



### A THESIS

### FOR THE DEGREE OF MASTER OF SCIENCE

# Effects of various legumes on physiological, morphological, and life-history traits in the bean bug, *Riptortus pedestris* (Hemiptera: Alydidae)

다양한 콩이 톱다리개미허리노린재(*Riptortus pedestris*)의 생활사, 형태적, 생리학적 형질에 미치는 영향에 관한 연구

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## UNDER THE DIRECTION OF ADVISER KWANG PUM LEE SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL OF SEOUL NATIONAL UNIVERSITY

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### ABSTRACT

# Effects of various legumes on physiological, morphological, and life-history traits in the bean bug, *Riptortus pedestris* (Hemiptera: Alydidae)

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The performance and fitness of an individual insect herbivore are strongly influenced by the nutritional quality of its food plant. There are multiple dietary factors determining the plant's nutritional quality, but the composition of macronutrients (e.g., protein, carbohydrate, lipid) is considered to be the most influential of all. Legumes are the seeds of plants belonging to the family Fabaceae and are one of the most widely cultivated crop plants worldwide. A number of legume species and varieties are currently cultivated in Korea and they are known to vary considerably in their composition of macronutrients. Among various insects attacking leguminous plants, the bean bug *Riptortus pedestris* (Fabricius) (Hemiptera: Alydidae) is one of the most important hemipteran pests on legumes and other crops in Korea, Japan, and China. Despite being a generalist-feeder, *R. pedestris* nymphs cannot complete their development without consuming legumes, indicating that legumes are essential for successful growth and development in this species.

The objective of this thesis was to explore how the consumption of different legume seeds or beans would influence the outcome of life-history, morphological, and physiological traits expressed within and across life-stages in *R. pedestris*. To achieve this aim, I performed two separate experiments using both the nymphs and adults of *R. pedestris*. In the first experiment (Experiment 1), the direct effects of 15 different types of beans on aforementioned traits were investigated separately for nymphs and adults. In the second experiment (Experiment 2), I examined how the nutritional quality of the diet consumed during the nymphal stage was carried over to affect adult fitness traits (lifespan and reproduction) and also how this carry-over effect of nymphal diet interacted with the quality of the diet consumed during the adult stage.

In Experiment 1, various traits were recorded from *R. pedestris* subjected to one of 15 different legume treatments. Nymphs fed on soybean, chickpea, and adzuki bean exhibited the highest survivorship. However, none of nymphs fed on kidney and white kidney bean survived to adulthood and this result is likely due to high concentrations of anti-nutritive compound (e.g., phytohaemagglutinin) present in these two kidney beans. Nymphs fed on sword bean showed the slowest development, while those on chickpea the fastest. The amount of lipids stored in the body of newly emerged adults was ca. 4-13 times higher in both male and female R. pedestris raised on peanut than in those on the other legumes. In consequence, body weight recorded at adult emergence was ca. 74.5-92.2% heavier in R. pedestris raised on peanut than on the others. Multivariate measurements of the size of various body parts were analyzed using the principal component analysis, with the first two principal components (PC1 and PC2) explaining ca. 72.3% of total variation. Males and females showed a major difference in the overall body shape (PC2), with females having wider thorax and abdomen and shorter hind leg femur than males. The overall body size (PC1) was larger in R. pedestris raised on mung bean. Unlike most other insects, males outlived females in R. pedestris. Lifespan was the longest on peanut in males and on palma bean in females. The total number of eggs produced over the lifespan was higher in females fed on protein-rich legumes, such as black soybean, soybean, and small black bean, than in those fed on the others. This result is consistent with the general notion that dietary protein is positively associated with fecundity in insects.

In Experiment 2, lifespan and reproductive output were recorded from adult *R. pedestris* assigned to one of nine treatments in which three nymphal and three adult diets were fully crossed. Three legumes used in this experiment were soybean, adzuki bean, and peanut, which were chosen based on their high nymphal survivorship and their contrasting difference in macronutrient composition. The

analyses revealed that there was a significant interaction between nymphal and adult diet for both lifespan and reproduction, indicating that diets consumed during different life-stages are not independent of each other. Males and females fed on adzuki bean as adult diet suffered substantially reduced lifespan compared to those fed on the other adult diets. In addition to this strong effect of adult diet, the carryover effect of nymphal diet on adult lifespan was also evident. For both males and females, *R. pedestris* that had been reared on peanut showed extended lifespan when they consumed the same peanut as their adult diet. Females fed on soybean during the adult stage produced more eggs per day and also over their lifespan than those on adzuki bean and peanut regardless of nymphal diet. It was noteworthy that this positive effect of soybean adult diet on egg production was most pronounced in females that had been raised on soybean during the nymphal stage. This indicates the presence of a significant carry-over effect of nymphal diet on adult reproduction although it remains to be determined whether this effect is due to environmental matching or silver spoon effect in *R. pedestris*.

This thesis offers the most detailed description of the impact of various legume beans on the individual performance and fitness in *R. pedestris*. The individual-based data obtained from this study will advance our fundamental understanding of how this important pest species responds to variation in dietary quality in the agricultural ecosystem, let alone improving our predictive power of its dynamics at population level.

Key words: Agricultural pest, Carry-over effect, Fitness, Leguminous plant, Life-history traits, Nutrition, *Riptortus pedestris* 

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### **INTRODUCTION**

Insects are the most diverse and the largest group of organisms on Earth (Schoonhoven et al., 1998). More than half of insects are phytophagous, feeding on diverse plant-derived materials, including leaves, roots, stems, flowers, fruits, and saps. The nutritional quality of food plant is determined by a number of dietary factors (e.g., fiber, secondary metabolites), but the compositions of macronutrients (e.g., proteins, carbohydrates, and lipids) are considered to be the most critical (Bernays and Chapman, 1993; Schoonhoven et al., 1998; Awmack and Leather, 2002; Singer et al., 2004). Especially, protein and carbohydrates are the two most predominant macronutrients present in the food of insect herbivores. The amount and mixture of these two macronutrients are known to vary considerably within and across plant parts, individuals, populations, and species and changes across time and space (Mattson, 1980; Yeoh et al., 1992; Bernays and Chapman, 1993; Awmack and Leather, 2002; Deans et al., 2016). Carbohydrates are the primary energy substrate for most insects while proteins are the major building blocks for somatic tissues, enzymes, and hormones and also serve as the metabolic energy. Experimental evidence accumulated over the last two decades has demonstrated that many insect herbivores have evolved a suite of behavioural and physiological mechanisms which enable them to regulate the intake of these two macronutrients independently to an optimal mixture (reviewed in Simpson and Raubenheimer, 2012; Simpson et al., 2015). There are ample studies describing the impact of dietary ratio of protein to carbohydrate on various measures of performance and fitness in a diverse range of insect herbivores (Behmer, 2009; Simpson and Raubenheimer, 2012).

Legumes refer to plants belonging to the family Fabaceae and, more specifically, their seeds. Legumes are one of the most widely cultivated crops worldwide and are an important source of protein, carbohydrate, and lipid for human consumption (Gebrelibanos et al., 2013; Smýkal et al., 2015; Maphosa and Jideani, 2017). There are a variety of legume plants currently cultivated in Korea, including soybean (*Glycine max*), mung bean (*Vigna radiata*), adzuki bean (*Vigna angularis*), cowpea (*Vigna unguiculata*), kidney bean (*Phaseolus vulgaris*), and so forth (Choung et al., 2003; Bae et al., 2009; Woo et al., 2016; Kim et al., 2018). These legumes are reported to vary substantially in their macronutrient composition (Sharma et al., 2014; Tang et al., 2014; O'Keefe et al., 2015; Kim et al., 2018). For example, soybean comprises 36% protein, 30% carbohydrate, and 20% lipids while adzuki bean is made up with 20% protein, 63% carbohydrate, and 1% lipids. Such variation in macronutrient composition is likely to result in different performance consequences for those insects feeding on legumes.

There are numerous insect herbivores causing serious damages on legumes plants in Korea, including those species belonging to the order Hemiptera, more

specifically the suborder Heteroptera: Halvomorpha halvs, Piezodorus hvbneri, Nezara antennata, and Dolycoris baccarum (Bae et al., 2007; 2008). Heteropterans or true bugs have sucking mouthparts through which they secrete saliva containing digestive enzymes to break down plant tissues and suck digested or liquified foods (Rahman and Lim, 2017). Among these heteropterans, the bean bug, Riptortus pedestris (Fabricius), is considered to be one of the most important pests on legumes and other orchard or crop plants in Korea, Japan, and China (Kim et al., 2012; Lim, 2013; Kim et al., 2014; Kai et al., 2019). This pest status had led to a number of studies conducted to understand the general biology of R. pedestris (Sakura, 1998; Bae et al., 2009; Okada, et al., 2011; Lim, 2013; Lim et al., 2015). R. pedestris has three generations per year: the first emerging from early July to early August, the second from mid-August to late September, and the third from early October to mid-November (Huh et al., 2005). As a hemimetabolous insect, R. pedestris undergoes five nymphal instars before molting into the adult stage (Fig. 1). Both the adults and nymphs of R. pedestris are the generalist herbivore, attacking a broad range of host plants (Lee at al., 2004; Youn and Jung, 2008), including many leguminous (G. max, Vicia villosa, Astragalus sinicus, Trifolium pretense, etc.), non-leguminous plants (Oryza sativa, Sesamum indicum, etc.), and fruit trees (Mizutani et al., 2011; Seo et al., 2011; Lim, 2013). Despite their versatile appetite, the nymphs of *R. pedestris* have been reported to be unable to complete their juvenile development when consuming fruits (apples and sweet persimmons) only (Kim and Lim, 2012). It is well established that *R. pedestris* nymphs must feed on legume seeds, especially soybean, for their successfully development (Bae et al., 2009; Mainali et al., 2014).

In this thesis, I investigated how different types of legume seeds or beans with differing nutritional quality would affect the performance and components of fitness expressed during and across the nymphal and adult stage in R. pedestris. Two separate but interrelated experiments were conducted in this thesis. The first experiment (Experiment 1) was aimed at testing the direct impacts of 15 different types of beans on various traits expressed in both R. pedestris nymphs and adults. Previous studies have repeatedly demonstrated that soybean (Glvcine max) is a high-quality food source that supports full development in R. pedestris (Kim and Lim, 2010; Kim et al., 2014). In addition to soybean, I chose 14 different species and varieties of legume seeds commonly cultivated and commercially available in Korea to compare their effects on various life-history, morphological, and physiological traits in R. pedestris. Life-history traits are those characters that define the lifetable of an organism and include traits such as longevity, the number of offspring produced, generation time, growth rate, body size, juvenile survival, and so forth (Stearns, 1992, 2000; Roff, 2002). Life-history traits are generally considered to be strongly linked to Darwinian fitness and the phenotypic expressions of these traits are strongly dependent upon nutrition (Lee et al., 2008; Simpson and Raubenheimer, 2012). Besides those key life-history traits, I recorded

the length and width of various body parts (head, thorax, abdomen, hind femur) from individual insects raised on these 15 legume diets. The composite measurement of these morphological traits was then used to analyze the effect of diet on body size. Since the amount of lipid reserves stored in the body is an important determinant of life or death under starvation (Rion and Kawecki, 2007; Lee and Jang, 2014), I also quantified this physiological trait from the experimental insects in Experiment 1.

The results obtained from Experiment 1 allowed me to have the general information regarding the direct effects of dietary legumes on adult and nymphal traits, but these effects were investigated independent of each other. However, in reality, the diet consumed early in life or during development is known to have profound consequences for traits expressed later in life or during adulthood. This so-called carry-over effects are reported to occur in a wide range of organisms, including insects (Zwaan et al., 1991; Tu and Tatar, 2003; Barrett et al., 2009; May et al., 2015; Stefana et al., 2017; Davies et al., 2018), and are often manifested by the presence of a significant interaction between developmental and adult environments (Boggs and Freeman, 2005; Bath et al., 2018; Nguyen et al., 2018). Currently, there are two major hypotheses proposed to explain the role play by the interaction between early and adult environments in shaping adult phenotype (Monaghan, 2008; Scharf et al., 2015): "environmental matching" vs. "silver spoon hypothesis". Environmental matching hypothesis predicts that the performance or

fitness of an individual animal is highest when the environment in which it lives as an adult matches that in which it has developed. Some evidence for this hypothesis has come from humans. When born or developed under nutritionally poor conditions, an individual is thought to develop into a "thrifty phenotype" which has enhanced capacity to store lipids and maintain high blood glucose (Hales and Barker, 2001; Bateson et al., 2004; Spencer et al., 2006). This metabolic adjustment in response to nutritionally poor environment encountered early in life may confer fitness advantages if poor adult conditions follow. However, if it encounters nutritionally rich environment later in life, this mismatch may lead an individual raised under poor condition to become prone to diabetes and obesity, thereby suffering fitness losses (Hales and Barker, 2001, Bateson et al., 2004; Gluckman et al., 2005; Fernandez-Twinn and Ozanne, 2006). On the other hand, silver spoon hypothesis posits that the performance and fitness of an individual raised under nutritionally rich environments is permanently better than those raised under nutritionally poor environments no matter whether the quality of adult environments is good or bad (Monaghan, 2008, Min et al., 2021). For example, it has been generally observed that animals grown or born under nutritionally favorable conditions exhibit larger body size, healthy body conditions, greater reproductive success, and longer longevity than those experienced nutritionally unfavorable conditions during development (Pigeon et al., 2019). There are ample empirical evidence in support of this hypothesis found in a wide range of insects, including damselflies (Block and Stoks, 2005), ladybird beetles (Dmitriew and Rowe, 2011), etc. This hypothesis assumes that the condition experienced during development is likely to impose a major constraint on adult phenotype.

The second experiment (Experiment 2) of this thesis was designed to test these two competing but not mutually exclusive hypotheses by investigating how the diet environments experienced by R. pedestris during the nymphal stage interact with those experienced during the adult stage to affect the two of adult life-history traits directly related to Darwinian fitness. Based on the results of Experiment 1, I selected three legume diets that support high larval survivorship but contained contrastingly different macronutrient compositions (soybean, aduzki bean, and peanut) and used these diets to create nymphal and adult nutritional conditions. In this experiment, I employed 3 x 3 fully factorial experimental design in which lifespan and various measures of reproductive success were measured form adults that were allocated to one of nine treatments representing a full combination of three nymphal and three adult environments. If R. pedestris follows the "environmental matching hypothesis", the fitness is expected to be higher in *R. pedestris* individuals when the diets consumed during the adult and nymphal stage match than when they mismatch. The cross-over interaction between adult and nymphal diet is therefore expected in this case. If there exists a silver spoon effect, the fitness is predicted to be higher in those individuals fed on high-quality nymphal diets regardless of adult diet, either resulting in a non-significant interaction term between the two diets. If adult diet affects the magnitude of the silver spoon effect without altering the order, a significant non-cross over interaction term is also predictable.



Figure 1. The overview of the life-cycle of *Riptortus pedestris*.

## **MATERIALS AND METHODS**

#### 1. Stock culture

The stock culture of R. pedestris used in this study came from the Pest Management Institute (PMI), Baekam, Gyeonggido Province, and was originally established from individual insects derived from the laboratory population kept at Andong University, Andong, Gyeongsangbukdo Province, in 2016. Throughout the stock maintenance, numerous individuals collected from the soybean field around Baekam area were added to the population to maintain the genetic variations of the stock culture. The new stock culture was established at Seoul National University, Seoul, in 2018 using more than 500 individual nymphs arrived from the PMI. Throughout the culturing period, adults and nymphs were kept separately, each in different-sized acrylic cages (adult cage: 45 cm × 45 cm × 45 cm; nymph cage: 20  $cm \times 40 cm \times 20 cm$ ). Soybeans were provided in 20-cm and 9-cm petri-dishes for adults and nymphs, respectively. Plastic cups  $(3 \text{ cm} \times 3 \text{ cm})$  filled with water and capped with sponges were presented to both adults and nymphs as water source. The insects were maintained at  $25^{\circ}$  under a 16:8 L:D photoregime. An average of 80 adults and 500 nymphs with mixed sex were maintained for each cage. To collect the eggs to start off the new generations of R. pedestris, I placed 8-10 cotton pads (2 cm x 3 cm) inside each adult cage as oviposition substrate and allowed females

to lay their eggs on these pads for three or four days. Eggs laid on these pads were collected and subsequently transferred into the nymph cages where they were hatched and grow until adult emergence.

### 2. Experimental design

Two separate experiments were performed in this thesis, each with different objective and experimental setup. The first experiment (Experiment 1, henceforth) was designed to examine the effect of various legumes cultivated in Korea on multiple physiological, morphological, and life-history traits in the nymph and adult of *R. pedestris*. In this experiment, *R. pedestris* nymphs or adults were fed on one of 15 different types of legume beans (see detailed information summarized in Table 1) until adult emergence or death, respectively. The composition of macronutrients present in these 15 legumes varied substantially, as summarized in Fig. 2. Experiment 1 was mainly focused on investigating the direct and independent impact of legume diet on larval and adult performance in *R. pedestris*. However, the objective of the second experiment (Experiment 2, henceforth) was to test whether the nutritional quality of the diet ingested during early development would be carried over to influence the key adult life-history traits that are directly linked to Darwinian fitness, lifespan and reproduction. Based on the data obtained from Experiment 1, I selected three legume diets that differed markedly in their

macronutrient composition (e.g. protein, carbohydrate, lipid) but supported the full development of R. pedestris nymphs. Three legumes used in Experiment 2 were soybean (Glycine max), adzuki bean (Vigna angularis), and peanut (Arachis hypogaea), which were known to be rich in protein, carbohydrates, and lipids, respectively. According to the results of Experiment 1, the survivorship of R. pedestris nymphs fed on soybean, adzuki bean, and peanut was ca. 75, 72.5, and 60%, respectively. In Experiment 2, I employed a complete  $3 \times 3$  factorial design where three nymphal diets and three adults were fully crossed to yield a total of nine nymphal and adult diet combinations. In brief, adult insects newly emerged from one of three nymphal diets were randomly allocated to either the same diet on which they were raised or the other two diets different from their nymphal diet. The schematic summary of the experimental setup employed in Experiment 2 is illustrated in Fig. 3. This full factorial experimental design enabled me to statistically assess how the interactions between development and adult food environments would shape the adult lifespan and reproduction in R. pedestris. All experiments were conducted in incubators set at a constant temperature of  $25\,^\circ\!\mathrm{C}$ under a 16:8 L:D photoregime and 50-60% relative humidity.

Common name	Genus	Species
Soybean	Glycine	max
Var. Black soybean		
Var. Seonbijabi bean		
Var. Palma bean		
Chickpea	Cicer	arietinum
Small black bean	Rhynchosia	nulubilis
Adzuki bean	Vigna	angularis
Mung bean		radiata
Cowpea		unguiculate
Peanut	Arachis	hypogaea
Kidney bean	Phaseolus	vulgaris
Var. White kidney bean		
Sword bean	Canavalia	gladiata
Lentil bean	Lens	culinaris
Pea	Pisum	sativum

 Table 1. The list of leguminous plants used in the present study.



Figure 2. Summary of the macronutrient compositions in legumes used in the current study.



Figure 3. Schematic outline of the  $3 \times 3$  full factorial experimental design employed in Experiment 2.

#### 3. Experiment 1: the effects of various legumes on nymphal and adult traits

#### 3.1. Nymphal traits

Newly laid eggs were harvested from the stock culture by placing cotton pads (oviposition substrate) inside the adult cage containing more than 80 adults for 24 h, as described above. Collected eggs were placed in 20-cm petri-dishes and then kept in an incubator set at 28°C. Upon hatching, the first-instar nymphs were individually transferred to 5-cm petri-dishes and randomly allocated to one of 15 diet treatments. In each diet treatment, the insects were presented to their respective beans or seeds, which were fixed on the floor of their experimental area by polymer clay. Throughout the experiment, microcentrifuge tubes (1.5 mL) filled with water and capped with cotton plug were provided to individual insects as water source. Food and water were replaced every three or four days. When nymphs reached their fourth instar, the lid of the petri-dish was replaced with a plastic cup  $(3 \text{ cm} \times 3 \text{ cm})$ . This was done to ensure that nymphs would have sufficient space to complete their molting into the fifth instar or into the adult stage successfully. The nymphs that died before the second instar in a treatment were removed from the experiment and newly hatched nymphs were added to that treatment to replace the dead ones. There were 40 replicates per treatment, resulting in a total of 600 insects used in this experiment.

Newly emerged adults were collected within 24 h, sexed, weighed to the nearest 0.1 mg, and frozen to death in  $-20^{\circ}$ C fridge. In order to measure the length and width of body parts (e.g., hind leg femur, head, thorax, and abdomen), hind legs were carefully separated from the main body using dissection scissors. Each body was then photographed using a Canon EOS 600D digital camera (Canon Inc., Tokyo, Japan) mounted with a Cannon EF 100mm f/2.8 USM macro lens (Fig. 4). The morphological parameters of each insect were measured from these photographed images using Image J software (National Institute of Health, NIH). Photographed insects were dried in an oven set at  $40^{\circ}$ C for three days and re-weighed to obtain dry body weight. Dried carcasses were sealed in polyethylene bags and lipidextracted by soaking these sealed carcasses in 10-mL of fresh chloroform for two days, twice. After this lipid extraction, the carcasses were re-dried and re-weighed. The lipid content for each insect was calculated as the mass difference before and after lipid extraction. Larval survivorship was determined as the proportion of the first-instar nymphs that successfully molted into the adult stage for each diet treatment. Development time was calculated as the number of days from egg hatch to adult emergence for individual insects.



Figure 4. Body parts of *R. pedestris* measured as morphological traits. All measurements were calibrated to the nearest 1 mm as well as the hind femur area.

#### 3.2. Adult traits

Experimental insects used for measuring adult life-history fitness traits were reared as nymph on soybean in acrylic cages at the density of 500 per cage, as previously described. Upon adult emergence, newly emerged adults were sexed and unrelated males and females were randomly paired in breeding dishes (10-cm diameter  $\times$  4 cm) which had fine mesh for ventilation in the lid. Each pair then received one of the same 15 diet treatments that were used for quantifying nymphal traits. There were 20 replicate pairs used for each treatment, thus resulting in a total of 300 male-female pairs in this experiment. In each breeding dish, each pair of male and female R. pedestris received ad libitum supply of legumes or beans placed in a 3-cm petri-dish and water-soaked cotton wool dangled from the ceiling of the breeding dish. I ensured that cotton wool was always moistened by adding extra water daily. Food dishes were replaced every five days. In order to provide oviposition substrate, a cotton pad was attached on the wall in each breeding dish. The number of eggs deposited in the oviposition substrate was counted at five-day intervals. Counted eggs were transferred into 9-cm petri-dishes and checked daily for hatched nymphs. The number of hatched nymphs was also counted and egg hatchability was calculated as the percentage of hatched eggs. The lifespan of individual insects was determined by checking their death daily. Lifetime egg production was counted as the total number of eggs produced by each female over the course of its lifetime and egg production rate was estimated by dividing lifetime

egg production by lifespan.

#### 3.3. Statistical analysis

All statistical analyses were carried out using SAS v 9.4 (SAS Institute, Cary, NC, USA). The effect of diet treatment on nymphal or pre-adult survivorship was analyzed using the generalized liner model with logit link function and binomial distribution (survived=1, dead=0) while that on female egg production was analyzed using one-way analysis of variance (ANOVA). The effect of diet treatment, sex, and their interaction on lifespan, development time, body weight, and lipid content was analyzed using two-way ANOVA.

In this study, the body size of *R. pedestris* was measured from various body parts in an individual insect, including the width and length of head, thorax, abdomen, and hind leg femur. Since a large number of body size measures were examined (n=8), I applied principal component analysis to extract principal components (PCs) for all male and female individuals raised on 15 diet treatments. According to the Eigen values calculated, the first two PCs (PC1 54.7% and PC2 17.6%) explained ca. 72.3% of total variation in body size parameters and therefore were used for further analyses. Since all of its Eigen values or coefficients were positive (Table 2), the PC1 was interpreted as a vector associated with body size. The PC2 comprised both positive and negative Eigen values, having positive
loading for abdomen width and length, head width and length, and thorax width and negative loading for hind femur width and length, and thorax length (Table 2). The PC2 was thus considered as a vector associated with body shape. For example, higher values on PC2 correspond to wider abdomen and shorter hind femur. Twoway ANOVA was used to analyze the effect of diet treatment, sex, and their interaction on PC1 and PC2.

	Eigenvectors			
Trait Pr	in1	Prin2		
Femur length 0.2	478	-0.6577		
Femur width 0.4	129	-0.2084		
Head length 0.2	613	0.2188		
Head width 0.4	125	0.1417		
Thorax length 0.4	221	-0.2120		
Thorax width 0.4	127	0.1395		
Abdomen length 0.3	686	0.0903		
Abdomen width 0.2	132	0.6193		

**Table 2.** Summary of the Eigen-vector values of the two principal components (PC1 and 2) extracted from the principal component analysis conducted on eight body parts.

## 4. Experiment 2: the interaction between nymphal and adult diet

Eggs were harvested from the stock culture, as previously described. The first instar nymphs freshly hatched from these eggs were randomly transferred into nymph cages (see above) in which they were reared on either soybean, adzuki bean, or peanut with *ad libitum* supply of water. Upon molting into adults, males and females emerged from the same diet treatment were randomly paired in breeding dishes (see above) used for measuring adult traits in Experiment 1. Each male-female pair was then randomly allocated to one of three adult diet treatments (soybean, adzuki bean, or peanut). The identical protocol used in Experiment 1 was also followed in Experiment 2 to provide sufficient food and water to insects and also to record their lifespan and reproductive outcome. There were 40 replicate pairs per each treatment, resulting in a total of 360 pairs used in this experiment. Just like in Experiment 1, the effect of diet treatment encountered during the nymphal and adult stage and their interaction on male and female lifespan and female reproduction was analyzed using two-way ANOVA.

# RESULTS

## 1. Experiment 1: the effects of various legumes on nymphal and adult traits

### 1.1. Nymphal traits

Nymphal survivorship was significantly affected by diet treatment ( $\chi^2 = 55.82$ , df = 12, P < 0.001), with nymphs fed on soybean exhibiting the highest survivorship followed by chickpea, adzuki bean, seonbijabi bean, cowpea, mung bean, peanut, palma bean, small black bean, pea, black soybean, and lentil bean (Fig. 5). It is noteworthy that none of nymphs fed on kidney and white kidney bean survived to the adult stage. The survivorship curves describing the age-dependent decrease in nymphal survival are shown in Fig. 6. *R. pedestris* nymphs fed on soybean, chickpea, and adzuki bean maintained high survivorship across the nymphal period while those fed on kidney and white kidney bean showed rapid drop in survivorship during their early development, with all dying by an average of 12.3 and 11.2 days after egg hatch, respectively (Fig. 6). Diet treatment also had a significant impact on nymphal development time (Table 3). Neither sex nor the interaction between sex and diet treatment influenced the duration of nymphal stage in *R. pedestris* (Table 3). Nymphs fed on sword bean showed the most delayed development (mean  $\pm$  SE: 26.8  $\pm$  0.65 days) whereas those on chickpea were the

fastest to reach the adult stage  $(23.3 \pm 0.35 \text{ days})$  (Fig. 7). Dry body weight recorded at adult emergence was significantly affected by diet treatment (Table 3). Females tended to be heavier than males, but the effect of sex on dry body weight marginally missed the statistical significance (Table 3). For both sexes, nymphs fed on peanut were the heaviest among all 13 treatments (Fig. 8). There was no significant interaction between the two main factors (Table 3), indicating that this association between diet and dry body weight was consistent across sex. Lipid content at adult emergence was significantly affected by diet treatment (Table 3), with nymphs fed on peanut accumulating more than 4-13 times higher body lipid (15.3 mg) than those on the other diets (1.2-3.9 mg) (Fig. 9). Males and females accumulated similar amounts of lipids in their body and they responded to diet treatments in a qualitatively similar manner, as indicated by non-significant effect due to sex and the interaction between sex and diet treatment (Table 3).



Figure 5. Effect of legume diet treatment on nymphal survivorship in *R. pedestris*.



Figure 6. Survivorship curves fitted for *R. pedestris* raised on 15 diet treatments during the nymphal stage.

Trait	Source	DF	MS	F	Р
Development time	Diet	12	20.08	3.35	< 0.001
	Sex	1	3.55	0.59	0.443
	Diet × Sex	12	7.58	1.26	0.241
	Error	259	6.00		
Dry body weight	Diet	12	204.17	25.72	< 0.001
	Sex	1	25.46	3.21	0.075
	Diet × Sex	12	7.05	0.89	0.560
	Error	259	7.94		
Lipid content	Diet	12	266.72	163.61	< 0.001
	Sex	1	$3.80 \times 10^{-3}$	0	0.962
	Diet × Sex	12	1.56	0.96	0.488
	Error	259	1.63		

**Table 3.** Results of two-way ANOVA testing the effects of diet, sex, and the interaction on nymphal development time, body weight, and lipid content at adult emergence in *R. pedestris*.



Figure 7. Effect of legume diet treatment on nymphal development time in R. *pedestris*. The values are mean  $\pm$  standard error.



Figure 8. Effect of legume diet treatment on nymphal dry weight in *R. pedestris*. The values are mean  $\pm$  standard error.



Figure 9. Effect of legume diet treatment on nymphal lipid content in *R. pedestris*. The values are mean  $\pm$  standard error.

#### 1.2. Morphological traits

Various morphological characters were measured from newly emerged R. pedestris adults. The length and width of head were significantly affected by diet treatment and sex (Table 4). Overall, females had 2.9% longer and 2.3% wider head compared to males (Fig. 10, 11). The interaction between diet treatment and sex was significant for head length but not for head width (Table 4). The length and width of thorax were significantly affected by diet treatment (Table 4). Males and females did not show any significantly difference in their thorax length (Table 4), but females had 4.6% wider thorax than males, as indicated by a significant effect of sex on thorax width. For both thorax length and width, the interaction between the two main factors was not significant (Table 4). Abdomen length was significantly affected by diet treatment, but not by sex (Table 4). While the length of abdomen did not differ between the sexes, females had 10.0% wider abdomen than males, as indicated by a significant effect of sex (Table 4). There was no significant interaction between diet treatment and sex for both measured aspects of abdomen (Table 4). The length of hind femur was significantly affected by diet treatment and sex, but its width was significantly affected by diet treatment only (Table 5). Males had 13.2% longer hind femur than females, but the width of hind femur was not significantly different between the two sexes (Fig. 16, 17). There was no significant interaction between the two main factors for both hind femur length and width (Table 5).

The PC1, which was a vector associated with body size, was significantly affected by diet treatment, but neither by sex nor by the interaction between the two factors (Table 6). Regardless of sex, *R. pedestris* showed the highest PC1 value when raised on mung bean (Fig. 18). The lowest PC1 was observed on sword bean in males and on lentil bean in females (Fig. 18). The PC2, which was a vector representing body shape, was significantly affected by diet treatment, sex, and the interaction between the two factors (Table 6). Females had a higher PC2 than male (Fig. 18), suggesting a shape difference between the sexes. Higher PC2 for females indicates a wider abdomen and shorter hind femur in comparison to males.

Trait	Source	DF	MS	F	Р
Head length	Diet	12	0.03	2.44	0.005
	Sex	1	0.15	10.66	0.001
	$Diet \times Sex$	12	0.03	2.1	0.018
	Error	259	0.01		
Head width	Diet	12	0.04	6.58	< 0.001
	Sex	1	0.22	38.18	< 0.001
	$Diet \times Sex$	12	$2.98 \times 10^{-3}$	0.53	0.897
	Error	259	$5.66 \times 10^{-3}$		
Thorax length	Diet	12	0.15	2.32	0.008
	Sex	1	0.25	3.78	0.053
	$Diet \times Sex$	12	0.05	0.82	0.627
	Error	259	0.07		
Thorax width	Diet	12	0.17	3.89	< 0.001
	Sex	1	1.42	32.91	< 0.001
	$Diet \times Sex$	12	0.04	0.86	0.587
	Error	259	0.04		
Abdomen length	Diet	12	0.27	3.25	< 0.001
	Sex	1	0.04	0.51	0.474
	$Diet \times Sex$	12	0.10	1.14	0.325
	Error	259	0.08		
Abdomen width	Diet	12	0.45	7.85	< 0.001
	Sex	1	5.78	102.02	< 0.001
	$Diet \times Sex$	12	0.07	1.23	0.260
	Error	259	0.06		

**Table 4.** Results of two-way ANOVA testing the effects of diet, sex, and the interaction on the width and length of head, thorax, and abdomen in *R. pedestris*.



**Figure 10**. Effect of legume diet treatment on (A) male and (B) female head length in *R. pedestris*. The values are mean  $\pm$  standard error.



**Figure 11**. Effect of legume diet treatment on (A) male and (B) female head width in *R. pedestris*. The values are mean  $\pm$  standard error.



**Figure 12**. Effect of legume diet treatment on (A) male and (B) female thorax length in *R. pedestris*. The values are mean  $\pm$  standard error.



**Figure 13**. Effect of legume diet treatment on (A) male and (B) female thorax width in *R. pedestris*. The values are mean  $\pm$  standard error.



**Figure 14**. Effect of legume diet treatment on (A) male and (B) female abdomen length in *R. pedestris*. The values are mean  $\pm$  standard error.



**Figure 15**. Effect of legume diet treatment on (A) male and (B) female abdomen width in *R. pedestris*. The values are mean  $\pm$  standard error.



**Figure 16**. Effect of legume diet treatment on (A) male and (B) female hind femur length in *R. pedestris*. The values are mean  $\pm$  standard error.



Figure 17. Effect of legume diet treatment on (A) male and (B) female hind femur width in *R. pedestris*. The values are mean  $\pm$  standard error.

Trait	Source	DF	MS	F	Р
Length	Diet	12	0.44	3.50	< 0.001
	Sex	1	31.06	250.08	< 0.001
	Diet × Sex	12	0.18	1.44	0.147
	Error	259	0.12		
Width	Diet	12	0.02	3.66	< 0.001
	Sex	1	$4.1 \times 10^{-3}$	0.61	0.435
	Diet × Sex	12	$4.3 \times 10^{-3}$	0.65	0.803
	Error	259	$6.7 \times 10^{-3}$		

**Table 5.** Results of two-way ANOVA testing the effects of diet, sex, and the interaction on the width and length of hind leg femur in *R. pedestris*.

Trait	Source	DF	MS	F	Р
PC1	Diet	12	11.26	2.83	0.001
	Sex	1	10.50	2.64	0.106
	Diet × Sex	12	3.23	0.81	0.640
	Error	259	3.98		
PC2	Diet	12	2.84	9.74	< 0.001
	Sex	1	197.88	678.59	< 0.001
	Diet × Sex	12	0.64	2.20	0.012
	Error	259	0.29		

**Table 6.** Results of two-way ANOVA testing the effects of diet, sex, and the interaction on the PC1 and PC2 in *R. pedestris*.



**Figure 18**. Scatter plot describing the distribution of the two principal components (the PC1 and 2). In (A), the mean values of PC1 and PC2 averaged from male and female *R. pedestris* raised on 15 legume diet treatments are plotted. In (B), individual components for all males and females are presented as green and purple symbols, respectively.

#### 1.3. Adult traits

Lifespan was significantly affected by sex, diet treatment, and the interaction between the two main factors (Table 7). Overall, males had longer lifespan than females (mean  $\pm$  SE: male, 28.9  $\pm$  0.75 days *versus* female, 21.2  $\pm$  0.59 days), but the presence of the interaction between sex and diet treatment indicated that the way in which diet treatment exerted its effect on lifespan was different between males and females. This interaction led me to analyze the effect of diet treatment on lifespan separately for each sex (Table 7). The separate one-way ANOVAs confirmed the significant effect of diet treatment for both sexes (male:  $F_{14,285} = 12.32$ , P < 0.001; female:  $F_{14,285} = 6.36$ , P < 0.001). Lifespan was the longest on peanut in males and on palma in females (Fig. 19A, B).

Lifetime egg production was significantly affected by diet treatment ( $F_{14,268}$  = 8.50, P < 0.001). The total number of eggs laid over the lifespan was high in female *R. pedestris* on black soybean (mean ± SE: 144.3 ± 14.18 eggs), soybean (135.6 ± 16.09 eggs), seonbijabi bean (130.4 ± 18.04 eggs), and small black bean (127.0 ± 22.24 eggs) and low in those on white kidney (14.0 ± 2.19 eggs) and kidney bean (20.3 ± 4.23 eggs; Fig. 20). Egg production was also affected by diet treatment ( $F_{14,268} = 9.73$ , P < 0.001), showing a pattern similar to that of lifetime egg production (Fig. 21). Egg hatchability was the highest percentage on black soybean (mean ± SE: 87.80 ± 2.85%) and the lowest on pea (71.5 ± 6.72%), but was not significantly different across diet treatments ( $F_{14,268} = 0.63$ , P = 0.842; Fig. 22).

 Table 7. Results of two-way ANOVA testing the effects of diet, sex, and the interaction on adult lifespan in *R. pedestris*.

Source	DF	MS	F	Р
Diet	14	1701.24	17.72	< 0.001
Sex	1	8816.67	91.83	< 0.001
Diet × Sex	14	172.70	1.80	0.036
Error	570	96.02		



**Figure 19**. Effect of legume diet treatment on (A) male and (B) female lifespan in *R. pedestris*. The values are mean  $\pm$  standard error.



Figure 20. Effect of legume diet treatment on lifetime egg production in R. *pedestris*. The values are mean  $\pm$  standard error.



Figure 21. Effect of legume diet treatment on egg production rate in *R. pedestris*. The values are mean  $\pm$  standard error.



Figure 22. Effect of legume diet treatment on hatchability in *R. pedestris*. The values are mean  $\pm$  standard error.

# 2. Experiment 2: the interaction between nymph and adult diet

### 2.1. Adult lifespan

There was also a significant overall effect due to sex (Table 8), showing that: males lived 34.6% longer than females (mean  $\pm$  SE: male, 33.1  $\pm$  1.33 days versus female,  $24.6 \pm 1.01$  days). Full model ANOVA in which sex, adult diet, nymph diet, and their two- and three-way interactions were included detected a significant twoway interaction between sex and adult diet ( $F_{2,596} = 3.27$ , P = 0.039), leading me to analyze the effect of the nymphal diet, adult diet, and their interaction separately for males and females. The lifespan of male R. pedestris was most strongly affected by adult diet (Table 9), with male bugs fed on adzuki bean having substantially shorter lifespan ( $15.0 \pm 0.99 \sim 21.7 \pm 1.79$  days) than those fed on soybean ( $32.0 \pm$  $3.23 \sim 41.6 \pm 2.90$  days) and peanut ( $39.1 \pm 4.91 \sim 51.7 \pm 5.63$  days). The effect of adult diet on male lifespan was dependent upon the type of diet consumed during the nymphal stage, as indicated by a significant interaction between adult and nymphal diet (Table 9). The lifespan of males consumed soybean and peanut during their adult stage was similar when they consumed soybean and adzuki bean during their nymphal stage. However, when peanut was consumed during their nymphal stage, lifespan was longer in male R. pedestris fed on peanut at the adult stage than in those fed on soybean (Fig. 23A).

The pattern of female lifespan expressed across diet treatments was

qualitatively similar to that of male lifespan. Just like male lifespan, female lifespan was most profoundly affected by adult diet (Table 9). Compared to those fed on soybean ( $25.8 \pm 2.28 \sim 36.4 \pm 2.57$  days) and peanut ( $27.4 \pm 3.97 \sim 36.6 \pm 4.56$  days) as their adult diet, female *R. pedestris* fed on adzuki bean during the adult stage showed substantially reduced lifespan ( $10.9 \pm 0.74 \sim 15.0 \pm 1.14$  days) regardless of the type of nymphal diet. A significant interaction between adult and nymph diet was also detected for female lifespan (Table 9), which indicates that the effect of adult diet was not independent of the diet consumed during the nymphal stage. When soybean was consumed during their nymphal stage, lifespan was longer in female *R. pedestris* fed on soybean at the adult stage than in those fed on peanut (Fig. 23B). However, the reverse was observed when female *R. pedestris* consumed peanut during their nymphal stage (Fig. 23B).

DF	MS	F	Р
2	1141.95	3.54	0.030
2	28700.98	88.93	< 0.001
1	11341.38	35.14	< 0.001
4	1529.22	4.74	< 0.001
2	60.82	0.19	0.828
2	1055.83	3.27	0.039
4	233.42	0.72	0.576
596	322.75		
	DF 2 2 1 4 2 2 4 596	DFMS21141.95228700.98111341.3841529.22260.8221055.834233.42596322.75	DF         MS         F           2         1141.95         3.54           2         28700.98         88.93           1         11341.38         35.14           4         1529.22         4.74           2         60.82         0.19           2         1055.83         3.27           4         233.42         0.72           596         322.75         322.75

**Table 8.** Results of three-way ANOVA testing the effects of nymphal diet, adult diet, sex, and their interactions on adult lifespan in *R. pedestris*.

Trait	Source	DF	MS	F	Р
Male lifespan	Nymphal diet	2	411.79	1.02	0.363
	Adult diet	2	19534.39	48.20	< 0.001
	Nymphal × Adult diet	4	1101.07	2.72	0.03
	Error	298	405.26		
Female lifespan	Nymphal diet	2	790.98	3.29	0.039
	Adult diet	2	10222.42	42.55	< 0.001
	Nymphal × Adult diet	4	661.57	2.75	0.028
	Error	298	240.23		

**Table 9.** Results of two-way ANOVA testing the effects of nymphal diet, adult diet, and the interaction on male and female lifespan in *R. pedestris*.



**Figure 23**. Effect of nymphal and adult diets on (A) male and (B) female lifespan in *R. pedestris*. The values are mean  $\pm$  standard error.
#### 2.2. Adult fecundity

Lifetime egg production was affected by nymphal diet, adult diet, and the interaction between them (Table 10). Regardless of the diet consumed during the larval stage, female R. pedestris fed on soybean as their adult diet produced significantly more eggs over their lifetime than those fed on peanut and adzuki bean (Fig. 24). It is important to note that this positive effect of soybean adult diet on lifetime egg production was most pronounced in female *R. pedestris* that had been fed on soybean during the nymphal stage (Fig. 24). This resulted in the highest lifetime egg production when soybean was constantly provided as both nymphal and adult diet. Overall, lifetime egg production was generally higher in female R. pedestris fed on peanut as adult diet than those on adzuki bean. Egg production rate was significantly influenced by nymphal diet and adult diet, but there was no significant interaction between them (Table 10). Egg production rate was the highest in female R. pedestris fed on soybean as their adult diet, the intermediate on adzuki bean, and the lowest on peanut and this pattern was consistent regardless of nymphal diet (Fig. 25). Female R. pedestris developed on soybean produced more eggs per day than those developed on the other diets. Egg hatchability was influenced by nymphal diet and adult diet, but not by the interaction between them (Table 10). The proportion of eggs that hatched was reduced when adzuki bean was consumed during the nymphal and adult stage. The lowest egg hatchability was thus observed when adzuki bean was presented as both nymphal and adult diet (Fig. 26).

Trait	Source	DF	MS	F	Р
Lifetime egg production	Nymphal diet	2	28919.45	7.16	< 0.001
	Adult diet	2	304766.60	75.44	< 0.001
	Nymphal × Adult diet	4	15236.90	3.77	0.005
	Error	253	4039.68		
Egg production rate	Nymphal diet	2	10.76	4.30	0.015
	Adult diet	2	175.77	70.26	< 0.001
	Nymphal × Adult diet	4	4.09	1.64	0.166
	Error	253	2.50		
Egg hatchability	Nymphal diet	2	3279.85	7.63	< 0.001
	Adult diet	2	3962.26	9.21	< 0.001
	Nymphal × Adult diet	4	316.96	0.74	0.568
	Error	243	430.07		

**Table 10.** Results of two-way ANOVA testing the effects of nymphal diet, adult diet, and the interaction on various measures of female reproductive parameters in R. *pedestris*.



**Figure 24**. Effect of nymphal and adult diets on lifetime egg production in *R*. *pedestris*. The values are mean  $\pm$  standard error.



Figure 25. Effect of nymphal and adult diets on egg production rate in *R. pedestris*. The values are mean  $\pm$  standard error.



Figure 26. Effect of nymphal and adult diets on egg hatchability in *R. pedestris*. The values are mean  $\pm$  standard error.

## DISCUSSION

As a generalist herbivore, *R. pedestris* includes a wide range of plant families as its diet, but must consume legumes to complete the nymphal development (Mizutani et al., 2011; Seo et al., 2011; Lim, 2013). The main objective of this thesis was to explore comprehensively the effects of the nutritional quality of legume seeds on multiple life-history, morphological, and physiological traits directly and indirectly associated with fitness in *R. pedestris*. I first investigated the direct effect of dietary legumes on adult and nymphal traits separately (Experiment 1) and then tested the potential carry-over effect of nymphal diet and its interaction with adult diet on adult traits in *R. pedestris* (Experiment 2).

The results of Experiment 1 showed that *R. pedestris* nymphs had the highest survivorship on soybean and chickpea despite contrasting difference in macronutrient composition between these two beans. On the other hand, no nymphs survived to adulthood when raised on two types of kidney beans (kidney and white kidney). The detailed analysis of the age-dependent survivorship pattern revealed that insects on these kidney beans started to die rapidly after 12.3 and 11.2 days post-hatching, the day upon which they started to feed after molting into their 2<sup>nd</sup> nymphal instar (Fig. 6). Since kidney beans are known to contain higher protein content than cowpea, chickpea, and adzuki beans which support high nymphal

survivorship, high mortality on these kidney beans is likely to be associated with possible toxicity caused by plant secondary metabolites (PSM) present in kidney beans. Although most legumes contain some degree of toxic compounds defending themselves from seed consumers (Anderson and Wolf, 1995; Fields et al., 2001; Choi et al., 2020; Petroski and Minich, 2020), kidney beans are particularly well known for their possession of an anti-nutritive, phytohaemagglutinin (PHA), which is a type of lectin found in high concentrations in red and white kidney beans. When ingested in high amounts, the PHA is known to cause severe mortality through disrupting the epithelial cell of guts and digestive enzymes in diverse insect herbivores (Janzen et al., 1976; Gatehouse et al., 1984; Habibi et al., 2000).

The development of those nymphs that survived to adulthood was generally faster on soybean, chickpea, and adzuki bean than on the other beans, with little difference in their final body mass at adult emergence. Together with the survivorship data, these results collectively suggest that the overall nutritional quality was high in these three beans. However, the most striking result found in this study was the way in which the ingestion of peanut during development shaped the body weight and lipid content of *R. pedestris*. *R. pedestris* raised on peanut had nearly 1.7 times heavier body weight compared to those raised on the rest of diet treatments and this was largely contributed by 4-13 times more fat accumulation by *R. pedestris* on peanut than on the others. Although interesting, this result is not unexpected given that peanut is rich in lipid, comprising 48% (Pae et al., 2011;

Adhikari et al., 2018). Lipids are energy dense substrates mainly used by organisms to store energy. It is therefore predictable that an extremely obese phenotype arising from eating peanuts may have survival advantages under a prolonged period of food deprivation or diapause. Since peanut seeds grow in hard-shelled seedpods beneath the soil surface, it is however questionable whether *R. pedestris* can actually eat the seeds of peanut plants in the natural condition.

In this study, I have also recorded the length and width of different body parts from newly emerged male and female *R. pedestris* individuals that had been raised on one of 15 legume diets. These multivariate morphological data were analyzed using the principal component analysis to extract the principal components and the first two of these extracted components were used to test whether the overall size and shape of *R. pedestris* differed among diet treatments and between the sexes. I found that *R. pedestris* raised on mung bean developed into larger adults than those on the others and this pattern was more pronounced in females than in males. However, the most distinctive differences in morphology were found between male and female *R. pedestris*. Although there was no marked difference in the overall body size between the two sexes, male individuals generally had a thinner abdomen and longer hind leg femur than female individuals regardless of the diet treatment. This sex-specific shape difference was consistent with those reported from previous studies comparing the morphologies of *R. pedestris* (Okada et al., 2011, 2012; Suzaki et al., 2013). In *R. pedestris*, hind leg

femur is considered to be male sexual selective traits, with males possessing larger hind leg femur tending to have higher reproductive success (Suzaki et al., 2015, 2018).

It is important to note that the nutritional quality of the legume diets was stage-specific in *R. pedestris*. In other words, the way in which a certain legume diet influenced the overall performance of R. pedestris differed depending on whether it was ingested during the nymphal or adult stage. Figure 27 summarizes how the relative overall performance of *R. pedestris* expressed in each legume diet differs across the life-stages (nymphal survivorship vs. adult lifetime egg production), with the lines connecting the nymphal and adult performance for each legume indicating the direction and magnitude of the rank changes. For example, while none of nymphs survived on two kidney bean varieties (kidney and white kidney bean), adults fed these beans lived normally and more or less comparable to those fed the other treatments. A likely explanation for this result is that adults became more tolerable to the anti-nutritive compounds present in kidney beans (PHA) than did nymphs as they grew larger. Another example of such disparity between life-stages can be seen from those nymphs and adults that consumed adzuki bean. On adzuki bean, R. pedestris nymphs grew well and exhibited high survivorship, but R. pedestris adults had reduced fecundity compared to those on other beans. Whatever the exact nutritional mechanism underlying such different effects, the finding that a certain diet does not have the consistently beneficial or detrimental effects on insect performance across different life-stages may explain why polyphagy is maintained in this species.

Multiple studies have reported sex-specific differences in lifespan in a number of insects, with the general trend being that females living longer than males (Fox et al., 2003). Sexual selection theories predict that a high male-male competition for mates would favor a 'live fast, die young' strategy in males, leading them to age more rapidly and die earlier than females (Vinogradov, 1998; Bonduriansky et al., 2008). I anticipated this to occur in *R. pedestris* since male adults are reported to show intense male-male competition in this species as in other heteropterans (Okada et al, 2011). Against the prediction, however, the present data showed that the lifespan of adult R. pedestris was longer for males than females across diet treatments. Why males outlived females in this species remains elusive, but may be explained by the experimental setup used for measuring lifespan and reproduction in this study. In this study, I housed a pair of male and female insects in the same experimental arena throughout their reproduction period, thereby precluding any possible competitions between multiple males. It is possible that the absence of any intra-sexual competition might have allowed male individuals to allocate more energetic resources into somatic repair and maintenance rather than reproductive efforts. Under the current experimental setup, female individuals on the other hand were constantly allowed to mate and thus be stimulated to produce eggs throughout their lifespan, a situation that is unlikely to take place in nature. It is conceivable that this increased mating and reproductive opportunities might have led to female insects to invest their resources more to reproduction at the expense of somatic repair and maintenance.

The total number of eggs and hatched offspring produced over the course of lifetime represents the reproductive success of an individual and so is the most widely used surrogate measure of individual fitness in insects and other organisms (Clutton-Brock, 1988; Newton, 1989; Brommer et al., 2002). The present data showed that this measure of female reproductive success was the highest in R. pedestris consuming three varieties of soybeans (Glycine max; black soybean, soybean, and seonbijabi bean) and small black or rat's eye bean (Rhynchosia volubilis). These beans comprise high concentrations of protein, with the average protein content being 41.8, 36, 33.6% for black soy, soybean, and small black bean (http://www.google.com), respectively. When the relationship between the approximate protein content of 13 legumes and the lifetime egg production was analyzed using a linear regression, I found a significant and positive linear association between the two, showing that lifetime egg production increases as a function of increasing dietary protein across diet treatments (Fig. 28). In this analysis, the dietary protein content present in legume explained ca. 40% of total variation in lifetime egg production. The explanatory power of dietary protein further increased up to 58% when two kidney beans which contain high concentrations of anti-nutritive PHA were removed from the analysis. These results

are in accordance with the well-established view in insect nutritional ecology which emphasizes the importance of dietary protein content as a key determinant of egg production or fecundity (Mattson, 1980; Awmack and Leather, 2002).

In Experiment 1, I studied the impact of different nutritional quality of 15 diet treatments on various traits expressed during the nymphal and adult stage independent of each other. In Experiment 2, I investigated how the nutritional quality of diets ingested during juvenile development would be carried over to exert an indirect influence over adult fitness traits in *R. pedestris*. Not surprisingly, the effect of adult diet on lifespan and reproductive performance was much stronger than that of nymphal diet. No matter what diet they consumed early in life, for example, the consumption of soybean during the adult stage resulted in the best reproductive outcome in terms of both lifetime fecundity and the rate of daily egg production. On the other hand, lifespan and lifetime reproductive success were substantially reduced in *R. pedestris* adults consuming adzuki bean as compared to those consuming soybean and peanut. These results are consistent with those patterns observed from Experiment 1, confirming that the overall nutritional quality is higher for soybean compared to adzuki bean.

Despite being much smaller in its effect size, the diet consumed during the nymphal stage had a significant carry-over or lasting effect on adult lifespan and reproductive output. It is important to note that this significant effect of nymphal diet was strongly modulated by the type of adult diet eaten, as indicated by the significant interaction between nymphal and adult diet for both lifespan and reproduction. In case of lifespan, I found that the lifespan of R. pedestris adults, particularly males, was extended when the diets consumed during the nymphal and adult stage were matched to peanut. It is possible that this lifespan benefit is the outcome of the physiological adjustments made by those individuals anticipating the match between developmental and adult diet (Monaghan, 2008; Dmitriew and Rowe, 2011; Hayward et al., 2013). Another and probably more likely possibility is that high accumulation of body fat caused by constantly feeding on high fat diet throughout the adult and nymphal stages is responsible for such extended lifespan, considering that the amount of lipids stored in the body is positively associated with lifespan in insects (Hansen et al., 2013). The number of eggs laid per day and also over the lifetime was significantly improved in female R. pedestris that had been raised on soybean early in life compared to those on adzuki bean and peanut, indicating that high nutritional quality of soybean diet consumed during development had a beneficial effect on adult fitness. This positive across-stage effect of early soybean was more pronounced when adult diet was soybean than when it was adzuki bean or peanut. Although some aspects of adult traits performed better when developmental and adult environments matched, the results of this study do not necessarily support the environmental matching hypothesis because the occurrence of this matching effect was strictly restricted to specific diet treatment.

The present study focused on investigating how the nutritional quality of legume diet consumed early in life influenced individual fitness expressed later in life, but the examination of such long-term diet effect was restricted within a generation and not beyond. There are ample evidence showing that the nutritional quality of foods consumed by parents has profound consequences for the performance and fitness of their offspring in insects (Opit and Throne, 2007; Priest et al., 2002; Benton et al., 2008). If silver spoon effect occurs, one can expect that offspring hatched from eggs laid by mothers consuming nutritionally high-quality diets will show higher juvenile performance than those laid by mothers consuming poor quality diets. From an adaptationist point of view, it is predictable that mothers experiencing nutritionally poor environments may allocate more nutritive reserves into their eggs, thereby producing offspring that are more robust to nutritional stress. To have a complete understanding of the effect of nutritional quality on insect performance and fitness, it is necessary to investigate transgenerational effects of parental dietary quality on offspring performance in the upcoming research.

There are several other caveats to be addressed in this study. First, in the present study, I was not able to assess the impact of macronutrients present in these legumes on insect performance because it was not feasible to experimentally control for the chemical compositions of the non-defined oligidic diet used in this study. Another confounding dietary factor that was not controlled in this study was the PSM, which is known to present in legumes in different forms and amounts

(Anderson and Wolf, 1995; Choung et al., 2003; Amarowicz et al., 2008; Wink, 2013). Further studies are therefore required using the chemically defined, holidic diets in which the quantity and balance of major macronutrients can be experimentally manipulated and also the effect of PSM are removed. Second, the experimental insects were confined to feed on the same legume diet throughout the experiment. This does not reflect the real nutritional environment experienced by *R. pedestris* under the natural condition considering that the nymphs and adults of *R. pedestris* are highly mobile and are known to eat a variety of food, including a variety of seeds, fruits, plant saps, etc. Just like other insect herbivores (Behmer, 2009; Simpson and Raubenheimer, 2002), R. pedestris nymphs and adults are likely to mix between multiple legumes so as to achieve the ideal balance of macronutrients, but this possibility was not taken into account in this study. Future research is thus necessary to examine the diet preference and the fitness consequences of dietary mixing of R. pedestris. Last, it is important to note that the stock culture used in this study was maintained on soybeans for several hundred generations before the onset of this study. This suggests that the insects might have been subjected to genetic selection on this particular diet. It is possible that the better performance of individuals fed on soybean over those on the other beans may be largely contributed by their long-term genetic adaptions to soybean. In order to separate the effect of dietary selection history from that of the diet consumed in the present generation, it is required to conduct a large-scale natural selection

experiment in which the performance measures of *R. pedestris* on legumes are quantified from the populations of *R. pedestris* selected under the same and different legumes for multiple generations.

In this thesis, I have conducted a comprehensive analysis on the immediate and long-term effects of dietary legumes on multiple life-history, physiological, and morphological traits in *R. pedestris*, an important crop pest in Korea. The results demonstrated in this thesis will provide useful baseline data for advancing our knowledge on the biology of this economically important species and have valuable implications for predicting its population dynamics and more ultimately its integrated pest management. My thesis has provided more questions than answers and it is my hope that these questions emerged from my study will become a fertile area for future research on the nutritional ecology of this important hemipteran pest.



**Figure 27**. The relative overall performance of *R. pedestris* expressed in each diet treatments across nymphal and adult stages. Nymphal survivorship and adult lifetime egg production were standardized to represent nymphal and adult performance, respectively. For each performance, higher values indicate higher performance ranks. Solid lines connecting two symbols per each diet represent the direction and magnitude of ranks changes across the life-stages.



**Figure 28**. The relationship between lifetime egg production by female *R. pedestris* and protein content (%) present in legume across 13 diet treatments. Solid line represents the least-squares regression line fitted between the two variables.

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## **ABSTRACT IN KOREAN**

다양한 콩이 톱다리개미허리노린재(*Riptortus pedestris*)의 생활사, 형태적, 생리학적 형질에 미치는 영향에 관한 연구

#### 성 재 민

#### 초 록

곤충의 발육, 생존, 그리고 번식은 먹이 식물에 함유된 영양분에 따라 크게 영향 받는다. 식물의 영양적 질은 다양한 요인에 의해 결정되며, 그 중 에서도 거대영양소(단백질, 탄수화물, 지방)의 비율에 의해 가장 크게 영향 받 는다. 콩은 콩과 식물(Family Fabaceae)의 종자로 현재 세계적으로 널리 재배 되고 있다. 다양한 종류의 콩이 현재 한국에서 재배되며, 이들이 함유한 거대 영양소의 양과 질은 콩 종류 마다 매우 다른 양상을 보인다. 콩과 식물을 가 해하는 여러 노린재목(Order Hemiptera) 곤충 가운데, 톱다리개미허리노린재 는 한국, 일본, 중국 등에서 콩과 기타 원예작물에 가장 큰 피해를 주는 농업 해충이다. 톱다리개미허리노린재는 광식성 해충으로 다양한 식물을 섭취할 수 있지만, 콩과 식물의 섭취 없이는 정상적으로 발달할 수 없다. 본 논문의 연구목표는 다양한 종류의 콩 섭취가 톱다리개미허리노린 재의 생활사, 형태, 생리학적 형질에 어떠한 영향을 미치는지 분석하는데 있 다. 이를 위해 본 연구는 톱다리개미허리노린재의 약충과 성충을 이용해 두 가지의 실험을 실시하였다. 첫 번째 실험에서는 15가지 콩에서 약충과 성충이 보이는 반응을 각각 조사하였다. 두 번째 실험에서는 약충 기간 동안 섭취한 먹이가 성충기에 발현하는 수명과 생식과 같은 중요 적응도 형질에 미치는 이 월효과를 조사하였으며, 성충 기간 동안 섭취한 먹이와 약충기 섭취한 먹이 간에 존재할 수 있는 교호작용을 분석하였다.

실험 1에서는 15가지 콩 중 한 가지를 섭취한 톱다리개미허리노린재의 다양한 형질을 측정하였다. 백태, 병아리콩, 그리고 팥을 섭취한 약충들은 가 장 높은 생존율을 보였다. 하지만 강낭콩과 흰강낭콩을 섭취한 약충들은 전 개체 사망하였고, 이는 두 강낭콩 품종이 고농도로 함유하는 것으로 알려진 반영양성 식물이차대사물질 때문인 것으로 추측된다. 약충의 발달은 작두콩 섭취시 가장 느렸으며, 병아리콩에서 가장 빨랐다. 성별의 구별 없이, 우화한 성충의 지방량은 땅콩을 섭취한 개체에서 다른 처리군들에 비해 4-13배 가량 높게 축적되었으며, 그 결과 땅콩을 섭취한 개체들은 타 처리군 개체들에 비 해 몸무게가 74.5-92.2% 정도 무거웠다. 노린재 개체들로부터 측정된 외부 형태 관련 측정치들은 주성분분석을 통해 분석되었으며, 그 결과 주성분1과 주성분2은 전체 분산의 72.3%를 설명하는 것으로 나타났다. 암수 간에는 몸 형태 상의 차이가 관찰되었는데, 암컷은 수컷에 비해 넓은 가슴과 복부를 가

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진 반면 짧은 다리를 지니는 것으로 나타났다. 전체적인 몸의 크기는 녹두를 섭취한 개체들에게 큰 경향을 보였다. 다른 곤충들과 달리, 톱다리개미허리노 린재의 경우 수컷이 암컷보다 오래 사는 것으로 나타났으며, 수컷은 땅콩을, 암컷은 아주까리밤콩을 섭취하였을 때 가장 긴 수명을 보였다. 일생 동안 낳 은 알의 수는 높은 함량의 단백질을 함유한 서리태, 백태, 그리고 쥐눈이콩을 섭취할 경우, 높게 나타났으며, 이러한 결과는 곤충의 생식에 있어 단백질 영 양이 갖는 중요성을 강조하는 기존의 가설에 부합되는 결과이다.

실험 2에서는 세 가지의 약충먹이와 세 가지의 성충먹이가 조합된 총 아홉 가지 실험조건을 제공받은 성충의 수명과 생식력을 조사하였다. 사용된 세 가지 콩은 백태, 팥, 땅콩으로, 앞서 수행된 실험1의 결과, 노린재 약충은 이들 콩에서 비교적 높은 생존율을 보이는 것으로 나타났다. 수명과 생식 모 두, 약충과 성충 먹이 간에는 유의한 교호작용이 존재하는 것으로 확인되었는 데, 이는 이 두 시기에 섭취한 먹이의 효과는 서로 독립적이지 않다는 것을 의미한다. 암컷과 수컷 모두, 팥을 섭취한 성충은 다른 콩을 섭취한 성충들에 비해 수명이 아주 짧았다. 이와 같은 성충 먹이 효과 외에도, 약충기에 섭취 한 콩이 성충 수명에 미치는 유의한 이월효과 또한 확인되었는데, 수명은 약 충과 성충 시기에 일관되게 땅콩을 섭취한 개체들에서 가장 긴 것으로 나타났 다. 일생 동안 암컷이 생산한 알의 수는 이들이 성충기에 백태를 섭취하였을 때 가장 많았는데, 산란과 관련된 이러한 백태섭취 효과는 약충기에 백태를 섭취한 개체들에서 더 크게 강화되는 것으로 나타났다. 이러한 결과 또한 산

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란과 관련된 약충 먹이의 이월효과가 존재함을 시사한다. 하지만 이러한 이월 효과가 발달기와 성충기 간의 환경일치로 인해 적응적으로 유도된 것인지, 아 니면 단지 좋은 환경에서 자란 '은수저' 효과의 결과인지에 대해선 추후 연구 를 통해 확인되어야 할 것이다.

본 논문은 다양한 콩의 섭취가 톱다리개미허리노린재의 생활사, 형태, 그리고 생리적 형질에 미치는 영향을 개체 수준에서 분석하였다. 이러한 연구 는 대표적인 농업해충인 톱다리개미허리노린재가 어떻게 다양한 영양환경의 변화에 반응하는지를 이해하는 기초 자료를 제공할 것이며, 개체군 수준에서 의 동향을 예측하는데 중요한 지표가 될 것이다.

주요어 : 농업 해충, 이월효과, 적응도, 콩과 식물, 생활사 형질, 영양, 톱다리개 미허리노린재

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# 감사의 글

처음 단순히 생물이라는 과목이 좋아서 시작했던 공부가 이제는 더 돈독해져버린 지금은 성재민이라는 사람의 한 켠에 크게 자리잡았습니다. 아직도 많이 부족합니다. 공부가 더욱 필요하지만 좋은 사람들과 함께 좋은 이야기를 나누며 좋은 경험과 배움을 얻어갈 수 있었습니다.

지난 2 년간의 대학원 생활 동안 많은 가르침을 주신 이광범 교수님께 감사의 말씀을 드립니다. 항상 열성을 다해 제자들의 실험을 도와주시고 아낌없는 조언을 해주심에 더욱 열심히 공부에 임할 수 있었습니다. 대학원 동안 많은 지식을 가르쳐주신 곤충학전공 교수님들께도 감사의 말씀을 드립니다. 앞으로도 이러한 지식을 바탕 삼아 사회에 나가서 전문적인 인재가 되도록 노력하겠습니다.

그리고 저희 실험실원에게도 감사의 말씀을 드립니다. 실험과 논문을 준비하는데 있어 많은 지식과 조언을 전달해주신 태환이형, 실험실 생활동안 함께 공통 주제로 이야기하고 실험 과정에서 유익한 기술을 가르쳐주신 명석이형, 초기 초파리 관리하는 법 및 설거지하는 법도 알려주신 조용한 건형, 항상 열심히 해왔고 지금도 누구보다 열심히 하고 있을 건희, 지금은 많이 보지 못하지만 일하면서도 석사과정을 열심히 준비하신 두암이형, 2년 동안 많은 생각들과 조언을 교환해준 대학원 생활 내내 함께 했던 경운이형, 졸업 막바지를 달려가고 있는, 하지만 현재도 실험 진행중인 항상 열심히 하는 혜진이, 어린 나이에도 불구하고 생각이 아주 깊고 배울 점이 너무나도 많은

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용수, 다음 노린재를 이어갈 열정적으로 가끔은 전투적으로 임하는 것 같은 우민이, 밝은 에너지로 보는 사람도 기분 좋게 하는 현서 정말로 고맙습니다. 2 년 어떻게 갔는지도 모를 즐거운 시간이었습니다.

마지막으로 저희 가족에게 감사의 말씀을 드립니다. 항상 아들 때문에 가게 일로 고생하시는 부모님, 아이들을 가르치느라 매번 고생하는 큰누나와 작은누나, 그리고 시골에서도 같이 놀아주지 못해 미안한 별이, 달이, 머루 정말 감사합니다. 그 누구보다도 존재 자체만으로 소중한 가족입니다. 지금까지 받은 사랑 보답하는 아들 되겠습니다.

2021년 2월

성재민 올림