Biology of *Blaptica dubia* (Blattodea: Blaberidae)

by

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Abstract

The Dubia Cockroach, *Blaptica dubia* Serville, has become a popular food choice among amphibian and reptile enthusiasts. However, there has been little scientific research conducted on this species. My research consists of three topics: instar determination, parental care, and temperature-dependent development.

Instar determination is fundamental to both basic entomological research and its application. A new method using Gaussian mixture models to determine the number of instars in this species was developed. Application of the method is illustrated by analysis of data collected on *B. dubia*. The analysis indicates that there are seven instars in *B. dubia* and that the growth ratio follows the Brooks-Dyar rule. The growth ratio of pronotal length, pronotal width, and head width are 1.26, 1.24, and 1.19, respectively. Since *B. dubia* shares a similar growth pattern with other paurometabolous insects, this method may be applicable to other species as well.

The social behavior of cockroaches is less studied compared to other insect groups. Most information about cockroaches is from brief notes made during field studies. For parental care, most of these studies have been focused on the family Cryptocercidae and no studies have been conducted on the parental care behavior of the Blaberidae. The objective of this study was to measure the possible effects of parental care on the offspring of *Blaptica dubia*. In this experiment, no parental care effects were detected although parental care behavior was observed in dubia.

Temperature-dependent development of the nymph of *dubia* was described using data

collected from constant-temperature laboratory experiments. Simple linear regression was used for the data from each instar. Degree-days required to complete each instar were estimated as 457.5, 668, 1031 .1317, 1515, 2071 for 1~7 instar, respectively. The results could be used to control the development rate and find the optimal rearing condition for *B. dubia*.

Acknowledgments

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Chapter 1 Introduction

Cockroaches are insects of the order of Blattodea. The order name for cockroaches sometimes is given as Blattaria. They are closely related to the praying mantids (Mantodea), and often are grouped with them (as suborders) to form the order Dictyoptera. Termites (Isoptera) can also be placed in the order Dictyoptera, and are considered by some to social cockroaches.

Cockroaches are mostly secretive, primarily nocturnal, typically ground-dwelling insects. Cockroaches originated in the Carboniferous, approximately about 359.2 million years ago. Modern cockroaches are believed to appear in Cretaceous approximately about 100 million years ago (Grimaldi and Engel 2005). Most cockroach species live in tropical and subtropical area. It is estimated there are about 4000 species worldwide (Roth 2003). Few of them, about 30 species, successfully enter into urban environment and become very import urban pest.

Blaptica dubia Serville (commonly known as the Dubia cockroach, Guyana spotted cockroach, or Orange-spotted cockroach) is a large (up to ca. 4.5 cm in length), sexually dimorphic blaberid cockroach. Males have wings, but they rarely fly. Because they lack developed arolium between their claws, neither adults nor nymphs can climb smooth surfaces. *B.dubia* is an ovoviviparous species. This reproduction pattern is typical in family Blaberidae of Blattodea (Mckittrick 1964, Roth 2003). More importantly, this species is used by pet lovers as a food source for many reptiles and amphibians. Compared to other foods such as crickets, *B. dubia* contains a higher percentage of protein, is easier to maintain, and unlike other cockroaches, produces little odor. Consequently, they have become an increasingly popular food

among amphibian and reptile enthusiasts.

My study is focused on the instar determination, parental care and temperature development of the Dubia cockroaches.

Instar determination

Growth of insect larva is continuous, but the growth of the exoskeleton is not (Gullan and Cranston 2005). Heavily sclerotized structures, such as headcapsules, remain approximately the same size during a stadium. The most measurable changes in size occur following molts. These special characters can be used to differentiate instars. There is a long history of entomologists trying to quantify changes in the size of body parts for a wide range of insects. One of the earliest attempts in this area was that of Dyar, who developed a rule from observations on the caterpillars of 28 species of Lepidoptera in 1890. Dyar's measurements showed that the width of the head capsule increased by a ratio (range 1.3-1.7) that was constant for a given species.) (Dyar, H. G. 1890)

A related empirical "law" of growth is Przibram's rule, which states that an insect's weight is doubled (2.09) during each instar, and at each molt all linear dimensions are increased by a ratio of 1.26 (Wigglesworth 1984, Solow and Faber 1995). However, the growth of most insects shows no general agreement with this rule, which assumes that the dimensions of a part of the insect body should increase at each molt by the same ratio as the body as a whole, which is not the case in most situations.(Gullan and Cranston 2005) Besides, the food consumption of different individuals and the differences between their development stages also make their

weight vary greatly. These factors may be the primary reason that Przibram's law is not as widely used as Dyar's law. There is an area of research called allometry that address these problems.

Morphological measurements can be viewed as normal distributions in each instar. Thus, samples from a given population of insects are a mixture of several normal distributions, one for each instar. This assumption is reasonable because measurements from individuals from the same ontogenetic stage are often distributed normally (Sokal and Rohlf 1994). In recent years, the development of technology makes many computer intensive analytical methods possible.

Parental care

Parental care involves any sort of parental behavior that increases the survival of the offspring. Parental care has been described in most animal phyla, but is especially well developed in numerous species of insects, crustaceans, and vertebrates. The amount and type of parental care provided to offspring is quite diverse. The most common parental care is to assist the offspring in living through harsh physical conditions, making resources available, which are otherwise difficulty or impossible to obtain, and decreasing risk of predation on eggs or young (Clutton-Brock and Scott 1991, Glazier 2002). Although levels of complexity are various, parental care can be divided into three major categories: (a) those that physically protect the young from danger, (b) those that protect resources vital to offspring, and (c) those that facilitate offspring feeding (Glazier 2002).

Most cockroaches show some form of parental care in the broad sense, because even the

most primitive cockroaches have ootheca (egg case) to protect the young before they hatch. If only the post-hatch parental care is considered, most of the information on cockroaches is from brief notes made during field work (Bell et al. 2007) and more of these are focused on species of Cryptocercidae (Seelinger and Sellinger 1983, Nalepa 1987, 1988, Park and Choe 2003). There is currently no information concerning *B.dubia*.

Temperature on insect development

The effect of temperature on insect development is rather a basic topic in insect biology. In the last century, numerous studies developed appropriate developmental rates functions for phonological or population models that can be used under a variety of conditions to predict important events in insect life cycles, or to estimate insect abundance for specific control strategies. The development of specific functions for each developmental stage is determined by rearing insects at range of constant temperatures (Damos and Savopoulou-Soultani 2012)

The earliest and still widely used model was the linear degree-days model (Arnold 1959). The calculations of lower developmental thermal thresholds and degree-day have long been used for the estimation of developmental times under different constant temperatures. This method has the advantage of simplicity and allows estimation of the developmental threshold and the degree-day requirement of insect species. The principle and the detailed methods are well described in general entomology textbooks. e.g. Gullan and Cranston 2005.

While the degree-days method, which assumes a linear relationship between developmental rate and temperatures, is often suitable for intermediate temperature, this model

does not consider nonlinearity at high and low temperatures. Therefore, it will produce considerable error when temperature conditions are toward the extremes under variable conditions.

Various nonlinear models also have been developed to improve these results. Many nonlinear models have been developed by entomologists, such as, the Belehradek model and the logistic equation of Davidson (Briere et al. 1999). More models are provided by Logan (Logan et al. 1976), Schoolfield (Schoolfield et al. 1981), and Lactin(Lactin et al. 1995).

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Chapter 2 Instar Determination of *Blaptica dubia* (Blattodea: Blaberidae) using Gaussian Mixture Models

ABSTRACT Instar determination is fundamental to both basic entomological research and its application. The cockroach, *Blaptica dubia* Serville, is a popular pet and an excellent feeder insect for many reptiles and amphibians. A new method using Gaussian mixture models to determine the number of instars in this species is developed. Application of the method is illustrated by analysis of data collected on *B. dubia*. The analysis indicates that there are seven instars in *B. dubia* and that the growth ratio follows the Brooks-Dyar rule. The growth ratio of pronotal length, pronotal width, and head width are 1.26, 1.24, and 1.19, respectively. Since *B. dubia* shares a similar growth pattern with other paurometabolous insects, this method may be applicable to other species as well.

Introduction

Instar determination is fundamental to both basic entomological research and application (Logan et al. 1998). Determining instar distributions from a given population is required for life table analysis, key factor analysis, and other important ecological investigations. The number of instars is useful in the development of phenology models, or in the refinement of existing models (Godin et al. 2002). In paleobiology, understanding the growth, evolution, and ecology of extinct species often depends on calibrating ontogeny with respect to either absolute time or relative

developmental age (Hunt and Chapman 2001). In forensic studies, instar determination helps to estimate the minimum postmortem interval (Velásquez and Viloria 2010).

The number of instars varies widely across insect species. Instar number is frequently considered to be invariable within species, although intraspecific variability in the number of instars is not an exceptional phenomenon (Esperk et al. 2007). In many species of Arthropoda, heavily sclerotized structures, such as the head capsule, remain approximately the same size during a stadium (Daly 1985). Measurable changes in size occur following molts. These sclerotized characters, particularly head capsule, have been used to differentiate instars.

For instar determination, direct observation may be most accurate, but in many cases, observations are not possible or it may take too much time to obtain the results (In our observation, it takes about 6 months at $30 \pm 2°C$). There is a long history of entomologists quantifying changes in size of particular structures for a wide range of insects. It has long been supposed that the ratio of measurements of well-sclerotized structures in any instar to the equivalent measurement in the preceding instar tends to be constant throughout the life history (Hutchinson and Tongring 1984). Many different techniques have been proposed for estimating either the growth stage (instar) or the age of insects (Daly 1985). The original observations on arthropod sizes and developmental stages were made by W. K. Brooks on stomatopod larvae collected by the H.M.S. Challenger (Brooks 1886). In Brooks's observation, the length of an instar is five-fourths of its predecessor. Dyar later independently came to the same conclusion of equivalent size ratios from observations on the caterpillars of 26 species of Lepidoptera in 1890. Dyar's measurements showed that the width of the head capsule increased by a ratio, ranging

from 1.3-1.7, that was constant for a given species (Dyar 1890):

$$\frac{postmolt\ size}{premolt\ size} = constant$$

However, the sizes of different instars may overlap, making instar determination more difficult.

Morphological measurements can be viewed as normal distributions in each instar. Thus,

samples from a given population of insects are a mixture of several normal distributions, one for

each instar. The probability density function of a mixture of G normal distributions can be

written as:

$$f_{Mix}(x) = \sum_{k=1}^{G} p_k f_k(x)$$

where p_k is the mixing proportion, $p_k \geq 0$, $\sum_{k=1}^{G} p_k = 1$, $f_k(x)$ is the p-dimensional normal density

function $N_p(\mu_k, \Sigma_k)$, with mean vector μ_k, and covariance matrix Σ_k. This assumption is

reasonable because measurements of individuals from the same ontogenetic stage are often

distributed normally (Hunt and Chapman 2001).

Distribution based methods have been adopted by many entomologists for a variety of

species (Logan et al. 1998, Hunt and Chapman 2001, Hammack et al. 2003, Gullan and Cranston

2005, Delbac et al. 2010). Almost all of these methods are based on the widths of head capsules.

There are some efforts to use different characters to determine instars (McClellan and Logan

1994, Velásquez and Viloria 2010), but the characters are used independently. Since different

characters of insects are highly correlated, it is more statistically appropriate to adopt a

multivariate approach.

Blaptica dubia Serville (commonly known as the Dubia cockroach, Guyana spotted cockroach, or Orange-spotted cockroach) is a large (up to ca. 4.5 cm in length), sexually dimorphic blaberid cockroach that is often kept as a pet. More importantly, this species is used as a food source for many reptiles and amphibians. Compared to other food sources such as crickets, *B. dubia* contains a higher percentage of protein, is easier to maintain, and produces little odor. Consequently, they have become an increasingly popular food among amphibian and reptile enthusiasts. In *B. dubia*, the head capsule width, pronotal width, and body length are the most convenient characters to measure in all nymphal instars. These three characters are used together in the analysis to determine instars. Since the three characters can be seen as a multivariate normal distribution in each instar, they form a Gaussian mixture when combined (Hastie et al. 2003). As a result, a new method using Gaussian mixture models to determine the number of instars in this species is developed. The results are assessed by the Brooks-Dyar rule and verified by direct observations on the development of *B. dubia* in the laboratory and corroborated with results of a previous study (Hintze-Podufal and Nierling 1986).

Material and Methods

Insect Rearing. *Blaptica dubia* were reared in clear gallon glass jars (3.8 L, 15.24 cm width by 25.4 cm height) with cardboard harborage at $30 \pm 2°C$. They were exposed to a photoperiod of 12:12 (L:D) h and supplied with water and dry dog Chow (Purina® Dog Chow®, Ralston Purina, St. Louis, MO) *ad libitum*.

Measurements. A total of 1925 nymphs of *B. dubia* were measured from Jan 2012 to Apr 2012. Head capsule width (the widest distance between the two compound eyes) was measured to 0.01 mm using a microscope (Leica MZ 6, Solms, Germany) with an ocular micrometer. The length and width of the pronotum were measured to 0.01mm using an electronic digital caliper (Model: 62379-531, Control Company, Friendswood, TX).

Statistical Model. In model-based clustering, it is assumed that data are generated by a mixture of underlying probability distributions $f_k(x)$, in which each component k represents a different group or cluster (Johnson and Wichern 2007). If there are G clusters, the observation of variables is modeled as arising from the mixture distribution:

$$f_{Mix}(x) = \sum_{k=1}^{G} p_k f_k(x)$$

where p_k is the mixing proportion, $p_k \geq 0$, $\sum_{k=1}^{G} p_k = 1$, $f_k(x)$ is the p-dimensional normal density function $N_p(\mu_k, \Sigma_k)$, with mean vector μ_k, and covariance matrix Σ_k. The Gaussian mixture model for one observation with G components is:

$$f_{Mix}(x|\mu_1, \Sigma_1, ..., \mu_G, \Sigma_G) = \sum_{k=1}^{G} p_k \frac{\exp\left\{-\frac{1}{2}(x - \mu_k)^T \Sigma_k^{-1}(x - \mu_k)\right\}}{(2\pi)^{\frac{p}{2}} |\Sigma_k|^{\frac{1}{2}}}$$

Inference is based on the likelihood, which for N objects and a fixed number of clusters G is (Johnson and Wichern 2007):

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$$L(p_1, ..., p_k; \mu_1, \Sigma_1, ..., \mu_G, \Sigma_G) = \prod_{j=1}^{N} f_{mix}(x_j | \mu_1, \Sigma_1, ..., \mu_G, \Sigma_G)$$

The Bayesian Information Criterion (BIC),

$$BIC = 2ln(L_{max}) - 2ln(N)(G\frac{1}{2}(p+1)(p+2) - 1)$$

Where L_{max} is the maximum of likelihood function, N is the number of observations, G is number of component, and p is number of variables. It has been used to determine the number of clusters (Fraley and Raftery 1998). In instar determination, the BIC gives a quantitative method to determine the number of instars for a given insect.

Fraley and Raftery (2012) developed the R (R Development Core Team 2012) package "mclust" for model-based clustering based on a parameterized Gaussian mixture model (Fraley and Adrian 1998). The software package "mclust" was used in the data analysis.

Results and Discussion

Model Selection. After comparing the BIC of every cluster (sub-model) and all the parameterized models, the unconstrained model with seven clusters was selected because it has the largest BIC value, -6232.165 (range -25282.88 ~ -6232.165). Therefore, there are seven instars in *B. dubia* when reared as described above.

Cluster Analysis. Gaussian mixture model based clustering was conducted based on the model selection using the BIC. Figs. 1-3 show the cluster results of three characters. From the scatter plots, there are seven instars in *B. dubia* and the characters of each instar cluster together. The means and 95% simultaneous T^2-intervals (similar to confidence intervals, but for

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multivariate data) of the three characters in each instar are shown in Table 1. The pronotal length

is in the range of 2.41 to 9.37 mm; the pronotal width is between 4.18 mm and 15.17 mm and the

range of head width is 1.79 to 5.04 mm, for first and seventh instars, respectively (Table 1).

Based on the result in the cluster analysis, predictions can be made on new observations, using

the function provided in the mclust package.

Result Assessment. According to the Brooks-Dyar rule (Dyar 1890):
$$\frac{I_n}{I_{n-1}} = constant$$
where $I_n = postmolt\ size$ and $I_{n-1} = premolt\ size$. This equation can also be written

as:

$$y = ae^{bx}$$

Which is equivalent to:

$$lny = lna + bx,$$

a linear function. We can, therefore, assess the cluster results using linear regression models. The

constant growth ratio is e^b. From Fig. 4 and Table 2 the linear relationships of the three

characters agree with the Brooks-Dyar rule. In our analysis, the growth ratios of pronotal length,

pronotal width, and head width are 1.26, 1.24, and 1.19, respectively (Table 1). The growth rates

of pronotal length and width are different and the shape of the pronotum changes slightly during

development. According to Cole (1980), the median growth ratio for hemimetabolous insects is

1.27 , and the mean is 1.27 ± 0.011 (n = 50, $\alpha = 0.05$). For holometabolous insects the median is

1.52, and the mean is 1.55 ± 0.033 (n = 55, $\alpha = 0.05$). This indicates that all instars are

represented in the samples and that the model-based approach gives reasonable results.

In another study, the development of *B. dubia* was monitored at the same conditions as

stated above [$30 \pm 2°C$, 12:12 (L:D) h]. We directly observed seven instars in *B. dubia* over a period of 6 months. In addition, Hintze-Podufal and Nierling (1986) also indicated that there are seven instars in *B. dubia* reared at $28 \pm 2°C$.

Although the results of our analyses support the Brooks-Dyar rule, they do not rely on it. Model-based clustering can also be used for other species even though their growth may not follow Brooks-Dyar rule (Bliss and Beard 1954, Klingenberg and Zimmermann 1992), as long as there are size differences between different instars.

All of the studies about instar determination that use statistical techniques are based on the normal distribution of the given characters in each instar (Gullan and Cranston 2005, Hunt and Chapman 2001, Godin et al. 2002, Logan et al. 1998, Delbac et al. 2010). Our approach uses the multinormal distribution of characters in each instar and this is the only assumption in our analysis. Distributions were examined visually using the bivariate plot of the characters.

Compared to the widely adopted method of McClellan and Logan (1994), model-based clustering provides a better approach to solve the instar determination problem. Instar determination using Gaussian mixture models successfully solved the problem of determining the number of instars using the BIC. In the McClellan and Logan (1994) method, the number of instars is based on direct observations and the initial value of the mean and variance of each instar are based on this observation. This approach may produce some bias because different observers may have various initial values.

The clustering method based on Gaussian mixture models described above illustrates that it can be successfully used to determine instars in *B. dubia*. Since no characters in our analysis

are specific to *B. dubia*, the same cluster methods should be applicable to other species as well.

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Table 2-1 The means and 95% simultaneous T2-intervals of three characters in each instar (mm) of *B. dubia*

	Instar 1	Instar 2	Instar 3	Instar 4	Instar 5	Instar 6	Instar 7	Ratio
N	127	178	146	280	394	408	285	
Pronotal length	2.41(2.37-2.45)	2.97(2.91-3.03)	3.82(3.77-3.87)	4.91(4.84-4.98)	6.17(6.11-6.23)	7.54(7.46-7.63)	9.39(9.30-9.47)	1.26
Pronotal width	4.18(4.14-4.22)	5.21(5.13-5.30)	6.47(6.40-6.54)	8.01(7.89-8.13)	9.95(9.86-10.04)	12.28(12.15-12.41)	15.17(15.04-15.30)	1.24
Head width	1.79(1.78-1.80)	2.08(2.06-2.11)	2.49(2.47-2.51)	2.97(2.94-3.00)	3.55(3.53-3.58)	4.24(4.21-4.28)	5.04(5.00-5.07)	1.19

Table 2-2 Linear regression models for three characters in the seven instars of *B. dubia*

Character	Slope ± SE	Ratio*	Intercept ± SE	R^2	F (df = 1,5)	P (> |t|)
Pronotal Length	0.2278 ± 0.0039	1.26	0.6573 ± 0.0176	0.9988	4082	< 0.0001
Pronotal Width	0.2136 ± 0.0011	1.24	1.2235 ± 0.0047	0.9999	108300	< 0.0001
Head Width	0.1734 ± 0.0014	1.19	0.3979 ± 0.0064	0.9996	12990	< 0.0001

*Ratio is e^{slope}
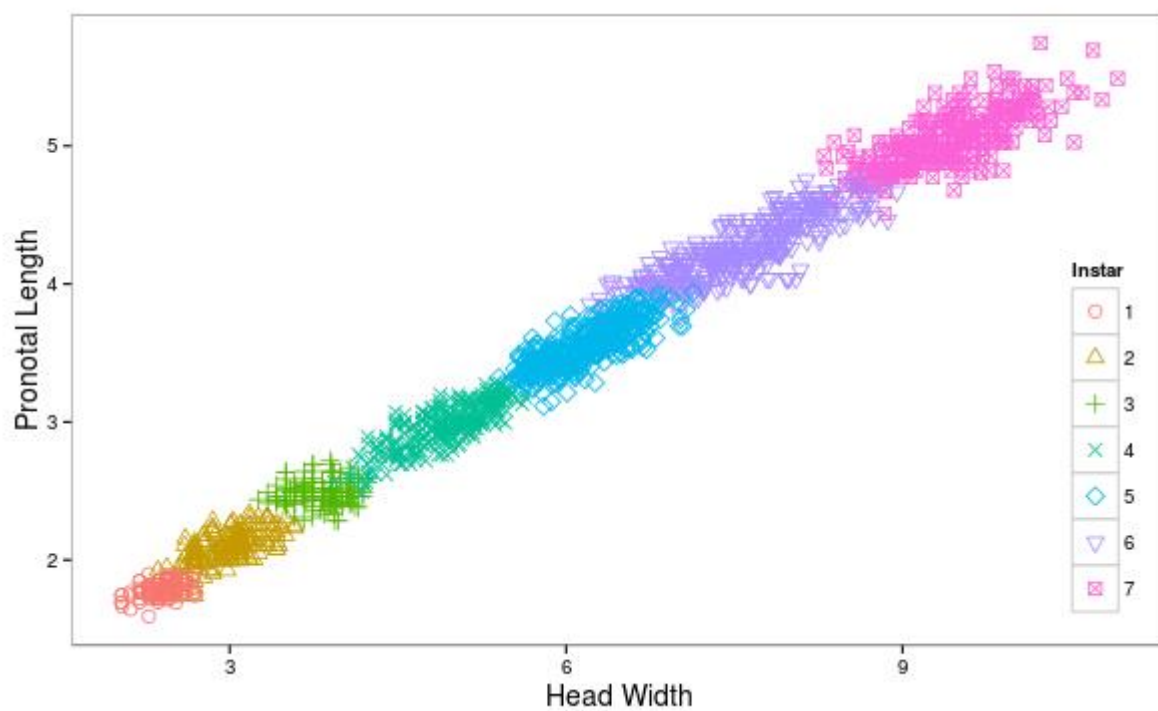
Figure 2-1. The seven instars of pronotal length (mm) and head width (mm) of *B.dubia*.
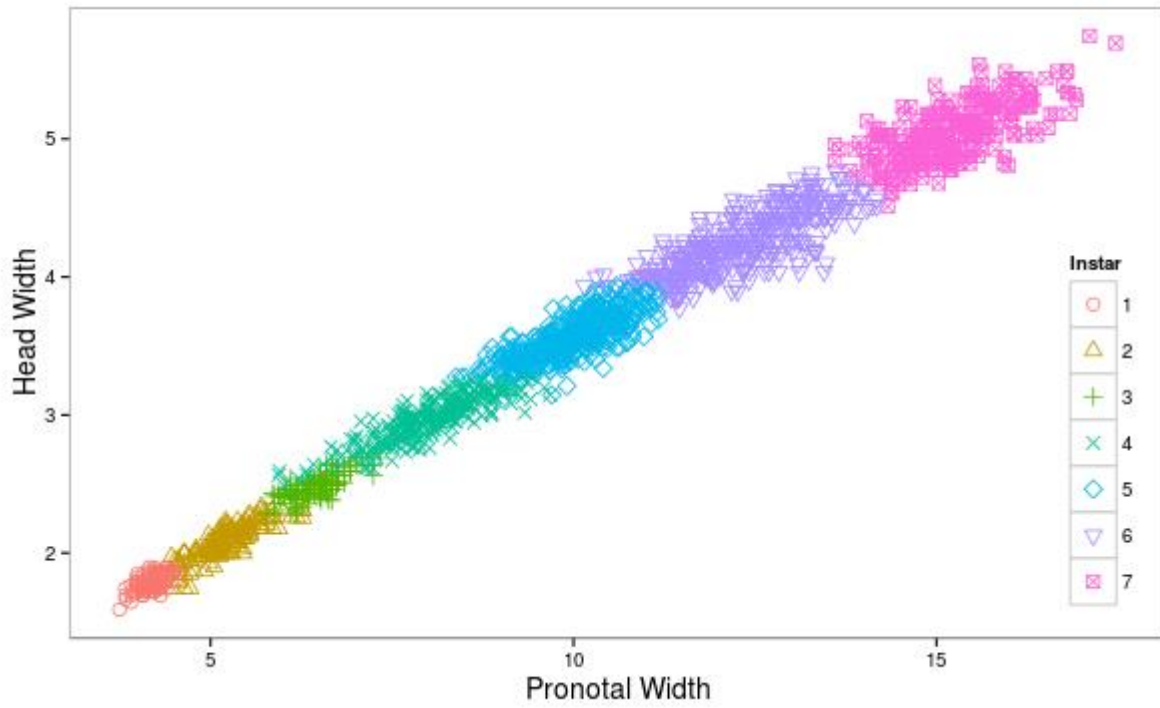
Figure 2-2. The seven instars of pronotal width (mm) and head width (mm) of *B.dubia.*
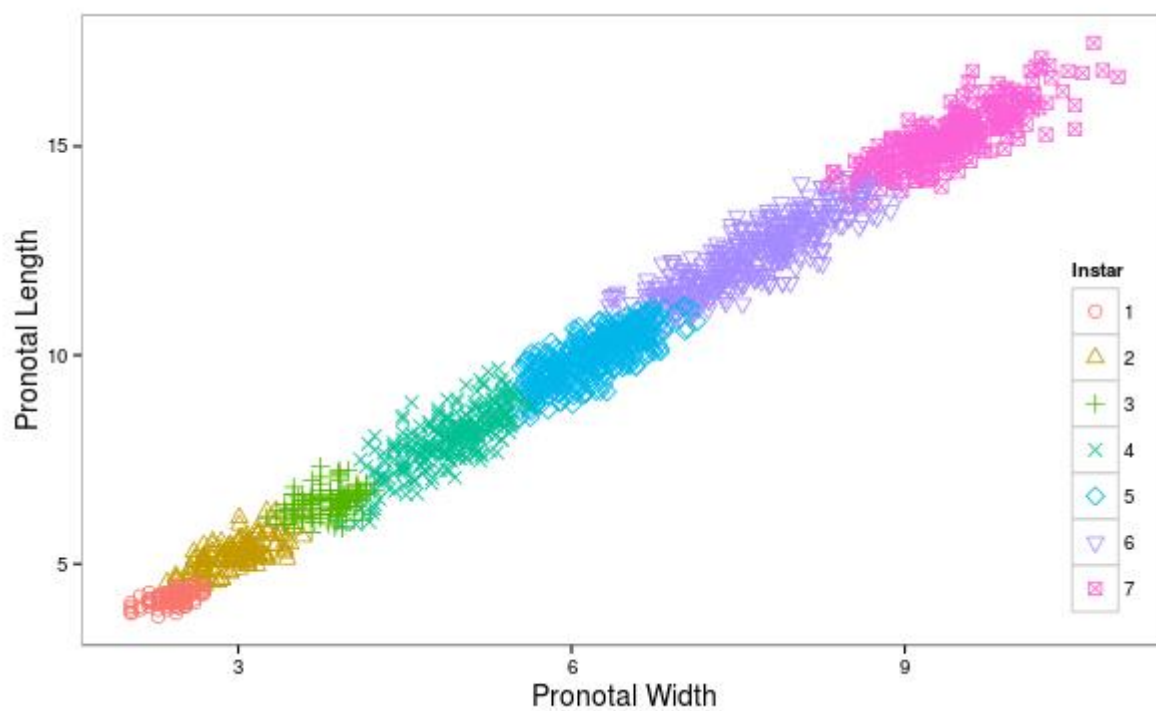
Figure 2-3. The seven instars of pronotal width (mm) and pronotal length (mm) of *B.dubia*.

Figure 2-4. The linear regression of three characters with 95% CI region in each instar. Pronotal length (Length), Pronotal width (Width), Head width (Head).

Chapter 3 The parental care of Dubia cockroaches

ABSTACT: The social behavior of cockroaches is less studied compared to that of other insect groups. Most of the information of cockroaches is from brief notes made during field observation. For parental care, most of observations are focused on species of Cryptocercidae and no study has been conducted o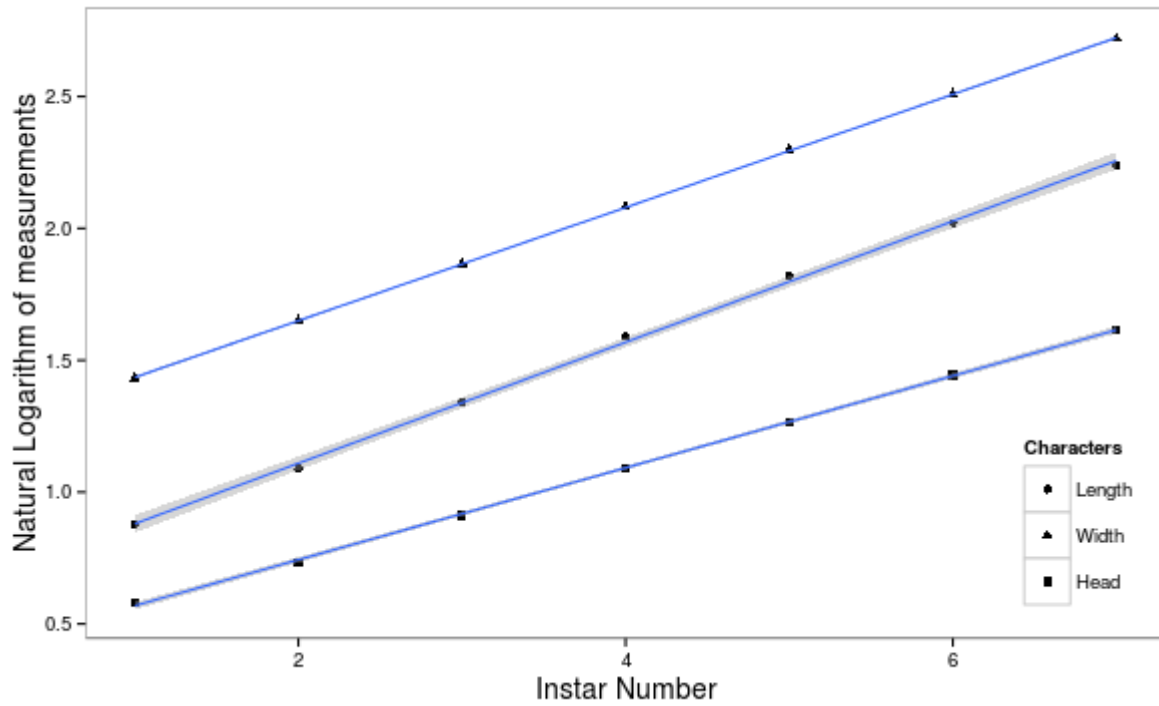n the parental behavior of Blaberidae so far. The objective of this study was to measure the possible effects of parental care on offspring of Dubia cockroaches *Blaptica dubia* Serville. No parental care effects are detected although parental care behavior is observed in Dubia cockroaches.

Keywords: *Blaptica dubia*, Parental care

Introduction

Parental care involves any sort of behavior that increases the survival of the offspring. Parental care has been described in most animal phyla, but is especially well developed in numerous species of insects, crustaceans, and vertebrates. There is great diversity in the amount of parental care provided to offspring by different species. The most common ways of parental care are to assist the offspring to live through harsh physical conditions, make resources available for offspring, which are otherwise difficulty or impossible to obtain, and decrease risk of predation of eggs or young (Clutton-Brock and Scott 1991) .Although levels of complexity are various, parental care can be divided into three major behavior categories: (a) those that

physically protect the young from danger, (b) those that protect resources vital to offspring, and (c) those that facilitate offspring feeding (Glazier 2002, Roldan and Soler 2011).

It is difficult to find other group of animals that are as diversely social as cockroaches. Most cockroaches show some form of parental care in the broad sense, because even the most primitive cockroaches have ootheca or egg case to protect the young before they hatch (Mckittrick 1964). If only the post-hatch parental care is considered, most of the information about cockroaches is from brief field notes (Bell et al. 2007). Most of these are focused on species of Cryptocercidae (Seelinger and Sellinger 1983, Nalepa 1987, 1988, 2010, Park and Choe 2003).

Few laboratory observations of parental care behaviors are focused on species of the Blaberidae. Until recently, a new form of maternal provisioning of newly hatched nymphs was described in the ovoviviparous hissing cockroach *Gromphadorhina portentosa* Schaum. Shortly after expelling the hatching egg case, the female exudes from her abdominal tip a whitish, translucent material on which neonates actively feed (Perry and Nalepa 2003).

This study is focused on the parental behavior in *Blaptica dubia* Serville (commonly known as the Dubia cockroach, Guyana spotted cockroach, or Orange-spotted cockroach). *B. dubia* is a large (up to ca. 4.5 cm in length), sexually dimorphic blaberid cockroach that is often kept as a pet. It is ovoviviparity and has post-hatch behavior. More importantly, this species is used as a food source for many reptiles and amphibians. Compared to other food sources such as crickets, *B. dubia* contain a higher percentage of protein, are easier to maintain, and unlike other cockroaches, produce little odor. As a result, they have become an increasingly popular food

among amphibian and reptile enthusiasts. The objective of the present study is to investigate the effect of parental care on nymphs in *B.dubia*. If nymphs show difference in development time, body weight, head length or head width between groups with or without parents, it is an indication that parental care has an effect on the development of *B.dubia*.

Material and Methods

Insect Rearing: *Blaptica dubia* were reared in clear gallon glass jars (3.8 L, 15.24 cm width by 25.4 cm height) with cardboard harborage at $30 \pm 2°C$. They were exposed to a photoperiod of 12:12 (L:D) h and supplied with water and dry dog Chow (Purina® Dog Chow®, Ralston Purina, St. Louis, MO) ad libitum.

Experiment Design: The development of nymphs with the presence or absence of parents was compared. In the control, there were 10 neonates, and in one test group, there is one extra adult female and in the other test group, there is one extra adult male. All the 30 neonates were from the same mother and the experiment were set up on the second day of getting the neonates. There were 6 replications in a randomized complete block design. We monitored the development of the cockroaches in daily basis. For every instar, the following characters were measured: the width of the head capsule, the pronotal length and width, the body weight, and development time.

Measurements: Head capsule width (the widest distance between the two compound eyes) was measured to 0.01 mm using a microscope (Leica MZ 6, Solms, Germany) with an ocular micrometer. The length and width of the pronotum were measured to 0.01mm using an

29

electronic digital caliper (Model: 62379-531, Control Company, Friendswood, TX). Body weight was measured to the nearest 0.1mg using an electronic scale (Serial No 0074082, Denver Instrument Company).

 Data Analysis. Shapiro-Wilk nomality test (Shapiro and Wilk 1965) was used to test the normality of the data collected. The mean and standard deviation of each variable was calculated. For each group, ANOVA were conduct after the normality tests. (R Development Core Team 2011, Ramsey and Schafer 2012).

<div align="center">

Results

</div>

<div align="center">

Effect of parental care on measurable characters

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 In table 1~4, the mean of head width, pronotal length, pronotal width and body weight are compared. The results of ANOVA indicate that there is no difference between the three groups.

<div align="center">

Effect of parental care on development time

</div>

 In Table 5, the mean of development time is compared. The results of ANOVA indicate that there is no difference between the three groups.

<div align="center">

Discussion

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 In Park and Choe's (2003) manipulation experiments with *Cryptocercus kyebangensis*, offspring separated from their parents could survive independently, but grow more rapidly when

<div align="center">30</div>

they remained with their parents. In particular, the effects of parental care on offspring growth were found to be stronger in groups with both parents than those with single parents.

However, our results indicate that there is no difference in the head size, pronotal length and width, body weight and developmental time. That is rather counter-intuitive. Why do we not observe a "parental effect" in our study? One possible reason may be that: the environment is so good (30°C is a very good temperature for dubia cockroaches), so parental behavior is hided. If the temperature were very low, the Dubia cockroaches will huddle around the mother and it will decrease the heat loss. Since we only measured the last stage and the development time from birth to adult. There is possibility that there were differences in the early stages and the difference were hidden by later development.

To get more accurate results, there are some possible ways to improve: (a) change the temperate to less comfortable, such as 25°C or 20°C. (b) Redesign the experiment and put two groups. One is neonate only and another is neonates and its parents. (c) Further observation of parental behavior of Dubia cockroaches is also necessary.

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Table 3-1 Effect of parental care on head width of 7th instar (mm)

Group	Neonate only	With adult female	With adult male	P value
1	5.079±0.1451	5.062±0.1737	5.033±0.2315	0.89
2	5.409±0.1384	5.367±0.1370	5.444±0.0967	0.49
3	5.233±0.1175	5.215±0.1428	5.244±0.0760	0.87

Table 3-2 Effect of parental care on pronotal length of 7th instar (mm)

Group	Neonate only	With adult female	With adult male	P value
1	9.693±0.2693	9.732±0.3798	9.631±0.4897	0.89
2	10.10±0.3466	10.25±0.2518	10.07±0.2362	0.40
3	9.933±0.3678	9.787±0.3298	9.876±0.2452	0.62

Table 3-3 Effect of parental care on pronotal width of 7th instar (mm)

Group	Neonate only	With adult female	With adult male	P value
1	15.22±0.2531	15.17±0.6773	15.37±0.6217	0.77
2	16.25±0.3955	16.05±0.3808	15.87±0.7114	0.33
3	15.49±0.5815	15.70±0.3813	15.63±0.4670	0.66

Table 3-4 Effect of parental care on body weight of 7th instar (g)

Group	Neonate only	With adult female	With adult male	P value
1	2.070±0.2062	2.122±0.2615	2.046±0.2952	0.86
2	2.644±0.1862	2.743±0.1750	2.487±0.2739	0.076
3	2.057±0.2271	2.165±0.2291	2.163±0.2734	0.57

Table 3-5 Effect of parental care on development time from birth to adut (days)

Group	Neonate only	With adult female	With adult male	P value
1	142.9±9.662	139.7±11.715	141.4±9.985	0.810
2	165.3±6.873	147.0±13.476	158.1±17.201	0.083
3	202.5±8.536	210.4±34.122	222.5±28.224	0.330
4	167.6±12.293	161.5±19.206	160.3±11.056	0.590
5	130.4±9.812	130.8±9.203	132.9±7.120	0.830
6	175.7±25.050	171.8±15.106	158.8±25.246	0.280

Chapter 4 Temperature-dependent development of Dubia Cockroaches (Blattodea: Blaberidae)

ABSTACT Temperature-dependent development of the nymphs of the Dubia Cockroaches *Blaptica dubia* Serville was described using data collected from constant-temperature laboratory experiments. Simple linear regression methods was developed based the data from each instar. Degree-days required to complete a particular life stage were estimated as 457.5, 668, 1031 .1317, 1515, 2071 for 1~7 instar, respectively. The results could be used to control the development of Dubia cockroaches rate and find the optimal temperature to rear *B.dubia* in a given situation.

Keywords Temperature-dependent development, *Blaptica dubia*

Introduction

Blaptica dubia Serville (commonly known as the Dubia cockroach, Guyana spotted cockroach, or Orange-spotted cockroach) is a large (up to ca. 4.5 cm in length), sexually dimorphic blaberid cockroach that is often kept as a pet. More importantly, this species is used as a food source for many reptiles and amphibians. Compared to other food sources such as crickets, *B. dubia* contains a higher percentage of protein, is easier to maintain, and produces little odor. Consequently, they have become an increasingly popular food among amphibian and reptile enthusiasts (Wu et al. 2013). Development of the temperature-dependent development model for *B. dubia* is essential to control the growth rate of *B.dubia* and find the optimal solution

to rear this species under a given situation.

The effect of temperature on development and growth of ectotherms has been well studied (Dixon et al. 2009). In the last century, numerous studies developed appropriate developmental rates function for phonological or population models that can be used under a variety of conditions to predict important events in insect life cycles, or insect abundance for specific control strategies. The development function for each stage is determined by rearing insects at range of constant temperatures (Briere et al. 1999, Trugill et al. 2005). The earliest and still most widely used model was the linear degree-days model. The calculations of lower developmental thermal thresholds and degree-day have long been used for the estimation of developmental times under different constant temperatures. This method has the advantage of simplicity and allows estimation of the developmental threshold and the degree-day requirement of insect species. The principle and the detailed methods are well described in Gullan and Cranston (2005).

Various nonlinear models also have been developed to improve these results. Many nonlinear models have been developed by entomologists, such as, the Belehradek model and the logistic equation of Davidson (Briere et al. 1999). More models are provided by Logan (Logan et al. 1976), Schoolfield (Schoolfield et al. 1981), and Lactin(Lactin et al. 1995).

While the degree-days method, which assumes a linear relationship between developmental rate and temperature, is often suitable for intermediate temperature, this model does not consider nonlinearity at high and low temperatures. Therefore, it will produce considerable error when temperature conditions are toward the extremes,especailly under

variable conditions (Gullan and Cranston 2005). However, in our analysis, degree days are still used due to the limited available data.

Material and Methods

Insect Rearing: *Blaptica dubia* were reared in clear gallon glass jars (3.8 L, 15.24 cm width by 25.4 cm height) with cardboard harborage at $30 \pm 2°C$. They were exposed to a photoperiod of 12:12 (L:D) h and supplied with water and dry dog Chow (Purina® Dog Chow®, Ralston Purina, St. Louis, MO) ad libitum.

Experiment Design: Couples of adult females and males were kept separately in clear half gallon glass jar (1.9 L, Dimensions: 5.5" width x 6.25" height) in condition stated above. After neonates were born, they were separated from their parents and divide them into group of 10 nymphs. Six groups (60 nymphs) were set up in every temperature. In this study, temperature was set at 20°C, 25°C, 30°C, 35°C, and 40°C and exposed to a photoperiod of 12:12 (L:D). The period of each instar was recorded and the development time (days) was calculated. Simple linear regression models of development were developed.

Data Analysis: Development rates for each instar were calculated using reciprocal of the average number of days (i.e., 1/days) required to complete a particular life stage. The relationship between development rate and temperature was described by a linear model. (Gullan and Cranston 2005). Linear regression (Faraway 2004, Ramsey and Schafer 2012) were conducted on data collected for each instar using the R software package (R Development Core Team 2011).

Results

At 40°C, in the first two days, 100% of them survive, but100% died in the 4th day. At 35°C, the nymph started to die in 3rd instar and all died in 4th in star. At 15°C, after 277 days, one nymph molt into 2nd instar. After 467 days, some nymphs are still in first instar and no nymph enters into 3rd instar.

At 20°C, 25°C, 30 °C, the nymph grew normally for 1st instar into adults. Table.1 shows the description statistics on the development time of each instar in 20°C, 25°C, 30 °C, and 35°C. Simple linear model are developed using the data available as shown in Table. 4 and Fig.1 ~ 5. The degree days required to complete 1-7 instar are 457.5, 668, 1031 .1317, 1515, 2071. The Lower development temperature threshold is 7.02°C, 11.56°C, 11.83°C, 12.66°C, 13.65°C, 16.48°C, 16.12°C for 1~7 instar, respectively.

Discussions

In our analysis, only simple linear models were used due to the limited date available. It is obvious that there are many more temperature –development models available (Damos and Savopoulou-Soultani 2012) and probably new models can be developed if more data are available (Bentz et al. 1991). In our situation, the simple linear model is the only choice. As stated in above, the linear model works well between 20°C ~30°C.

The same Degree-days methods was adopted in a temperature-dependent development study on *Lacanobia subjuncta* (Lepidoptera: Noctuidae) by Doerr (2002). In Doerr's study,

larvae successfully completed development of all instars in constant-temperature regimes of 10.0 to 30.0°C. No larval development occurred at 37.5°C. Lower developmental threshold for larvae is 9.9°C, 7.8°C, 6.6°C, 5.1°C, 6.9°C, 7.3°C for 1~6 instar. The degree days required to complete 1-7 instar are 52.1, 53.2, 62.9, 72.5, 50.0, 142.9 for 1-6 instar, respectively.

Compared to Doerr's result, the lower development threshold for B. dubia are high and the degree-days for *B.dubia* are larger. Bothe species develop fastest in 30°C. In both study, insect can grow normally only in a temperature rage. The lower limit for *B.dubia* is higher than *L. subjuncta,* probably because the *B.dubia* originates from tropical areas.

One of the short coming of this study is that we do not have enough different temperatures to model. Although 6 temperatures were set, no validate data were recorded in 15°C, 35°C, and 40°C. The only valid temperatures were 20°C, 25°C, and 30°C. If more data in other temperature are available, more accurate linear or none linear models can be developed.

In order to fully understand the temperature-development pattern in *B. dubia*, it might be useful to compare constant temperature development data with fluctuating temperature development data (Doerr et al. 2002). However, in most situations, *B. dubia* are reared by pet lovers and they do not exist in the wild. As a result, there is probably not enough incentive to observe in fluctuating temperature experiment.

The information presented here forms the basis for developing a more complete understanding of *B.dubia.* These data will be useful in control the development of Dubia cockroaches through temperature. It might also useful to compare life history of different group of cockroaches.

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Blaberidae) using gaussian mixture models. Ann Entomol Soci Am. 106: 323–328.

Table 4-1 Development time of each instar in different temperature. (mean (SD) n)

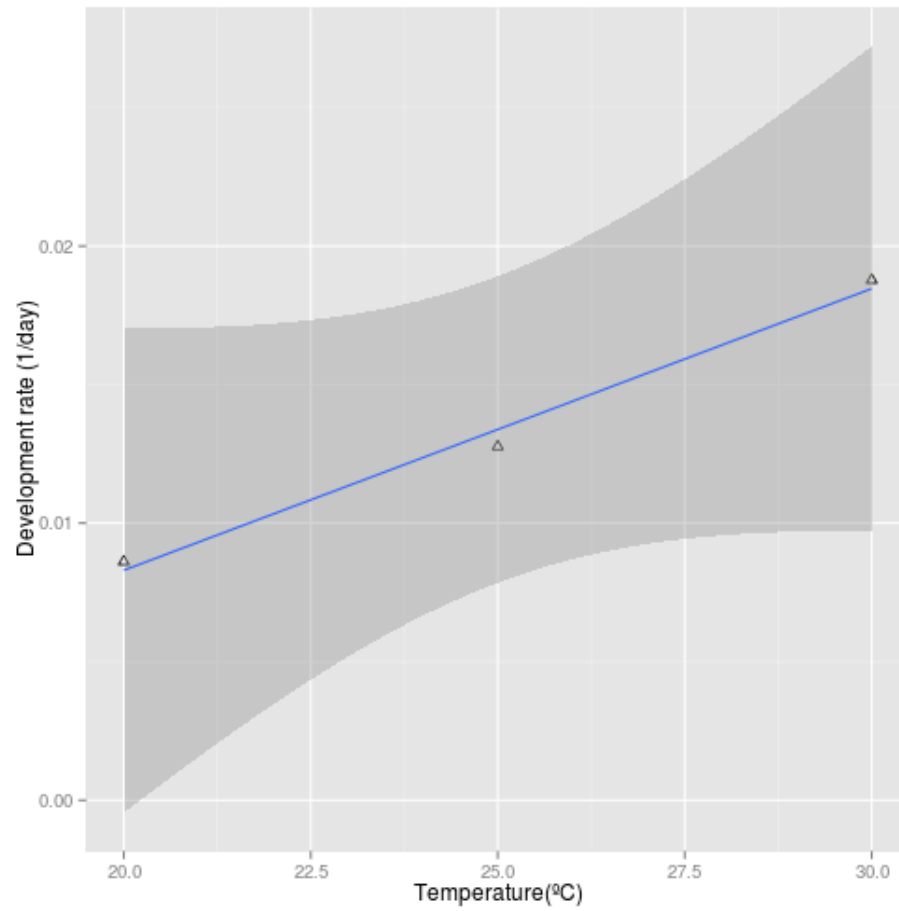| instar	20°C	25°C	30°C	35°C
1	38.8 (3.96) 28	25.4(2.84) 36	20.08(3.23) 38	17.46(5.19) 59
2	79.9(5.931) 28	51.18(3.79) 34	36.06(4.63) 36	NA
3	116.15(8.60) 26	78.32(4.83) 31	53.26(7.28) 34	NA
4	161.74(13.91)	106.76(7.10) 33	70.28(8.41) 36	NA
5	220.48(24.54)	133.45(7.71) 33	87.56(8.91) 36	NA
6	NA	167.80(10.48)	105.70(17.00)	NA
7	NA	233.20(17.55)	149.20(15.69)	NA
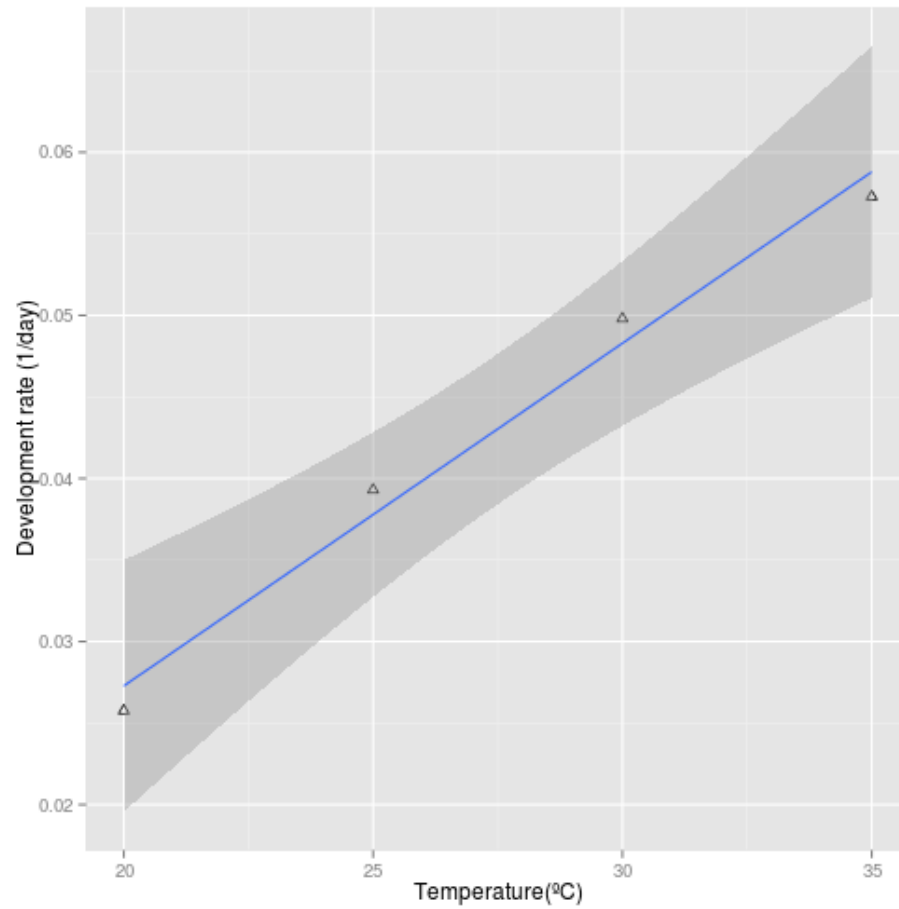
Figure 4-1 The linear regression of development rate and temperature (20°C, 25°C, 30°C, 35°C) of 1st instar
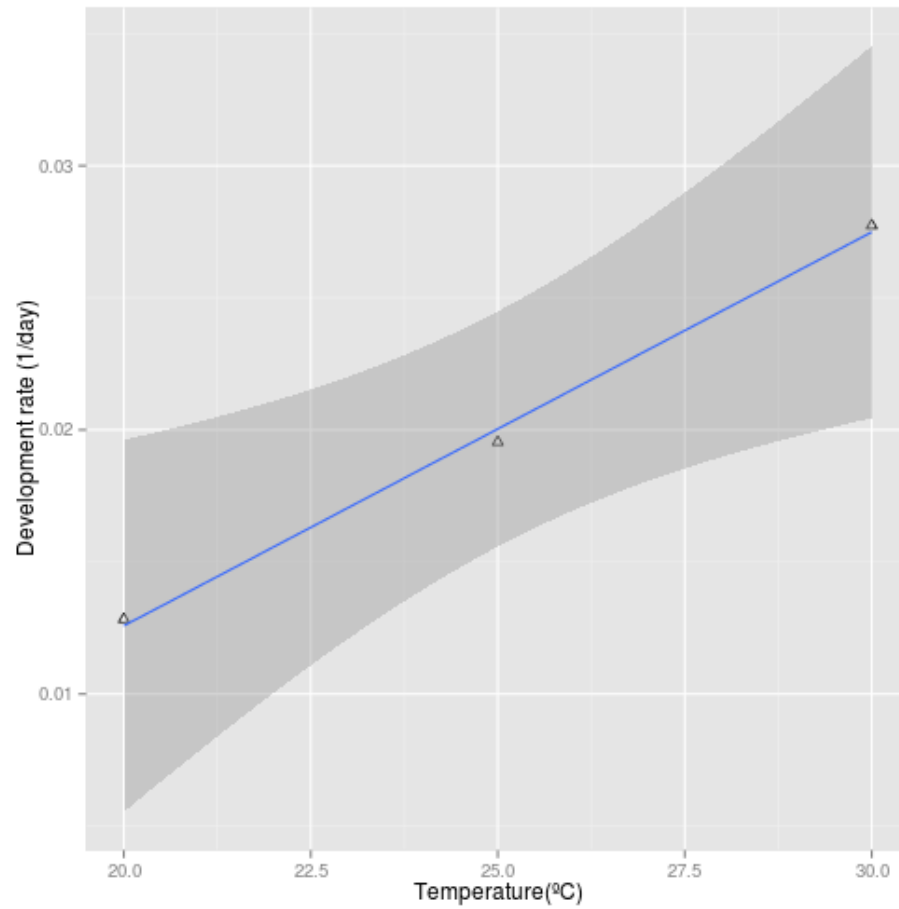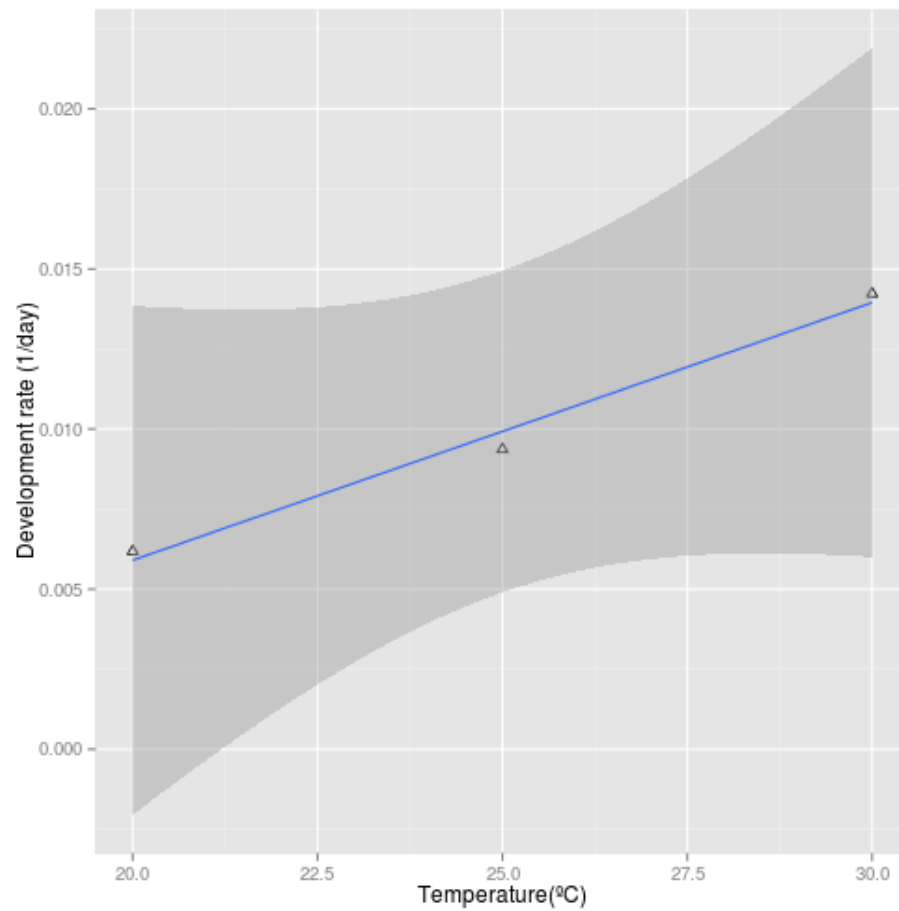
Figure 4-2 The linear regression of development rate and temperature (20°C, 25°C, 30°C) of 2nd instar
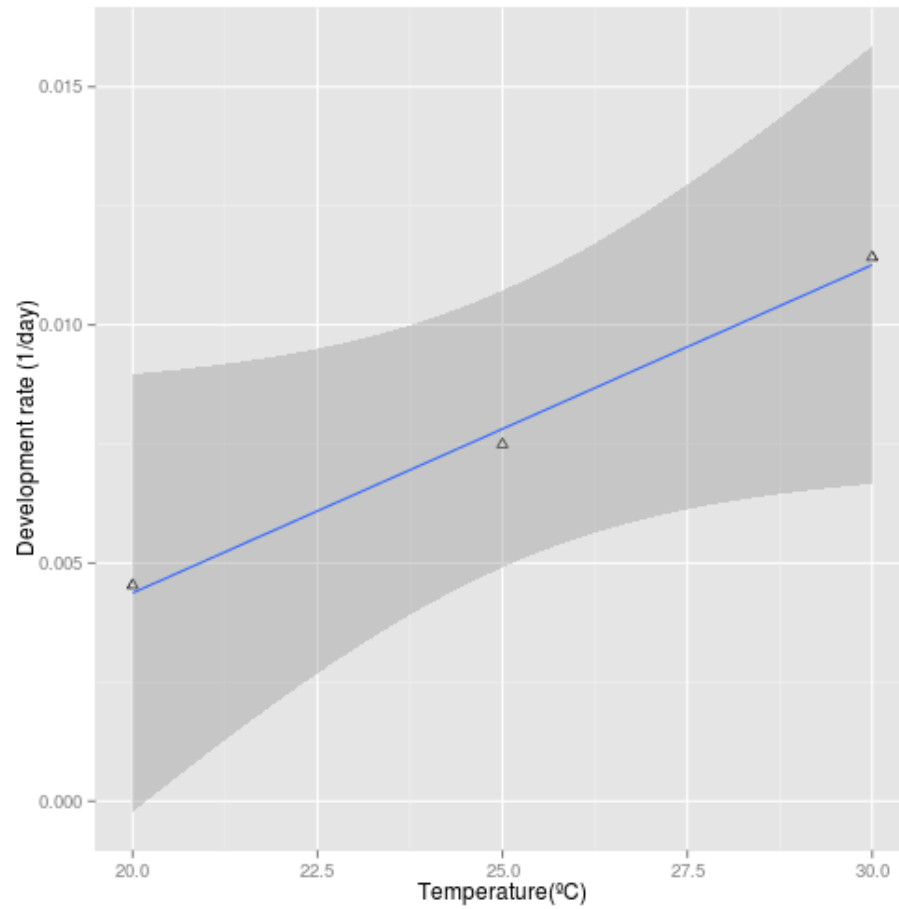
Figure 4-3 The linear regression of development rate and temperature (20°C, 25°C, 30°C) of 3rd instar

Figure 4-4 The linear regression of development rate and temperature (20°C, 25°C, 30°C) of 4rd instar

Figure 4-5 The linear regression of development rate and temperature (20°C, 25°C, 30°C) of 5rd instar

Table 4-2 Linear regression models of temperature-dependent development in the seven instars of *B. dubia*

Instar	Model	R squred	F	P (> |t|)	Lower Development Threshold (°C)	Degree days
1st instar	Y = 0.0021X − 0.0148	0.984	120	< 0.01	7.02	457.5
2nd instar	Y = 0.0149X − 0.0172	0.997	301	0.0366	11.56	668
3rd instar	Y = 0.001023X − 0.01203	0.989	90.8	0.0666	11.83	1031
4th instar	Y = 0.000805X − 0.010190	0.986	69	0.0763	12.66	1317
5th instar	Y = 0.000689X − 0.009398	0.993	151	0.0157	13.65	1515
6th instar	Y = 0.0007X − 0.0117	NA	NA	NA	16.48	1429
7th instar	Y = 0.000483X − 0.007783	NA	NA	NA	16.12	2071