total eggs were significantly different (Fig. 4-4a; N = 36, r^2 = 0.55, F = 7.43, P = 0.0001), but the interaction of these two factors was not significant (Table. 4-2). Similarly, for mature eggs, the effect was also significant (Fig. 4-4b; N = 36, r^2 = 0.63, F = 10.34, P < .0001), but not in interaction (Table. 4-2).

4.3.2.2 Body weight

The body weight at emergence was significantly and positively correlated to female fecundity regardless of food type. The effect of both body weight and food types on total eggs was significant different (Fig. 4-5a; N = 36, r^2 = 0.70, F = 13.97, P < .0001), and the interaction of these two factors was also significant (Table. 4-3). Similarly, for mature eggs, the effect was significant (Fig. 4-5b; N = 36, r^2 = 0.77, F = 19.73, P < .0001), also in interaction (Table. 4-3).

Thus, the body weight of parasitoid at emergence may be a better predictor of body size of female *I. naranyae*.

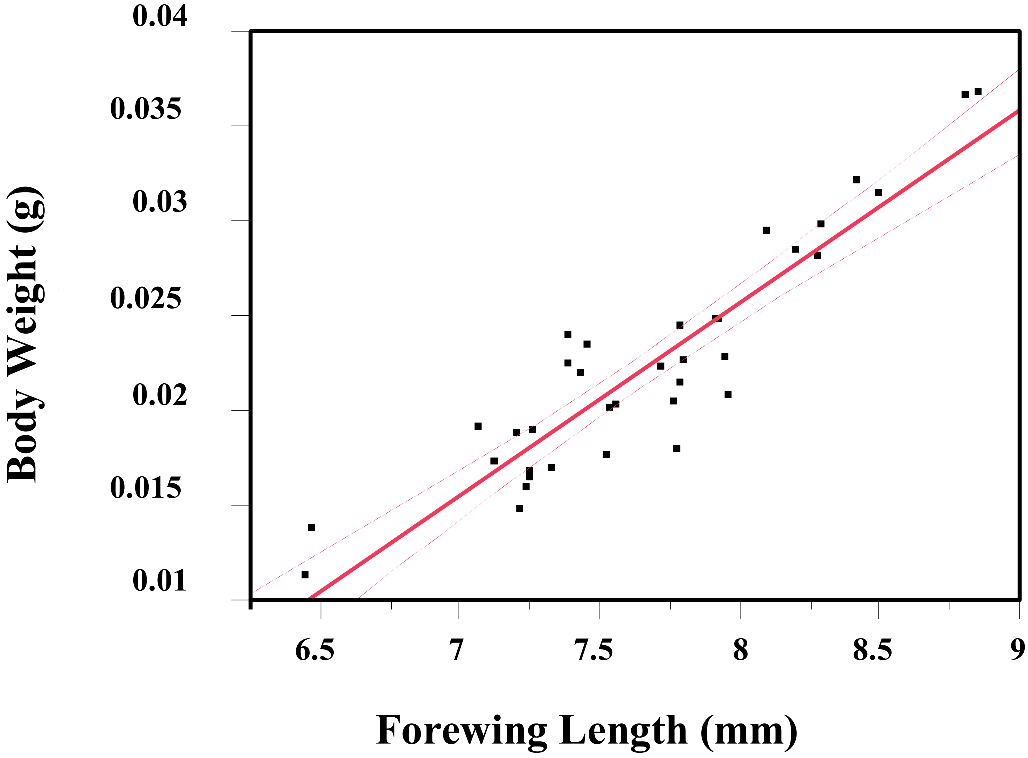

Fig. 4-3. The regression analyses between body weight at emergence and forewing length of *I. naranyea*. The two parameters are significantly correlated with each other ($P < 0.0001$).

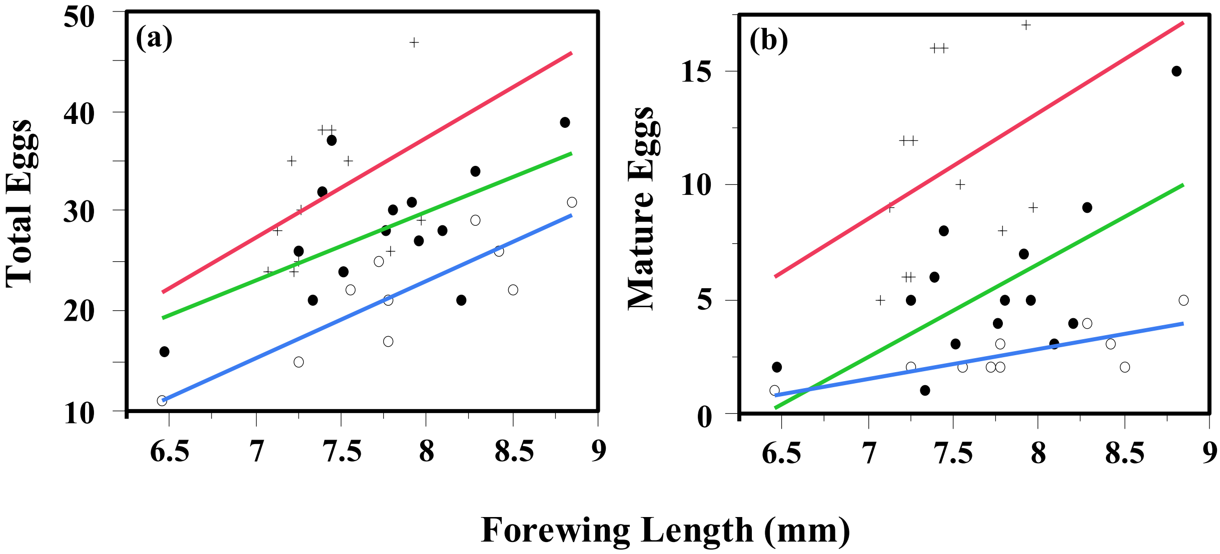

Fig. 4-4. The interaction of forewing length and food types on the number of total eggs (a) or mature eggs (b) of *I. naranyea*. Food types: Host Blood (—+—); Sucrose (—●—); Water (—○—).

Table. 4-2. The effect tests of Generalized Linear Model (GLM) for accessing factors affecting the number of total eggs and mature eggs of *I. naranyea*.

Factors	df	F Ratio	P values
Total Eggs			
Forewing Length (mm)	1	13.0685	0.0006
Food Type	2	731.0974	<.0001
Interaction	2	0.1418	0.8684
Mature Eggs			
Forewing Length (mm)	1	7.9800	0.0083
Food Type	2	20.2974	<.0001
Interaction	2	1.1270	0.3373

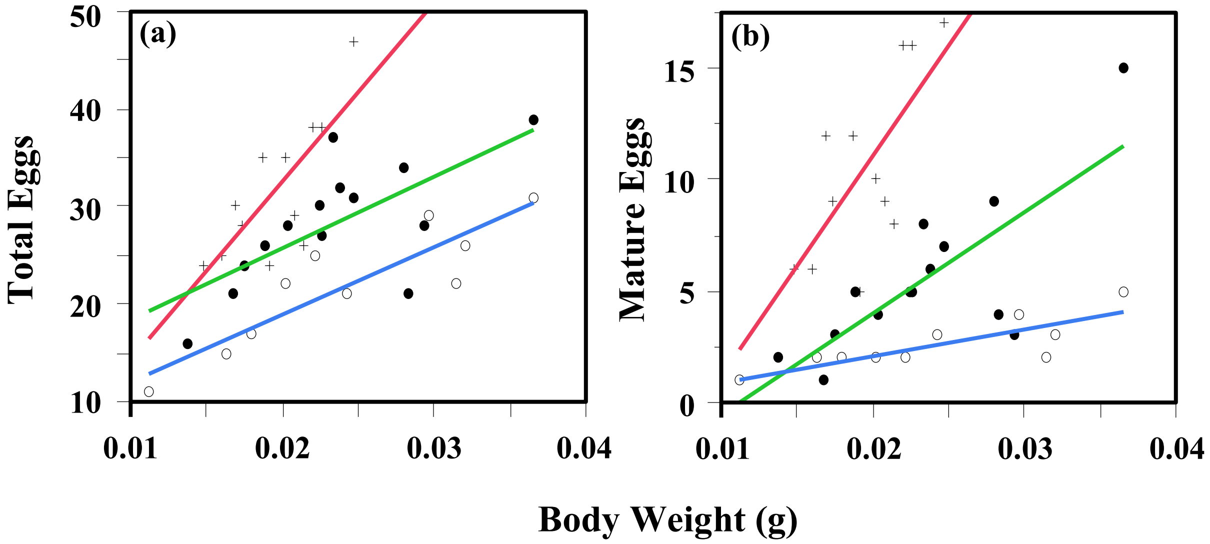

Fig. 4-5. The interaction of body weight at emergence and food types on the number of total eggs (a) or mature eggs (b) of *I. naranyea*. Food types: Host Blood (—+); Sucrose (—●—); Water (—○—).

Table. 4-3. The effect tests of Generalized Linear Model (GLM) for accessing factors affecting the number of total eggs and mature eggs of *I. naranyea*.

Factors	df	F Ratio	P values
Total Eggs			
Body Weight (g)	1	37.7932	<.0001
Food Type	2	24.5481	<.0001
Interaction	2	2.8195	0.0755
Mature Eggs			
Body Weight (g)	1	30.2172	<.0001
Food Type	2	40.6008	<.0001
Interaction	2	6.6594	0.0040

4.4 Discussion

The results of this study have revealed that, in *Itoplectis naranyae*, like other parasitoids, the fecundity is strongly affected by the present of food source. Both the number of total eggs and mature eggs were critically increased after fed with host hemolymph or with sucrose solution. However, comparing with carbohydrates, host-feeding appeared to be the most important in egg maturation of *I. naranyae*. Without food sources, because of egg resorption, both the number of total eggs and mature eggs decrease sharply.

Parasitoids larval reserves are usually the most important source of nutrients for egg production in the early adult stage (Rivero *et al.*, 2001). The fat body is accumulated during larval feeding inside hosts and carried over to the adult stage where it may be metabolized for egg production (Rivero *et al.*, 2001; Bezemer *et al.*, 2005). Recent physiological studies have given evidence that adult parasitoids can not synthesize new lipids from carbohydrates (Casas *et al.*, 2003; Giron and Casas, 2003). Comparative studies have further suggested an absence of lipogenesis during the adult stage may exist across all Hymenoptera (Jervis *et al.*, 2001; Jervis and Harvey, 2008). Thus, the lipid and protein in adult diet may be the key factor affecting the reproductive strategies of parasitoids.

It is common that adult parasitoids exploit food sources, such as honeydew, pollen and nectar, in the field (Jervis and Kidd, 1986). Also host tissue and blood contain a rich source of nutrients useful for egg production such as proteins and fats that are either scarce or absent in other foods (Jervis and Kidd 1986; Heimpel and

Collier 1996; Rivero and Casas 1999; O'Brien *et al.*, 2004). Thus, parasitoids during the immature stage should store such nutrients by feeding on host tissue or blood and carry over the nutrients to the adult stage. Instead, synovigenic parasitoids can gain such nutrients by feeding on hosts during the adult stage. The fact that only females exhibit host-feeding indicates its importance in female reproduction, i.e., egg production (Jervis and Kidd, 1986). Host-feeding has been demonstrated to improve the fecundity in many parasitoids including *I. naranyae* (Heimpel and Collier 1996; Ueno, 1999b; Burger et al., 2005; Ueno and Ueno, 2007). Likewise, in the present study, test females that orally fed with host blood carried much more mature eggs than other groups. This result demonstrates that female *I. naranyae* maturate eggs by using the nutrients from host blood. The mean difference between 3μL blood-fed and control females was 5.5 for mature eggs. This implies that 1μL host blood contains nutrients equivalent to 1.8 mature eggs.

In contrast, the number of immature eggs of *I. naranyae* was not different between females fed with sucrose solution and with host blood. Although the number of immature eggs tended to be low in control group, the diversion was not significant. In fact, I observed abnormally formed eggs in the ovary when dissected the females of control group. Because of accumulation of such eggs, the number of non-matured eggs did not differ among female groups. This result further demonstrated the importance of carbohydrates in egg production and host-feeding in egg maturation.

In solitary parasitoids, the host may limit their body size (Sequeira and Mackauer, 1992; Harvey and Strand, 2002; Harvey *et al.*, 2004). A large number of researches have employed body size in the research of biology and ecology of parasitoids life time traits (Sequeira and Mackauer, 1992; Rivero *et al.*, 2001; Ueno and Ueno, 2004; Bezemer *et al.*, 2005; Harvey, 2008; Nijhout *et al.*, 2010; Granger *et al.*, 2011). Most of them used indexes of size such as tibia length (Rivero *et al.*, 2001), hind tibia length (Granger *et al.*, 2011), body weight (Ueno and Ueno, 2004; Bezemer *et al.*, 2005; Harvey, 2008), dry weight (Sequeira and Mackauer, 1992). In the present study, I used two indexes, which were forewing length and body weight at parasitoid emergence, to represent the body size of *I. naranyae*. Comparing with forewing length, body weight impacted the egg production and maturation much more significantly, especially when interacted with food types. The body weight of an individual can directly reflect the capability of resource acquiring and/or storing, and may be more suitable in the research of nutrition acquisition and allocation of parasitoids. Thus, I hypothesize that the body weight should be a better predictor of body size of *I. naranyae* in this research.

In general, synovigenic parasitoids produce relatively large, yolk-rich eggs, but emerge only a small fraction of their potential egg complement and continue to mature eggs throughout the adult life (Jervis and Kidd, 1986; Ueno, 1999b). Thus, the diet of adult females critically affects the lifetime reproductive success (Jervis *et al.*, 1996). I have verified that the adult food strongly impacts both survival and reproduction in *I. naranyae*. Moreover, adult parasitoids display marked variation in their reproductive and associated traits both within and among species. Optimal progeny size models suggest that an increase in progeny fitness should be archived with an increase in parental investment (Smith and Fretwell, 1974). These models also predict a trade-off between the number and size of eggs, but this assumption holds true only when females have a fixed amount of resources to allocate to reproduction. The further research should focus on the variation in parental investment among female individuals.

Chapter 5. Food and investment in egg size of *I. naranyae*

5.1 Introduction

Egg size evolution is an important life-history trait that has been a central subject in evolutionary biology and ecology (Fox and Czesak, 2000). Because egg size affects the survival and growth of offspring, the amount of maternal investment to each egg can be critical to the reproductive success of both mother and offspring (Giron and Casas, 2003). The optimal egg size however is not fixed because of ecological and physiological constraints. A major constraint is trade-off between egg size and number, which has been found in numerous species of arthropods (McGinley and Charnov 1988; Braby, 1994; Fox and Czesak, 2000; Fischer *et al.*, 2002; Steigenga and Fischer, 2007; Liefting, 2010). This constraint emerges because the amount of resources available for mother is limited (Smith and Fretwell, 1974).

Egg size variation is induced by both internal and external factors. Egg size within a given species can differ in temporally and spatially different environments, such as age, latitude, altitude, seasons and temperature (Fox and Czesak, 2000; McIntyre and Gooding, 2000). On the other hand, the pattern that mother size is strongly correlated with egg size has been found in many animal taxa (Bernardo, 1996; Roff, 2002; Bezemer *et al.*, 2005). Body size, which represents the nutrients they have taken previously, is generally correlated with life-history traits such as survival, dispersal capability, and reproductive success (Bezemer *et al.*, 2005). Such, body size would be crucial for species exhibiting variation of adult size in the egg size differences among individuals.

Parasitoids are known as excellent organisms to test a variety of evolutionary theory (Godfray, 1994); egg size evolution has rarely been examined for parasitoids, however, although it is known that egg size in parasitoids varies among species (Price, 1973; O'Neill *et al.*, 1990). Price (1973) showed that parasitoid species that carry more, smaller eggs in the ovary use hosts of younger stage to cover higher mortality of younger hosts and to cope with higher host encounter rate. Thus, at species level of parasitoids, the risk of offspring mortality can determine the optimal number of eggs stored, which in turn determines the optimal egg size through the trade-off between egg size and number.

Likewise, egg size can vary among individuals within a species of parasitoids; Bezemer *et al.* (2005) have found that the egg size of a non-host-feeding ectoparasitoid *Mastrus ridibudus* is correlated with body size and food availability. It is however not known whether such egg-size variation is limited to a certain parasitoid and whether the variation depends on parasitoid life history strategies. In synovigenic species that produce eggs throughout the adult stage, food available for adult females should strongly determine the amount of resources allocated to egg production, and females may hence modify the egg number and size in response to external factors, i.e., food availability. Instead, in synovigenic parasitoids, the effect of internal factors, i.e., body size, can be weakened because disadvantage of being small may be balanced with food intake. However, very few studies have given evidence that parasitoids can control the resource allocation to eggs by modifying the number and size of eggs being produced.

In this chapter, I examined the effects of body size versus adult nutrition in later adult stage on the egg size in the solitary endoparasitoid *I. naranyae*. Basing on the results obtained, I discussed the importance of food on egg size variation and the adaptive significance of egg size control depending on physiological conditions.

5.2 Materials and Methods

Newly emerged females were anesthetized using CO_2 and were weighed on a microbalance individually. They were then placed individually in plastic containers (10 cm in diameter, 4.5 cm in height) together with a male. The male was removed after 24 hours because the mating was ensured during this period. Cotton saturated with 20% sucrose solution was placed in the container as a food source, and was replaced twice a week. The containers were kept at 20 ± 1℃ under a 16:8 h L:D regime.

Female *I. naranyae* emerge with no mature eggs, and oocytes start to develop and maturate after emergence (Ueno and Ueno, 2007). Thus, from the first 3 days since emergence, females for testing were allowed access to hosts, each of which was placed in a plastic tube with 40 - 50 small holes on the surface to allow oviposition but to prevent from host feeding. The females were allowed to attack the hosts for four hours every day.

From the 4th day to 14th day, each test female was offered two hosts inside the tube as above for 4 hours every day. On 15th day since female emergence, the cotton was removed from the containers. 24 hours later, females were orally fed with a micro-injector 3μL of one of the following food sources; 20% sucrose solution, host blood, 20% sucrose solution with 5% animo-acid mixture, and water as control treatment. Then females were offered with diluted water only. In *I. naranyae*, host feeding allowed females to produce additional eggs, but there was a time delay between the feeing behavior and new egg production. For this reason, test wasps were dissected on the 3rd day after the treatment, and the length of mature eggs was measured under a microscope. The forewing length of test females was also recorded as a parameter of female size. Statistical analyses were made with the aid of JMP 7.0.1 (SAS Institute 2007).

5.3 Results

The present study has revealed that egg size of *I. naranyae* varies among individuals (mean = 1238.70 ± 84.35μm), a range from 1044.05 μm to 1415.8 μm where rearing conditions other than female size and food are standardized (Fig. 5-1).

One way ANCOVAs were performed to access the effect of different food sources on the egg size of *I. naranyae* (Fig. 5-2). The egg size of control group (water only) (mean ± SE: 1138.38 ± 25.7 μm) was significantly smaller than those of the other treatment groups. When fed with host blood, egg size (1234.03 ± 21.5 μm) was relatively smaller than two treatments with sucrose (20% sucrose solution and 20% sucrose & 5% amino acid). The difference between 20% sucrose solution (1272.54 ± 21.5 μm) and mixture of 20% sucrose & 5% amino acid (1284.32 ± 22.7 μm) was very tiny, even though the egg size was bigger when amino acid was added.

The generalized liner model was performed to examine the effects of food sources and mother size (body weight and forewing length) on the egg size of *I. naranyae*, but the interaction was not significant (Table. 5-1). In all treatment, the length of mature eggs proportionately rose with the size of females (for body weight, $r^2=0.78$, $p<0.0001$, Fig. 5-3a; for forewing length, $r^2=0.81$, $p<0.0001$, Fig. 5-3b).

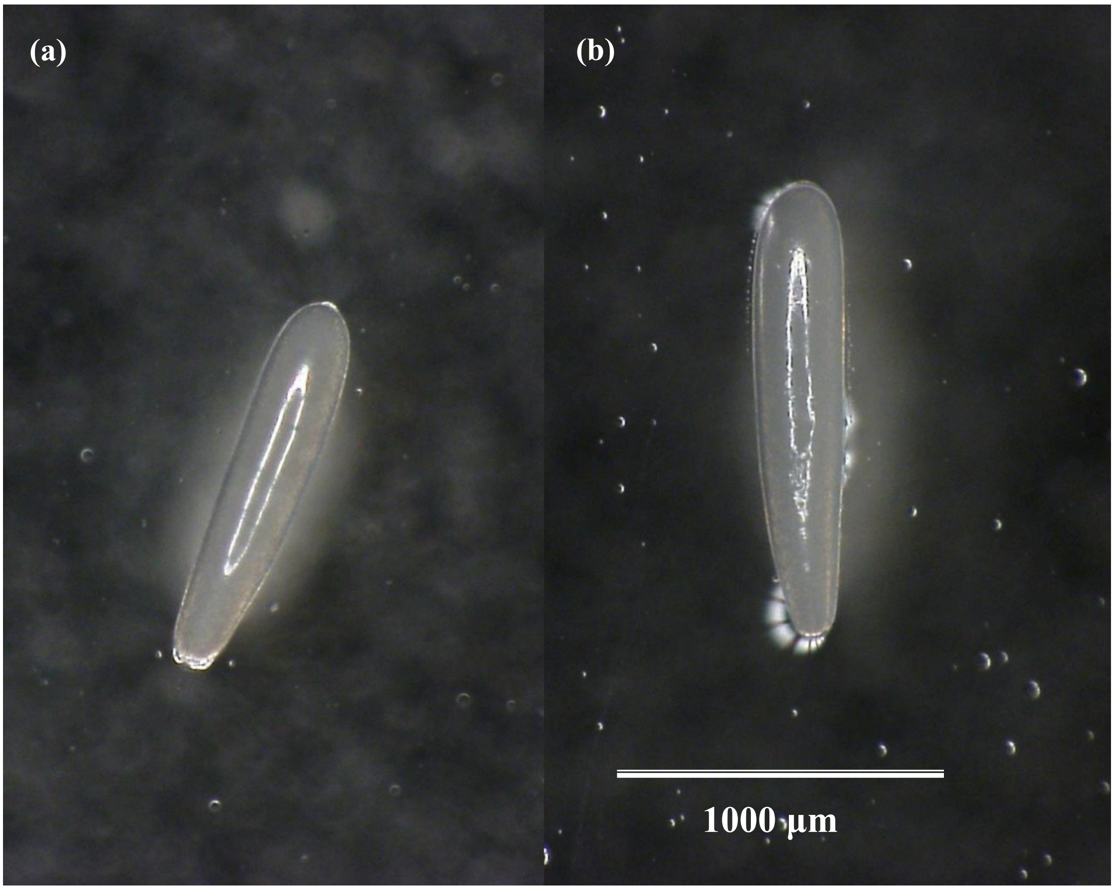

Fig. 5-1. A comparison of big egg and small egg of *I. naranyae* under a microscope. The size of eggs is apparently different. The length of egg (a) is 1194.33 µm, and egg (b) is 1370.98 µm.

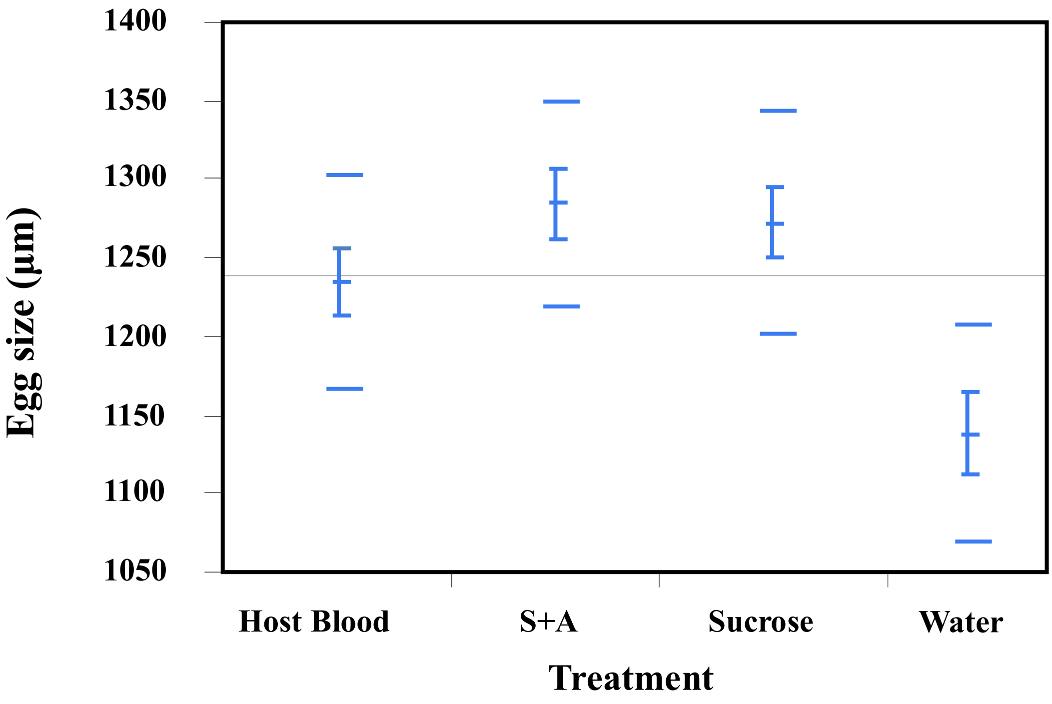

Fig. 5-2. One way ANCOVAs were performed to access the effect of different food sources on the egg size of *I. naranyae*.

Table. 5-1. The result of generalized liner model for assessing factors affecting the egg size of *Itoplectis naranyea*.

Factors	df	F	P values
Body Weight			
Body Weight (g)	1	41.2737	<.0001
Treatment	3	10.4362	<.0001
Interaction	3	0.4506	0.7189
Forewing Length			
Forewing Length (mm)	1	64.4840	<.0001
Treatment	3	12.1447	<.0001
Interaction	3	1.0171	0.3999

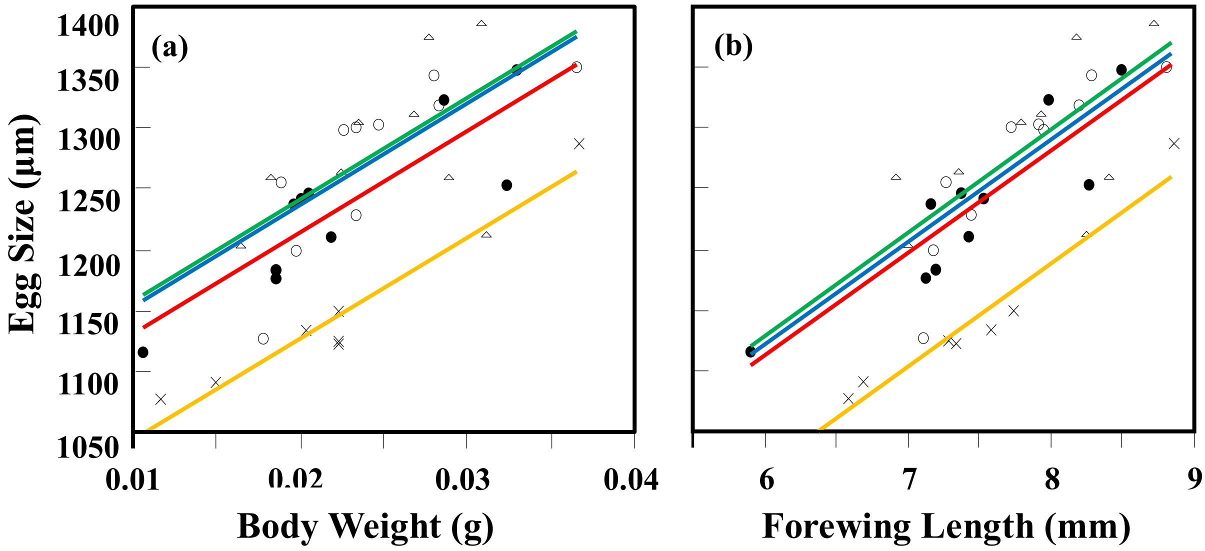

Fig. 5-3. Generalized linear model was performed to check the effect of body size and food source on egg size. (a) Body weight (R^2=0.78, $p<0.0001$); (b) forewing length (R^2=0.81, $p<0.0001$); (—●) Water treatment; (—○) Host Blood treatment; (—×) Sucrose treatment; (—△) Sucrose & Amino acid treatment.

5.4 Discussion

I. naranyae is a typical synovigenic species which produce relatively large, yolk-rich eggs (Ueno and Ueno, 2007). Moreover, they exhibited the egg size variation among individuals in this study. Given that egg size can affect the offspring fitness and performance critically (Lalonde, 2005; Boivin and Gauvin, 2009), the presence of egg size variation in *I. naranyae* suggests that investment to an egg varies among female individuals due to physiological and ecological constraints.

The factors inducing the egg size variation have been studied in numerous insect species (Fox *et al.*, 2000), but similar studies are rare in parasitoids. To my knowledge, Bezemer *et al.* (2005) is the sole study in which factors affecting egg size have been examined in an ectoparasitoid wasp. In their study, the egg size of *Mastrus ridibundus*, which is not a host-feeder, was shown to increase with mother size. In addition, starved female *M. ridibundu* produced smaller eggs than fed wasps. *Itoplectis naranyae* is a typical host-feeder, and its host-feeding plays an important role in egg production (Ueno and Ueno, 2007). In my study, the factors causing the egg size variation can be assumed from two aspects. First, the strategies to allocate the energy reserves should be different among species. The body size of *I. naranyae* varies in a large range, because it is directly determined by the size of host pupae, which are variable in size. The result that female size (both body weight and forewing length) significantly affected the egg size showed that body size of *I. naranyae* can also affect the reproductive success in later stage of adult. Second, the strategies of maternal nutrient allocation mainly depend on environmental factors. When food source is limited, the trade-off between survival and egg production occurs in life-history traits (Harvey, 2008). To optimize the resource utilization, *I. naranyae* allocates a relatively great amount of nutrients to activity and maintenance at a cost of egg production when food condition is limited, which lead to the egg size of water treatment was apparently smaller than other groups.

Here, when fed with host blood only, egg size was slightly smaller than two treatments with sucrose, though the difference was not significant. Egg size did not differ between sucrose and sucrose-amino acid treatments. Although fed parasitoids mature more eggs (Rivero and West, 2002), some parasitoids are able to acquire additional nutrients for egg production via host-feeding, as with the case for *I. naranyae* (Ueno and Ueno, 2007). Nonetheless, carbohydrates are still the mainly component of parasitoid eggs, so the sugar source availability may affect the egg size more significantly than the other elements.

Similarly to Bezemer *et al.* (2005), in the present study, I have found (1) smaller *I. naranyae* produce smaller eggs and (2) food types affect the size of eggs produced. Thus, regardless of parasitoid life history traits, i.e., ecto- or endo-parasitoids, or host feeders or non-host-feeders, flexible allocation of resources to egg may be present in parasitoids in general.

Moreover, super-parasitism and multi-parasitism are common in parasitoids including *I. naranyae*. In such cases, the offspring have to compete for the limited resource inside the host, especially for solitary parasitoids, in which only one individual can survive in one host (Ueno, 1997). Larger eggs should carry more resources and can allocate more to competitive capacity than smaller eggs. Thus, production of larger eggs may increase the probability of an offspring overcoming in larval competition under super- or multi-parasitism. If so, the production of large eggs should be adaptive to improve the reproductive success.

In conclusion, my study gives evidence that *I. naranyae* can adjust egg size in response to nutritional state, and body size. But the reason leads to this variation should focus on their offspring fitness. Larval fitness such as development time, larval size, survival rate etc. should be measured in relation to egg size in future studies, which would reveal the resource allocation strategy of females under variable food conditions.

Chapter 6. Egg size and reproductive success of *I. naranyae*

6.1 Introduction

Reproductive success is one of the most important biological parameters to evaluate a natural enemy in biological control system (Heimpel *et al.*, 1997). Parasitoids are a species-rich group of insects that lay their eggs on or in the body of other insects, i.e., hosts, and the larvae developed by feeding on the host and eventually kill it (Price, 1973). Parasitoid wasps have long been considered as an excellent model organism in the research of optimal resource allocation to different fitness functions, such as body size and development time (Stearns, 1989; Godfray, 1994; Roff, 2001).

In the preimaginal stages including embryo, larvae and pupae, holometabolic parasitoid species develop completely within a host individual, in which the nutrients are limited inside the host (Heimpel *et al.*, 1997). During this time, parasitoids rely on a limited amount of resources that are obtained from a single source (the host), i.e., 'capital resources' (Heimpel *et al.*, 1997). It has found that capital resources profoundly impact the adult life in many aspects such as longevity and reproductive success (Doyon and Boivin, 2005; Jervis *et al.*, 2008). Strong synovigeny is associated with weak capital reserves compared to weak synovigeny or pro-ovigeny, so nutrient reserves must be viewed in terms of individual nutrient classed (Jervis *et al.*, 2008).

Theoretically, to maximize fitness, an organism should grow rapidly and achieve early sexual maturity and a large adult size, enjoy a long life expectancy, and produce many offspring. However, in nature these conditions are never met because of different constraints including metabolic limitations, lack of sufficient resources, environmental heterogeneity, and other factors (Kraaijeveld and Vanderwel, 1994; Begon *et al.*, 1998; Roff, 2001). Therefore, developmental patterns between traits can show trade-offs (Murphy *et al.*, 1983; Pennacchio and Strand, 2006), such as between body size and developmental time (Stearns, 1989, 1992). Consequently, patterns of variation between fitness traits may be indicative of the life-history strategy employed by different species (Jervis *et al.*, 2008). Numerous studies have found the developmental differences among species, or under different conditions, such as temperature or host qualities (Liu and Stansly, 1996).

Most species exhibit a range of adult body size owing to environmental variation, for example, temperature (Roff, 2000). Therefore, adult body size is one of the key determinants in life-history traits, such as mating efficiency, dispersal capability and reproductive success (Ueno, 1998, 1999b; Ellers *et al.*, 1998). A massive literature has shown the information regarding the effect of body size on the life time strategies, such as longevity and fecundity (Bezemer *et al.*, 2005; Fischer *et al.*, 2006; Nijhout *et al.*, 2010). What more, the relationship of adult size and development time was also discussed in some research, but rarely connected the development time with the body size of mothers.

Egg size variation is common among parasitoid species, and may potentially affect the fitness of offspring in many aspects. Basing on studies with arthropods excluding parasitoids, it is assumed that bigger eggs provide higher fitness to offspring (Smith and Fretwell, 1974; Avelar, 1993). Optimal progeny size models hypothesize that the more eggs a female produces, the lower amount of resources should be allocated per progeny (Smith and Fretwell, 1974). The positive associations between egg size and offspring fitness have been found only in a few studies (Boivin *et al.*, 2009).

For *Itoplectis naranyae*, a solitary, internal parasitoid of a variety of lepidopterous pupae, I have shown that there is an egg size variation (Chapter 5). However, the effect of this variation on the offspring fitness is still unknown. So here, I examined the performance of immature *I. naranyae* before emergence from the mother with different body size.

6.2 Materials and Methods

6.2.1 Insect culturing

All experiments here were conducted within laboratory populations of *Itoplectis naranyae*. Pupae of the greater wax moth *Galleria mellonella* were used as hosts here. Hosts were reared following an artificial diet (Ueno and Tanaka, 1994). All experiments in this study were carried out under controlled conditions in the laboratory (20 ± 1℃, 60-70 RH, 16:8 h L:D regime).

Newly emerged females for testing were collected from rearing containers (10 cm in diameter, 4.5 cm in height) and were anesthetized with CO_2 to weigh on the microbalance. The females were then individually placed in plastic containers (10 cm in diameter, 4.5 cm in height) and were paired with a male. The male was removed after 24 hours.

6.2.2 Hatching time, egg size and larvae size

The time interval between oviposition and hatching was investigated. First, the approximate time of hatching was determined by preliminary observations, and then the exact timing of hatch was examined. As a pre-experimental treatment, females were presented with two fresh host cocoons every day, and 7 days old females were selected as the mother here. Each individual was allowed to contact with a fresh host for one hour. After that, the host pupa was dissected in a drop of distilled water under a microscope to obtain the eggs. Parasitoid eggs were separately put into a culture dish (35 * 10 mm) with 2ml insect Ringer's Solution (contents: 10.93g NaCl, 1.57g KCl, 0.83g $CaCl_2*2H_2O$, 0.83g $MgCl_2*6H_2O$ per liter solution), and were incubated under a control condition in the laboratory (25 ± 1℃, 24 H Dark). The length and width of eggs were recorded as parameters of egg size. After 18 H, the eggs were checked every hour until the larvae hatched to determine the hatching time, i.e., the time from oviposition to 1st instar hatching. When the larvae hatched, the head width of larvae was also measured under a microscope.

6.2.3 Developmental time

The interval from oviposition to adult emergence was recorded. In this part, I randomly selected mated females in different ages, and two treatments were set up. In the first group, mated females were individually placed in a plastic container (10 cm in diameter, 4.5 cm in height), and was offered two fresh host cocoons for two hours. In the second group, two mated females were put in a plastic container; the other condition was the same with the first group. The parasitized pupae were collected and incubated under a control condition (25 ± 1℃). After 10 days, I daily checked the pupae for emerging female parasitoids for the next 10 days, and when parasitoids emerged, the fresh body weight was measured on a microbalance.

6.2.4 Mother size and developmental time

The effect of mothers' body size on developmental time of the offspring was examined in this part. The offspring sex ratio and survival rate were also recorded. Newly emerged, mated females were collected and individually put in a container (10 cm in diameter, 4.5 cm in height). Each female was offered 3 fresh host pupae every day, and from the 3rd day I collected the parasitized hosts. The interval from parasitism to emergence of female offspring was recorded. Moreover, I noted the sex of emerging offspring, and the number of parasitized pupae and offspring of each mother.

6.3 Results

6.3.1Hatching time, egg size and larvae size

Eggs normally developed and the larvae successfully hatched in insect Ringer's Solution (Fig. 6-1). In all, 62 larvae hatched from 71 eggs; thus the hatching rate is 87%. Hatching time of *I. naranyae* ranged between 20 and 26 hours period, and the mean hatching time (mean ± SE) was 22.3 ± 0.19 hours.

Twenty females were tested as mothers, and each female in this test oviposited more than one egg in the host during the testing time of one hour, so superparasitism could be common in *I. naranyae*. I applied simple regression analyses and the random effect model was employed to test the effect of egg length on the hatching time. There was a significantly positive relationship between egg length and hatching time ($P < 0.0001$, $r^2 = 0.72$, $t = 6.75$; Fig. 6-2). Head width of 1st larval instar was positively correlated to the egg width ($P < 0.0001$, $r^2 = 0.66$, $F = 116.25$; Fig. 6-3).

6.3.2 Developmental time

The developmental time of *I. naranyae*, which is from oviposition to adult emergence, exhibited a range of 12-19 days. The developmental time was apparently different between the two groups (for one female group mean ± SE was 14.62 ± 0.14; for two females group mean ± SE was 15.61 ± 0.26). The developmental time of two females group was one day longer in average. No significant effect of body size on their developmental time was detected (P = 0.0295, r^2 = 0.047, F = 4.88; Fig. 6-4).

6.3.3 Mother size and developmental time

We tested 16 females *I. naranyae* and each female was offered totally 21 fresh pupae. In all 336 pupae were given, and 151out of the 336 host pupae produced offspring parasitoids, which means the overall emergence rate is 45%. Among the 151 individuals, there were 75 females and 76 males, such the sex ratio was nearly half and half, but the sex ratio was apparently different among individuals. The female body weight positively affected the offspring sex ratio (= proportion of females) (r^2 = 0.4, P = 0.0087, F = 9.3; Fig. 6-5). Mothers' body weight did not significantly affect the developmental time of offspring (P = 0.5036, r^2 = 0.0061, F = 0.45; Fig. 6-6).

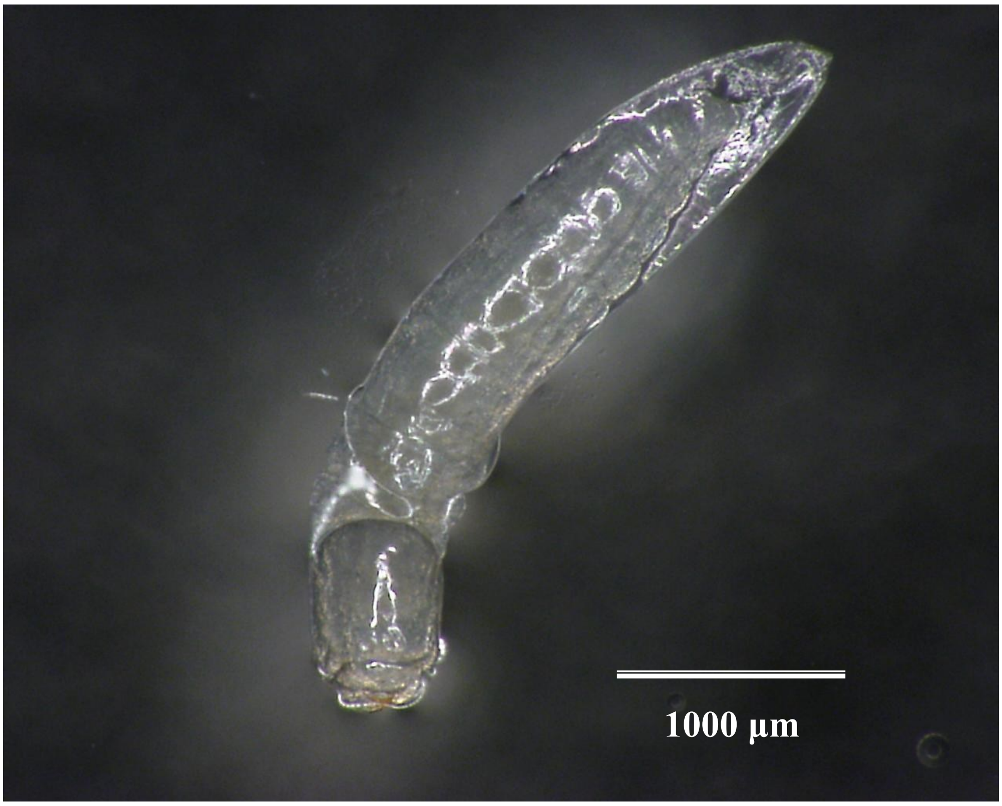

Fig. 6-1. Newly hatching larva of *I. naranyae* in insect Ringer's solution.

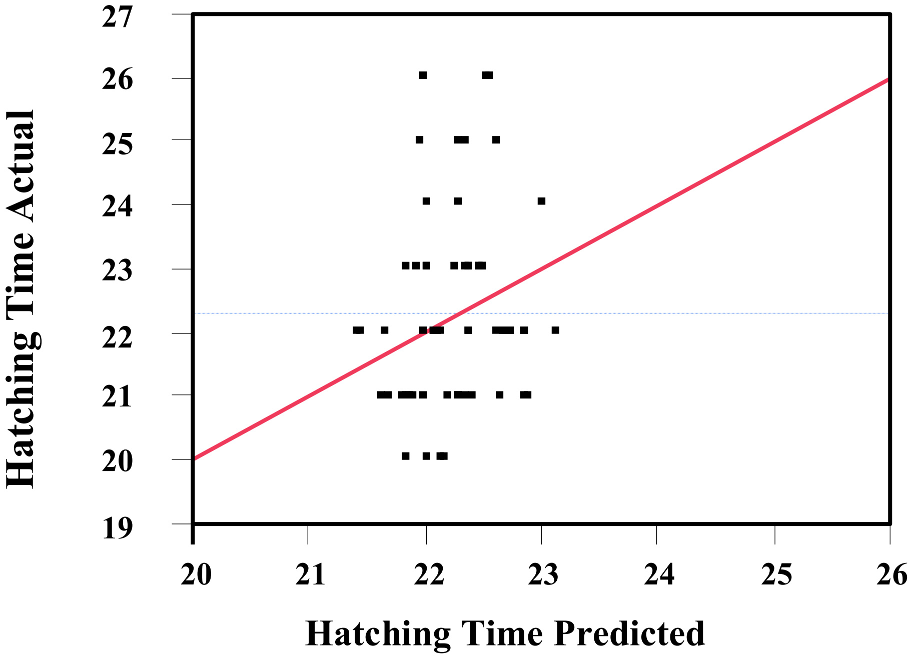

Fig. 6-2. Simple regression analysis and the random effect model were employed to test the effect of egg length on the hatching time of *I. naranyae*. The relationship between egg length and hatching time was positively significant ($P < 0.0001$).

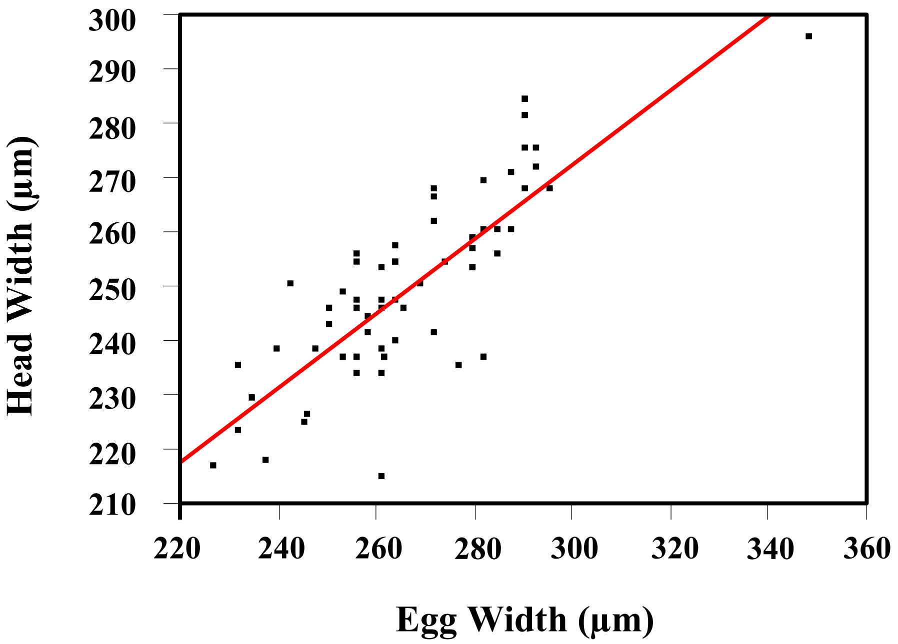

Fig. 6-3. The regression analysis between egg width and larva head width at hatching of *I. naranyea*. The two parameters are significantly correlated with each other ($P < 0.0001$).

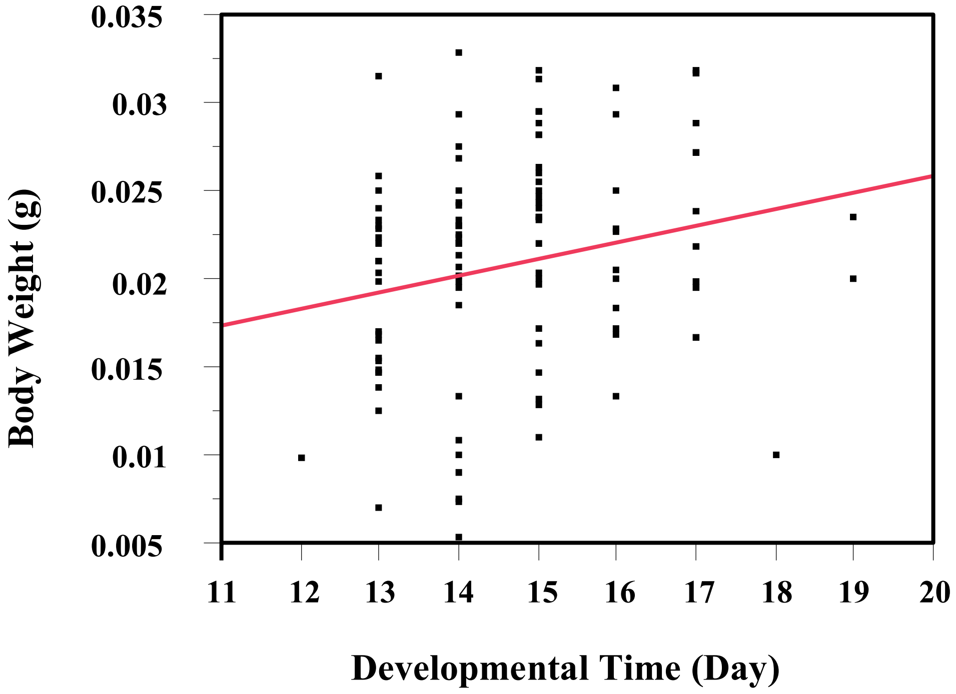

Fig. 6-4. The regression analyses between developmental time and body weight at emergence of *I. naranyea.* No significant effect correlated with each other (P = 0.0295).

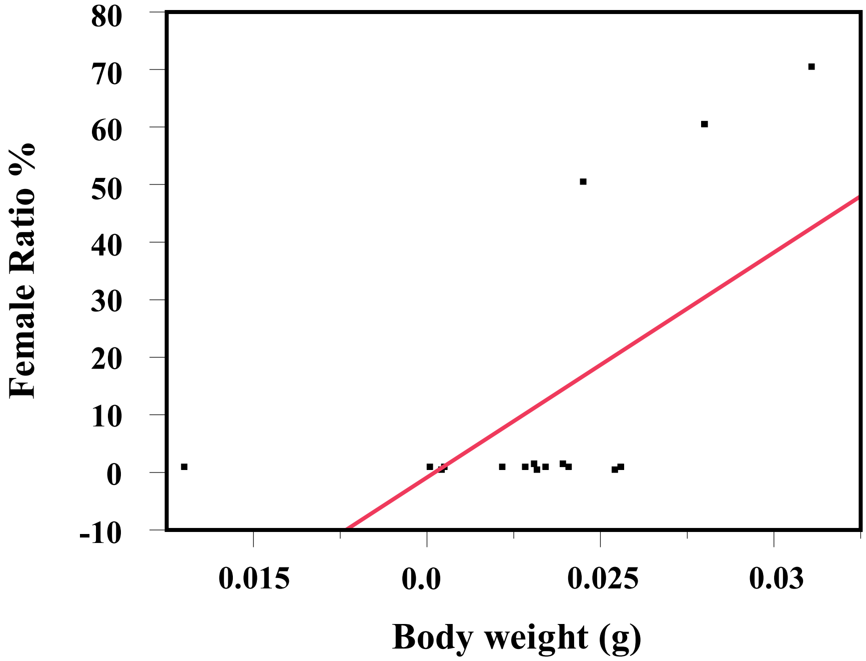

Fig. 6-5. The regression analyses between mothers' body weight and female ratio in offspring of *I. naranyea*. The two parameters are significantly correlated with each other (P = 0.0087).

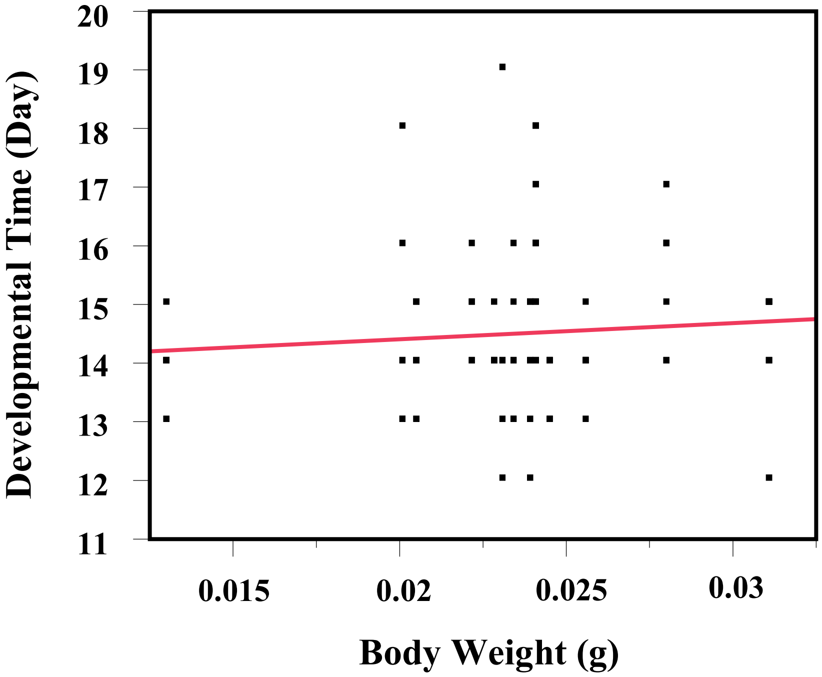

Fig. 6-6. The regression analyses between mothers' body weight at emergence and their offspring developmental time of *I. naranyea*. No significant effect correlated with each other (P = 0.5036).

6.4 Discussion

The egg size of *I. naranyae* is variable, which has discussed in chapter 5. It has been reported in Satyrinae butterflies that the egg size positively affected larval survival and fitness (Braby, 1994). Development of larvae has been found different among different stages of host (Mansaray and Sundufu, 2010) or among congeneric species (Donnell and Hunter, 2002). Smaller eggs have been found to have longer developmental time in one species of parasitoids (Bezemer *et al.*, 2005), but rarely concerned with the hatching of larvae. Donnell and Hunter (2002) found that the hatching time differ when offered different instar hosts. Available nutrients for embryonic development are constrained within eggs, so the egg size should significantly impact the embryonic fitness such as hatching time and hatching rate. *I. naranyae* produce relatively large, yolk-rich eggs (Ueno and Ueno, 2007) which can bring enough nutrients to complete embryonic development (hatching rate is 87%).

In the present research, the egg length of *I. naranyae* exhibited a positive correlation with hatching time. Thus, female *I. naranyae* face a trade-off between maternal investment in eggs and survival of larvae. With the constraint of investment to reproduction, when females try to optimize their reproduction by increasing the number of eggs, the conflict between mother and offspring can arise (Harvey, 2005). The fitness of offspring would increase as mothers reduce the number of eggs and aim at a preference of larger eggs (Fox and Czesak, 2000).

A positive relationship was found between the egg size and the size of the hatching larva. Similar relationships have been found in numerous insect species

(Braby, 1994; Fischer *et al.*, 2002), including several Coleoptera species (Wallin *et al.*, 1992; Guntrip *et al.*, 1997), and also in an ectoparasitoid *Aleochara bilineata* (Boivin and Gauvin, 2009). The present study is the first to show that such a relationship has been discovered in a synovigenic endoparasitoid species.

I. naranyae is a solitary parasitoid, which indicates that only one individual can successfully emerges from one host. Moreover, during the interval of one hour, one female oviposited more than one eggs in one host, so the phenomena of superparasitism should be common for *I. naranyae*. Thus, larger larvae have more resources to sustain themselves to overcome competition under super- and multiparasitism. When intraspecific competition occurs, large larvae may be advantageous in a solitary species, thus most evolutionary life history models suppose that the intense larval competition have a preference for larger offspring (Parker and Begon, 1986). Lower mortality in large larvae has been reported in some insect species (Wallin *et al.*, 1992; Fox, 1994; Fox and Czesak, 2000) including parasitoids (Boivin and Gauvin, 2009). All behavioral studies that were carried out tend to prefer larger larvae, similar results are also found in adult parasitoids (Boivin and Gauvin, 2009).

Adult size is often correlated with the amount of resources carried, and further reflects the ability of nutrient reservation (longevity) and egg production (reproductive success) (Bezemer *et al.*, 2005). Large size benefits parasitoids directly in competing fitness functions, such as survival and reproduction (Ellers *et al.*, 1998; Rivero and West, 2002), but it is also assumed that large size would have negative impacts on some life-time traits, such as energy consumption, mobility and development. For solitary parasitoids, body size is marginally restricted by the host they emerge, so the variation of body size is common in these species including *I. naranyae*. However, the developmental time was not significantly affected by their body weight at emergence (Fig. 6-4), but it differed between one female group and two females group. Thus, the multi-parasite may affect the development inside the host. Here, we conclude that (1) the development of *I. naranyae* before emergence is not restricted by their body size; (2) the interspecies competition for larvae inside host is intensive; (3) the competition leads to a delay of their development.

In a situation of low host density, larger larvae are more likely to survive and emerge from hosts (Harvey, 2005). Because pupal hosts are much less abundant than egg or larval hosts in the field, pupal parasitoids may have difficulty in finding enough hosts during their lifetime (Ueno, 1999). In species where larval mortality is high, selection pressure to avoid long developmental time may be strong enough to prevent a clear correlation between egg size and developmental time to appear (Bernardo, 1996). Although correlations between egg size and developmental factors have been described in several species (Fox, 1993, 1994) including an ecto-parasitoid (Mayhew and Heitmans, 1999), weak effects between egg size and developmental time have also been found in ecto-parasitoid parasitoid *A. bilineata* (Boivin and Gauvin, 2009). Although I did not include the egg size to test the developmental time of *I. naranyae*, the result in Chapter 5 has shown a positive correlation between female body size and their egg size. According the result that there was no relationship between the body size of mothers and developmental time of their offspring larvae, I hypothesize that the egg size may not affect the development of *I. naranyae*.

Offspring sex ratio is supposed to be under strong natural selection (Kobayashi *et al.*, 2003). Trivers and Willard (1973) suggest that females can adjust the sex of their offspring in response to environmental conditions. The haplodiploid sex determination makes it possible for females to control the sex of their offspring according to whether to fertilize depositing eggs, because males develop from unfertilized eggs and females develop from fertilized eggs (Godfray, 1994). Sex ratio adjustment is common in hymenopteran insects (ants, bees, and wasps), but the strategies that they employ are different among species (Trivers and Willard, 1973). In the early stage of adult life, *I. naranyae* produced nearly the same number of males and females, but larger mother tended to produce more female offspring (Fig 6-5). The size and sex of the larva may have an impact on the issue of a fight (van Baaren *et al.*, 1999) and on the reproductive success.

Chapter 7. General conclusion

Variable patterns of life-history strategies that exist inter- and intra-species have fascinated numerous evolutionary ecologists (Partridge and Harvey, 1988; Stearns, 1992). Parasitoid wasps that can be an important factor in regulating insect population dynamics are often considered to be an excellent model organism in these realms. Female parasitoids lay their eggs in or on other organisms (usually are insects) and the immature parasitoids develop by feeding on host tissues (Harvey, 2005). Unlike predators which require many preys to complete their maturity, development of parasitoids rely on the resources contained in a single host, and they often emerge only marginally smaller than the host as an adult (Sequeira and Mackauer, 1992, 1993; Harvey *et al.*, 1994, 1999; Harvey and Strand, 2002; Harvey *et al.*, 2004). As a result, host resources for parasitoid development are often highly limited (Slansky, 1986). On the other side, adult parasitoids exhibit apparent variation in their reproductive and associated traits, both within and among species (Jervis *et al.*, 2008). The potential impact of a parasitoid on the population depends, on the parasitoid's longevity, its fecundity, and the efficiency of host location (Harvey, 2005).

The external factors that affect egg production of parasitoids are adult nutrition (Ellers *et al.*, 1998; Giron *et al.*, 2004; Bezemer *et al.*, 2005), host availability (Ellers *et al.*, 2000; Carbone and Rivera, 2003; Zhang *et al.*, 2004; Harvey, 2008), temperature (Lysyk, 1998; Sagarra *et al.*, 2000), and age. Nutrients in adult diet of parasitoids are mainly including sugars, proteins and lipids (Jervis *et al.*, 2008), and both the acquisition and allocation of these nutrients are also different.

The majority of adult parasitoids require sugar sources, such as nectar or honeydew, as their main source of energy in the field, while the food source for rearing parasitoids in the laboratory is replaced by honey solution or sugar solution (Jervis and Kidd, 1986). Sugar feeding can increase parasitoid longevity (Wäckers, 1998, 2001; Siekmann *et al.*, 2001) as well as fecundity (Olson and Andow, 1998). Many studies have found that a lack of sugar availability may temporarily or permanently limit the reproductive success of parasitoids (Jervis and Kidd, 1986). In addition, well-fed parasitoids are usually also more active in host seeking (Wäckers, 1998).

The longevity and fecundity of female *I. naranyae* was significantly enhanced when feeding with carbohydrate than those without. Female *I. naranyae* lived more than 1.5 months with sugar source, but no more than two weeks when given water only (Fig. 2-2). Dissecting experiment revealed that both of the egg production and maturation were improved under the condition of sufficient carbohydrates (Fig. 2-5). In addition, they invested more to each egg and produced larger eggs (Fig. 5-2). Between different sugar sources, sucrose solution is a better food source for *I. naranyae* than honey solution because of a longer life-time. Due to the complex contents of honey, some of which may be harmful or bring a trouble to metabolite, honey may further affect the survival and reproduction, while the content of sucrose solution is much simpler and more easily to metabolize and convert to energy they need.

Some hymenopteran parasitoid species feed on host fluids or tissues as a part of adult nutrient sources that are directly utilized for egg production, a behavior known as ‘host-feeding’ (Chan and Godfray, 1993). The fact that only females consume host materials implied that the host-feeding may significantly correlate with the reproductive traits. Host-feeding is tightly associated with parasitoid nutritional ecology, because it is the primary method of obtaining nutritional resources such as proteins and lipids for most female parasitoids (Jervis and Kidd, 1986; Heimpel and Collier, 1996; Jervis *et al.*, 1996). It has been demonstrated that the adult nutrition, especially host-feeding, enhances both the egg production and longevity in synovigenic parasitoids (Jervis and Kidd, 1986). According to the research of Ueno and Ueno (2007), *I. naranyae* is a typical synovigenic host-feeding species, and the diet from host fluid plays a very important role in its egg production during the early adult life.

In the present study, the fecundity of *I. naranyae* increased when host fluid was given (Fig. 4-2). Especially the number of mature eggs was improved a lot with host-fluid, but the number of immature eggs was not significantly different comparing with individuals fed on sucrose solution (Fig. 4-2). However, the longevity of female *I. naranyae* was lower in the present of hosts than those deprived of hosts (Fig. 3-1). This finding indicates the presence of a trade-off between reproduction and life expectancy, i.e., cost of reproduction. The differing effects of host access on longevity may be based on the strategies that parasitoids employ to obtain resources for maintenance versus reproduction (Harvey, 2005; Jervis *et al.*, 2008). Female *I. naranyae* offered with host blood only produced smaller eggs than those with carbohydrates in their diet (Fig. 5-2).

Another confounding factor when considering reproductive resource allocation is female body size (Jervis *et al.*, 2008). Body size, which can directly reflect the resource acquiring and/or storing capability of an individual, is one of the key biological traits because of the critical correlation with life-history traits such as mating efficiency, dispersal capability and most importantly, reproductive success (Ueno, 1998, 1999b; Ellers *et al.*, 1998; Traynor and Mayhew, 2005). *I. naranyae* is a solitary parasitoid, which indicates that only one individual can successfully emerge from one host. Thus, the body size of this species is marginally restricted by the host they emerge. Because there is a great variation in the size of lepidopterous pupae, body size is commonly varied among *I. naranyae* individuals. In this study, body size of female *I. naranyae* positively affected their lifetime traits from different aspects. The longevity increased as the body size increase, no matter of food source types or the availability of host pupae (see Fig. 2-3 and Fig. 3-2). Larger females produced more eggs in their ovary and also mature more eggs (see Fig. 4-4 and Fig. 4-4). Moreover, the body size of *I. naranyae* also significantly and positively affected on their egg size. Larger females tend to produce more female offspring, but no effects on the fitness of their offspring were found.

In this dissertation, I employed two indexes for body size of *I. naranyae*, which were body weight at parasitoid emergence and forewing length. Although these two parameters were significantly correlated with each other, the effects were not. In the study of longevity, the forewing length seemed to be a better predictor of body size of female *I. naranyae*; while in the study of fecundity and egg size, the body weight at emergence was better. Large size benefits parasitoids directly in competing fitness functions, such as survival and reproduction (Ellers *et al.*, 1998; Rivero and West, 2002), but it is also assumed that large size would have negative impacts on some life-time traits, such as energy consumption, mobility and development. Forewing length is directly correlated with ability and consume of flight and other daily activities, so it would be more suitable in the test of life span. On the other hand, the body weight of an individual can directly reflect the resource stored during the larval stage, so here it may be more suitable in the research of nutrition acquisition and allocation of parasitoids.

The egg size affects both the survival and growth of offspring (Lalonde, 2005; Boivin and Gauvin, 2009), so the amount of maternal investment to each egg can be critical for the reproductive success of both mother and offspring (Giron and Casas, 2003). *I. naranyae* produce relatively large, yolk-rich eggs (Ueno and Ueno, 2007) which can bring enough nutrients to complete embryonic development. In this research, the egg size of *I. naranyae* exhibited a positive correlation with hatching time (Fig. 6-2), and a positive relationship was found between the egg size and the size of the hatching larva (Fig. 6-3). Because the super- and multi-parasites are common inside hosts of *I. naranyae*, faster development may be the superiority to overcome the competition of both inter- and intra-species.

Before emergence as an adult, parasitoids generally rely on a limited amount of resources that are obtained from a single source (the host), which was known as ‘capital resources’. Natural selection should prefer reproductive strategies that optimize acquisition and efficient metabolic allocation of these resources (Slansky, 1986; Mackauer and Sequeira, 1993). It has found that capital resources profoundly impact the adult life in many aspects such as longevity and reproductive success. Here, the development of *I. naranyae* before emergence is not restricted by egg size, but the inter-species competition leads to a delay of their development. This may be because of the intensive interspecies competition for larvae inside host.

Life-history strategies of *I. naranyae* are complex and flexible, and are affected by both internal (i.e. body size, egg production, age) and external factors (i.e. nutrition both prey and non-prey, host availability, environment). These factors combine together, restrict and improve with each other, influence the traits throughout life time.

SUMMARY

1. The importance of food for female reproduction of *I. naranyae*

Nutrient acquisition and allocation can critically impact the fitness of parasitoids (Rivero and Casas, 1999; Jervis *et al.*, 2008). Carbohydrates obtained by adults from host or non-host resources dramatically impact on the activity, longevity and lifetime fecundity of parasitoids (Boggs, 1981; Ho and Ueno, 2011). In this part, I examined: longevity and fecundity of *I. naranyae* with or without food source, and the relationship between female body size and longevity. Both fecundity and survival were strongly affected by the availability of food source. The result that the mean longevity of female *I. naranyae* is beyond 1.5 months demonstrates that *I. naranyae* has a considerably long lifetime among parasitoid species. The sharp decrease of lifetime in the absence of carbohydrate sources indicates that internal storage in *I. naranyae* is rapidly depleted when resources are allocated to both physical and metabolic activities. Moreover, fecundity sharply decreased when fed with water only. This result indicates that egg resorption may take place in *I. naranyae* under food limitation. Body size always positively and significantly affects longevity of female *I. naranyae*, no matter food conditions. However, in the effect tests, comparing with body weight, the forewing length may be a better predictor of body size of female *I. naranyae*. The resource re-allocation may be advantageous for this species with long lifetime because environments (host and food availability) should change temporarily during their lifetime. Thus future studies will then focus on the interaction between food and cost of reproduction in *I. naranyae*.

2. The cost of reproduction of *I. naranyae*

Trade-offs among life history traits plays an important role in shaping the reproductive strategy of parasitoids. *I. naranyae* is a typically synovigenic species and produces relatively large yolk-rich eggs (Ueno, 1998). Host-feeding has been demonstrated to be important in their lifetime egg production. In this chapter, I compared the female longevity when different food sources were given, and the effect of host availability. The longevity of female *I. naranyae* was lower when provided with hosts than those deprived of hosts, no matter of food types. This may indicate the cost of reproduction in *I. naranyae.* Besides, individuals fed with sucrose solution exhibited a relative longer lifetime than those with honey solution, implying a metabolic cost of the non-prey food sources in the field. If food limitation occurs, the degree of the cost may be much higher depending on the size of females. Here, body size of females had a positive effect on the longevity, but the interaction was significant only in the present of hosts or feed with sucrose solution.

3. Food and egg production of *I. naranyae*

Both of the capital resources storage during larval stage and the diet during the adult stage can have important effects on lifetime reproductive success of parasitoids (Hagan, 1986; Jervis *et al.*, 1996). The mode of egg production is one of life-history traits, which strongly links to the nutrient acquisition and allocation strategies of parasitoids (Roff, 1992). *I. naranyae* emerges with no mature eggs, indicating that it is extremely synovigenic. Thus, the adult nutrition seems to be important in egg maturation and reproductive success of *I. naranyae*. The adult diets of *I. naranyae* include two types, which are non-prey food (sugar sources) and host-feeding (proteins and lipids), and both of these nutrients are important for their reproduction. I examined the effects of a fixed amount of food on the egg production and egg maturation in the later life stage of *I. naranyae*. Both total eggs and mature eggs were critically increased after fed with host haemolymph, or with sucrose solution. Comparing with carbohydrates, host-feeding appeared to be most important in egg maturation of *I. naranyae*. Thus, female *I. naranyae* maturate eggs by using the nutrients from host blood. The mean difference between 3μL blood-fed and control females was 5.5 for mature eggs. This implies that 1μL host blood contains nutrients equivalent to 1.8 mature eggs. The body weight at emergence impacted the egg production and maturation more strongly than forewing length did, especially when interacted with food types. The body weight directly reflects the capability of resource acquiring and/or storing, so here it may be more suitable in the research of parasitoids fecundity.

4. Food and investment in egg size of *I. naranyae*

The egg size, which directly reflects the nutrient investment of mothers to their offspring, affects the survival and growth of offspring, so the amount of maternal investment to each egg can be critical to the reproductive success of both mother and offspring (Giron and Casas, 2003). It is assumed that large eggs bring higher fitness to offspring, but large eggs are more costly to produce (Bernardo, 1996; McIntyre and Gooding, 2000; Roff, 2002). The present study examined the effects of body size versus adult nutrition in later adult stage on the egg size of *I. naranyae*. The egg size of *I. naranyae* varied among individuals, which suggested that maternal investment to an egg varied among female individuals due to physiological and ecological constraints. Smaller *I. naranyae* produced smaller eggs, and food types affected the size of eggs produced. Sugar source availability affected the egg size more significantly than the other elements, because relatively larger eggs were produced comparing with those from individuals fed with host blood. *I. naranyae* thus can adjust egg size in response to nutritional state, and body size. In addition, large eggs were supposed to improve the fitness of offspring by overcoming the competition and mortality risk.

5. Egg size and reproductive success of *I. naranyae*

Reproductive success is one of the most important parameter to evaluate a natural enemy in biological control system. Egg size variation is common in parasitoid species, and may affect the fitness of offspring in many aspects. In *I. naranyae,* maternal investment to each egg may affect the development and survival before adult stage. Available nutrients for embryonic development are constrained within eggs, so the egg size should impact the embryonic development such as hatching time and hatching rate. While the food sources in larval stage are constrained within hosts, the quality and quantity of these resources would affect the development of larvae. Here, I examined the effect of egg size on the fitness traits of larval *I.*

naranyae. The egg length of *I. naranyae* exhibited a positive correlation with hatching time, and the size of 1st instars. Because the super- and multi-parasites are very common inside the hosts of *I. naranyae*, larger eggs may favor larvae to overcome the competition and survive successfully. The development of *I. naranyae* before emergence was not influenced by egg size, but the interspecies intensive competition inside host may lead to a delay of their development. Thus, I hypothesize that the egg size may not affect the development of *I. naranyae*.

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