University of Kentucky
UKnowledge

Theses and Dissertations--Biology | Biology

2012

EMPIRICAL AND CONCEPTUAL APPROACHES TO LIFE-HISTORY TRADE-OFFS: THE SIZE AND NUMBER OF OFFSPRING IN BROODS OF A PARASITOID WASP

Yoriko Saeki
University of Kentucky, yoriyoripp@gmail.com

Right click to open a feedback form in a new tab to let us know how this document benefits you.

Recommended Citation
Saeki, Yoriko, "EMPIRICAL AND CONCEPTUAL APPROACHES TO LIFE-HISTORY TRADE-OFFS: THE SIZE AND NUMBER OF OFFSPRING IN BROODS OF A PARASITOID WASP" (2012). *Theses and Dissertations--Biology*. 8.
https://uknowledge.uky.edu/biology_etds/8

This Doctoral Dissertation is brought to you for free and open access by the Biology at UKnowledge. It has been accepted for inclusion in Theses and Dissertations--Biology by an authorized administrator of UKnowledge. For more information, please contact UKnowledge@lsv.uky.edu.

STUDENT AGREEMENT:

I represent that my thesis or dissertation and abstract are my original work. Proper attribution has been given to all outside sources. I understand that I am solely responsible for obtaining any needed copyright permissions. I have obtained and attached hereto needed written permission statements(s) from the owner(s) of each third-party copyrighted matter to be included in my work, allowing electronic distribution (if such use is not permitted by the fair use doctrine).

I hereby grant to The University of Kentucky and its agents the non-exclusive license to archive and make accessible my work in whole or in part in all forms of media, now or hereafter known. I agree that the document mentioned above may be made available immediately for worldwide access unless a preapproved embargo applies.

I retain all other ownership rights to the copyright of my work. I also retain the right to use in future works (such as articles or books) all or part of my work. I understand that I am free to register the copyright to my work.

REVIEW, APPROVAL AND ACCEPTANCE

The document mentioned above has been reviewed and accepted by the student's advisor, on behalf of the advisory committee, and by the Director of Graduate Studies (DGS), on behalf of the program; we verify that this is the final, approved version of the student's dissertation including all changes required by the advisory committee. The undersigned agree to abide by the statements above.

Yoriko Saeki, Student

Dr. Philip Crowley, Major Professor

Dr. Brian C. Rymond, Director of Graduate Studies

EMPIRICAL AND CONCEPTUAL APPROACHES TO LIFE-HISTORY TRADE-OFFS:

THE SIZE AND NUMBER OF OFFSPRING IN BROODS OF A PARASITOID WASP

DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Arts and Sciences
at the University of Kentucky

By
Yoriko Saeki

Lexington, Kentucky

Director: Dr. Philip Crowley, Professor, Department of Biology

Lexington, Kentucky

2012

Copyright © Yoriko Saeki 2012

ABSTRACT OF DISSERTATION

EMPIRICAL AND CONCEPTUAL APPROACHES TO LIFE-HISTORY TRADE-OFFS:
THE SIZE AND NUMBER OF OFFSPRING IN BROODS OF A PARASITOID WASP

Trade-offs in resource allocation underlie key life history traits of organisms. My dissertation focuses on the size-number trade-off in clonal broods of offspring using the polyembryonic wasp, *Copidosoma bakeri* parasitizing immature stages of the moth *Agrotis ipsilon*. I aim to characterize responses of wasp brood size and individual body mass by manipulating the environments in order to understand the allocation pattern in the size number trade-off. In reviewing the functional forms of trade-off relationships in relation to resource constraints, I distinguish among three main trade-off types based on graphical representations of the relationship between the trade-off variables: linear, convex (inverse), and concave. The size-number trade-off in *C. bakeri* shows convex relationship. Characteristics of the trade-off are sex specific: female broods have larger body mass but smaller brood size than do male broods. When food intake of the host was increased, the trade-off between wasp body mass and brood size for both sexes shifts toward both higher wasp brood size and higher body mass. When the host has better access to food late in development, the size-number trade-off curve moves up and to the right on the graph. However, the trait combinations shift along the same trade-off curve toward greater wasp body mass but smaller brood size when the host development time is shorter due to more resources in early in development. I also investigate temperature effects on the size-number trade-off. *C. bakeri* brood size significantly increases with high temperature early in host development. There is no shift in the allocation pattern of the size-number trade-off with temperature. Finally, I test effects of body mass on longevity, fecundity, and mating competitiveness of *C. bakeri*. Larger body size increases female longevity, and mated females produce more eggs than unmated females. There are no significant relationships between male body mass and longevity or mating competitiveness. Mating reduces male longevity independent of body mass. The different impact of body mass on fitness between male and female wasps suggests the observed sex-specific allocation patterns of the size-number trade-off. Implications of the experiments and possible follow-up work are discussed.

Key words: clonal brood, host-parasitoid interaction, size-number trade-off, trade-off curve

Yoriko Saeki
May 21, 2012

EMPIRICAL AND CONCEPTUAL APPROACHES TO LIFE-HISTORY TRADE-OFFS: THE SIZE AND NUMBER OF OFFSPRING IN BROODS OF A PARASITOID WASP

By

Yoriko Saeki

Philip H. Crowley, PhD
Director of Dissertation

Brian C. Rymond, PhD
Director of Graduate Studies

May 21, 2012

Table of Contents

TABLE OF CONTENTS III

LIST OF TABLES VI

LIST OF FIGURES VII

CHAPTER 1 INTRODUCTION 1

Study System *3*
Overview of the Chapters *4*

CHAPTER 2 ALLOCATION TRADE-OFFS AND LIFE HISTORIES: A GRAPHICAL APPROACH 7

OVERVIEW 7
DEFINITIONS AND TYPES OF TRADE-OFFS 8
THE CONSTRAINING VARIABLE THAT FORCES THE TRADE-OFF 10
THE RELATIONSHIP BETWEEN TWO LIFE-HISTORY TRAITS THAT TRADE OFF: SHAPES OF TRADE-OFF CURVES 13
Linear relationship (Figure 2.1A) *14*
Linear-bent relationship (Figure 2.1B) *15*
Concave relationship (Figure 2.1C) *16*
Convex relationship (Figure 2.1D) *16*
COST-BENEFIT FITNESS RELATIONSHIPS ASSOCIATED WITH TRAIT MAGNITUDES AND TRADE-OFF ALLOCATION PATTERNS 17
THE SHAPE OF TRADE-OFFS, ALLOCATION PATTERNS, AND ENVIRONMENTS 18
SUMMARY 20

CHAPTER 3 A SEX-SPECIFIC SIZE-NUMBER TRADE-OFF IN CLONAL BROODS 27

INTRODUCTION 27
MATERIALS AND METHODS 29
Focal system *29*
Empirical methods *30*
Data Analysis *32*
(i) Trade-off between the number and mass of progeny 32
(ii) Interaction with host resources 33
RESULTS 35
Basic brood data *35*
Trade-off between the number and size of progeny *36*
Interaction with host resources *37*
DISCUSSION 38

CHAPTER 4 THE SIZE-NUMBER TRADE-OFF IN CLONAL BROODS OF PARASITIC WASP: RESPONSES TO THE AMOUNT AND TIMING OF RESOURCE AVAILABILITY 50

INTRODUCTION 50
MATERIALS AND METHODS 53
Study species *53*
Empirical methods *54*
Analyses *58*
Effects of food treatments on host growth and its interaction with parasitism 58
Effects of food treatments on the parasitoid brood 58
Effects of food treatments on the precocious wasp larvae 59
RESULTS 60
Effects of food treatments on host growth and its interaction with parasitism *61*
Effects of food treatments on the parasitoid brood *61*
Effects of food treatments on the precocious wasp larvae *63*
DISCUSSION 64
Interpreting the results of hypothesis tests *64*
Shifts in trade-off curves and allocation patterns *65*
Mechanisms of brood size adjustment *67*
Precocious larvae *69*
Sex specific responses *69*

CHAPTER 5 TEMPERATURE EFFECTS DURING DEVELOPMENT ON A POLYEMBRYONIC PARASITOID AND ITS HOST 78

INTRODUCTION 78
METHODS AND MATERIALS 80
Study species *80*
Experimental design *81*
Analysis *84*
Survival 84
Temperature and parasitism effects on growth of moth larvae 84
Temperature effects on wasp brood size, wasp body mass and the size-number trade-off 84
RESULTS 85
Survival 85
Temperature and parasitism effects on growth of moth larvae 85
Temperature effects on wasp brood size, wasp body mass and the size-number trade-off 86
DISCUSSION 87
Temperature and parasitism effects on growth of moth larvae *88*
High temperature effects on parasitized and unparasitized larvae early and late in development *89*
Temperature effects on wasp brood size, wasp body mass and the size-number trade-off *91*

CHAPTER 6 THE SIZE-NUMBER TRADE-OFF AND COMPONENTS OF FITNESS IN CLONAL PARASITOID BROODS 96

INTRODUCTION 96
METHODS 98
Study species *98*

Empirical methods *98*
Longevity 98
Potential fecundity 99
Male mating competitiveness: 100
Analyses *100*
Longevity 100
Potential fecundity 101
Male mating competitiveness: 102
RESULTS 102
Longevity 102
Potential fecundity 102
Male mating competitiveness 104
DISCUSSION 105
CHAPTER 7 KEY FINDINGS AND FUTURE DIRECTIONS 114
APPENDIX STANDARDIZING BROOD SIZE AND BODY MASS OF PARASITOIDS 117
REFERENCES 118
VITA 132

List of Tables

TABLE 2.1 EMPIRICAL STUDIES OF ALLOCATION TRADE-OFFS: TRAITS, RESOURCES, AND RELATIONSHIPS. 25
TABLE 3.1 EFFECTS OF SEX AND PARASITISM ON HOST CHARACTERISTICS 45
TABLE 3.2 CORRELATIONS BETWEEN HOST RESPONSES AND BROOD SIZE 46
TABLE 4.1 MEANS OF MEASUREMENTS OF HOST AND WASP POLYGERM GROWTH AT EACH HOST LARVAL STAGE.....77

List of Figures

FIGURE 2.1 THE SHAPES OF HYPOTHETICAL TRADE-OFF CURVES 21
FIGURE 2.2 A HYPOTHETICAL LINEAR RELATIONSHIP BETWEEN TWO TRAITS UNDER THE TRADE-OFF, WITH FITNESS CONTOURS AND THE OPTIMAL ALLOCATION PATTERN 22
FIGURE 2.3 THREE WAYS THAT ENVIRONMENTAL CONDITIONS MAY ALTER A TRADE-OFF, USING A LINEAR EXAMPLE 24
FIGURE 3.1 RELATIONSHIP BETWEEN SECONDARY BROOD SIZE AND WASP BODY MASS 47
FIGURE 3.2 RELATIONSHIP BETWEEN HOST MASS AND BROOD MASS 48
FIGURE 3.3 RELATIONSHIP BETWEEN HOST MASS AND BROOD SIZE/WASP MASS 49
FIGURE 4.1 HOST DEVELOPMENT TIME AND MAXIMUM WET MASS OF PARASITIZED AND UNPARASITIZED LARVAE OF ALL TREATMENT 72
FIGURE 4.2 RELATIONSHIP AND BEST-FIT LINES FOR THE TRADE-OFF BETWEEN LOG-TRANSFORMED STANDARDIZED WASP MASS AND BROOD SIZE 74
FIGURE 4.3 WASP BROOD SIZE AND DRY MASS FOR 4 FEEDING TREATMENT COMBINATIONS 76
FIGURE 5.1 DEVELOPMENT TIME AND MAXIMUM WET MASS OF PARASITIZED AND UNPARASITIZED LARVAE REARED AT EACH TEMPERATURE TREATMENT 93
FIGURE 5.2 WASP SECONDARY BROOD SIZE AND DRY BODY MASS AT EACH TEMPERATURE TREATMENT 94
FIGURE 5.3 RELATIONSHIP BETWEEN STANDARDIZED WASP BODY MASS (MG) AND STANDARDIZED WASP SECONDARY BROOD SIZE 95
FIGURE 6.1 RELATIONSHIPS BETWEEN WASP DRY MASS AND LONGEVITY FOR FEMALES AND MALES 110
FIGURE 6.2 MEAN NUMBER OF EGGS, MEAN EGG VOLUME, AND MEAN TOTAL EGG VOLUME IN ML AT 1ST, 3RD, AND 7TH DAYS AFTER EMERGENCE 112
FIGURE 6.3 EFFECTS OF MATING ON MEAN EGG MATURATION OR PRODUCTION 113
FIGURE 6.4 EFFECTS OF MATING ON MALE LONGEVITY IN DAYS 113

Chapter 1 Introduction

Life history traits are important features and processes during the life cycle, such as growth rate, development time, age at maturity, and adult body size. These traits are fundamentally important in determining phenotypes in different environments (e.g. Stearns and Koella 1986; Nylin and Gotthard 1998; Juliano et al. 2004) and ultimately the evolutionary fitness of organisms (Roff 1981; Roff 1992; Stearns 1992; Stearns 2000). The direction and magnitudes of changes in life history traits in response to the environment generally reflect ways that the traits contribute to fitness (Stearns 2000). Therefore, the expression of different traits may be species-, condition-, and sex-specific (Hedrick and Temeles 1989; McNamara & Houston 1996)--and may interact in important ways with other such traits.

Life histories of organisms are shaped by trade-offs, where each trade-off is the allocation of a limiting resource between alternative structures or functions that both increase fitness. Life-history trade-offs have been well documented, providing abundant data and numerous conceptual frameworks (e.g. Roff 2002). A trade-off can be illustrated as a negative relationship between variables on a graph in terms of fitness; but the full potential of empirical trade-off data to express the underlying biological relationships have not been often realized.

I refer to the characteristic of trait combinations of a trade-off relationship, illustrated graphically, as the trade-off curve; when the empirically observed trait combinations are highly localized on the graph, I refer to the distribution as an allocation pattern. The allocation pattern may be adaptive, inviting evolutionary interpretations. To understand the dynamics of life history traits in a trade-off relationship, my dissertation

examines trade-offs in various environments using graphical approaches, because the graphs express the conceptual framework of the system containing the relationships.

In particular, I focus on the size-number trade-off in clonal broods. The size-number trade-off of progeny within a single reproductive bout occurs when the body sizes of offspring and the number of individuals in the brood are both positively related to fitness but constrained by the resources available (Stearns 1992; Messina and Fox 2001). Clonal broods consist of genetically identical individuals resulting from asexual replication, removing some sources of variation in the trade-off relationship. My overall goal is to characterize and understand the trade-off and the allocation pattern between the size and number of progeny, with their changes under different environments. Subsequently, the interpretation of the trade-off characteristics to the lifetime reproductive success is also examined.

Many factors such as sibling competition (Godfray and Parker 1991), variation in parental effort among offspring (Mayhew 1998, Mayhew and Glaziot 2001), or variation in resource acquisition (Noordwijk & de Jong 1986), and interactions among traits involved in the trade-off (e.g. development time: Klingenberg and Spence 1997) can obscure existing trade-off relationships. These factors are unavoidable in most natural systems; but to minimize these complications, I select a research system in which clonal broods are asexually replicated in the host. Here the trade-off relationship is more apparent: the host is serving as a well-defined limiting resource, no competition is expected among clonal offspring, and no parent-offspring conflict is expected because asexual division is under control of the developing embryo.

Therefore, I conduct a series of studies to demonstrate and analyze a size-number

tradeoff of progeny, which differs between the two sexes and shifts depending on environment, using the polyembryonic parasitoid, *Copidosoma bakeri*. I also examine the effect of the body size determined by the trade-off during development on subsequent reproductive success to address evolutionary consequences of the trade-off.

Study System

Copidosoma bakeri has been found in the central to northern United States and Canada (Schaaf, 1972; Cheng, 1977; Byers et al., 1993). *C. bakeri* is a polyembryonic egg-larval parasitoid wasp whose hosts are reported to include at least 19 lepidopteran species (Schaaf, 1972). *C. bakeri* parasitizes the black cutworm *Agrotis ipsilon* (Hufnagel) in Kentucky, USA (Y.S. personal observation). In Kentucky, *A. ipsilon* parasitized by *C. bakeri* is found from July to October, whereas *A. ipsilon* appears in April, migrating from the south, and disappears in October, migrating southward (Showers, 1997).

A female *C. bakeri* usually lays one egg when she locates a host egg. The parasitized host egg hatches and develops in the same way as an unparasitized host egg. The parasitoid egg inside the host also develops and divides. The wasp embryo divides while the host larva is between its 1st and 4th instars and becomes a polyembryo. The resulting polyembryo may contain hundreds to thousands of individual embryos. Wasp larvae hatch when the host enters the final larval stage and then consume the host (Strand 1989; Baehrecke and Strand 1990). The development of polyembryonic wasps is thus synchronized with host development (Baehrecke and Strand 1990).

Since thousands of clonal larvae are produced from one embryo through divisions resulting from asexual reproduction, all individuals constitute a single genetic entity

(genet) (Hughes 1987; Harper 1977; Pearse et al. 1989; Hughes et al. 2005). Therefore, fitness is determined at the level of entire broods rather than individual offspring when fitness is attributable to a single genotype shared with brood mates. Another life history characteristic of polyembryonic wasps may include polyphenism, polymorphism within genetically identical individuals, indicated by the presence of both precocious and reproductive larvae (Strand and Grbić 1997). Precocious larvae are sterile and hatch before reproductive larvae emerge. The function of precocious larvae is apparently to damage or kill intra- and inter-specific competitors in the same host (Cruz 1981). There are several different patterns of producing of precocious larvae in the genus *Copidosoma*, and the number of precocious larvae in *C. bakeri* seems to be small.

Overview of the Chapters

In chapter 2, I review shapes of trade-off relationships and their graphical representations. I summarize studies that attempted to predict trade-off curves created by different life-history components and analyze patterns of the shapes based on the types of constraints on the trade-offs, types of components of the trade-offs, and effects of environmental factors on the shapes of the trade-off curves.

In chapter 3, I examine the sex-specific size-number tradeoff in *C. bakeri*. I collect observational data of the growth of parasitized *A. ipsilon*, such as mass gain and development time. I also measure the body size and brood size of *C. bakeri* progeny that emerge from the hosts to examine the difference in the way that size and number of offspring traded off in female and in male broods. With the measurement of the host growth, I investigate how parasitoid broods balanced the size-number trade-off and fit the

body mass of the host.

In chapter 4, I examine effects of reduced food resources on the size-number trade-off by manipulating access to food by the host caterpillar at two different phases of developmental stages, early and late. By shifting the food access treatment between developmental intervals, I attempt to shift the development time and the total mass of the wasp brood independently.

In chapter 5, I investigate responses to high vs moderate temperature in the size-number trade-off, with particular interest in stage-, species-, and sex-specific temperature effects on the host-parasitoid interaction. Three focal points of this study are (1) temperature effects on the growth of the host, and the effect of the timing of the stress imposed, (2) temperature effects on the unparasitized caterpillar, and (3) temperature effects on the size-number trade-off in broods of *C. bakeri* offspring. I expose *A. ipsilon* larvae parasitized by *C. bakeri* to high temperature when *A. ipsilon* was in early or late larval stages and measured their development time and body mass.

In chapter 6, my focus shift to the fitness of the adult parasitoid to interpret the balance of the size-number trade-off I observed in the previous chapters. I examine several possible effects of body mass on male and female reproductive success, including longevity, fecundity of females, mating competitiveness for males. I also investigate whether *C. bakeri* is proovigenic (i.e. all eggs produced on emergence) or synovigenic (i.e. eggs matured during the adult stage).

In chapter 7, I summarize the findings from the previous chapters and discussed opportunities for additional studies to further understanding of the theme. In a retrospective manner, I identify problems raised during the studies and unknown factors requiring

follow-up investigation. I also integrate these issues and results from the series of the studies to discuss future work on life-history trade-offs.

Chapter 2 Allocation Trade-Offs and Life Histories: A Graphical Approach

Overview

A *trade-off* is a relationship between the magnitudes of two (or more) variables such that changes in the net benefit derived from one implies opposite changes in net benefits derived from the other(s). Trade-offs play a fundamental role in shaping life history traits in nature (Stearns, 1992, Roff, 2002, Stearns & Hoekstra, 2005, Roff & Fairbairn, 2007).

Here I distinguish among some types of life-history trade-offs and then focus on the large subset associated with the allocation of a limiting resource between conflicting demands. I then review this literature with particular emphasis on the types of relationships (and their graphical expression) between the trade-off variables. I suggest that the shapes of trade-off curves help us understand the ecological and evolutionary forces acting on the traits and the resulting combination of trait magnitudes (Schaffer 1974), referred to here as the *allocation pattern*. Although the importance of understanding shapes of trade-off curves and associated mathematical relationships has occasionally been noted in the literature, empirical examinations of the trade-off curves and allocation patterns have received little attention on a comparative basis (Stearns 1992; Roff, 2002). The shapes of trade-off curves have been used to predict population dynamics (e.g. Bowers et al. 2005; deMazancourt & Dieckmann 2004). However, in the field of life histories, the studies of trade-offs have mainly focused on trade-off detection, often based simply on whether or not correlations between traits are negative (see Agrawal et al. 2010).

Here I review the shapes of trade-off curves and associated allocation patterns that have been proposed or demonstrated previously, and I discuss their implications for the ecology and evolution of life history traits and for future theoretical studies.

Definitions and types of trade-offs

In life-history trade-offs, fitness-related net benefits accrued from one trait come at the expense of net benefits from another trait because of some constraining linkage between them. This definition is consistent with most currently in use in biology (Stearns 1992; Roff 2002) and closely analogous with usage in economics (Fama & French 2002). The seemingly incongruous concept of a “one-trait trade-off” arises when the allocation pattern is considered to be the focal trait (e.g. see Agrawal et al. 2010), but in that case the trait is not the trade-off but the outcome. For instance, the shell of a freshwater snail can be either elongated or rotund, each of which is specialized to resist against from the predators, crayfish or fish, but not both (DeWitt et al. 2000). Here protection from crayfish predation and from fish predation are traded off under constraints on the architecture of the snail's shell. Fitness can be maximized by finding the shape allocation pattern that maximizes lifetime reproductive success, taking into account any costs associated with shell characteristics.

Trade-offs between traits have been categorized as: (1) allocation trade-offs, in which limited resources are allocated to different traits, (2) acquisition trade-offs, in which acquisition of resources carries fitness costs through other traits such as reduced probability of survival, and (3) specialist-generalist trade-offs, in which organisms specialized for a given environment perform less well in other environments (Roff 2002; Angilletta et al. 2003; Malausa et al. 2005).

In allocation trade-offs (sometimes known rather restrictively as physiological trade-offs: Stearns 1992; Zera & Harshman 2001), the trade-off arises from the limited

amount of some resource available for use in producing traits (reviewed in Stearns 1992). Male songbirds allocating their time between territorial defense and foraging and long-lived plants dividing photosynthetically-derived energy between growth and reproduction provide prototypical examples.

Acquisition trade-offs balance the accumulation of some resource against the cost of obtaining it (e.g. Werner & Anholt 1993; Biro et al. 2006). For example, diurnal foraging for Lepidopteran larvae incurs greater predation risk but higher energy intake compared to nocturnal foraging (Berger & Gotthard 2008). Allocation trade-offs and acquisition trade-offs often underlie life history traits, where the allocation trade-off occurs after the acquisition trade-off (Angilletta et al. 2003; Berner & Blanckenhorn 2007; Boggs 2009; King et al. 2010; King et al. 2011). Hence, allocation and acquisition trade-offs are often coupled, and resources thus acquired can qualitatively and quantitatively alter the associated allocation trade-off (e.g. King et al. 2011).

Specialist-generalist trade-offs have mainly been considered from the viewpoint of evolutionary physiology (reviewed in Angilletta et al. 2003). For instance, herbivores specialized to feed on a particular plant species are able to metabolize the secondary compound produced from the plant but may be unable to metabolize different secondary compounds from other plant species. On the other hand, generalist herbivores may not be able to metabolize a certain kind of secondary compound efficiently but may be able to metabolize, sequester, or excrete various types of secondary compounds to a limited extent (Torregrossa et al. 2012).

In the remainder of this chapter, to keep the subject matter manageable and to focus on situations with one or a small number of resource-related constraining variables, I turn

attention exclusively to allocation trade-offs.

The constraining variable that forces the trade-off

The adaptation of a trait to a given environment is ultimately subject to constraints (e.g. Maynard Smith et al. 1985; Charnov & Skinner 1988; Arnold 1992; Ghalambor et al. 2004). In the context of allocation trade-offs, there is usually a dominant factor, often a limiting resource that ties the trade-off variables together in a mutually restrictive fashion (Mole 1994; but see Roff & Fairbairn 2007). The form taken by these restrictions, the nature of the constraints for examples of life-history allocation trade-offs, and the associated fitness components are summarized in Table 2.1.

Constraints may include physiological resources such as energy, nutrients, food biomass (i.e. both energy and nutrients), and space (e.g. total available volume or shelter) (Cody 1966; Sibly 1991; Stearns 1992;Sinervo & Svensson 1998; Ketterson & Nolan 1999; Zera & Harshman 2001). Energetic constraints are often the basis for trade-offs in natural systems (Zera & Harshman 2001). Energetic resource allocation has been investigated in numerous studies, mainly via phenotypic and/or genetic correlation. Such allocation studies frequently involve life history traits, primarily reproduction, growth, and somatic maintenance. The corresponding trade-offs are usually between reproduction and somatic maintenance (e.g. Roff & Gélinas 2003; Karlsson et al. 2008; King et al. 2011), where somatic maintenance includes behavioral performance (Ghalambor et al. 2004), survival (e.g. Rosenheim 1996), future reproduction (Stearns & Hoekstra 2005), or growth (e.g. Bolmgren & Cowan 2008); or between growth and behavioral performance as somatic maintenance (e.g. Billerbeck et al. 2001; Pfenning et al. 2007).

For example, consider the trade-off between size and age at maturity. Early maturation implies a shorter development time and usually a smaller body size at maturation. Shorter development time is advantageous in terms of increased survival to maturity, shorter generation time (increasing intrinsic growth rate), and sometimes a longer time for reproduction, but is disadvantageous in terms of the decreased body size, typically reducing reproductive potential. In this circumstance, organisms may allocate energy (1) to additional growth to produce larger body mass and thus increase fecundity, or (2) to early reproduction to increase survival and shorten generation time (Crowley 2000; Stearns & Hoekstra 2005; Bolmgren & Cowan, 2008). Though allocation to reproduction and growth may be concurrent, the allocation pattern may shift, in which allocation to growth for maturing may precede allocation to reproduction from an ontogenetic perspective (Berner & Blanckenhorn 2007).

Nutrients and certain other chemical components such as hormones (e.g. Sinervo 1999; Nager et al. 2000) are non-energetic resources that can cause and constrain trade-offs. Oxygen and its allocation can sometimes play this role through competing physiological processes. For example, increasing the growth rate of the fish *Menidia menidia* requires oxygen for metabolism at the expense of oxygen needed for locomotion (Arnott et al. 2006). Another example involves nitrogen allocation: caddisflies traded off larval survival via construction of the protective larval case against allocation to the development of potential reproductive structures (Stevens et al. 1999).

Space limitation can arise through maternal body size as a physical constraint that produces size-number trade-offs among progeny (Sinervo & Svensson 1998; Glazier 2000; Uller & Olsson 2005). For example, amphipod broods from smaller mothers had a stronger

trade-off relationship between the size and number of offspring within the brood (Glazier 2000), changing the shape and position of the trade-off curve (Glazier 2000; see below). The volume of maternal body cavity is often believed to constrain the reproductive output of both viviparous and oviparous species (Qualls & Shine 1995; Vitt & Congdon 1978), thus imposing a size-number trade-off of progeny (Sinervo & Svensson 1998). Space for progeny could be constrained by host size in some internal parasite species. For example, the size and number of offspring in parasitoid wasps growing within the limited resource cache of the host are in a strong trade-off relationship (Saeki et al. 2009), though this may depend more strongly on host biomass than host volume.

Space may also constrain trade-offs among organs in the body cavity, which may "compete" for the space to function better (Sibly 1991; Hulsey et al. 2007). For example, exaggerated sexual traits resulting from sexual selection have been reported to limit the size of other organs, especially neighboring organs (e.g. male beetle horns and eyes; Emlen 2001). However, these may be consequences of resource allocation during development, such as imaginal-disk tissues during metamorphosis in insects (Nijhout & Emlen 1998; Emlen 2001; Moczek & Nijhout 2004). As another example of internal space limitation, Clauss et al. (2003) hypothesized that the larger digestive organs, such as ruminoreticulum of grazing ruminant species, reduce the size of other organs such as the colon descendens, which is responsible for water absorption. The body size of ruminant species is limited by digestive physiology, and this creates intra-abdominal space competition. These hypothesized constraints, energy and space, are not mutually exclusive and raise issues for future investigations.

The relationship between two life-history traits that trade off: shapes of trade-off curves

Constraints force the allocation of resources to traits to be negatively related: allocation of the resources to one trait decreases the allocation of the resources to the other trait. When each increment of resource allocated to one trait alters the magnitudes of both traits by constant amounts, the trade-off relationship is linear (Figure 2.1A), implying a constant negative slope in the graph; on the other hand, when the slope of the relationship changes with trait magnitudes, the trade-off is non-linear (Figures 2.1C, 2.1D). When greater magnitudes of one trait are associated with *greater* shifts in magnitude of the other trait, the graph is concave (Figure 2.1C); when greater magnitudes of one trait are associated with *reduced* shifts in magnitude of the other trait (Figure 2.1D), the graph is convex (Stearns1992). (Note: concave and convex refer to curve shapes from the perspective of the origin.) It is important to note that these shapes of trade-offs can change depending on how the trait is defined. For example, the trade-off curves between seed size and number differ depending on whether size is based on seed length or seed mass. In some cases, understanding the nature of the constraint suggest the most insightful way of measuring the trait. For instance, if the plant is constrained in the total mass of seeds it can produce, then assessing seed size as mass leads directly to the prediction of a simple inverse relationship between traits.

Theoretical studies include various assumptions about relationships between life history traits, showing different shapes of trade-off curves (e.g. Bell 1980). These curve shapes may be assumed to follow one of these general patterns (e.g. Schaffer 1974; Charnov & Skinner 1984; Creel & Christianson 2008) or may be established empirically

(e.g. Sibly & Calow 1989; Ellers et al. 2000). In a study of the relationship between parental reproductive effort and parental survival, Charnov (1986) noted that the corresponding graphs could be concave, linear, or convex. When the adult survival P is expressed as $P = P_m(1-(E/E_m)^c)$, where P_m is maximum adult survival due to the absence of parental reproductive effort, E is actual parental effort, E_m is maximal parental effort, and c is the shape coefficient. The shape of the P curve depends on the coefficient c, yielding concave, linear, and convex relationships when $c > 1$, $c = 1$, and $c < 1$, respectively. Among species, this coefficient c is expected to vary in magnitude and among these categories of curve shape (Charnov 1986).

Linear relationship (Figure 2.1A)

A negative linear relationship between the two traits can arise in two main ways. (1) Under the assumptions (a) that the total amount of resource available is independent of its allocation between the two traits, (b) that each increment of resource allocated to one trait reduces resource available to the other by the same amount, and (c) that the trait magnitude increases in proportion to the amount of resource allocated to it, the relationship between traits is linear with negative slope. These assumptions are rarely stated or evaluated. (2) A negative linear relationship can be a first approximation for small data sets and those containing considerable random variation. Note that a negative correlation between the two traits is necessary but not sufficient to demonstrate a trade-off, nor sufficient to demonstrate linearity. In trade-offs between life history traits, where energetic resources are subdivided among growth, somatic maintenance, and reproduction, their relationships are generally thought to produce linear trade-off curves (reviewed in Stearns 1992). Energy,

nutrients, time, and space are resources often associated with linear trade-off relationships, at least for a restricted range of trait magnitudes. For example, Sevenster et al.(1998) and Ellers et al. (2000) examined the trade-off between egg production and survival based on time allocation, the relationship between variables was consistent with linearity (but see Rosenheim 1996).

Linear-bent relationship (Figure 2.1B)

When there are two different potentially limiting resources shared by the two traits (multi-currency), the trade-off curve may be a hybrid combination of the two trade-off relationships. For example, when two nutritionally demanding traits are limited in a linear fashion by the abundances of two nutrients, two linear relationships, each of which is based on a single nutrient, come into play. When the two lines intersect, it is the line segments nearest the origin that define the trade-off relationship based on alternative limiting nutrients, expressing in each case the responses to the strongest constraint. This is a concave linear-bent function based on stoichiometry (Yoshida 2006). When important life-history traits (e.g. growth and reproduction) have differing demands for different nutrients in short supply (Boggs 2009), these linear-bent trade-off relationships could result. In principle, such relationships may actually contain multiple intersecting linear segments derived from several alternative limiting nutrients or other constraints; the result is still a piecewise concave linear-bent relationship in an overall concave pattern. To my knowledge, however, no such piecewise trade-off functions have been empirically demonstrated.

Concave relationship (Figure 2.1C)

Curved concave relationships between the two traits of a trade-off are commonly assumed in many theoretical studies (e.g. Schaffer 1974; Bell 1980; Charnov & Skinner 1984; Charnov 1986). Yet there were apparently no empirical demonstration of curved concave relationships in the literature (Rueffler et al. 2004; Michod et al. 2006) until a recent study of bacteria and bacteriophage (Jessup & Bohannan 2008), described in more detail below. In theoretical studies, the constraint generating a concave graph is often assumed to be energy (e.g. Hernandez and León 1995). For example, Schaffer (1974) predicted that iteroparity would evolve when the increase in reproductive effort accelerates the decrease in the parental survival and growth. Curved concave relationships could result from diminishing returns or accelerating costs associated with a high level of allocation to either of the two traits. For example, energy allocated almost exclusively to parental investment or to parental survival and growth may be used less efficiently in achieving these responses than at intermediate levels of allocation to each.

Convex relationship (Figure 2.1D)

A convex relationship arises when allocating the limiting resource to either trade-off trait increases the trait magnitude in an accelerating fashion. An important category here is when the relationship between two traits is constrained in magnitude by their multiplicative product.

For example, the size-number trade-off in broods of offspring is a well-studied life-history trade-off (Smith & Fretwell, 1974) based on constrained total resources invested into reproduction, and conventionally expressed as total resource mass = (number

of offspring)×(mass per offspring) (e.g. Sinervo & Licht, 1991; Sadras, 2007; Paul-Victor & Turnbull, 2009). This implies an inverse relationship between offspring number and size (as in the seed size example mentioned earlier). In the progeny size-number trade-off, the mother optimizes individual offspring mass and total number within a finite total mass of resources so that she maximizes the number of her grand-offspring by producing the highest-fitness combination of offspring size and number (Smith & Fretwell, 1974, Saeki et al. 2009).

Another example (Rosenheim 1996) is that investment in reproduction (R) may trade-off with investment in somatic maintenance, resulting in a linear relationship to mortality rate: $R = a\mu$, where a is a positive constant. When mortality is assumed to be independent of time, then expected longevity $L = 1/\mu$, and so $L = a/R$ (Ellers et al. 2000). Thus longevity and reproductive investment graph as the convex shape of this inverse trade-off relationship (but see Ellers et al. 2000).

In general, inverse and other convex relationships should often be associated with trade-offs between intensity and duration (e.g. locomotion speed vs time spent moving), between quality and quantity (e.g. offspring size and number), or between rates and efficiencies (e.g. rate of food processing by an animal's gut vs the fraction of the food assimilated).

Cost-benefit fitness relationships associated with trait magnitudes and trade-off allocation patterns

Trade-off curves are simple representations of the negative relationships between the traits under the constraint and do not inherently express fitness. When fitness is considered in

association with the trade-off in theoretical studies, the fitness curve or contour is usually combined with the trade-off curve to identify the optimal allocation pattern (Levins 1962; Stearns 1992; Roff 2002; Rueffler et al. 2004; Figure 2.2). Empirical work lags far behind theory in linking trade-off curves to fitness contours or other fitness measures, a priority for future studies.

Organisms are presumably under selection to balance the two trade-off traits at the best combination of magnitudes. This balancing process may reflect many features of the organisms and their environment (Arendt 1997). For example, in the trade-off between growth and reproduction in the context of size and age at maturity, sexual selection favoring protandry may help shape the fitness contour for the species, resulting in a particular allocation pattern with shorter time of development and smaller body size than might otherwise be expected (e.g. Fischer & Fiedler, 2002). Also, increased predation risk causes the energy allocation in the trade-off between growth and somatic maintenance to shift toward growth efficiency, facilitating rapid growth at the cost of decreased reproductive success in the future (Stoks et al. 2005).

The shape of trade-offs, allocation patterns, and environments

Shapes and magnitudes of trade-off relationships may vary among environments (Sibly & Calow 1989; Jessup & Bohannan, 2008), life stages (Schaffer 1974), sexes (Saeki et al. 2009) and species (Charnov & Skinner 1984). Environmental conditions may alter (1) the magnitude of the constraint(s) on the trade-off relationship (Bohannan et al. 1999; Saeki & Crowley, submitted), (2) the curve shape or slope instead of or in addition to any effects on the constraint(s) (Bohannan et al. 2002), and/or (3) the allocation pattern generated along

the trade-off curve (Stearns 1992; Saeki & Crowley, submitted) (see Figure 2.3). All three of these types of responses and three of the four trade-off curve shapes from Figure 2.1 (2.1A, 2.1C, 2.1D) were documented in work on *E. coli* B and the bacteriophage T2 (Jessup and Bohannan 2008). Under three different culture conditions in that study (glucose-limited batch, trehalose-limited batch, and glucose-limited continuous culture), 86 genetically distinct isolated of bacteria documented the trade-off between competitive ability and resistance to phage T2. This appears to be the most comprehensive empirical demonstration of graphical patterns and environmental effects to date for a single trade-off relationship.

Plastic responses to different environments are crucial traits for species developing in unpredictable conditions (Fischer *et al.* 2011). These plastic responses may be genetically-based (reviewed in Via et al. 1995, Moczek et al. 2011), and plasticity of the two traits settling at an allocation pattern is subject to natural selection (see Nylin & Gotthard, 1998). For example, higher temperature can cause ectothermic organisms to develop faster but with smaller adult body sizes (e.g. damselflies in De Block & Stoks, 2003). High food availability can shorten development time, resulting in smaller adult body size (Johansson *et al.*, 2001), while low food availability lengthens development time and decreases body size and longevity (Agnew *et al.*, 2002).

When the acquired resources are allocated to different traits, the allocation that maximizes fitness may vary among environments (Noordwijk & de Jong 1986; Roff 2002; Worley et al. 2003; Malausa et al. 2005) as the fitness contour shifts (Stearns 1992). Exploring of optimal points and trade-off curves in various environments would provide us empirically testable predictions and help us to understand complex responses of two traits

of the trade-off in dynamic environments.

Summary

Measuring and demonstrating of trade-offs and estimating the shapes of trade-offs are generally challenging. For example, continuous acquisition of resources during reproduction (Fox & Czesak, 2000), parent-offspring and sibling conflict (Godfray, 1986; Godfray & Parker, 1991, 1992), and many other factors influencing trade-offs (e.g. van Noordwijk & de Jong, 1986; Pease & Bull, 1988; Klingenberg & Spence, 1997) may obscure trade-off relationships. Since shapes of trade-offs provide predictions of ecological and evolutionary consequences but are difficult to determine empirically, theoretical studies can generate and sharpen predictions to make them more empirically accessible (e.g. Schaffer 1974; Charnov & Skinner 1984; Charnov 1986; Partridge & Sibly 1991; Ellers et al. 2000). Understanding of the shape of a particular trade-off relationship allows us to predict adaptive life-histories of organisms, but documenting and accounting for the shapes of the trade-off relationships and associated allocation patterns are surprisingly rare. I encourage more focus on this in future work.

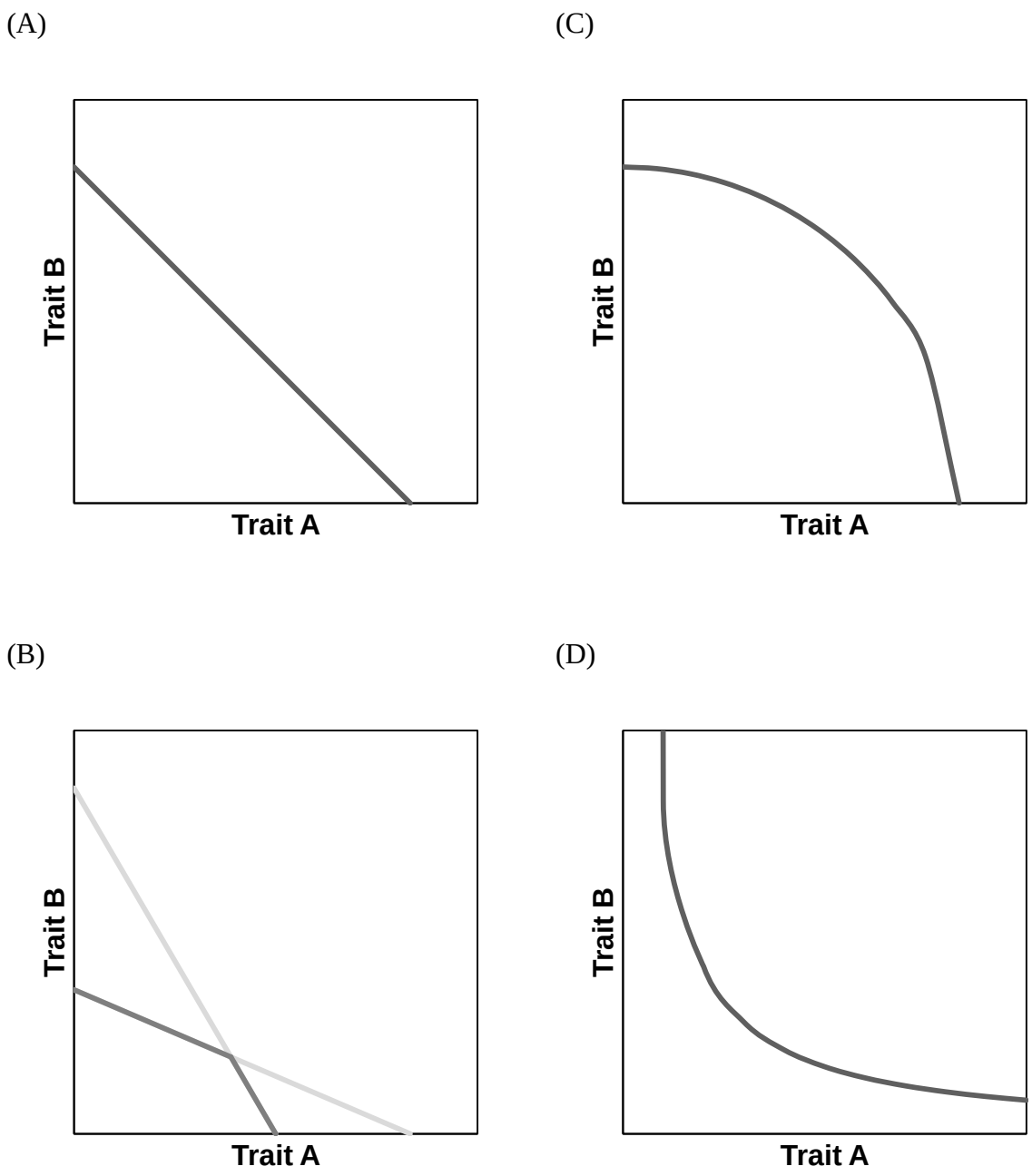


Figure 2.1
The shapes of hypothetical trade-off curves, indicated in dark grey. Higher fitness is assumed here to be associated with higher trait magnitudes. (A) linear; (B) linear-bent, showing the light grey parts of the linear relationships that do not limit the trade-off because of stronger limitation by the other trait; (C) convex; and (D) concave.

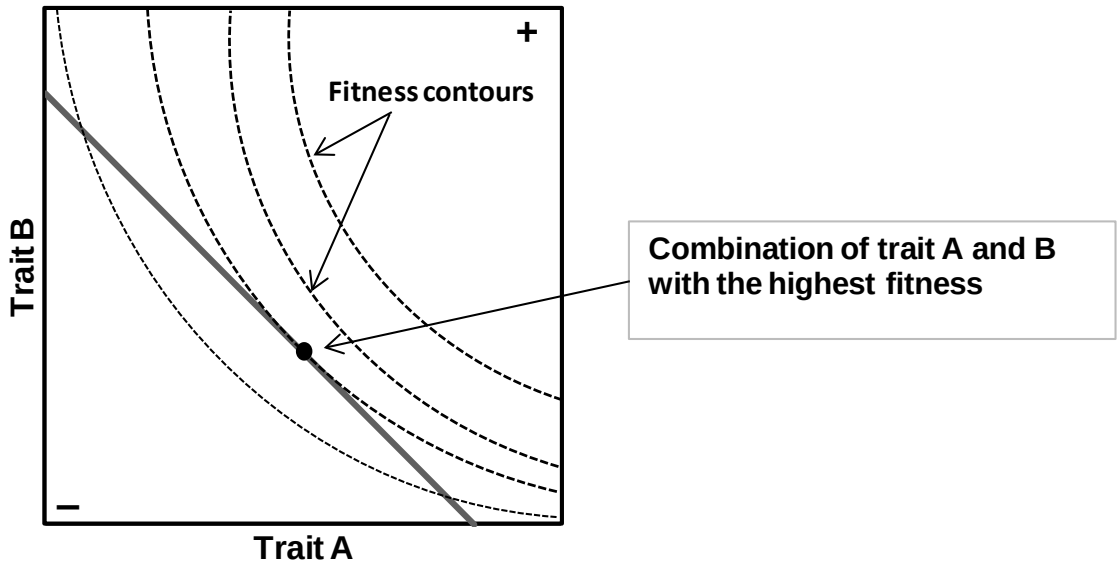
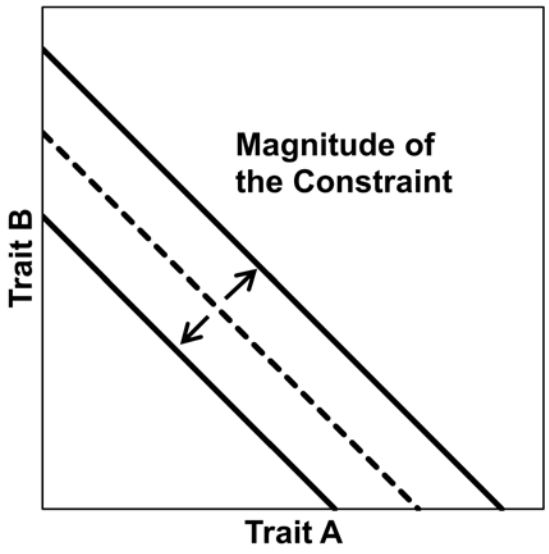
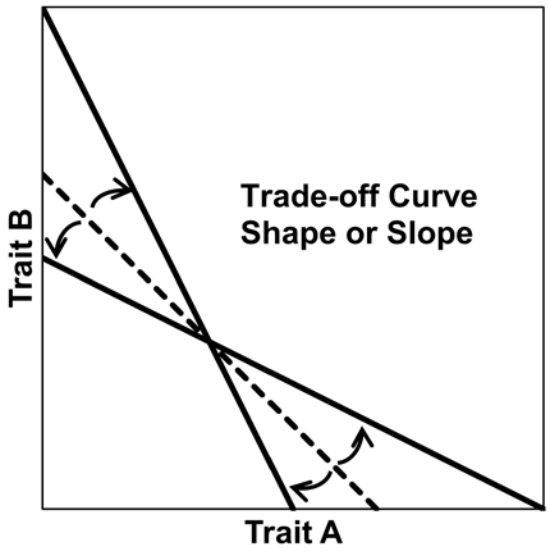


Figure 2.2
A hypothetical linear relationship between two traits under the trade-off, with fitness contours and the optimal allocation pattern (black dot) indicated. The solid line represents the linear trade-off relationship and dashed lines represent fitness contours (Levins 1962; Stearns 1992; Roff 2002). Each contour line illustrates combinations of trait magnitudes of equal fitness; the lines increase in fitness value at higher magnitudes of the two traits toward the upper right and decrease toward the lower left.

(A)


Magnitude of
the Constraint
Trait B
Trait A

(B)


Trade-off Curve
Shape or Slope
Trait B
Trait A

(C)

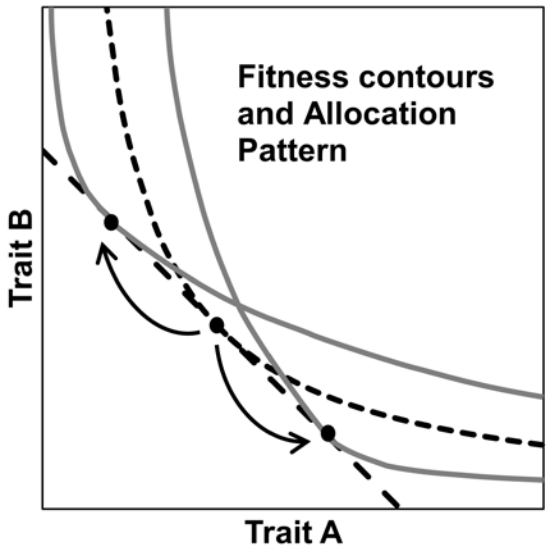


Figure 2.3
Three ways that environmental conditions may alter a trade-off, using a linear example. The dashed lines in each panel are the standard against which the implications of different environments (solid lines) are compared. (A) Altering the magnitude of the constraint generally moves the trade-off line closer to or farther from the origin. (B) Environmental changes can alter the slope or shape of the trade-off line. (C) Allocation patterns (black dots) may shift along trade-off lines in different environments, presumably in response to shifts in fitness contours.

Table 2.1 Empirical studies of allocation trade-offs: traits, resources, and relationships.

Trait 1	Contriubtion to Fitness	Trait 2	Contribution to Fitness	Limiting factor/resource	Type of limiting factor/resource	Relationships (Shape)	References
Offspring size	Reproduction	Number of offspring	Reproduction	Energy/Nutrients	Physiological	Multiplicative (convex/inverse), or non-linear	Sinervo & Licht 1991; van Noordwijk and de Jong 1992; Christians 2000; Stuefer et al. 2002; Uller and Olsson 2005; Saeki et al. 2009
Future reproduction	Reproduction/Somatic maintenance	Current reproduction	Reproduction	Energy/Nutrients	Physiological	Negative correlation only	Stearns 1992; Stearns and Hoekstra 2005
Growth rate	Growth	Reproductive rate	Reproduction	Energy/Nutrients	Physiological	Additive (linear)	Stearns 1992; Fischer & Fiedler 2002; Stearns & Hoekstra 2005; Jorgensen & Fiksen 2006; Bolmgren & Cowan 2008
Survival	Somatic maintenance	Current reproduction	Reproduction	Energy/Nutrients	Physiological	Additive (linear)	Tatar & Carey 1995; Sevenster et al. 1998; Karlsson et al. 2008

Trait 1	Contriubtion to Fitness	Trait 2	Contribution to Fitness	Limiting factor/resource	Type of limiting factor/resource	Relationships (Shape)	References
Reproductiive rate	Reproduction	Locomotor performance	Somatic maintenance	Energy (via metabolism)	Physiological	Additive (linear; King et al. 2010), or negative correlation only	Stirling et al. 2001; Roff & Gelinas 2003; Ghalambor et al. 2004; Nespolo et al. 2008; Saglam et al. 2008; King et al. 2010; King et al. 2011
Growth rate	Growth	Locomotor performance	Somatic maintenance	Energy/Nutrients	Physiological	Negative correlation only	Billerbeck et al. 2001; Pfenning et al. 2007
Egg quality	Reproduction	Egg number	Reproduction	Energy/Nutrients (Lipids)	Physiological	unknown	Nager et al. 2000
Growth rate	Growth	Locomotor performance	Somatic maintenance	Oxygen	Physiological	Linear with log-transformed data	Arnott et al. 2006
Reproductive rate	Reproduction	Locomotor performance	Somatic maintenance	Nitrogen	Physiological	unknown	Stevens et al. 1999
One body part	Growth	Another body part	Growth	Tissues	Physiological	unknown	Nijhout & Emlen 1998; Moczek & Nijhout 2004
One body part	Growth	Another body part	Growth	Space	Mechanical	unknown	Clauss et al. 2003
Offspring size	Life history (Reproduction)	Number of offspring	Reproduction	Hormone	Physiological	Negative correlation only	Sinervo 1999

Copyright © Yoriko Saeki 2012

Chapter 3 A Sex-Specific Size-Number Trade-off in Clonal Broods

Introduction

The number and body size of progeny within broods are among the most studied traits in life history trade-offs (Smith and Fretwell 1974; Stearns 1989) and should generally be inversely related when resources are limited (Sibly and Calow 1986). This number-size trade-off has been documented in plants (e.g. Stuefer et al. 2002), invertebrates (reviewed in Fox and Czesak 2000), and vertebrates (e.g. Mappes and Koskela 2004). But attempts to detect a size-number trade-off may prove unsuccessful because of (1) the absence of a trade-off (e.g. when brood size is genetically fixed), or because of masking of the trade-off by (2) resource variation (van Noordwijk and de Jong 1986; Roff and Fairbairn 2007), (3) an interaction between resource variation and parental reproductive behavior (Mayhew 1998, Mayhew and Glaziot 2001), (4) effects of another trait mediating the trade-off (e.g. development time: Klingenberg and Spence 1997), (5) other fitness components influencing the trade-off (e.g. future reproduction: Lack 1947), (6) continuous acquisition of resources during reproduction (Fox and Czesak 2000), or (7) competition among offspring (Godfray and Parker 1991).

In this study, I am able to by-pass many of these issues to address possible sex-differences in the size-number trade-off of offspring broods. Sex-specific trade-offs have been documented in previous work (e.g. Fischer and Fiedler 2000) but not to my knowledge for the size-number tradeoff, though two studies have demonstrated declines in parasitoid body size with greater brood size. The usual genetically diverse and mixed-gender broods, involving parent-offspring and sibling conflicts (Godfray 1986) and sometimes differential provisioning of offspring by gender (West and Sheldon 2002; Young and Badyaev 2004), can obscure or render moot any such gender

differences in the size-number trade-off.

Here, I focus on the polyembryonic parasitoid *Copidosoma bakeri* (Howard), which produces large broods of clonally identical (and thus all male or all female) offspring. For endoparasitic wasps in general, food intake and biomass of the host constitute the resources used to produce a wasp brood. Interactions between endoparasitoids and their hosts affect parasitoid fitness through survival to the adult stage, development time, and adult body mass (Mackauer and Sequeira 1993; Godfray 1994). To increase their fitness, koinobiont endoparasitoids, which develop inside a host that continues to grow, may actively manipulate host growth ("host regulator"; Vinson and Iwantsch 1980) or may passively adjust their own development to the host's growth ("host conformer"; Lawrence 1986). Parasitoids may interfere with the host's endocrine system (Beckage and Riddiford 1982), increase the host's dietary assimilation efficiency (Slansky 1978), alter its nutritional status (Vinson and Iwantsch 1980; Slansky 1986), and ameliorate or neutralize host defenses (Beckage 1997). Also, parasitoids may adjust their own development and/or emergence in response to host physiology (Lawrence 1986; Harvey 1996), allowing these wasps to maximize the available host resource (Mackauer and Sequeira 1993).

With these strategies, parasitoids may generally be able to shape various key traits to their advantage, especially their development time, body mass, and growth rate (Mackauer and Sequeira 1993). For gregarious parasitoids, in which multiple offspring of different genotypes emerge from one host, brood size may be adjusted by the ovipositing female (Charnov and Skinner 1985) or by siblicide or larval competition within a host (Godfray 1987). In these cases, selection may often favor increased body size due to the typical advantages of greater size in competition for resources (Mayhew and Glaizot 2001). In contrast, for polyembryonic broods that produce multiple genetically-identical offspring arising from a single egg, these complexities of gregarious

development do not apply. In polyembryonic species, brood size and body size are presumably adjusted by the brood itself and not by the ovipositing female (Godfray 1994). Also, when broods are exclusively of genetically identical offspring, selection favors cooperation among larvae rather than competition (Godfray 1994) and should tend to maximize reproductive success of the clonal brood as a whole (Godfray and Parker 1991). In addition, multiple genetically identical offspring may provide advantages when a female wasp cannot predict host quality at the time of oviposition, because the brood itself may more accurately assess the host's ultimate resource value later in the development of brood and host – and without generating the parent-offspring conflict typical of gregarious parasitoids (Godfray 1994).

In the present study, I examined the way that wasp brood size and body mass trade off in *C. bakeri* and how the fit between host and parasitoid brood is achieved. I postulated that male and female broods would achieve similar total masses in hosts of similar size but that males and females may differ in how the trade-off is balanced between individual wasp body mass and brood size. I also postulated that wasps might fit the brood to the host by adjusting brood size based on host characteristics early in development, manipulating host size to accommodate the brood size late in host development, or adjusting wasp body size to fit the brood into the host at its maximum mass.

Materials and Methods

Focal system

Copidosoma bakeri (Howard) is a polyembryonic egg-larval parasitoid that oviposits in eggs of the host. The range of *C. bakeri* covers the central and northern United States into Canada, parasitizing at least 19 lepidopteran (Noctuidae) species (Schaaf 1972; Byers et al. 1993).

The genus *Copidosoma* (Hymenoptera: Encyrtidae), especially the species *Copidosoma floridanum* (Ashmead), has been extensively used to explore the biology of polyembryony (e.g. Hunter and Stoner 1975; Jones et al. 1982; Strand 1989a, b). The development of a polyembryonic egg to produce hundreds to thousands of clonal individuals is synchronized with host development (Strand 1989a). After *Copidosoma* parasitizes a host egg, the host hatches and starts to develop in the same way as an unparasitized larva, while the wasp egg divides multiple times (Ivanova-Kasas 1972). During the first several host instars, some portion of the parasitoid embryo develops into precocious larvae (~10; unpublished data) that may perform defense functions but die before the embryo matures. When the host reaches the penultimate larval stadium, the wasp larvae start morphogenesis and growth (Baehrecke and Strand 1990). Divisions of eggs in *C. bakeri* seem to be consistent with those of *C. floridanum* as indicated by the presence of the same- sized embryos of both species at the beginning of the penultimate stadium but not thereafter (unpublished data). During the host's final larval stadium, the wasp larvae devour the host and pupate (Strand 1989b). When the wasps form cocoons, the host is mummified and dies (Strand 1989a). Thus, the features of host development before the cessation of wasp polyembryo formation by the start of the host's penultimate stadium may cue or otherwise influence wasp brood size, whereas host development during and after the host' penultimate stadium may respond in part to the active influence of wasp brood size on the host.

Empirical methods

Copidosoma bakeri parasitizing the black cutworm *Agrotis ipsilon* (Hufnagel) were collected at the University of Kentucky Turfgrass Research Facility (Fayette County, KY; 37.988N, -84.477W) from July to September, 2005, and maintained for about three generations before the

start of the experiments. The black cutworm hosts were reared on a pinto-bean based diet in an environmental chamber maintained at 27 ± 0.1 ºC (daytime) and 25 ± 0.1ºC (night time) and light:dark regime L14:D10.

Thirty newly emerged and mated female wasps were individually introduced into a Petri dish that contained host eggs (< 24 h old) and were left until they parasitized one to four eggs. Oviposition behavior was observed, and there was no superparasitism. Non-parasitized eggs were prepared in a Petri dish in the same manner, without wasps. Both parasitized and non-parasitized eggs were placed singly on pre-weighed diet in a plastic cup [3.8 (top)-2.8 (bottom) cm diameter, 3.5cm depth] with a cardboard lid. The cutworms were reared as described above and fed *ad libitum*.

Head capsule widths of cutworms were measured at every molt under a dissection microscope (25× magnification). Host larvae were weighed to the nearest 0.1 mg every 24 h beginning in the 3rd stadium, until the parasitized larvae formed mummies or non-parasitized larvae pupated. Black cutworms cease feeding about 2 d before pupation, expel their gut contents, and wander to find a place to pupate (wandering phase). Therefore, the last stadium was considered finished at the start of the wandering phase. Frass was removed and the uneaten diet was weighed to determine the amount eaten after estimating the amount of water loss from the artificial diet. Diet mass was corrected for water loss by weighing diet maintained without larvae under the same condition.

Wasps emerging from the host mummy were frozen within 24 h after emergence. All wasps, including adults, undeveloped larvae, and pupae that remained inside the mummy (hereafter total brood size) were counted. The adult wasps found inside appeared to have eclosed, because they occupied the middle of the cross section of the mummy with appendages liberated; individuals that

had not eclosed were found between the layers of the integument of the host, the site of pupation (Strand 1989b), and their appendages were not liberated. The total number of wasps produced from an egg (including larvae that did not survive to adult) is the "total brood size". The sum of the number of adult wasps from both inside and outside the host is my measure of "secondary brood size" (wasps inside the host had eclosed but were trapped inside the host). Secondary brood size has been termed "secondary clutch size" in some earlier literature (Strand 1989c); however, in this paper brood size refers the number of wasp larvae that hatched from a single egg. Size of the adult wasps was estimated from 40 randomly chosen wasps per brood by collectively weighing them, 20 at a time, to the nearest 0.1 μg.

Data Analysis

All data were analyzed with SPSS (SPSS Inc., version 15.0, Chicago, IL, USA) unless otherwise indicated.

(i) Trade-off between the number and mass of progeny

Differences in secondary brood size and wasp body mass between male and female broods were tested with ANOVA. I also examined whether there is a difference in brood mass (total brood size × wasp body mass) between male and female wasp broods. To examine whether the number of wasp undeveloped larvae was related to wasp total brood size, I regressed the number of undeveloped larvae on total brood size. Then, to evaluate whether wasp body mass and wasp secondary brood size vary with sex and have an inverse relationship overall, I first adjusted the two variables for differences in host mass (Appendix). With these standardized data, I conducted a MANOVA of wasp brood size and wasp body mass vs. wasp sex and then examined the brood size-body mass regression. Since both wasp brood size and wasp body mass were variables

measured with error and since neither was obviously causal with respect to the other, I used model II regression to test for an inverse relationship by log-transforming both variables and determining whether the slope was close to –1 (Warton et al. 2006).

Also, using model II regression, I tested whether relationships between host mass and wasp brood mass (brood size × wasp mass), wasp brood size, or wasp mass differ between male and female wasps. When neither slopes nor intercepts differed between sexes, data were combined, and confidence intervals of slopes and intercepts were calculated for the statistically distinct relationships (Warton et al. 2006). To determine whether wasp brood mass was proportional to and correlated with host mass, I examined whether the model II confidence interval for the slope included one. I calculated the coefficient of determination r^2 and the confidence interval for the intercept using model II on untransformed data, predicting that r^2 should be high (>0.5) and the intercept should approximate zero.

(ii) Interaction with host resources

Survival rates of unparasitized and parasitized larvae were analyzed with Cox regression (failure time analysis). Broods from hosts with supernumerary stadia (>6) were excluded from analyses described in this section, since I did not know when the parasitoids' egg clutch was fully formed within the host undergoing supernumerary instars. The remaining analyses distinguished between the developmental interval through host stadium 4 (before brood size was set) and the interval including stadia 5 and 6 (after brood size was set).

To evaluate effects of parasitism and parasitoid sex on hosts, I conducted a repeated measures ANOVA, with these two developmental intervals constituting the time variable; a brood treatment reflecting whether the host was parasitized by a male wasp brood, a female wasp brood,

or neither; and the mass gained by the host during each developmental interval as the response variable. Statistical significance of time, treatment, and time × treatment (see below) then justified separate analyses by developmental interval to test two orthogonal contrasts: parasitized vs unparasitized and male vs female wasp broods.

I investigated six different host characteristics as response variables: mass gain during the developmental interval, duration of the interval, total food consumption during the interval (natural log-transformed to improve normality), assimilation efficiency (mass gain divided by total food), growth rate (mass gain divided by duration), and feeding rate (total consumption divided by duration). I protected the statistical power of the mass gain contrasts, because the experiment was designed to focus on this variable most closely linked to available parasitoid resources. To test the other contrasts, I used the Bonferroni correction, dividing the critical p-value of 0.05 by the total number of contrasts (including those based on the mass gain response) to obtain a much smaller and more conservative critical p-value.

To examine whether wasp brood size might be influenced by host characteristics early in development or whether wasp brood size might influence host mass, I conducted bivariate correlation analyses between wasp brood size and each of the six host characteristics used in the previously described analysis. Once again I separated the analyses by developmental interval and protected the power to detect the mass gain relationship. Relationships between brood size and each of the other host characteristics were again evaluated using the Bonferroni correction for the total possible number of comparisons. For each developmental interval and host characteristic, I tested first for a significant difference between relationships for male and for female wasp broods. If that difference was significant, then I tested the relationship separately by sex of wasps; otherwise, I tested the relationship for the data pooled across sexes.

Results

Basic brood data

Wasps from 25 broods successfully emerged as adults (Fig. 3.1). Eight wasp broods produced all females, 16 broods were all males, and 1 brood was a mixture of males and females with a male fraction of 0.44. The sex ratio among broods did not differ from 1:1 ($\chi^2 = 2.67$, p = 0.10). There were two outliers showing unusually small brood sizes (258 and 666; both male broods) with unusually low survival of wasp larvae (<50%; larval survival for all other broods was >80%), and I eliminated them from all analyses. The single mixed brood was eliminated from analyses as well.

There was no difference in wasp brood mass (brood size × wasp body mass) between male and female broods (unstandardized: $F_{1,20} = 0.34$, p = 0.57; standardized: $F_{1,20} = 0.04$. p = 0.85). Secondary wasp brood sizes range from 1119 to 3522 wasps, averaging (mean ± SE) 1719 ± 124 (n = 8) wasps for female broods and 2411 ± 168 (n = 14) for male broods. Total brood sizes (which include undeveloped pupae/larvae) range from 1171 to 3743 wasps, averaging 1862 ± 41 wasps (n = 8) for female broods and 2530 ± 46 (n = 14) for male broods. The percentage of adults emerging successfully [(the number of adults/(numbers of undeveloped larvae + pupae + adults)) × 100] averaged 94.1± 1.4 % (n = 22). Total wasp brood size was not correlated with the number of undeveloped larvae or pupae ($r^2 = 0.01$, p = 0.60). Male broods were significantly larger (contained more offspring) than female broods in both total brood and secondary brood size (Total brood: $F_{1,20} = 7.33$, p = 0.01; Secondary brood: $F_{1,20} = 8.16$, p = 0.01). Female wasps were significantly larger than males ($F_{1,20} = 16.7$, p < 0.01, Females = 37.4 ± 1.6μg, Males = 29.3 ± 1.2μg). The MANOVA indicated that standardized brood size (the number of emerging adult wasps corrected

with host mass) and individual wasp mass for males and females were significantly different ($F_{2, 19}$ = 8.97, $p < 0.01$); in other words, males and female wasps had different combinations of brood size and offspring size, with females having smaller brood size and larger body mass but males having larger brood size and smaller body mass.

Trade-off between the number and size of progeny

There was an inverse relationship between standardized secondary wasp brood size and wasp body mass (Fig. 3.1B). Model II was used for all of the remaining regression analyses reported below. This regression yielded a negative linear relationship between natural log-transformed standardized secondary brood size and wasp body mass (slope = -0.86, 95% CI = [-1.15, -0.65], intercept = 2.70, 95% CI = [1.15, 4.24], $r^2 = 0.61$); back-transforming produced the power function illustrated in Fig. 3.1B. These results are consistent with an inverse relationship between the variables.

Wasp brood mass (brood size x wasp mass) was proportional to host mass (Fig. 3.2; r^2 = 0.60, slope = 0.054, 95% CI = [0.040, 0.072], intercept = -16.0, 95% CI = [-40.9, 8.98]). Neither slopes nor intercepts differed significantly between males and females (slopes: $\chi^2 = 0.01$, $p > 0.05$; intercepts: $\chi^2 = 0.50$, $p > 0.05$). Slopes of relationships between secondary brood size and host mass differed significantly between male and female wasps (Fig. 3.3A; $\chi^2 = 4.62$, $p = 0.03$; Male: slope = 2.46, 95% CI = [1.73, 3.51], intercept = -1.54×10^3, 95% CI = [-2.98×10^3, -0.10×10^3], r^2 = 0.67, $p < 0.05$; Female: slope = 1.02, 95% CI = [0.50, 2.11], intercept = 2.24×10^2, 95% CI = [-9.84×10^2, 14.3×10^2], $r^2 = 0.38$, $p > 0.05$). Slopes of relationships between wasp body mass and host mass also differed significantly between male and female wasps (Fig. 3.3B; $\chi^2 = 122.2$, $p < 0.001$; Male: slope = -1.74×10^{-5}, 95% CI = [-3.07×10^{-5}, -0.98×10^{-5}], intercept = 5.72×10^{-2}, 95% CI =

[4.02×10^{-2}, 7.42×10^{-2}], $r^2 = 0.09$, $p > 0.05$; Female: slope = 1.32×10^{-5}, 95% CI = [0.69×10^{-5}, 2.55×10^{-5}], intercept = 1.81×10^{-2}, 95% CI = [0.42×10^{-2}, 3.20×10^{-2}], $r^2 = 0.51$, $p < 0.05$). Therefore, wasp brood mass in both sexes was proportional to host mass; however, male wasps significantly increased only brood size, whereas female wasps significantly increased only body mass to fit to the host.

Interaction with host resources

The probability of surviving to the final larval instar did not significantly differ between parasitized and non-parasitized hosts ($p = 0.67$, $\chi^2 = 0.19$, 30.3% overall mortality). Five out of 57 surviving black cutworm larvae had supernumerary stadia (>6), of which 4 were parasitized and 1 was unparasitized. Five out of the 30 total host larvae presumed to have been parasitized pupated and emerged as a moth. Since it is unclear whether they were resistant to parasitism or wasps had actually failed to parasitize them, these larvae were eliminated from all analyses.

Repeated measures analysis of the brood data based on stadia (through stadium 4, and from stadium 5 through 6) as the time variable, parasitoid sex (female, male, or unparasitized) as the treatment variable, and mass gain as the response variable, yielded statistical significance for time ($p < 0.001$, $F_{1,42} = 2.53 \times 10^3$), treatment ($p < 0.001$, $F_{2,42} = 66.0$), and time × treatment ($p < 0.001$, $F_{2,42} = 68.5$). Orthogonal contrasts for female vs. male wasp broods and parasitized vs. unparasitized hosts for each stadium are shown in Table 3.1. By the end of stadium 4, parasitized host larvae were significantly larger (on average by 15%), and had longer development time (on average by 9%) than unparasitized larvae, but no other significant differences among host responses were detected. In particular, male and female wasp broods seemed to have similar effects on and responses to their hosts in these early stages of development. But for stadia 5

through 6, male wasp broods were associated with significantly larger hosts than were female broods (on average by 15%). During that interval, parasitized larvae consumed more food (on average by 36%) and accumulated more mass (on average by 50%) while taking longer to develop than did unparasitized larvae (on average by 33%).

Both total and secondary brood sizes were larger in hosts that underwent supernumerary instars than in hosts that did not (ANCOVA: total brood size: $F_{1,22} = 33.1$, $p < 0.001$; secondary brood size: $F_{1,22} = 27.2$, $p < 0.001$). I thus eliminated the broods whose hosts underwent supernumerary instars from analyses of the relationship between host development and brood size to avoid introducing another factor that could obscure the focal relationship.

Table 3.2 summarizes correlations and slopes of model II regression relationships between wasp brood size and host responses. Through stadium 4, only the positive correlation between wasp brood size and host mass gain pooled over sexes is statistically significant. For stadia 5 through 6, males and females differ significantly in the correlation between brood size and three of the host responses: mass gain, duration, and growth. Only for the protected variable host mass gain is there sufficient power to detect significant correlations within sex. For both sexes, the correlation is strongly positive, but mass gain increases more rapidly with wasp brood size for male broods than for female broods.

Discussion

In a species with many advantages for studying the size-number trade-off within broods of offspring, I was able to demonstrate the trade-off and clarify how the trade-off is balanced. Wasp brood mass was proportional to host mass with a relatively high coefficient of determination, indicating a tight fit of the polyembryonic wasp brood to the size of its host, and thus establishing

the basis for the trade-off.

Of the three ways I considered that wasps might achieve a fit to host mass, data were most consistent with host manipulation by the wasp brood. Both sexes extended late development by the host, resulting in greater total food consumption and mass gain, but there was a stronger relationship between brood size and host mass and a greater host mass gain for male broods than for females. The inverse relation between brood size and wasp body mass after correcting for host size suggested body mass adjustment to fill the host, particularly for males, with their apparently greater variation in brood size. Moreover, brood size was significantly correlated with host mass gain before the time of brood size fixation independent of brood sex, which could mean that mass gain by the host cues or otherwise leads to larger brood size, in anticipation of a larger final host mass. This interpretation seems consistent with transplant experiments on *Copidosoma floridanum*, in which brood embryos transplanted early in development to new hosts increased their ultimate brood sizes with the new host's age, but those transplanted late (apparently after brood size fixation) did not (Corley et al. 2005). But the combination of greater mass gain and a longer early developmental interval in parasitized vs unparasitized hosts could alternatively be explained as an early onset of host manipulation by the developing brood.

The two sexes clearly differed in the ways they balanced the size-number trade-off. Females tended to produce smaller broods of larger individuals, and they responded to different final host sizes mainly by adjusting the mass of individual offspring. In contrast, male wasps tended to produce larger broods of smaller individuals, and they responded to different final host sizes primarily by adjusting brood size. My results thus provide strong evidence of differences in allocation mechanisms, allocation patterns, and adjustments of allocation patterns to resource levels between broods of the two sexes.

That parasitism increased total food consumption by hosts appears to be a typical consequence of parasitism by polyembryonic wasps (Hunter and Stoner 1975; Jones et al. 1982; Strand et al. 1990; Byers et al. 1993). Parasitized hosts may grow larger by extending the feeding phase (Rahman 1970), by increasing the efficiency of metabolism (Hunter and Stoner 1975), or because the parasitoids have a lower metabolic rate than their host (Slansky 1978; but see Jones et al. 1982). In contrast to the patterns for early instars, parasitized black cutworms had a prolonged late developmental interval and more total food consumption but did not have significantly greater daily food consumption or food utilization than non-parasitized larvae. This suggests that *C. bakeri* does not manipulate food processing by the host at this stage but does prolong the host's last stadium and thus ensures greater mass gain by the host. In *Trichoplusia ni* (Hübner) parasitized by *Copidosoma floridanum* (Ashmead), the normal decline in juvenile hormone titer and corresponding increase in ecdysteroid hormone titer are delayed by 24 h, resulting in delayed onset of the host's wandering phase (Strand et al. 1990).

Immediately before pupating and emerging from the host, the wasp brood consumes almost everything inside the cuticle of the caterpillar. This is consistent with the relatively tight proportional relationship between (dry) brood mass and (wet) maximal host mass in Fig. 3.2. From my preliminary data of dry host and brood mass, I estimate that the emerging brood accounts for roughly 53% of the host mass. The other 47% of the host mass may constitute an essentially irreducible residual: the few larvae that died shortly before pupation and pupae unable to emerge, host cuticle, wasp molting waste products, frass (meconium), and perhaps a very small amount of uneaten material inside the host. Thus, selection may have nearly maximized the mass of the emerging brood, which is the mathematical product of body mass and brood size.

The inverse relationship between standardized wasp body mass and wasp brood size

implies that a larger number of offspring in the host yields smaller individuals because of less host resource available per individual wasp (Slansky 1986). In genetically heterogeneous gregarious parasitoids, larger wasp mass in a larger host is predicted as an adaptive response by the wasps to more intense intra-brood competition (sibling conflict; Godfray and Parker 1991). In addition, gregarious parasitoids that halt host development (idiobionts) tend to have both *larger* wasp adults and larger broods in larger hosts, due to under-compensation for larger host size providing more resource per offspring (Mayhew 1998; Mayhew and Glaizot 2001; see also Charnov and Skinner 1985). But in clonal broods, compensation via a decrease in wasp body mass at large brood size can be advantageous, since intense larval competition is counterproductive. Clonal broods may maximize brood fitness by increasing host size regardless of the way that brood size and body mass trade-off.

Fig. 3.2 strongly suggests that most of the variation in brood mass of *C. bakeri* (=brood size × wasp body mass), and thus much of the variation in wasp brood size and body mass, is attributable to differences in host mass. Variation in the precise way that each brood trades off brood size against body mass, notably including differences between the sexes, accounts for another component of variation among broods in body mass and in brood size, which may be influenced by particulars of the host environment beyond host size alone. This is because brood size is almost certainly fixed when physiological and morphological cues to the ultimate host size could only be very rough and approximate (see West and Sheldon 2002), with body mass providing the later adjustment to achieve the tight fit between brood mass and final host mass. Selection on adult traits seems likely to determine the typical or average allocation pattern of the trade-off (see Mayhew and Glaizot 2001).

After correcting for differences in host mass, female broods tended to have fewer but larger

wasp individuals, whereas males broods had more individuals of smaller body mass. Female wasps were about 32% heavier than males, in general accord with the typical pattern for insects (Teder and Tammaru 2005). Larger females are assumed to have more eggs or sometimes larger eggs (Stearns and Koella 1986). In parasitoids, the typically female biased sexual size dimorphism may arise from selection on host finding ability (Bennett and Hoffmann 1998) or on fecundity (Visser 1994). Since female *C. bakeri* are larger bodied than males, female broods must necessarily contain fewer individuals than male broods to allow females to attain larger mass (Godfray 1994) – in contrast to smaller male broods than female broods or no differences in some other polyembryonic parasitoids of the families Encyrtidae, Platygasteridae, and Braconidae (Strand 1989c; Ode and Strand 1995).

Two patterns in the data that deserve further attention are the apparently higher variance in male than in female body mass and brood size (Fig. 3.1) and the increase in body mass with host mass in females but the increase in brood size with host mass in males (Fig. 3.3). Though the trend toward increasing brood size with host mass in females is not statistically significant, a positive relationship between these variables is consistent with the sub-proportional increase in female body mass with host mass. Taken together, these patterns suggest (1) weaker selection overall on the trade-off allocation pattern in males than females and (2) a difference between sexes in the relative benefits of body mass and brood size.

Effects of body size on fitness may differ between males and females, in which female body size has stronger positive association with their fitness than male body size does with male fitness (Charnov 1979; van den Assem 1989). Both males and females have a primary searching and handling problem to solve as adults – males must rendezvous with females and females with host eggs. One possible difference between sexes is that the ability to find and oviposit into hosts is

more positively related to body mass than is the ability to find and mate with females. Larger females may be more likely to find hosts than smaller females (Bennett and Hoffmann 1998). In contrast, the mating strategies of males may minimize size effects on mating success. For example, males of differing sizes may be equally able to locate females emerging in large aggregations, or may attract them by swarming (Thornhill and Alcock 1983), as documented for some encyrtids (e.g. Nadel 1987). But, larger females may be better able to insert the ovipositor into eggs more difficult or time-consuming to penetrate for smaller females. These factors may all yield more readily to larger female size, and larger females are able to produce more eggs as well. Thus, males may benefit more from increasing their numbers rather than their body size to maximize the number of females mated, while females may achieve more total oviposition by trading some brood size for body mass. These ideas need to be tested in additional laboratory, field, and perhaps greenhouse studies, which would also benefit from modeling work to assess the potential adequacy of proposed mechanisms and to sharpen the hypotheses to be tested.

Sex-specific allocation patterns have been documented in other life-history trade-offs (reviewed in Nylin and Gotthard 1998). For example, in species that are univoltine or territorial or both, males usually emerge earlier (protandry) with smaller body size than females. In the trade-off between age at maturity and body size, males prioritize early maturation while females may be under greater selection pressure to be larger for greater fecundity (Fisher and Fiedler 2000). These trade-offs may become more evident at high densities leading to intense sexual selection or under strong resource limitation environmental stress. Future studies will investigate how the sex-specific allocation patterns of the trade-off respond to these intensifying conditions.

My results on the size-number trade-off in *C. bakeri* highlight some significant advantages of working with clonal systems in identifying both the mechanisms and the selection pressures

involved in establishing and balancing trade-offs. Many other clonal species should prove amenable to this approach. But my results may also contribute to the analysis of genetically mixed broods and provide a way of measuring the cost of sibling conflict during development. For example, the sex of the eggs being laid within a host can sometimes be consistently determined from the female's oviposition behavior, and some females will lay multiple eggs into a host (Strand 1989b). Thus the size-number trade-off in clonal broods of each sex can potentially be compared with the way the trade-off works for sibling eggs of the same or different sexes and for unrelated eggs of the same or different sexes.

Table 3.1 Effects of sex and parasitism on host characteristics[1]

Host Response	Through stadium 4				Stadia 5 & 6			
	Females vs Males		Parasitized vs Unparasitized		Females vs Males		Parasitized vs Unparasitized	
Mass gain (g)	0.075 ± 0.004	0.085 ± 0.005	0.081 ± 0.003 *	0.071 ± 0.002	1.28 ± 0.08 *	1.48 ± 0.05	**1.41 ± 0.05** ***	**0.94 ± 0.02**
Duration (d)	8.86 ± 0.14	9.17 ± 0.17	9.05 ± 0.12 †	8.27 ± 0.10	10.00 ± 0.69	9.58 ± 0.19	9.74 ± 0.27 †	7.31 ± 0.15
Growth rate (g / d)	8.44×10-3 ± 0.48×10-3 9.33×10-3 ± 0.57×10-3		9.00×10-3 ± 0.40×10-3 8.56×10-3 ± 0.30×10-3		13.35×10-2 ± 1.27×10-2 15.54×10-2 ± 0.63×10-2		14.74×10-2 ± 0.64×10-2 13.02×10-2 ± 0.40×10-2	
Total food consumption (g)	0.725 ± 0.033	0.656 ± 0.026	0.682 ± 0.022	0.647 ± 0.025	7.06 ± 0.73	7.21 ± 0.60	7.15 ± 0.45 †	5.27 ± 0.17
Daily food consumption (g / d)	0.304 ± 0.012	0.278 ± 0.012	0.288 ± 0.009	0.319 ± 0.007	0.724 ± 0.082	0.757 ± 0.066	0.745 ± 0.050	0.725 ± 0.024
Food assimilation efficiency (g / g)	0.086 ± 0.004	0.108 ± 0.006	0.100 ± 0.005	0.090 ± 0.004	0.191 ± 0.019	0.219 ± 0.016	0.208 ± 0.012	0.184 ± 0.007

[1] Mean ± SE of each host response at each host developmental stage. We emphasize that mass gain was consistently uncorrelated (through stadium 4) or negatively correlated (for stadium 5 through 6) with duration, based on analyses not presented here; this indicates that neither significant duration contrast can be explained by correlation with mass gain. Similarly, total food consumption was uncorrelated with mass gain (for stadium 5 through 6). Significance levels are * = $0.05 > p \geq 0.01$, *** = $0.001 > p$, and † = $0.0021 > p$ for the Bonferroni corrected contrasts (equivalent to $0.05 > p$); no symbol = not significant.

Table 3.2 Correlations between host responses and brood size[1]

Host Response	Through stadium 4		Stadia 5 & 6
Mass gain (g)	F vs. M, NS $r = 0.47$, $b = 3.83 \times 10^4$, *	***	F: $r = 0.85$, $b = 1.46 \times 103$, * M: $r = 0.75$, $b = 3.25 \times 10^3$, **
Duration (d)	F vs. M, NS $r = 0.42$, $b = 1.11 \times 10^3$	†	F: $r = -0.91$, $b = -1.63 \times 10^2$ M: $r = 0.52$, $b = 8.37 \times 10^2$
Growth rate (g/d)	F vs. M, NS $r = 0.32$, $b = 3.32 \times 10^5$	†	F: $r = 0.89$, $b = 8.89 \times 103$ M: $r = 0.33$, $b = 2.57 \times 10^4$
Total food consumption (g)	F vs. M, NS $r = -0.21$, $b = -6.17 \times 10^3$		F vs. M, NS $r = 0.31$, $b = 2.94 \times 10^2$
Daily food consumption (g/d)	F vs. M, NS $r = -0.11$, $b = -1.49 \times 10^4$		F vs. M, NS $r = 0.30$, $b = 2.66 \times 10^3$
Food assimilation efficiency	F vs. M, NS $r = 0.55$, $b = 2.78 \times 10^4$		F vs. M, NS $r = 0.15$, $b = 1.09 \times 10^4$

[1] Correlation coefficients (r) and slopes (b) from model 2 regression for each developmental interval and host response. For the slopes, brood size was on the abscissa and host response on the ordinate. Significance levels are * = $0.05 > p \geq 0.01$, ** = $0.01 > p \geq 0.001$, *** = $0.001 > p$, † is equivalent to $0.05 > p$ following Bonferroni correction, and no symbol = not significant.

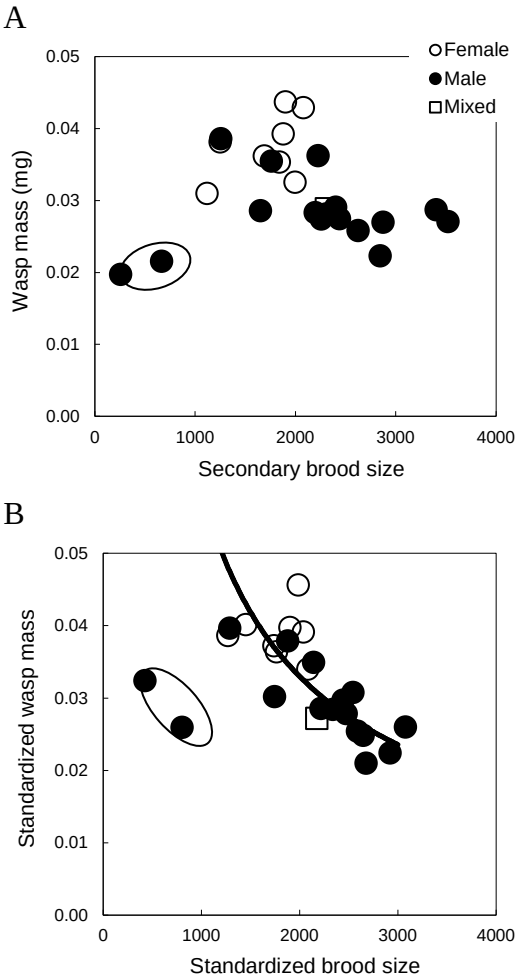


Figure 3.1
Relationship between secondary brood size and wasp body mass. The two outliers circled on the left side of the graphs and the mixed brood were not included in any of the analyses or curve fits because of high mortality.

A. Unstandardized data
B. Data standardized to remove the effect of host mass. The best-fit line, drawn without the two outliers and mixed brood, $Y = aX^b$, where Y is standardized wasp mass, X is standardized brood size, a = 14.8 (95% CI [3.15, 69.7]), and b = -0.86 (95%CI [-1.15, -0.65]).

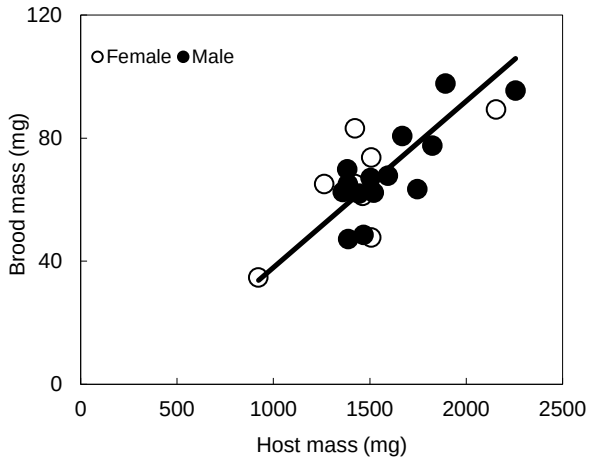
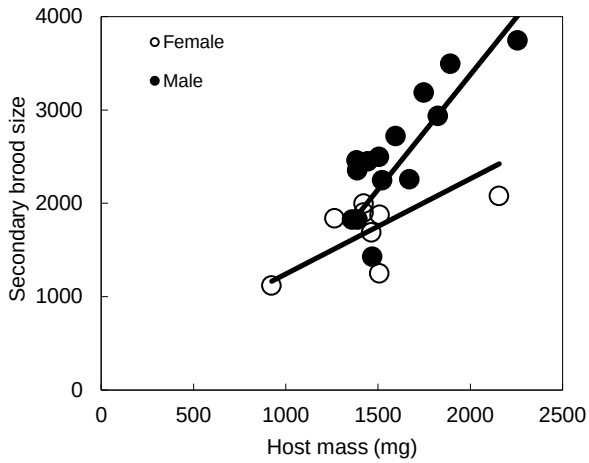


Figure 3.2
Relationship between host mass and brood mass (brood size × wasp mass). The line fit to the data by Model II regression is $Y = aX \pm b$, where Y is brood mass, X is host mass, $a = 0.054 \times 10^{-2}$ (95% CI = [0.040, 0.072]), and $b = -16.0$ (95% CI = [-40.9, 8.98]).

A



B

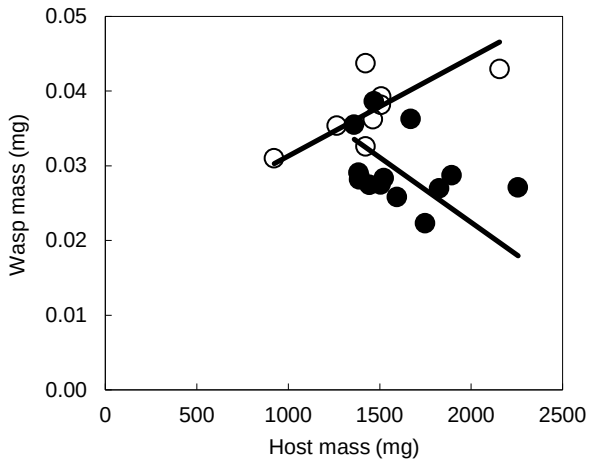


Figure 3.3

A. Relationship between host mass and brood size. The line fit to the data by Model II regression is $Y = aX \pm b$, where Y is brood size, X is host mass. Female: $a = 1.02$ (95% CI = [0.50, 2.11]), $b = 2.24 \times 10^2$ (95% CI = [-9.84×10^2, 14.3×10^2]); Male: $a = 2.46$ (95% CI = [1.73, 3.51]), $b = -1.54 \times 10^3$ (95% CI = [-2.98×10^3, -0.10×10^3])

B. Relationship between host mass and wasp mass. The line fit to the data by Model II regression is $Y = aX \pm b$, where Y is wasp mass, X is host mass. Female: $a = 1.32 \times 10^{-5}$ (95% CI = [0.69×10^{-5}, 2.55×10^{-5}]), $b = 1.81 \times 10^{-2}$ (95% CI = [0.42×10^{-2}, 3.20×10^{-2}]); Male: $a = -1.74 \times 10^{-5}$ (95% CI = [-3.07×10^{-5}, -0.98×10^{-5}]), $b = 5.72 \times 10^{-2}$ (95% CI = [4.02×10^{-2}, 7.42×10^{-2}])

Copyright © Yoriko Saeki 2012

Chapter 4 The size-number trade-off in clonal broods of parasitic wasp: Responses to the amount and timing of resource availability

Introduction

Trade-offs between fitness-related traits are fundamental to life histories in nature (Stearns 1992; Roff 2002; Roff & Fairbairn 2007). One of the best-studied life-history trade-offs—of importance across all organisms that produce multiple offspring simultaneously—is the relationship between the size and number of offspring in each seed set, clutch, or brood (Smith & Fretwell 1974; Paul-Victor & Turnbull 2009). The basic idea underlying the progeny size-number trade-off is that the mother optimizes offspring size and number within a fixed amount of resources so that she maximizes the number of her grand-offspring by producing as many high-fitness progeny as possible under resource constraints (Smith & Fretwell 1974).

However, the causal factors underlying the relationship between body mass and brood size of progeny generally remain elusive because of secondary influences on these traits during offspring development, such as; parent-offspring and sibling conflict (Godfray 1986; Pease & Bull 1988; Godfray & Parker 1991 and 1992; Klingenberg & Spence 1997). My approach is to isolate the trade-off from other factors by studying a clonal system that seems to avoid or minimize some of the most difficult issues and which can be experimentally manipulated to clarify the key underlying mechanisms. Features of these mechanisms can be revealed by observing the trade-off outcome or *allocation pattern* between two traits like body mass and brood size in response to different environmental conditions.

Many empirical studies have demonstrated phenotypic plasticity in life history traits in

response to environmental factors such as temperature, photoperiod, and food availability (reviewed in Nylin & Gotthard 1998). In particular, experimental manipulations of these factors have demonstrated influences on development rate and adult body size (e.g. damselflies: Johansson et al. 2001; De Block & Stoks 2003) and have the potential to make trade-off outcomes based on these important variables more interpretable.

Here I focus on changes in body mass and brood size in the parasitoid wasp *Copidosoma bakeri* (Howard) under different host food availabilities that alter host development time and body mass. *C. bakeri* is a koinobiont parasitoid that attacks moth eggs and develops in the host while the host itself continues to develop. The parasitoid is polyembryonic: the wasp embryo divides into multiple embryos and develops into a clonal brood under constraints associated with development of the host caterpillar. The wasp embryo completes its division at the beginning of the host penultimate instar; hence the maximum brood size (the initial number of developing wasp individuals produced from the embryo) is determined at this time (a key benchmark in my experimental design below). The wasp larvae hatch within the host at the final instar and consume the inside of the host before the host pupates; the wasps pupate inside the host carcass (Baehrecke and Strand 1990; Y.Saeki personal observation).

When parasitized, the host increases its maximum mass approximately 50% by prolonging the final instar about 2 days before the parasitoid larvae consume the host (Saeki et al. 2009). Male and female broods differ in trade-off allocation pattern. There is female-biased sexual size dimorphism in *C. bakeri*: adult female wasps are usually larger than adult males (mean dry mass $\pm$ 1 SE: female 0.037$\pm$0.001mg; male 0.029$\pm$0.001mg), while female brood size is smaller than male brood size (mean brood size at emergence $\pm$ 1

SE: female 1862±117; male 2530±173; Saeki et al. 2009).

The wasp brood mass, which is wasp body mass *multiplied by* the number of individuals in the brood (i.e. brood size), is proportional to the host mass, implying an inverse relationship between wasp body mass and brood size for a given host mass (Paul-Victor & Turnbull 2009; Saeki et al. 2009). When final host masses are similar, the wasp's size-number allocation pattern should lie along a single trade-off curve; different final host masses should produce allocation patterns lying along *different* trade-off curves. Other environmental shifts with little influence on final host mass may displace the allocation pattern along the *same* trade-off curve. Observed allocation patterns are combinations of wasp brood size and body mass for individual broods along the trade-off curve.

In this study, I manipulated the amount and timing of host and wasp resource availability, resulting in changes in total brood mass and in development rate, and I examined how the allocation pattern between the wasp brood size and body mass responded for a common host species and laboratory diet. The focal hypotheses addressed in this study are as follows.

(1) Effects of food treatments on host growth:

(a) Reducing food availability to the host *early* in development will increase host development time with little or no reduction of maximum host mass (consistent with other Lepidoptera: Nijhout & Williams 1974; Nijhout 1981).

(b) Reducing host food availability *late* in development will decrease maximum host mass (in accord with the geometric mass gain of Dyar's rule: Klingenburg & Zimmermann 1992).

(2) Effects of food treatments on the parasitoid brood:

(a) Reducing food availability to the host early in development will reduce wasp brood size but enlarge wasp body mass at emergence (fewer offspring should be more beneficial with fewer resources: Stearns 1992; Guisande et al. 1996; Stuefer, van Hulzen, & During 2002; Sadras 2007). (Reducing food availability to the host late in development should reduce wasp adult body mass to adjust to the pre-determined brood size and smaller host mass (see 1b).)

(b) The sexes of wasps will respond differently to changes in food availability early or late in host development or both. (For example, if body mass relates more strongly to fitness in female wasps than in male wasps, then female wasps may tend to maintain body mass at the expense of brood size; the opposite pattern is also possible.)

(3) Effects of food treatments on the precocious wasp larvae:

(a) Reducing food availability to the host early in development will increase the number of precocious wasp larvae (soldiers) produced from the wasp embryo. (Soldiers are sterile, play a role in brood defense, and die without consuming the host, thereby making no resource demands of their own (Giron et al. 2004). Slow host growth may suggest the presence of competing genotypes and thus result in more soldiers.)

Materials and methods

Study species

Copidosoma bakeri (Howard) is a minute (~1mm) wasp that parasitizes at least 19 noctuid moth species (Schaaf 1972). The host species of *C. bakeri* I used in this study is the black cutworm, *Agrotis ipsilon* (Hufnagel; Lepidoptera: Noctuidae), a major pest in turf and crop fields throughout much of the world (Forbes 1905; Crumb 1929; Harris, Mazurek & White

1962; Busching & Turpin 1977; Williamson & Potter 1997a, b). *A. ipsilon* larvae are found in Kentucky from April to October. The moths migrate from the south in Kentucky around the end of April, and then migrate back to the south in mid-October for the winter (Showers 1997). *C. bakeri* uses other host species as well, with which it generally produces smaller brood masses (see Snow 1925; Cheng 1977).

Empirical methods

I used *C. bakeri* from a laboratory colony of wasps and *A. ipsilon* hosts established in 2005. I reared the host with a pinto-bean-based diet following Hendrix, Gunnarson & Showers (1991) in a growth chamber held at 27 ± 0.1 ºC (daytime) and 25 ± 0.1ºC (night time) and light:dark regime L14:D10, simulating average summer conditions in Kentucky, USA.

I prepared parasitized host larvae by rearing from the host egg stage as follows. I first paired newly emerging male and female wasps and isolated the mated females overnight. The next day, I introduced the female parasitoid into a Petri dish containing more than 200 fresh cutworm eggs (<24h old) on filter paper. Once the female oviposited into a host egg, I marked the filter paper and monitored her oviposition so that parasitized eggs were not super-parasitized. Parasitized eggs were isolated by cutting out the piece of filter paper with the parasitized eggs and were placed in a small container with a cotton ball soaked with water until they hatched. Hatched larvae were therefore presumably parasitized. Unparasitized larvae were prepared in the same manner from the host egg stage but without exposing the cutworm eggs to the female parasitoid.

When larvae hatched, parasitized and unparasitized larvae were individually reared in plastic containers in growth chambers using the standard light and temperature regime

described above. Newly hatched larvae were then systematically allocated to four treatment combinations so that no host larvae parasitized by the wasp having the same mother were allocated to the same treatment combination. The food treatments were feeding levels early (FE) or late (FL) in host development. Within each developmental interval, food availability was low (L) or high (H), producing four treatment combinations of feeding levels: HL (high food availability early and low food availability late in development), HH (high food availability early and high food availability late in development), LH (low food availability early and high food availability late in development), and LL (low food availability early and low food availability late in development). For each of these combinations, I started with N_p parasitized and N_u unparasitized individuals: LL (N_p = 52, N_u = 15), HH (N_p = 45, N_u = 12), HL (N_p = 44, N_u = 15), and LH (N_p = 34, N_u = 14). Since I needed wasp brood samples for sex-specific responses, I prepared more samples of parasitized larvae than of unparasitized larvae. Also, differences in expected larval mortality and in availability of parasitized larvae due to variation in oviposition behavior in wasps accounted for the differences in numbers among the N_p groups and among the N_u groups.

In designing the experiment, I referred to the well studied development of the *Copidosoma floridanum* embryo in the host *Trichoplusia ni*. Embryos of *C. floridanum* complete division and form a polymorula on the second day of the host penultimate stadium (Baehrecke & Strand 1990). *A. ipsilon*, the host of *C. bakeri*, typically has 6 larval stages rather than 5 stages like *T. ni*, the host of *C. floridanum*. Nevertheless, my preliminary study allowed us to link this pattern to the timing of embryonic development of *C. bakeri* in *A. ipsilon*, as indicated by uniform sized wasp embyos on the first day of the

host penultimate stadium (YS, unpublished observations). I therefore categorized the interval before molting into the penultimate stadium as the “early” development phase, and the penultimate stadium and final stadium as the “late” development phase. For the few larvae undergoing supernumerary (seventh) instars as their final developmental stadia, especially starved larvae, I was able to identify larvae molting into the penultimate stadium from the current and previous head capsule width. In addition, to avoid confusing developmental stages of extra-molting larvae and how much of the last stadium is important in the development of host and wasps, I express the caterpillar’s larval stage as “F” for the final stadium, “F-1” for the penultimate stadium, “F-2” for two stages before the final stadium, and so on in this paper (Table 4.1).

To impose a low feeding rate (L), I alternated an empty container with a cotton ball soaked with water and a container containing the pinto-bean based diet between days; at the high feeding rate (H), host larvae were also transferred to different diet cups to provide equal handling stress with the larvae in the low food treatment but received the pinto-bean diet every day. Since the feeding amount of *A. ipsilon* is variable depending on age and molting cycle, I could not precisely regulate the amount eaten by a caterpillar each day, and so I manipulated caterpillar feeding via daily food access, with the caveat that this might have exerted extra stress on the larvae. For the LH treatment, larvae were fed at the lower rate (L) during the early development phase, and then switched to the higher rate (H) on molting into the F-1 stadium until the final day of the last stadium. For HL treatment, larvae were fed at the higher rate during the early development phase, and then switched to the lower rate during the late development phase. For LL and HH treatments, host larvae fed at the low rate and the high rate, respectively, throughout development.

I recorded host molting dates and head capsule width daily. In addition, I recorded the host mass on the day of molting into the F-3, F-2, and F-1 stadia, and daily mass in the F stadium. Once the mummy, which is actually the host cuticle containing wasp pupae, was formed, I transferred it to a test tube sealed with a cotton ball. After the wasps were allowed to emerge for 24 hours, I froze them, counted the number of wasp individuals, and determined their sex. After separating wasps, host carcass, and undeveloped larvae/pupae, I put them in the drying oven over night (57°C). I then weighed the whole wasp brood, host, wasp undeveloped larvae/pupae, and 2 groups of 20 individual wasps with a microbalance (CAHN, C31). Because individual wasp sizes within a brood were very similar (YS, unpublished observations), I addressed mean body mass of the brood and inter-brood variation.

I randomly pre-assigned several host larvae in each treatment to be dissected. The assigned larvae were dissected on the day of molting into the F-3, F-2, F-1, and F stadia. I dissected hosts from the LL and HH treatments at molting into the F-3, F-2, F-1, and F stadia to determine early- and late stage responses to the two feeding levels—and from all four treatments at molting into F-1 and F stadia to determine responses to the four treatment combinations in late host development. In dissection, I isolated the polygerm, the aggregation of embryos enveloped within membranes (Strand 1989), in Pringle's solution (Pringle 1938) and counted the number of soldiers under the dissection microscope. I also measured the diameter of 5 wasp embryos randomly selected from portions of center, right, bottom, left, and top at the moment of measurement and a head capsule of soldiers under a dissection microscope (50x). Isolated polygerms and host carcasses were placed on a piece of foil, dried over night in the drying oven (57°C), and weighed using the microbalance

within 0.1μg (CAHN, C31).

Analyses

I examined the effects of parasitism and food treatments on the probability of death of host larvae before the end of the final stadium with Cox-regression. Covariates were parasitism and food treatments early and late in host development.

Effects of food treatments on host growth and its interaction with parasitism

I analyzed effects of food treatments and parasitism on host development time and host maximum wet mass with 3-way ANOVAs (independent variables: parasitism, early feeding regime, and late feeding regime). In both ANOVA tests, dependent variables were log-transformed to eliminate heteroskedasticity.

Effects of food treatments on the parasitoid brood

I first obtained the fraction of wasps that emerged successfully by dividing secondary brood size (the number of adult wasps that actually emerged) by primary brood size (the number of adult wasps plus any undeveloped larvae and pupae left inside the host). Then, I analyzed the effects of host feeding treatments on the fraction of wasps that successfully emerged with 2-way ANOVA. I also tested the effects of the sex of wasps on the fraction of wasps that emerged successfully with logistic regression.

To confirm the existence of the trade-off between brood size and body mass of the wasp, I examined the curve fit of the data using Modell II regression after standardizing and log-transforming to remove the effect of host mass, following the procedure in Saeki et

al. (2009). Model II regression was used because brood size and wasp body mass were correlated variables without a direct causal relationship between them. Model II regression allows us to test whether these two variables are in an inverse relationship by determining the slope of log-transformed data is close to –1 (Warton et al. 2006). In addition, to test whether and how the wasp-mass/brood-size trade-off curves differed between the late-food treatments--in other words whether the high-food treatment late in host development significantly increased host maximum body mass--I compared slopes and elevations of the log-transformed curves of wasp-mass/brood-size trade-off for high food late in host development (HH and LH treatment combinations pooled) and that for low food late in host development (HL and LL treatment combinations pooled) with model II regression (Warton et al. 2006).

I examined responses of wasp body mass and secondary brood size to early and late feeding levels brood with 2-way ANOVAs. I included sex as an independent variable in the model to test for sex-specific responses. Secondary brood size data were log-transformed to meet the assumption of homogeneity for ANOVA. In addition, I used ANOVA to test whether brood size is different between HH and HL, and between LL and LH food treatment combinations to assess the effect of the food treatment late in development.

Effects of food treatments on the precocious wasp larvae

I tested whether the wasp embryo volume calculated from their diameters, polygerm dry mass, and host dry mass are correlated using samples of host larvae from all food treatments pooled. I examined the difference of polygerm dry mass, mean wasp embryo volume, and the number of soldiers between food treatments using ANCOVA, with host

dry mass as a covariate. Since dissections were conducted at 4 host larval stages, and hosts dissected at the beginning of the F-3, F-2, and F-1 stages received only the early food treatment, I divided the data into two groups: F-3, F-2, and F-1 (only early food treatment) and F (early and late food treatments). Hence each group was tested for differences with ANCOVA using the appropriate food treatments as independent variables.

All data were analyzed with SPSS (SPSS Inc., version 15.0, Chicago, IL, USA). Means are expressed with standard errors.

Results

Of 230 host eggs exposed to female wasps, 14 of the resulting host larvae pupated in the parasitism treatments, indicating that either parasitoid oviposition or development failed; 64 host larvae died, and 2 larvae parasitized by mixed broods. The remainder produced emerging wasp broods from LL (N_p = 26, N_u = 12), HH (N_p = 33, N_u = 11), HL (N_p = 26, N_u = 12), and LH (N_p = 19, N_u = 11) treatment combinations. In addition, 3 of these emerging broods (one female brood and a brood of unknown sex) escaped at emergence and were therefore eliminated from the wasp brood size and body mass analyses.

The probability of host mortality before the end of the final instar was (1) greater for parasitized hosts than for unparasitized hosts (odds ratio for parasitized: unparasitized = 2.34 [P = 0.03] and (2) greater for the low food treatment early in host development than for the high food treatment early in host development (odds ratio for low: high = 1.95 [P = 0.02]). There was no significant effect of food treatment in host late in development on the risk of death (P = 0.47).

Effects of food treatments on host growth and its interaction with parasitism

Parasitism and the low food level both early and late in development prolonged larval development time (3-way ANOVA; parasitism : $F_{1,141} = 67.3$, $P < 0.01$; food treatment early in host development (FE): $F_{1,141} = 419.7$, $P < 0.01$; food treatment late in host development (FL): $F_{1,141} = 26.9$, $P < 0.01$; Fig. 4.1a). The low food level early in development and high food level late in development resulted in greater host maximum mass, whether or not the host was parasitized (3-way ANOVA: parasitism effect: $F_{1,141} = 98.7$, $P < 0.01$; food treatment early in host development (FE): $F_{1,141} = 19.2$, $P < 0.01$; food treatment late in host development (FL): $F_{1,141} = 11.5$, $P < 0.01$, Fig. 4.1b). There were no significant interactions in any combinations of independent variables (all combinations: $P > 0.05$). Secondary brood size in the HH treatment was significantly greater than that in HL food treatment combination ($F_{1,54} = 6.05$, $P = 0.02$). Secondary brood size in the HL food treatment combination did not differ significantly from (but tended to be greater than) that in LL food treatment combination ($F_{1,54} = 3.09$, $P = 0.09$).

Effects of food treatments on the parasitoid brood

I obtained 20 male broods (mean brood size 2123.1±98.3) and 12 female broods (mean brood size 1558.3±136.2) in the HH treatment combination, 16 male broods (mean brood size 2270.0±218.8) and 3 female broods (mean brood size 1689.7±190.3) in the LH combination, 22 male broods (mean brood size 1741.4±73.6) and 4 female broods (mean brood size 1326.5±197.1) in the HL combination, and 19 male broods (mean brood size 1892.8±123.4) and 5 female broods (mean brood size 1679.2±196.4) in the LL combination. There were no effects of food treatment on the proportion of wasps that

emerged successfully (early stage food treatment: $F_{1,98} = 0.30$, $P = 0.59$; late stage food treatment: $F_{1,98} = 2.80$, $P = 0.10$). Sex of wasps did not significantly affect the fraction of successful wasp emergence (*Wald test* = 0.51, $P = 0.48$).

Two HH broods, 1 LL brood, and 1 LH brood of wasps were eliminated from the analysis either because the host was not completely consumed or because the fraction of successfully emerging wasps was much lower (i.e. < 0.7) than all other cases (i.e > 0.85). (These situations violated the assumption of a total brood mass directly constrained by final host mass.) Model II regression based on the remaining data showed that wasp brood mass (brood size × mass) was proportional to host mass; accordingly, the 95% confidence interval of the intercept overlapped zero (r^2=0.85, brood mass =0.54±95%CI [0.50, 0.59] ×host mass + 0.0014±95%CI [-0.0068, 0.0095]). The trade-off between brood size and body mass of the wasp was supported statistically: model II linear regression found an inverse relationship between secondary brood size and wasp body mass (Fig 4.2, r^2=0.51). In summary, brood mass is proportional to host mass and is the multiplicative product of brood size and wasp mass, which are inversely related.

Model II regression showed that the log-transformed wasp-mass/brood-size trade-off for high food late in host development (HH and LH treatment combinations pooled) and that for low food late in host development (HL and LL treatment combinations pooled) differed significantly in their elevations ($X^2 = 73.9$, df = 1, $P < 0.01$) but not in their slopes ($X^2 = 1.2$, df = 1, $P = 0.27$). Thus the two curves in Fig. 4.3a differ in location on the graph but not in shape. This reflects the differences in host and brood mass associated with the feeding treatments differences that were removed from Fig. 4.2 by standardization.

Wasp secondary brood size increased significantly with food level late in host

development ($F_{1,94}$ = 7.38, P < 0.01) but not with increased food level early in host development ($F_{1,94}$ = 3.50, P = 0.07; Fig. 4.3a), thought there was a non-significant trend consistent with a modest positive effect. Wasp body mass increased significantly with food level both early and late in host development (early stage food treatment: $F_{1,94}$ = 5.81, P = 0.02; late stage food treatment: $F_{1,94}$ = 9.41, P < 0.01; Fig. 4.3a). When sex was included as an independent variable, there was no significant interaction between sex and the feeding treatments (3-way ANOVA all combinations: P > 0.05; Fig. 4.3b), implying no sex-specific response to food resource levels. Though sexual dimorphism, in which female brood size was larger and male male body mass was greater, were observed (brood size: $F_{1,93}$ = 10.0, P < 0.01; body mass: $F_{1,94}$ = 29.0, P < 0.01).

Effects of food treatments on the precocious wasp larvae

Sample sizes and host and wasp polygerm growth determined from dissections are shown in Table 4.1. At the beginning of the host's final stadium, wasp mean embryo volume, polygerm dry mass, and host dry mass of all food treatment combinations pooled were positively correlated (wasp mean embryo volume vs. polygerm dry mass: P < 0.01, r = 0.72; wasp mean embryo volume vs. host dry mass: P < 0.01, r = 0.80; polygerm dry mass vs. host dry mass: P < 0.01, r = 0.86).

Polygerm dry mass at F-3 and F-2 host larval stages did not differ significantly between the food treatments ($F_{1,13}$ = 2.31, P = 0.15). However, in the host larval stages F-1 and F, polygerms were significantly larger in high food treatments than in low food treatments both early and late in host development (early food treatments: $F_{1,24}$ = 5.61, P = 0.03; late food treatments: $F_{1,24}$ = 7.20, P = 0.01). Too few wasp embryos had formed in

host larval stages F-3 and F-2, and wasp embyo volume data were not enough for analysis. Wasp embryo volumes in the host larval stages F-1 and F did not differ between food treatments (early food treatments: $F_{1,19} < 0.01$, $P = 0.96$; late food treatments: $F_{1,19} = 0.85$, $P = 0.37$). In the host larval stages F-2 and F-3, soldiers were more abundant in the low food treatment than in the high food treatment ($F_{1,13} = 7.14$, $P = 0.02$). The number of soldiers in the host larval stages F-1 and F was greater when the host received both the high food treatment early in host development and the low food treatment late in host development (early food treatments: $F_{1,22} = 4.93$, $P = 0.04$; late food treatments: $F_{1,22} = 7.94$, $P = 0.01$). In these ANCOVA tests, host mass varied significantly with polygerm mass and wasp embryo volume ($p < 0.01$), but the number of soldiers did not ($p = 0.26$).

Discussion

<u>Interpreting the results of hypothesis tests</u>

(1) Effects of food treatments on host growth:

(a) A reduced food supply early in host development (LH & LL) increased host development time independent of parasitism and increased maximum host body mass, as predicted (Fig. 4.1).

(b) The predicted strong dependence of final host mass on late-stage feeding was confirmed (LH & HH; Fig. 4.1b).

(2) Effects of food treatments on the parasitoid brood:

(a) Responses of wasp broods to reduced food availability early in host development (LH & LL) were inconsistent with the predicted brood-size reduction; instead there was a trend toward increased brood size with reduced food availability in the early stage (P =

0.07). Host development time was lengthened by early food reduction as I expected, but wasps responded to longer host development time mainly by reducing body mass (Fig. 4.3a). In response to reduced host food availability late in host development (HL & LL), the expected pattern of reduced both wasp mass and brood size was observed (Fig. 4.3a). (b) I did not detect sex specific responses in wasp brood size or body mass.

(3) Effects of food treatments on the precocious wasp larvae

The reduced amount of food early and late in host development increased the number of soldiers.

Shifts in trade-off curves and allocation patterns

My host feeding manipulation demonstrated how the size-number trade-off curves and allocation patterns in *C. bakeri* respond to resource availability at different stages of host development. More early host feeding mainly speeds host development and results in fewer, larger wasps but with total brood mass (and thus the trade-off curve) essentially unchanged; more late host feeding increases total brood mass and increases *both* brood size and body mass of wasps (thus shifting to a different trade-off curve).

Changes in the trade-off relationship between body mass and brood size with resource availability have been observed in wide range of taxa (reviewed in Stearns 1992; Roff 2002) because resource constraints are often the basis for trade-offs in natural systems (Smith & Fretwell 1974; Zera & Harshman 2001). Other gregarious parasitoids are known to increase brood size but decrease body size with the increase in host size (Dijkstra 1986; Le Masurier 1987; Hardy, Griffiths, & Godfray 1992). The polyembryonic parasitoid, *Ageniaspis fuscicollis* Dalman, increases brood size with host size though the change in

wasp body size is unknown (Cleary & van Ginkel 2004). Increases in *both* body mass and brood size with the increase in resources have often been found (but see Guisande et al. 1996), suggesting major contributions from both traits to the fitness of the lineage (Sinervo 1999; Kolm et al. 2006). This pattern is consistent with a recent modeling analysis based on *C. bakeri* (Crowley & Saeki 2009), under conditions in which fitness benefits from larger body mass and larger brood size accrue with diminishing returns. Note in Fig. 4.3a that greater final host mass and thus brood mass (i.e. HH vs HL and LH vs LL) yield approximately proportional increases in the two trade-off variables for *C. bakeri.* If this pattern of plasticity is adaptive, it indicates similar intensities of selection pressure on brood size and body mass. If instead selection strongly favors some particular body mass regardless of brood size as assumed in some models, (e.g. Smith & Fretwell 1974), the dashed lines through HH and HL and through LH and LL in Fig. 4.3a should be horizontal. Whether plasticity of allocation patterns is generally adaptive and how the trade-off traits relate to fitness will require additional analysis across a range of taxa.

In my results, faster development of the host produced larger wasp body mass and the trend of smaller wasp brood size, in contrast to the usual pattern of faster development decreasing body size (Stearns & Koella 1986; Fischer & Fiedler 2000). The pattern of larger brood size and smaller body mass in response to reduced host feeding early in development and the resulting slower host development rate could simply result from additional cell divisions within the wasp polygerm during the extended host development time and thus in more wasp embryos. However, this pattern may be non-adaptive since wasp brood size, with its implications for reproductive success of the brood, is likely to be under such strong selection, and decreasing body size, especially under poor environment

suggested by the host nutritional condition does not seem to be adaptive (Guisande et al. 1996; Stuefer et al. 2002). Two possible adaptive explanations for a larger brood size and smaller body mass in response to the poorer nutritional environment are: (1) Poor nutritional conditions may signal the need for greater emphasis on bet hedging, in which larger broods can help reduce the variance in brood reproductive success and thus increase lineage fitness (Crowley & Saeki 2009). (2) Increasing brood size under poor nutritional conditions may help ensure survival of the brood during wasp larval development.

Life history studies of plasticity in resource allocation trade-offs have focused on responses of traits to increased resource availability (e.g. Glazier 2000; Sadras 2007), including tests of the van Noordwijk-de Jong model (1986) to detect cryptic trade-offs (e.g. Jordan & Snell 2002; Malausa, Guillemaud & Lapchin 2005; King, Roff & Fairbairn 2011). In contrast to these, my study has demonstrated how the allocation pattern of traits reflects both available resources and constraints on the timing of development. The result is interpretable shifts both along and between trade-off curves showing the plasticity of traits to be anticipated in nature. Allocation patterns clearly respond not only to resource availability per se but also to environmental conditions resulting in different trait combinations along the same trade-off curve. Studying trade-offs in this way in future work should facilitate an understanding of the relationship between plasticity and fitness of key life-history traits.

Mechanisms of brood size adjustment

Comparisons of brood size between LH and HH and between LL and HL suggest that different resources levels early in host development may partially uncouple wasp

development from host development, resulting in different numbers of wasp individuals by the end of that early host development phase. Brood size is then adjusted further in the late host development phase. Comparisons between LH and LL and between HH and HL imply reductions in brood size if food availability is low (relative to high) late in host development. Thus the wasp polyembryo apparently produces an intitial number of embryos based on the host environment early in development and then *reduces* the brood size and determines wasp body mass as appropriate for the host final size (and total wasp brood mass). Brood size reduction might be accomplished through apoptosis, atrophy, or cannibalism (Polis 1980; Cushing 1992 on cannibalism as a “lifeboat strategy”). These cooperative behaviors are plausible among genetically identical individuals, and documenting both the mechanism and its coordination in producing a brood of individuals almost identical in size and a total mass based precisely on available host resources is a challenge for future work.

Various abilities of the polyembryonic wasp embryo in responding to the environment, such as adjustment of embryonic divisions with host developmental stage and host size (Cleary & van Ginkel 2004; Corley, White & Strand 2005) and attacking other parasitoids present in the same host and achieving desired sex ratio (Giron et al. 2004), have been reported. Plastic responses in accord with environmental variation are often crucial for species living in unpredictable environments (Fischer, Taborsky & Kokko 2011). In parasitic species, adjustment of the brood size according to the environment are usually done by the mother (e.g. Allen, Buckley & Marshall 2008; Leips et al. 2009), though the ability to predict her offspring's environment is usually limited. Polyembryony, on the other hand, may provide a more accurate ongoing assessment of the host

environment during development, because adjustment of brood size and body size to an allocation pattern by the parasitoid offspring brood itself (but see Morag et al. 2011).

Although the current study demonstrated that of the size-number relationship of a particular polyembryonic parasitoid is highly plastic, the extent to which this plasticity may be genetically determined (and thus subject to adaptation) requires additional study. So far, a gene involved in proliferation of embryo has been identified (Corey et al. 2005), but its relationship to allocation patterns is unknown.

Precocious larvae

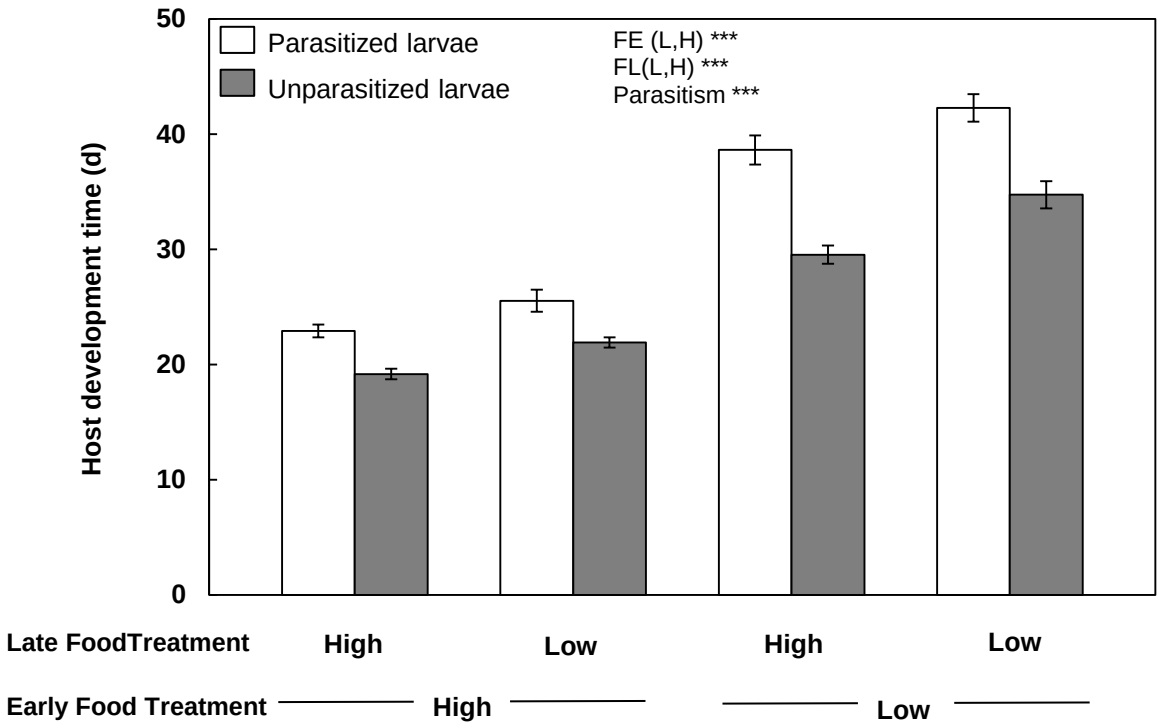
My data showed that the number of soldiers increased when the host resources were reduced early in development. More soldiers are produced when other parasitoids are present in the same host (Giron et al. 2004). Wasp embryo may be sensitive to host nutritional status for defense from other parasitoid inside the same host, and the pattern may be associated with the increase in brood size when host resource was low early in host development. Larvae dissected during late stadia (F-1 & F) showed that high food early in host development and low food late in host development increased the number of soldiers. Further investigation of the timing of soldier production in relation to resource availability and super-parasitism should help explain these results.

Sex specific responses

Selection pressures on body mass and brood size may differ between males and females (e.g. Vertainen et al. 2000; De Block & Stoks 2003). For example, females may differ from males in the optimal distribution of available brood mass into separate individuals; for

example, relationships between survival or fecundity and body mass may differ by sex (Charnov et al. 1981; Ode & Strand 1995; Fischer & Fiedler 2000; Fox & Czesak 2000). Different selection pressures by sex on body mass and brood size could result in different responses to resource levels, but no such differences were observed in the present study. Perhaps selection pressure for size compatibility between males and females helps account for the parallel responses by sex to resource manipulations.

(a)


Parasitized larvae
Unparasitized larvae
FE (L,H) ***
FL(L,H) ***
Parasitism ***
Host development time (d)
50
40
30
20
10
0
Late FoodTreatment
High
Low
High
Low
Early Food Treatment
High
Low

(b)

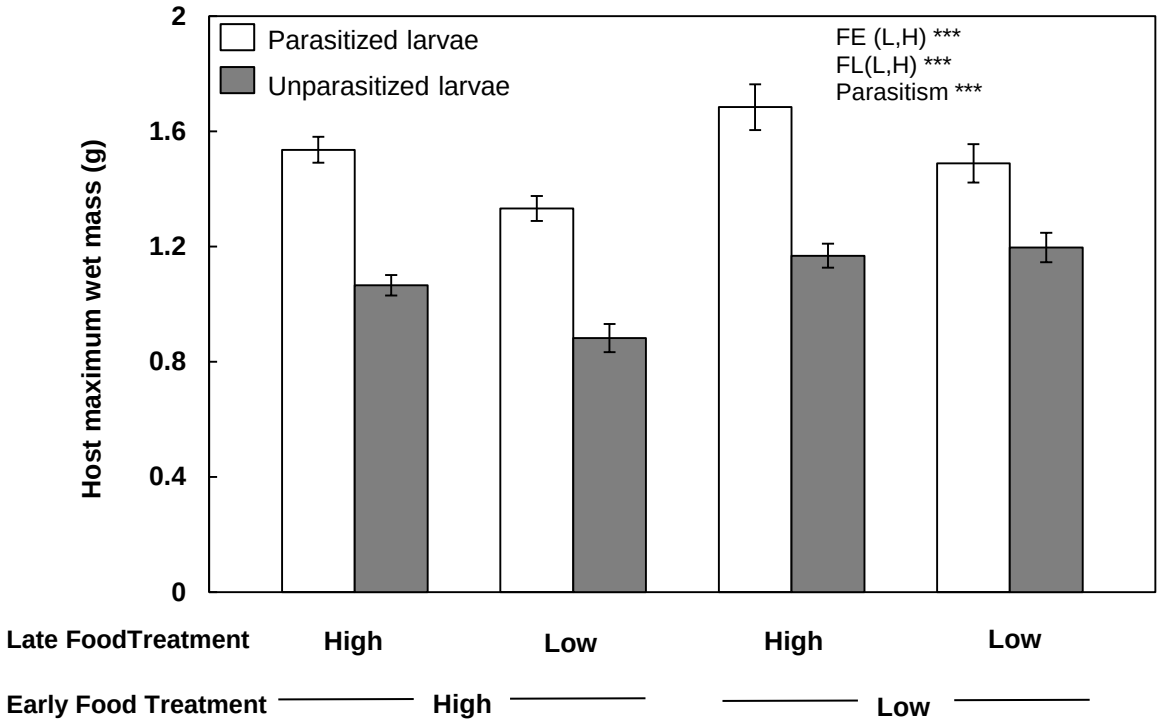
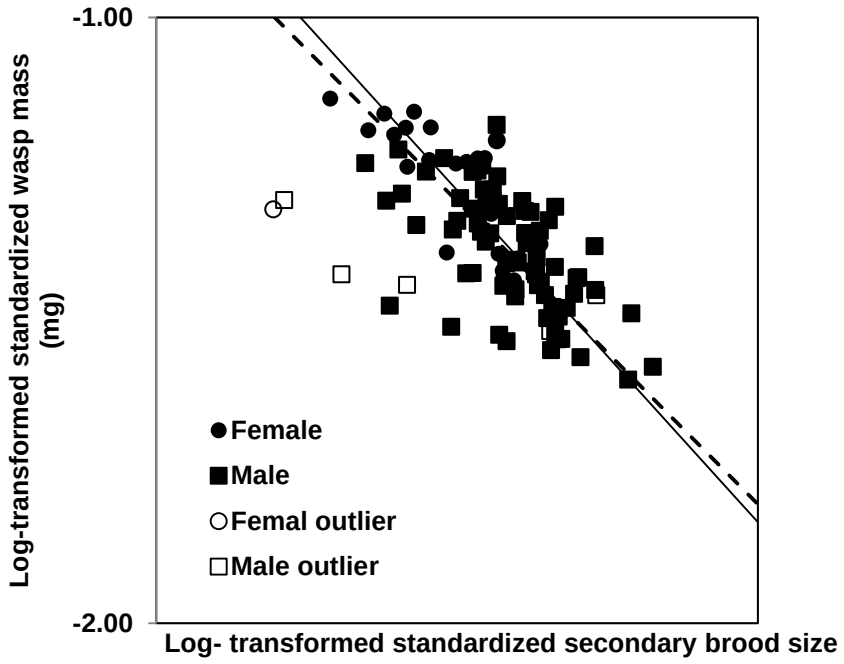

Parasitized larvae
Unparasitized larvae
FE (L,H) ***
FL(L,H) ***
Parasitism ***
Host maximum wet mass (g)
2
1.6
1.2
0.8
0.4
0
Late FoodTreatment
High
Low
High
Low
Early Food Treatment
High
Low

Figure 4.1

(a) Host development time (from hatching to wandering phase in days, and (b) host maximum wet mass of unparasitized and parasitized larvae of all treatments (mean±SE). Significance of each independent variable and their interactions from ANOVA are shown in upper left. FE (L,H) and FL (L,H) represent food treatment (low and high), early and late in host larval development respectively. "**" represents $P < 0.01$; "*" represents $0.01 \leq P < 0.05$, and "NS" represents $P \geq 0.05$ (non-significance).

(a)


-1.00
-2.00
Log-transformed standardized wasp mass (mg)
Log- transformed standardized secondary brood size
Female
Male
Femal outlier
Male outlier

(b)

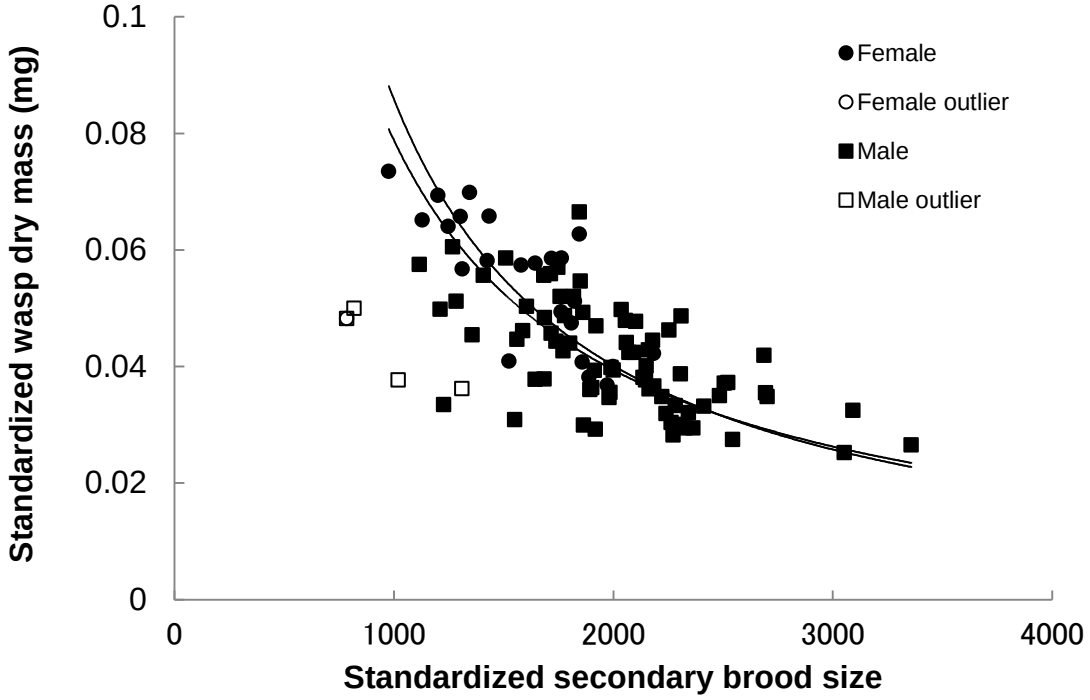
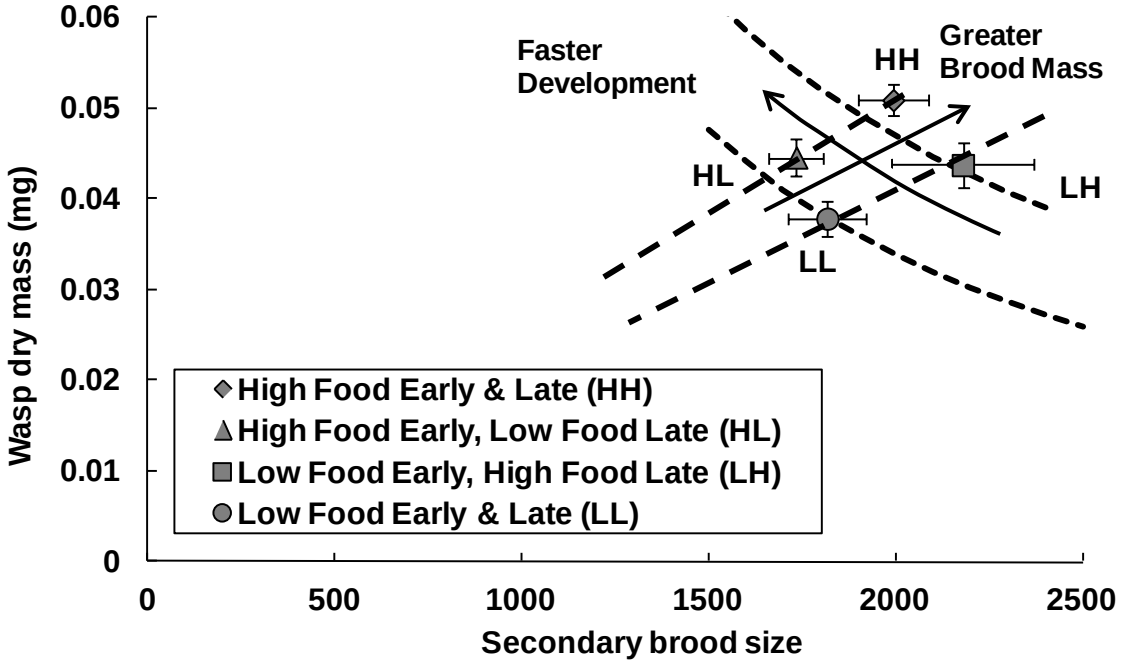

0.1
0.08
0.06
0.04
0.02
0
Standardized wasp dry mass (mg)
0
1000
2000
3000
4000
Standardized secondary brood size
Female
Female outlier
Male
Male outlier

Figure 4.2
(a) Best-fit lines for the trade-off between log-transformed standardized wasp mass and brood size. The solid line is the overall best fit to the data using model II regression (slope = -1.10, intercept = 2.22), and the dashed line represents best-fit line with the same intercept but slope set to -1. (b) Relationship between wasp brood size and dry mass (trade-off curves with back-transformed data from 2a). The best-fit line is the solid line obtained from the power function $Y = aX^b$, where Y is standardized wasp mass, X is standardized brood size, $a=1.66\times10^2$ (95%CI [6.68, 4.12×10^3]) and b= -1.10 (95%CI [-1.34, -0.90]). The dashed line is the same except with b= -1.

(a)


Faster
Development
Greater
Brood Mass
HH
HL
LH
LL
High Food Early & Late (HH)
High Food Early, Low Food Late (HL)
Low Food Early, High Food Late (LH)
Low Food Early & Late (LL)
Wasp dry mass (mg)
Secondary brood size
0.06
0.05
0.04
0.03
0.02
0.01
0
0
500
1000
1500
2000
2500

(b)

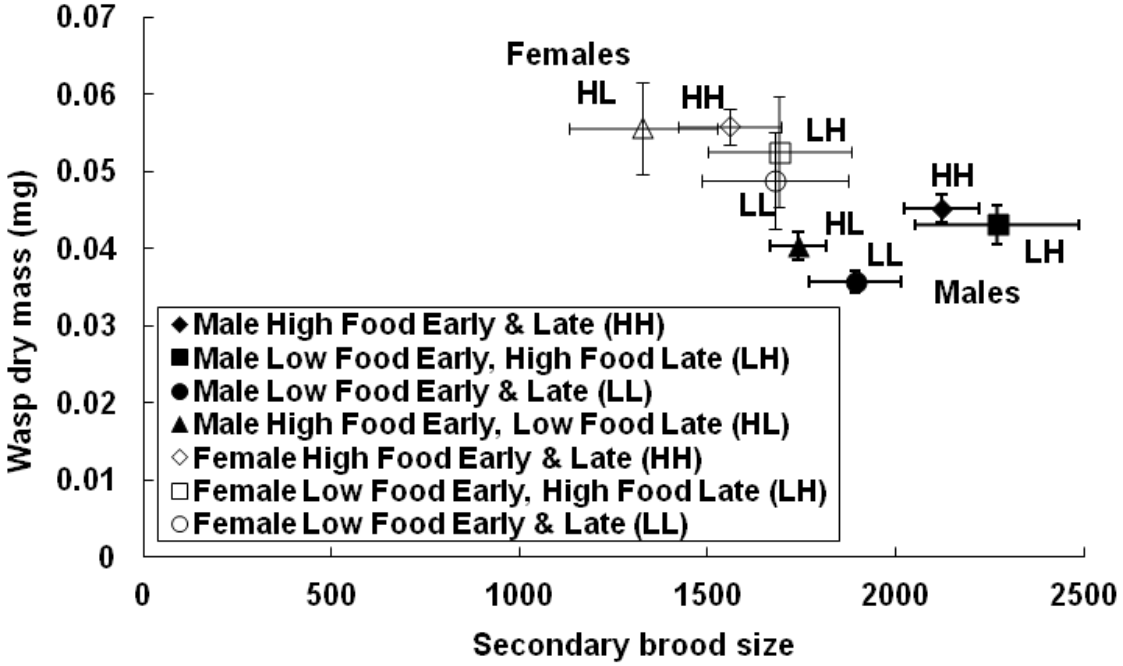

Females
HL
HH
LH
LL
HH
HL
LL
LH
Males
Male High Food Early & Late (HH)
Male Low Food Early, High Food Late (LH)
Male Low Food Early & Late (LL)
Male High Food Early, Low Food Late (HL)
Female High Food Early & Late (HH)
Female Low Food Early, High Food Late (LH)
Female Low Food Early & Late (LL)
Wasp dry mass (mg)
Secondary brood size
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
500
1000
1500
2000
2500

Figure 4.3
(a) Wasp brood size and dry mass for the 4 feeding treatment combinations (HH, LL, LH, and HL; mean ± SE). Female and males broods are combined by treatment combination. HH and HL treatment combinations are grouped as “fast host development”, and LL and LH treatment combinations are grouped as “slow host development”, reflecting the food treatment *early* in host development (see Fig. 4.1). Also, HH and LH treatment combinations are grouped as “large host”, and LL and HL are grouped as “small host” relflecting the food treatment *late* in host development (see Fig. 4.2). (b) Wasp brood size and dry body mass among 4 feeding treatment combinations (HH, LL, LH, and HL) of female and male broods (mean ± SE).

Table 4.1 Means of measurements of host and wasp polygerm growth at each host larval stage

Treatment		Larval Stage				
		F-3	F-2	F-1	[1]	F
High Food Early and Late (HH)[2]	N	3	5	6		8
	Host dry mass (g)	$0.67\times10^{-3}\pm0.03\times10^{-3}$	$2.72\times10^{-3}\pm0.05\times10^{-3}$	$15.6\times10^{-3}\pm0.21\times10^{-3}$		$61.3\times10^{-3}\pm1.95\times10^{-3}$
	Polygerm dry mass (mg)	$20.1\times10^{-3}\pm1.95\times10^{-3}$	$48.8\times10^{-3}\pm0.72\times10^{-3}$	$136.9\times10^{-3}\pm3.47\times10^{-3}$		$1874.9\times10^{-3}\pm36.5\times10^{-3}$
	Mean egg volume (mm^3)	NA	NA	$10.9\times10^{-3}\pm0.66\times10^{-3}$		$39.9\times10^{-3}\pm1.17\times10^{-3}$
	Number of soldiers	$1\pm1.92\times10^{-1}$	$1\pm0.72\times10^{-1}$	$5.33\pm1.87\times10^{-1}$		$6.67\pm2.31\times10^{-1}$
	Soldier head capsule (mm^3)	NA	NA	$1.32\times10^{-3}\pm0.04\times10^{-3}$		$0.70\times10^{-3}\pm0.02\times10^{-3}$
High Food Early, Low Food Late (HL)	N	-	-	-		6
	Host dry mass (g)	-	-	-		$40.1\times10^{-3}\pm0.61\times10^{-3}$
	Polygerm dry mass (mg)	-	-	-		$1340.0\times10^{-3}\pm24.5\times10^{-3}$
	Mean egg volume (mm^3)	-	-	-		$34.4\times10^{-3}\pm0.26\times10^{-3}$
	Number of soldiers	-	-	-		$11\pm4.97\times10^{-1}$
	Soldier head capsule (mm^3)	-	-	-		$1.65\times10^{-3}\pm0.05\times10^{-3}$
Low Food Early and Late (LL)	N	4	6	3		5
	Host dry mass (g)	$0.63\times10^{-3}\pm0.02\times10^{-3}$	$1.03\times10^{-3}\pm0.02\times10^{-3}$	$8.77\times10^{-3}\pm0.72\times10^{-3}$		$19.1\times10^{-3}\pm0.28\times10^{-3}$
	Polygerm dry mass (mg)	$23.6\times10^{-3}\pm1.51\times10^{-3}$	$38.0\times10^{-3}\pm0.48\times10^{-3}$	$138.6\times10^{-3}\pm10.7\times10^{-3}$		$455.8\times10^{-3}\pm8.03\times10^{-3}$
	Mean egg volume (mm^3)	NA	$3.37\times10^{-3}\pm0.29\times10^{-3}$	$10.7\times10^{-3}\pm0.13\times10^{-3}$		$17.3\times10^{-3}\pm0.19\times10^{-3}$
	Number of soldiers	$2\pm1.92\times10^{-1}$	$5.5\pm0.78\times10^{-1}$	$7.67\pm4.49\times10^{-1}$		$6.75\pm0.76\times10^{-1}$
	Soldier head capsule (mm^3)	$0.83\times10^{-3}\pm0.06\times10^{-3}$	$1.11\times10^{-3}\pm0.02\times10^{-3}$	$2.15\times10^{-3}\pm0.09\times10^{-3}$		$2.45\times10^{-3}\pm0.01\times10^{-3}$
Low Food Early, High Food Late (LH)	N	-	-	-		11
	Host dry mass (g)	-	-	-		$57.2\times10^{-3}\pm0.63\times10^{-3}$
	Polygerm dry mass (mg)	-	-	-		$1404.4\times10^{-3}\pm14.2\times10^{-3}$
	Mean egg volume (mm^3)	-	-	-		$35.0\times10^{-3}\pm0.26\times10^{-3}$
	Number of soldiers	-	-	-		$2.5\pm1.08\times10^{-1}$
	Soldier head capsule (mm^3)	-	-	-		$2.38\times10^{-3}\pm0.06\times10^{-3}$

Copyright © Yoriko Saeki 2012

[1] The gap between F-1 and F larval stages is colored in gray because effects of food treatments switched late in host development (i.e. HL and LH food treatment combinations) are expected to be seen only in the dissection on the molting day into F larval stage.

[2] At host larval stages of F-3, F-2 and F-1, combinations of LL and LH food treatments and combinations of HH and HL food treatments were treated the same, therefore, we measured the polygerm growth only in HH and LL food treatment combinations early in development.

Chapter 5 Temperature Effects During Development on a Polyembryonic Parasitoid and its Host

Introduction

Organisms adjust to heterogeneous thermal environments in time and space with flexible responses to improve performance (Angilletta et al., 2002). In ectotherms, temperature and growth rate are often positively related because the ambient temperature directly affects metabolic rate and thus growth rate and somatic maintenance. Increased growth rate with increased temperature, however, often decreases development time and body size at maturity (Stearns and Koella, 1986; Berrigan and Charnov, 1994; Sibly and Atkinson, 1994; Klingenberg and Spence, 1997; Nylin and Gotthard, 1998). The relationships between body size and temperature have been observed in wide range of taxa (Atkinson 1994; reviewed in Angilletta, 2009). Under various thermal environments, optimization of body size at maturity and development time are particularly important because they are core life history traits of insects directly affecting fecundity, mortality, and reproductive time (Sibly and Atkinson, 1994; Nylin and Gotthard, 1998).

In the present study, I examined growth responses to two different rearing temperatures in a koinobiont parasitoid-host system. Koinobiont endoparasitoids grow within other living arthropods while allowing the host to continue development. Optimal temperatures for development in these systems are species-specific due to different physiological mechanisms and selection pressures for each species (reviewed in Huey and Kingsolver, 2011). The nutrition of the host increasingly divertedt to the parasitoid causes host responses similar to starvation and it may alter the host's thermal plasticity (Godfray,

1994; Kingsolver et al., 2006; Diamond and Kingsolver, 2010). Moreover, because the parasitoid and host interact in complex ways (Vinson and Iwantsch, 1980; Slansky, 1986; Godfray, 1994; Harvey, 1996) potentially influenced by temperature, predicting responses to temperature is particularly difficult. So far, only a few studies have examined temperature effects on koinobiont parasitoids (Colinet et al., 2007; Le Lann et al., 2011). To my knowledge, no studies have investigated temperature effects on the interaction between koinobiont parasitoid and the host.

Responses of insects to temperature may be stage specific (Petersen et al., 2000; Folguera et al., 2010). Even within the same developmental stage, different thermal sensitivities have been reported. For example, *Manduca sexta* caterpillars are most sensitive to high temperature in their last instar, decreasing growth rate, development time, and mass gain at higher temperatures (Petersen et al., 2000). In addition, larval condition in one stage may influence growth and development in a later stage (Matcalfe and Monaghan, 2001). Thus, thermal sensitivity to rearing temperature may depend on the timing of the application of temperature, and the effects of rearing temperature may appear in later developmental stages.

In this study, I examined temperature effects on the koinobiont endoparasitoid *Copidosoma bakeri* (Howard; Hymenoptera: Encirtidae) parasitizing the caterpillar host, *Agrotis ipsilon* (Hofnagel; Lepidoptera: Noctuidae). *C. bakeri* is a polyembryonic parasitoid: a single wasp embryo laid in a moth egg divides into thousands of parasitoid embryos within the host, which continue to grow during the host's larval stage. In *C. bakeri*, the body mass and brood size of emerging wasps trade off, subject to the total brood mass limit imposed via maximum host mass (Saeki et al. 2009). The total wasp brood mass

should remain proportional to the host mass if the wasp individual body mass changes with temperature (see Saeki et al. 2009).

The size-number allocation pattern of the trade-off in *C. bakeri* was found to be sex-specific, in which female wasp brood sizes were usually smaller than male wasp brood sizes, but female wasp body size was larger than male wasp body size (Saeki et al. 2009). Since the female *C.bakeri* usually oviposits one egg per host, emerging adult broods are of one sex only (Saeki et al., 2009). Thus, I also examined here whether temperature effects on the parasitoid were sex-specific and influence the allocation pattern between wasp adult body size and brood size. Female body size is generally more plastic than male body size in insects (Stillwell et al., 2010). Also, the magnitude of sexual size dimorphism changes with different temperatures for some species (De Block and Stoks, 2003; Stillwell and Fox, 2007; Hu et al., 2011). Since different allocation patterns between body mass and brood size for the size-number trade-off in *C. bakeri* cause female-biased sexual size dimorphism, I wondered whether sexual size dimorphism and corresponding shifts in brood size are temperature-dependent.

Therefore, I asked 2 main questions regarding thermal sensitivity: (1) Does the growth response to temperature by *A. ipsilon* depend on whether the moth caterpillar is parasitized or on when during development of the temperature is experienced? (2) Does the allocation pattern of body size and brood size in *C. bakeri*, change with temperature?

Methods and Materials

Study species

The polyembryonic wasp *Copidosoma bakeri* (Howard) is a tiny parasitoid (~1mm) whose

hosts are the egg and larval stages of Noctuid moth species (Schaaf, 1972). *C. bakeri* parasitizes its host at the egg stage by ovipositing usually one egg; however, larval development of the parasitoid inside the host larva does not begin until the host's penultimate instar. During the early host instars, the parasitoid embryo grows larger and divides into multiple clonal embryos, resulting in up to 3500 wasp embryos. The parasitized moth larvae feed and develop similarly to unparasitized larvae, but the parasitized host's maximum body mass is enlarged approximately 50% by increased feeding over the extended larval stage of the parasitized host. During the last two host instars, the wasp larvae hatch, feed on the host, pupate forming a mummy, at then emerge from the host carcass (Saeki et al., 2009).

C. bakeri has been found in the central and northern United States into Canada (Schaaf, 1972; Byers et al., 1993). In Kentucky, *C. bakeri* parasitizes the black cutworm *Agrotis ipsilon* (Hufnagel) from July to October, and I used *A. ipsilon* as hosts in the present study. *A. ipsilon* moths migrating from the south appear in Kentucky around the end of April; adults migrate southward in mid-October (Showers, 1997). The season of *A. ipsilon* larvae in Kentucky is thus April-October, presumably based on several generations.

Experimental design

My samples of the host and the parasitoid were obtained from the colony maintained in my laboratory since I collected the host and the parasitoid at the University of Kentucky Turfgrass Research Facility (Fayette County, KY; 37.988N, -84.477W) from July to September in 2005 through 2007. The wasps were cultured within parasitized hosts reared with a pinto-bean-based diet following Hendrix et al. (1991). The hosts were housed

individually in plastic containers placed in growth chambers at 27±0.1 ºC (daytime) and 25±0.1ºC (night time) and a light regime of 14L:10D.

Newly emerging male and female wasps were paired, and the females whose copulation was observed were isolated. To prepare parasitized larvae, I introduced the mated wasp females into a petri dish with numerous moth eggs younger than 24 hours old glued on the filter paper with double-sided-adhesive tape. In this preparation, I allowed each female to parasitize about 50 eggs. I prepared unparasitized larvae in a manner identical to the parasitized larvae, except without exposing the eggs to the wasps. Once a moth larva hatched, I allocated it haphazardly to treatment, except that no two larvae from the same mother were allocated to the same treatment to avoid pseudoreplication. One hundred and thirty parasitized and 47 unparasitized moth larvae were prepared in this way.

The temperature treatment levels were 27±0.1 ºC (with lights on in the incubator = daytime) and 25±0.1ºC (with lights off = nighttime) for the low and 32±0.1 ºC (daytime) and 30±0.1ºC (night time) for high temperature. Low temperature conditions were based on mean summer (June-August) temperature observed in Kentucky, and high temperature was set at a level often experienced but rarely sustained across multiple days during Kentucky summers. The light regime was 14L:10D for both temperature regimes.

To investigate temperature effects on wasp embryo and host development stages, I divided moth larval development into two phases according to wasp embryo development. The wasp embryo divides repeatedly before the host enters the penultimate stadium and forms a polyembryo consisting of several thousand embryos (Baehrecke and Strand, 1990). In this study, larval stages before the penultimate stadium were grouped as the “early development phase”, and larval stages during and after the penultimate stadium were

grouped as the “late development phase”. Thus, the samples in the group of “early development phase” contained actively dividing embryos and those “late development phase” contained embryos after ceasing the divisions. I used a 2x2 design, crossing the two development phases with the two temperature levels to create four combinations. These treatment combinations were labeled with letters L or H at each phase: LL for the low temperature level in both early and late development phases, HH for the high temperature level in both early and late development phases, LH for low in the early development phase and high temperature in the late development phase, and HL for high temperature in the early development phase and low in the late development phase.

I reared the experimental larvae in growth chambers maintained at the designated temperatures. I monitored moth larval growth every 24 hours and recorded molting events, remeasuring the head capsule size of caterpillars at each stadium to confirm larval stages, since each larval stage has a distinct head capsule size. I also measured host body (wet) mass at the day of molting into the penultimate stadium, the final stadium, and mummification (a dead host containing wasp pupae).

When unparasitized larvae pupated, the pupal mass and the time to pupation were recorded. For parasitized larvae, when I observed mummification, I placed each mummy in a test tube sealed with cotton after recording the mummy mass. Approximately 3 weeks later, the wasps emerged and were then frozen until they could be sexed and counted. Close inspection indicated negligible variation in body size within broods (consistent with numerous observations of *C. bakeri* broods by YS). I dried the wasps in the drying oven overnight (57°C), weighed 20 randomly chosen individuals from each brood with an electrobalance (CAHN, C31), and calculated the average of wasp body mass for the brood.

Analysis

All data were analyzed with SPSS version 15.0 (SPSS inc., Chicago).

Survival

I examined and compared survival of parasitized and unparasitized larvae among treatment combinations with logistic regression.

Temperature and parasitism effects on growth of moth larvae

I calculated the proportion of development time and degree-days using a lower temperature threshold of 10.63 ºC for *A. ipsilon* (Dahi et al., 2009) in the early and late development phases of unparasitized and parasitized larvae. To determine whether the growth of unparasitized *A. ipsilon* moth larvae and those parasitized by polyembryonic wasps differ at different temperatures, I compared development time and maximum host mass with 3-way ANOVAs (independent variables: parasitism and temperature treatments early and late host larval developments; dependent variable: total development time of larvae or host maximum body mass). Both dependent variables were log-transformed to improve homogeneity of variance. Growth rate as maximum host mass divided by development time was also indicated by these two analyses.

Temperature effects on wasp brood size, wasp body mass and the size-number trade-off

To examine whether temperature treatments affected the trade-off between wasp brood size and body mass, and whether the responses are sex-specific, I conducted MANOVA with

wasp body mass and brood size as dependent variables, followed by ANOVA's for the two dependent variables and temperature treatment combinations as independent variables. I standardized the wasp secondary brood size (the number of wasps emerged) and wasp body mass following Saeki et al. (2009) to correct for differences in host mass, and I log-transformed the wasp variables to document the trade-off relationship and size-number allocation patterns with Model II regression analysis.

Results

Survival

Host survival was not affected by temperature treatments early or late in host development (Early: *Wald* = 0.36, *P* = 0.55; Late:*Wald* = 0.05, *P* = 0.93), but was affected by parasitism (*Wald* = 6.76, *P* < 0.01; odds ratio of parasitized to unparasitized = 0.33, $N_{\text{parasitized larvae}}$ = 130, $N_{\text{unparasitized larvae}}$ = 47). Probabilities of survival in the absence of insignificant terms of temperature treatments, were 83.0% for unparasitized larvae and 61.5% for parasitized larvae.

Temperature and parasitism effects on growth of moth larvae

The proportions of development time in the early phase were 54% for parasitized larvae and 56% for unparasitized larvae. The degree days were 268.5 for parasitized larvae and 241.7 for unparasitized larvae. For the late development phase, the proportions were 46% of the total development time for parasitized larvae and 44% for unparasitized larvae. Degree-days in the late development phase were 233.2 for parasitized larvae and 192.9 for unparasitized larvae. Development time increased in parasitized larvae relative to

unparasitized larvae ($F_{1,111}$ = 43.4, P < 0.01) but decreased at higher temperature both early and late in development (early: $F_{1,111}$ = 40.6, P < 0.01; late: $F_{1,111}$ = 9.83, P < 0.01). There were no significant interactions among these independent variables (P > 0.05; Fig. 5.1a). Larval maximum mass did not significantly change due to the temperature treatment (early: $F_{1,111}$ = 0.35, P = 0.55; late: $F_{1,111}$ = 2.22, P = 0.14) but did significantly increase with parasitism ($F_{1,111}$ = 40.8, P < 0.01). The significant interaction between temperature treatment early in development and parasitism showed that parasitized larvae increased final host mass —but unparasitized larvae decreased final host mass—at higher temperature early in host development ($F_{1,111}$=5.72, P = 0.02; Fig. 5.1b). I conducted two additional ANOVA's to examine the difference in host maximum mass among the 4 temperature treatment combinations separately both with and without parasitism. For unparasitized larvae, host maximum mass did not depend on temperature treatments ($F_{3,35}$=1.11, P = 0.36); but host maximum mass of parasitized larvae did differ significantly with temperature treatments ($F_{3,35}$=3.19, P = 0.03). A post-hoc test indicated that host maximum mass in the LH temperature treatment combination was smaller than in the others (all P < 0.02).

Temperature effects on wasp brood size, wasp body mass and the size-number trade-off

Eight broods had less than 70% of the wasps emerge successfully, leaving dead larvae/pupae inside the host carcass. Consequently, I eliminated those broods with less than 70% emergence to avoid possible confounding factors in trade-off analyses of adult wasps. The MANOVA test detected no significant effects of temperature or sex on wasp body mass and brood size (early temperature treatment: $F_{2,61}$ = 2.59, P = 0.08; late temperature

treatment: $F_{2,61} = 0.53$, $P = 0.59$; sex: $F_{2,61} = 2.78$, $P = 0.07$). There was no interaction between the temperature treatments and sex (all combinations $P > 0.05$) and thus no sex-specific response to temperature. However, wasp secondary brood size increased when the temperature treatment early in development was high ($F_{1,62} = 5.10$, $P = 0.03$), but there were no significant effects of temperature on brood size late in development ($F_{1,62} = 0.68$, $P = 0.41$) or between the two sexes ($F_{1,62} < 0.01$, $P = 0.98$; Fig. 5.2a). No interactions between brood size and temperature or sex were significant ($P > 0.05$). Wasp body mass did not differ with temperature early in development ($F_{1,62} = 0.04$, $P = 0.85$) or late in development ($F_{1,62} = 0.67$, $P = 0.42$), and no interactions were significant ($P > 0.05$). However, females were heavier than males ($F_{1,62} = 5.24$, $P = 0.03$; Fig. 5.2b).

There was a trade-off between log-transformed wasp secondary brood size and log-transfromed wasp body mass ($r = -0.53$, $t = 5.09$, $P < 0.01$, Fig. 5.3). However, the slopes of trade-off relationships did not differ between sexes ($\chi^2 = 1.8$, $P = 0.19$).

Discussion

Increased temperature during development decreased the development time of both unparasitized and parasitized larvae but not the developmental delay associated with parasitism (as in Saeki et al. 2009). Increased temperature did not reduce larval maximum mass overall, contrary to other studies (e.g. Atkinson 1994). When I examined stage-specific temperature effects, host enlargement by the parasitoid was significantly less at higher temperature late in host development (Fig. 5.1b); this is also considered further below. Female-biased sexual size dimorphism in the parasitoid was found in all temperature treatment combinations. Although wasp body mass did not change

significantly depending on temperature, brood size significantly increased when high temperature was applied early in host development. There was no sex-specific shift in the trade-off with temperature.

Temperature and parasitism effects on growth of moth larvae

A. ipsilon larvae reared at high temperature decreased development time but not final body size, indicating increased growth rate at high temperature. The shorter development time at high temperature may be beneficial in terms of reduced mortality risk and possibly a greater number of generations per season (Sibly and Atkinson, 1994; Fischer and Fiedler, 2002). Optimal temperatures for different traits vary widely across species (Dell et al., 2011), but optimal temperature for growth within species may be subject to strong selection pressure (reviewed in Angilletta et al., 2002). For example, growth rate of *Manduca sexta* reaches a peak at an intermediate temperature (34°C; Kingsolver and Woods, 1997). The increased growth rate with elevated temperature inferred from my results suggests that *A. ipsilon* may follow a pattern similar to *M. sexta,*but additional work is required to evaluate this possibility.

Insignificant differences in the final body size of the moth larvae between the temperature treatments seem to be inconsistent with other studies, which indicated a negative relationship between temperature and body size in ectotherms (Atkinson, 1994) , often termed the “temperature-size rule”. While abundant empirical documentation supports this relationship, some studies have showed that the rule was followed only under certain conditions (Walters and Hassall, 2006; Diamond and Kingsolver, 2010) or that an organism's thermal sensitivity is state-dependent (Gotthard et al., 2000). For example,

growth rate responses of *Pieris rapae* larvae to increased temperatures were greater when feeding on natural diet than when feeding on artificial diet (Kingsolver et al., 2006). Thus, negligible differences in body size at different temperatures in the present study may be because the ad lib diet I used obscured the thermal sensitivity of growth. It is also possible that differences in body size would have been detected with greater differences in temperature treatment levels.

Alternatively, maintaining the body size of *A. ipsilon* larvae in a heterogeneous thermal environment may be important for achieving high fitness, through effects on mortality and fecundity. For example, growth rate and development time are generally thought to be sensitive to temperature, while critical weight for metamorphosis is insensitive to temperature (reviewed in Chown and Gaston, 2010), consistent with my results here. Indeed, a recent mathematical model showed that body size and development time may be determined independently (Nijhout et al., 2010), in accord with other recent empirical results (Saeki and Crowley, submitted).

Parasitism did not alter the temperature effect on the host larvae in my experiment. Rearing temperature effects on insect growth may depend on the condition of the treatment recipient (Kingsolver et al., 2006; Diamond and Kingsolver, 2010). For example, *M. sexta* feeding on poorer-quality food increased final body size with elevated temperature, while individuals feeding on richer-quality food decreased final body size (Diamond and Kingsolver, 2010). Parasitism, which reduced the host's access to nutritional benefit from feeding, did not seem otherwise to influence temperature effects.

<u>High temperature effects on parasitized and unparasitized larvae early and late in</u>

development

Lower temperature early in their development followed by higher temperature later in development (LH temperature treatment combination) reduced the maximum body mass of parasitized larvae to approximately the maximum body size of unparasitized larvae under the same conditions. At low temperatures throughout the development, parasitized *A. ipsilon* larvae grow approximately 50% larger than unparasitized larvae, reflecting the extended period of host development attributable to manipulation by the parasite (Saeki et al., 2009). Reduced host enlargement in the LH treatment combination suggests that the transition to higher temperature at about the time of larval eclosion may have inhibited the ability of *C. bakeri* to manipulate the host feeding and development time. However, parasitized larvae in HH temperature treatment combination did not reduce the maximum host mass. The different responses in parasitized larvae between LH and HH temperature combination may suggest beneficial acclimation, in which organisms can perform best at the initial rearing temperature (Leroi et al., 1994; Huey and Berrigan,1996).

Though the higher temperature used in this study is within the natural range of conditions, I cannot entirely rule out the possibility that some of the observed responses reflected temperature stress. In fact, the optimal temperature for *C. bakeri* may be lower than for the host, *A. ipsilon*, stressing the parasite more than the host when temperature increases during development. *C. bakeri* is found in Canada and in the northern and central USA (Schaaf, 1972; Byers et al., 1993); Kentucky is the southern edge of the natural range. Studies across more different temperatures and a greater range could help clarify whether stress was involved in my results.

Temperature effects on wasp brood size, wasp body mass and the size-number trade-off

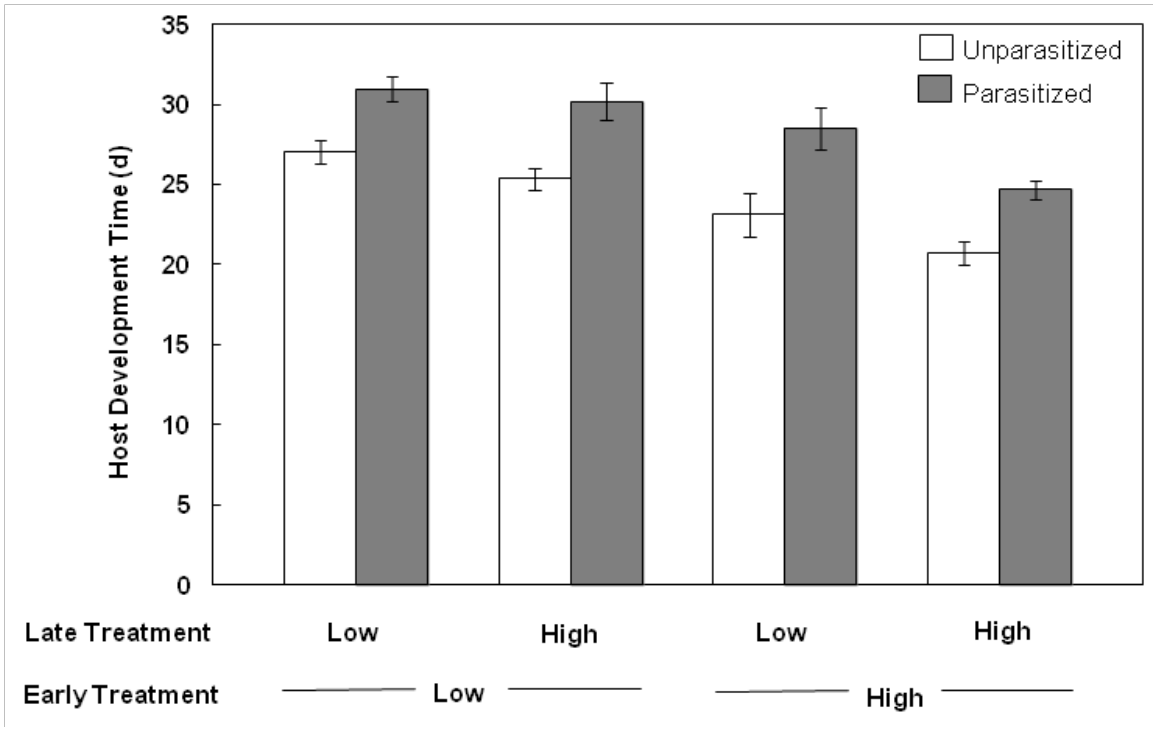
The body mass of *C. bakeri* did not differ significantly across temperatures, but, secondary brood size increased when temperature early in host development was high. High temperature may have stimulated the embryo, which undergoes divisions during early in host development, resulting in greater brood size.

There was no sex-specific shift in the allocation pattern for the trade-off between wasp body mass and brood size (in contrast to Saeki et al., 2009). In insects, females are often more plastic in body size (Stillwell et al., 2010), influencing the magnitude of sexual size dimorphism (e.g. Hu et al., 2011). The degree of phenotypic plasticity between the sexes may reflect different selection pressures on each sex (e.g. Vertainen et al., 2000; De Block and Stoks, 2003). For example, females under selection for larger body size and males under selection pressure for earlier emergence may respond differently to high temperature causing increased growth rate (Fischer and Fiedler, 2000). For *C. bakeri*, however, body size and development time are considerably constrained by the host's development. But the absence of a sex-specific response to temperature during development may result from strong selection on both body mass and brood size in both sexes, based on the post-emergence adult environment (see Crowley and Saeki, 2009).

Surprisingly, the exponent of the power function expressing the trade-off relationship illustrated in Fig. 5.3 was not -1, as expected for the inverse relationship found in previous work (Saeki et al. 2009, Saeki and Crowley, submitted); instead, the 95% confidence interval was [-0.96, -0.64]. The observed pattern suggests that in this case wasp broods may have been able to utilize the host more efficiently as the brood size increased (Malausa et al., 2005).

Overall, my results indicated that temperature effects on growth rate and development time in this host-parasitoid system seem to be de-coupled. Responses by *A. ipsilon* and *C. bakeri* to high temperature in my study were clearly stage-dependent, possibly with acclimation (see also Folguera et al., 2010; Potter et al., 2011). Moreover, temperature may affect performance in their next ontogenetic stage. For example, effects of temperature may extend to longevity, fecundity and host searching ability (Carrière and Boivin, 1997; van Baaren et al., 2005; Colinet et al., 2007; Niewiarowski and Angilletta, 2008; Boivin, 2010; Le Lann et al., 2011), which were not investigated here. Future work investigating performance in the adult stage of *C. bakeri* may provide a more thorough understanding of temperature effects in this interaction.

(a)



(b)

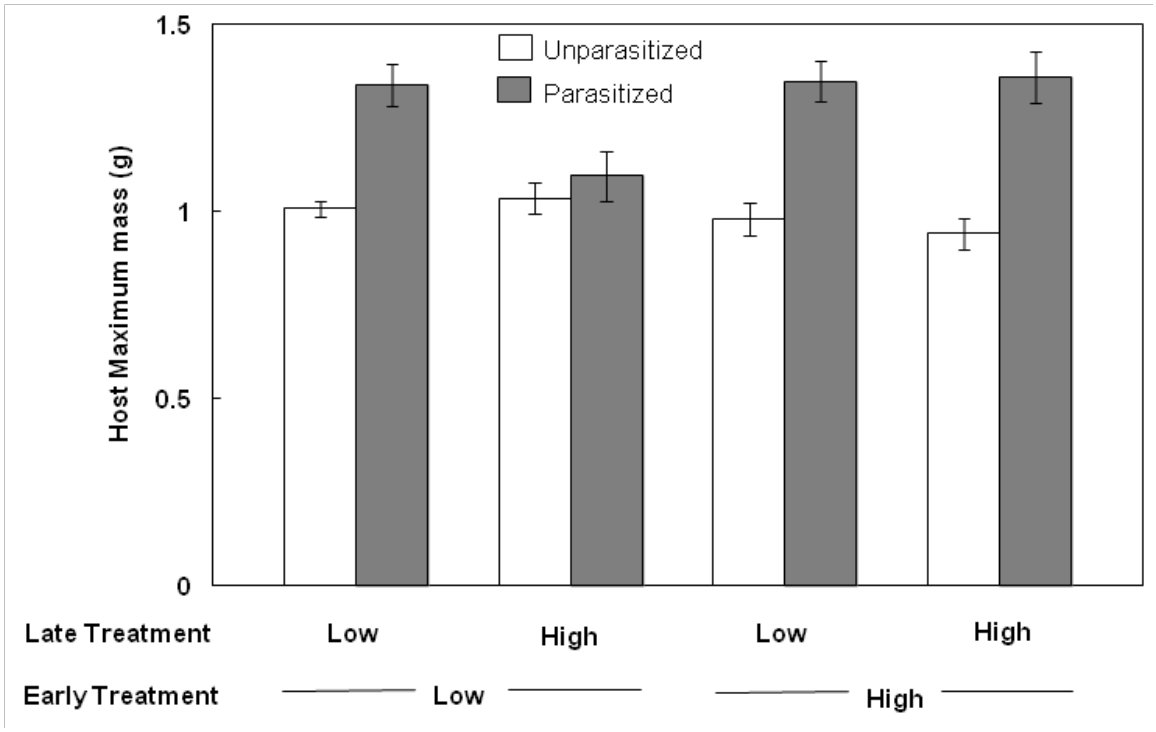
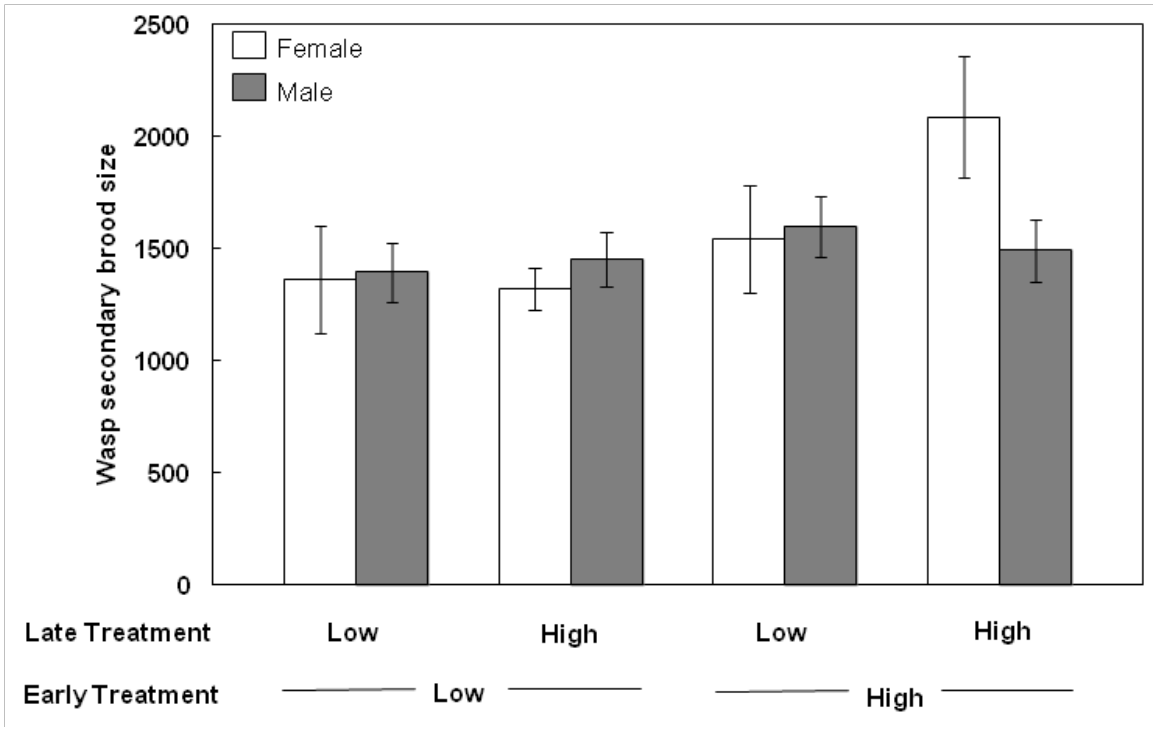


Figure 5.1

(a) Development time (from hatching to wandering phase in days) of parasitized and unparasitized larvae reared at each temperature treatment (mean±SE). (b) Maximum wet mass of parasitized and unparasitized larvae reared at each temperature treatment (mean±SE).

(a)



(b)

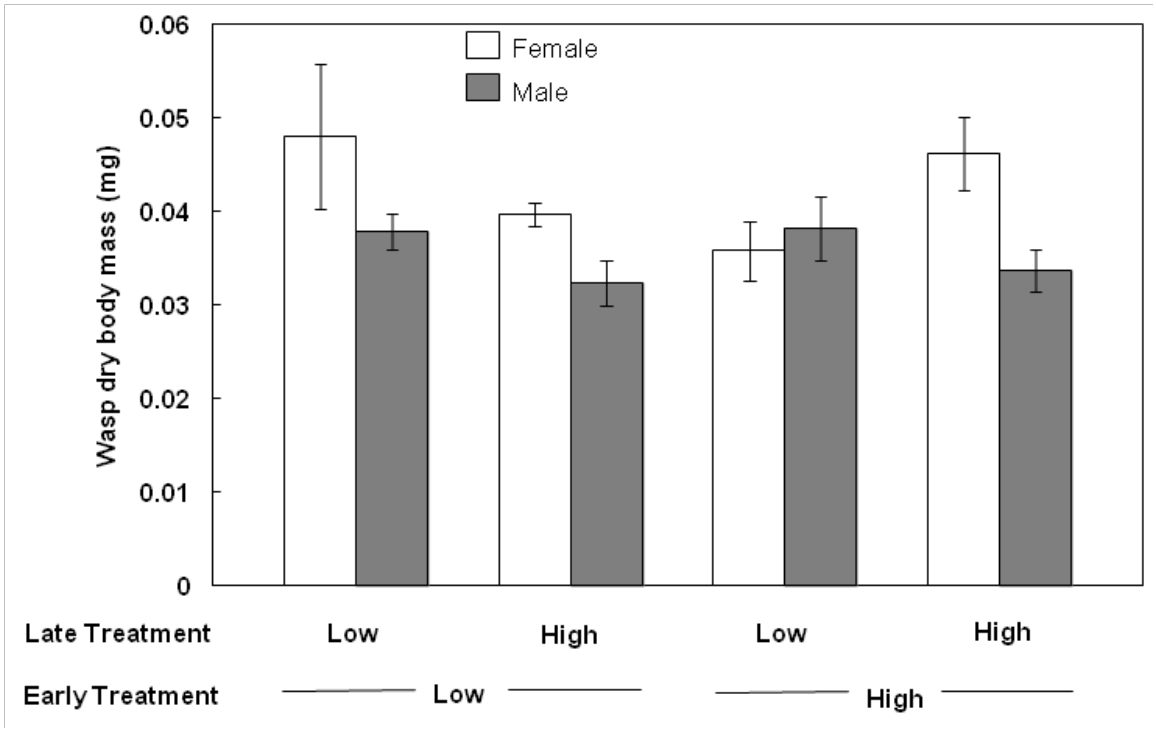


Figure 5.2
(a) Wasp secondary brood size each temperature treatment (mean±SE). (b) Wasp dry body mass at each temperature treatment (mean±SE).

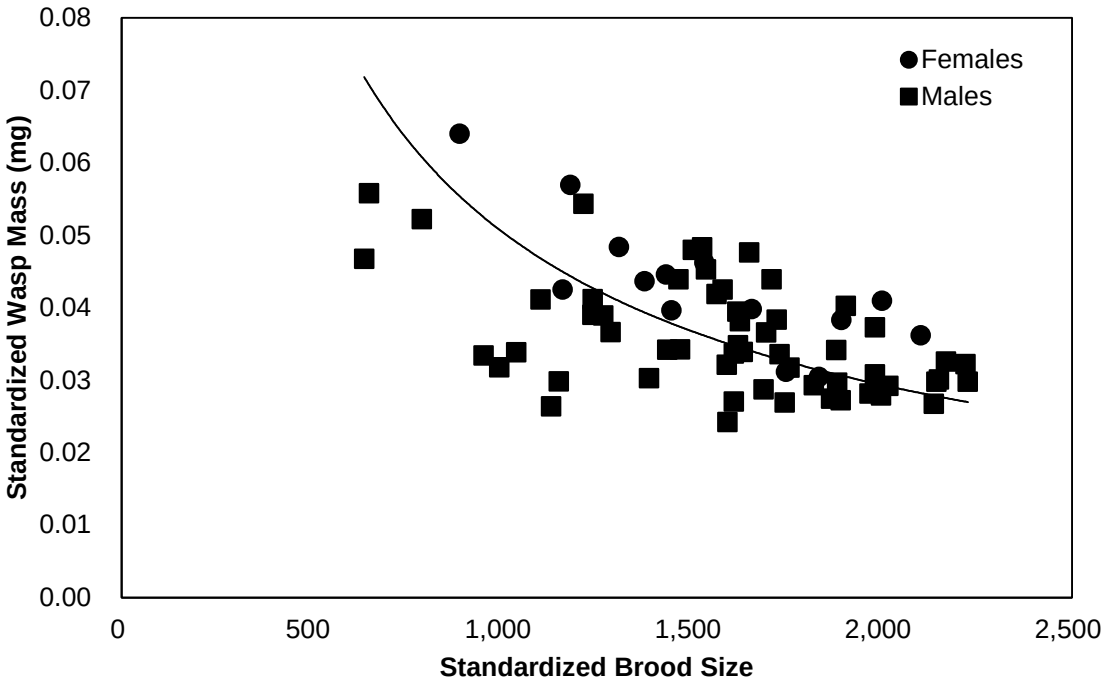


Figure 5.3
Relationship between standardized wasp body mass (mg) and standardized wasp secondary brood size. Squares represent females and filled circles represent males. The best-fit line drawn is obtained from the power function$Y = aX^b$, where Y is standardized wasp mass, X is standardized brood size, a=11.3 (95%CI [3.49, 36.4]) and b= -0.78 (95%CI [-0.96, -0.64]).

Copyright © Yoriko Saeki 2012

Chapter 6 The size-number trade-off and components of fitness in clonal parasitoid broods

Introduction

Body size at maturity is often highly correlated with lifetime reproductive success (= fitness) of organisms through close relationships with survival, fecundity, and mating competitiveness (reviewed in Stearns, 1992; Blanckenhorn, 2000). Moreover, body size at maturity plays a central role in trade-off relationships with other life history traits, such as time to maturity (Stearns, 1992). Body size may thus be under selection in nature.

Effects of body sizes on fitness may be sex-specific, resulting in sexual size dimorphism (reviewed in Blanckenhorn, 2000, 2005). Positive relationships between female body size and fecundity are well-documented in many ectotherms (Roff, 1981; Shine, 1988; Honěk, 1993). For males, selection may influence body size through mechanisms such as female choice and male-male competition (van den Assem, 1989). Larger body size may provide greater competitiveness in combating rivals to mate (Thornhill & Alcock, 1983), or there may be female preference for larger males (Uhl *et al.*, 2004). On the other hand, smaller body size may be favored when food supply is limited (reviewed in Blanckenhorn, 2005; Blanckenhorn *et al.*, 2011). Smaller body size may also be advantageous for males in the context of a trade-off with development time. For example, in protandrous species, smaller body size in exchange for early emergence often allows better access to females (Fischer & Fiedler, 2000).

Body size affects fitness of parasitoids via fecundity and longevity but also through components specific to parasitic wasps, such as searching efficiency for hosts (Visser, 1994). For example, in the hymenopteran parasitoid *Trichogramma carverae*, the body sizes of females found at oviposition sites are larger than those found at emergence sites, indicating that larger female parasitoids may locate hosts more efficiently or survive longer (Bennett & Hoffmann, 1998). Also,

larger *Anagyrus kamali* oviposited at a greater daily rate (Sagarra *et al.*, 2001).

Although body size is expected to affect fitness, parasitoid body size may partly depend on the host size (Vinson & Iwantsch, 1980; Charnov *et al.*, 1981; Lawrence, 1986; Reudler Talsma *et al.*, 2007; but see Harvey *et al.*, 2000; Harvey *et al.*, 2004). For example, males of the parasitoid wasp *Dinarmus basalis* emerging from smaller hosts were smaller, with reduced sperm storage and less success in competing for mates (Lacoume *et al.*, 2006).

In this study, I examined effects of body size of the polyembryonic parasitoid *Copidosoma bakeri* (Howard) on longevity, fecundity, and mating competitiveness. *C. bakeri* is a koinobiont parasitoid, undergoing its development in a developing host, and the need for synchronization constrains the timing (Baehrecke & Strand, 1990). *C. bakeri* usually produces a brood of approximately 2000 clonal offspring within a host, with body size and brood size in a strong trade-off relationship (Saeki *et al.*, 2009). My previous study found female-biased sexual size dimorphism (Saeki *et al.*, 2009), with the allocation pattern of the offspring size-number trade-off responding flexibly to maximum host mass (Saeki & Crowley, submitted). To understand how selection might influence fitness-related traits linked to body size after emergence, I addressed the following questions: (1) Is longevity positively related to body size in both sexes?, (2) Is potential fecundity positively related to female body size?, and (3) Is male mating competitiveness positively related to male body size?

To investigate fecundity, it is important to establish whether *C. bakeri* is pro-ovigenic or synovigenic. Some parasitoids, such as *Copidosoma floridanum*, are pro-ovigenic, in which all eggs for the female's life mature by the time of emergence (Grbić, 2003; Jervis *et al.*, 2003). In such cases, the body size at emergence may be the major influence on fecundity, whereas other factors come into play if the mature female continues to generate and mature new eggs (i.e. she is

synovigenic). Therefore, I also examined whether *C. bakeri* is pro-ovigenic or synovigenic and whether mating events induced egg production after emergence.

Methods

Study species

Copidosoma bakeri (Howard) is an egg-larval parasitoid of noctuid moth species (Schaaf, 1972). *C. bakeri* is polyembryonic: the wasp embryo divides into multiple embryos within the host. Wasp embryos continue to develop until they pupate within the host (see Saeki *et al.*, 2009 for details of the development of *C. bakeri*). Usually, the adult female lays one egg into a host at the egg stage; therefore wasps emerging from the host are usually all clonal individuals derived from one embryo. The approximate brood size, which is the number of adult wasps emerging from the host, is 1800 for females and 2200 for males, with females usually larger-bodied than males (Saeki *et al.*, 2009).

The host species I used to rear *C. bakeri* was the black cutworm, *Agrotis ipsilon* (Hufnagel; Lepidoptera: Noctuidae). I have cultured *C. bakeri* with the host *A. ipsilon* in a laboratory colony since 2005. I conducted all experiments in 2008.

Empirical methods

Longevity

I isolated 5 individual wasps from each newly emerging wasp brood in glass test tubes with a piece of filter paper soaked with water and sealed the tubes with cotton (female: 19 broods; male 21 broods). I incubated them in a growth chamber (daytime: 28±1°C; nighttime: 26±1°C) until they died. The water was refilled daily. After I observed death and recorded longevity in days, I dried the wasps in the oven (57°C; Yamato) over night and weighed them with a micro-balance (CAHN C31; wasp individual dry mass). I froze the host including the remaining wasp brood after taking

the samples and then isolated adult wasps, undeveloped larvae and pupae from the host carcass. The isolated wasp brood, host and undeveloped larvae and pupae were dried at 57°C overnight and weighed. I also weighed 2 groups of 20 adult wasps with the micro-balance and calculated mean wasp dry mass. For analyses, I calculated mean wasp dry mass from the measurement of 2 groups of 20 individuals.

Potential fecundity

On the day of female wasp brood emergence, I isolated 21 individuals from the brood in individual test tubes narcotized with carbon dioxide (N = 10 broods). The remaining wasps were frozen for the measurement of the whole brood and wasp dry mass. I introduced approximately 10 males into each of 9 test tubes containing a female wasp, and observed their mating (mated treatment). After mating, I removed the males. For the virgin treatment, 3 females were weighed (wet mass) with the microbalance (CAHN C31) while narcotized with carbon dioxide. Ovaries of these females were isolated in Pringle's solution, and ovarioles were separated with trypsin solution by adding a cover slide and gently pressing the ovaries. I counted the eggs and measured length and width of 3 randomly chosen eggs under a dissection microscope (50× magnification). Nine females from the virgin treatment and 9 females from the mated treatment were allocated to 3-day, 7-day, and total-lifespan treatments (3 females each), to be dissected in the same manner as above on the designated day. These females were separately incubated in test tubes with a piece of filter paper soaked with sugar solution (20%) in a growth chamber. The sugar solution and filter paper were replaced daily. For measurement of the whole brood, I counted the number of individuals in the brood and weighed the whole brood and 2 groups of 20 individuals with the micro-balance after drying them.

Male mating competitiveness:

I isolated 2 males from different newly emerging broods in a glass test tube. I then introduced a virgin female into the test tube. I recorded the time of mounting and of beginning and completing copulation (N = 20 groups of 3). I then isolated the mating and non-mating males individually in centrifuge tubes and froze them. I dried the males in the oven overnight and weighed them with the microbalance.

To examine the effects of mating on longevity, newly emerging male wasps were isolated in test tubes. Except for control males, I introduced five females per male and observed the mating behavior. Once the male paired up with a female, the other 4 females were removed. I recorded the times of mounting and of starting and completing copulation. After copulation, the female was removed. Another group of 5 females was introduced with each male 20 minutes later, and their mating was recorded in the same manner. Males were allowed to mate for either 2 or 3 times a day until death.

Analyses

All data were analyzed with SPSS (SPSS Inc., version 15.0, Chicago, IL, USA).

Longevity

I regressed the mean longevity with mean wasp dry mass of the brood, which was obtained from the dry masses of genetically identical siblings right after emergence, to complement the analyses above. To examine the association of longevity with body mass of female and male wasps, I also conducted an ANCOVA test (independent variable: sex and sex x wasp dry mass; random factor:

brood identity of the wasp; dependent variable: longevity).

Potential fecundity

Since eggs of dead female wasps were often ruptured, and some eggs might have been resorbed, I excluded dead females from the fecundity analyses. Egg volume (μl) was estimated as 0.52 × Length (mm) × $Width^2$ (mm) (Preston, 1974). I calculated means of 5 estimated egg volumes for each female.

I examined the correlation between the number of eggs and mean egg volume. Mean values of each brood were used to avoid pseudoreplication.

I conducted regression tests to analyze the effect of the female wet mass on the dependent variables number of eggs, egg volume, and total egg volume (the number of eggs × mean egg volume), including brood identity as a random factor. For these analyses, I used the data obtained immediately following emergence to exclude influences from the post-emergence environment.

To examine whether *C. bakeri* matures or produces eggs after emergence, I conducted ANOVA tests, using only unmated females on the 1st, 3rd and 7th days after emergence (dependent variables: egg number, egg volume, or total egg volume; independent variable: age; random factor: brood). I also examined age and mating effects on egg production and maturation with ANOVAs (dependent variables: the number of eggs, mean egg volume, or total egg volume; independent variables: mating status, days after emergence; and random factor: brood identity of the dissected female).

I also examined whether or not mortality increased with time after females emerged with logistic regression.

Male mating competitiveness:

I performed a paired t-test to test whether successfully mating males differed consistently in size from non-mating males. To test if mating was size-assortative, I examined whether the correlation coefficient for females and non-mating males was contained within the confidence interval of the correlation coefficient for females and mating males.

I examined whether time to mounting, time to copulation and copulation duration were correlated and then analyzed the relationship to male body mass with regressions. I also examined effects of mating on longevity in relation to male body mass.

Results

Longevity

Female longevity was positively related to mean wasp dry mass ($F_{1.17}$ = 16.0, P < 0.01, R^2 = 0.49; Fig. 6.1), but not for males ($F_{1.19}$ = 1.28, P = 0.27, R^2 = 0.06). The relationship between longevity and wasp dry mass was significantly different between the sexes (ANCOVA: $F_{2,136}$ = 3.6, P = 0.03). Wasp brood identity accounts for 41.6% of the variance in longevity.

Potential fecundity

There was no significant overall relationship between the number of eggs and mean egg volume (R = -0.52. P = 0.12; but note the negative trend) or between female wasp wet mass and the number of eggs, ($F_{1, 26.9}$ = 0.86, P = 0.36). Brood accounts for 72.0% of the overall variance in the number of eggs (P = 0.05). Mean egg volume had no significant relationship to female wasp wet mass ($F_{1,22.3}$ = 0.18, P = 0.67); 34.3% of the variance in mean egg volume was accounted for by brood (P = 0.23). Total egg volume was not related to female wasp wet mass ($F_{1,13.7}$ = 0.12, P = 0.74), and

brood accounts for little of the variance in the total egg volume (2.0%, $P = 0.93$).

Looking at the time course of these relationships proved instructive. Female wasps examined immediately after emergence had fewer eggs than females examined 3 days after emergence ($F_{2,59.5} = 4.07$, $P = 0.02$; Fig. 6.2a), and 46.1% of the variance in the number of eggs was attributable to brood identity (Wald Z test: $P = 0.06$). Post-hoc tests showed that the number of eggs on the third day was significantly greater than the first day after emergence ($P = 0.03$), but there was no significant difference in the number of eggs between the 3rd and 7th days ($P > 0.9$) or between the first and 7th days after emergence ($P = 0.18$). Egg volume increased significantly with time after emergence ($F_{2,54.5} = 7.62$, $P < 0.01$; Fig. 6.2b), but the brood effect was small (4.72%, Wald Z test: $P = 0.69$). Egg volume on the 7th day was significantly larger than that on the first and 3rd days after emergence ($P < 0.01$ in both cases) though those on the first day and the 7th day did not differ ($P > 0.9$). Total egg volume increased with age ($F_{2,53.7} = 11.0$, $P < 0.01$), especially by the 7th day (1st day vs 3rd day: $P = 0.45$; 1st day vs 7th day: $P < 0.01$; 3rd day vs 7th day: $P = 0.01$; Fig. 6.2c). The brood accounted for 16.7% of the variance in the total egg volume (Wald Z test: $P = 0.23$).

Mated females contained more eggs than unmated females independent of age (mating status: $F_{1,77.4} = 4.25$, $P = 0.04$, Fig. 6.3; age: $F_{1,77.9} = 0.01$, $P = 0.91$). There was no significant interaction between mating status and age ($P = 0.11$). Brood identity accounted for 57.9% of the variance in the number of eggs. On the other hand, regardless the mating status, females increased egg volume by the 7th day after emergence (mating status: $F_{1,66.1} = 0.02$, $P = 0.88$; age: $F_{1,72.5} = 5.99$, $P = 0.02$), and there was no significant interaction between mating status and time after emergence ($P = 0.17$). The broods contributed 8.1% of the variance in the mean egg volume. Total egg volume increased significantly with age ($F_{1,70.7} = 6.48$, $P = 0.01$) but not with mating status (F

$_{1,66.1}$ = 0.99, P = 0.32). The effect of broods was small (21.2%, Wald Z test: P = 0.12).

In the experiments regarding fecundity with time effects, mortality of samples increased with time after emergence, t (*Probability of death* = $1.45^t + 0.02$, $P < 0.01$).

Male mating competitiveness

Mating males were neither larger nor smaller in dry mass than non-mating males (Wilcoxon signed ranks test: Z = -0.28, P = 0.78). The correlation coefficient for dry masses of females and mating males was 0.35 with a 95% CI of [-0.13, 0.69] (P = 0.14) and for females and non-mating males was 0.40 with a 95% CI of [-0.06, 0.73] (P = 0.09). These broadly overlapping confidence intervals do not support size-assortative mating.

None of the pair-wise combinations of time to mounting, time to copulation, and copulation duration was significantly correlated (time to copulation and time to mounting: R = -0.01, P = 0.61; time to copulation and copulation duration: R = -0.14, P = 0.43; time to mounting and copulation duration: R = 0.04, P = 0.83). Time to mounting, time to copulation, and copulation duration were not significantly related to male body mass (time to mounting: $F_{1,17}$ = 1.77, P = 0.20, R^2 = 0.09; time to copulation: $F_{1,17}$ = 0.02, P = 0.89, $R^2 < 0.01$; copulation duration: $F_{1,17}$ = 1.03, P = 0.33, R^2 = 0.06).

Mating significantly decreased male longevity independent of body mass (ANCOVA; mating: $F_{2.42}$ = 40.7, $P < 0.01$; body mass: $F_{1.42}$ = 1.20, P = 0.28; Fig. 6.4). Post-hoc tests showed that the number of matings per day (2 vs 3 times) did not affect the longevity of mating males (P > 0.9), but non-mating males lived longer than mating males (0 times vs 2 times: $P < 0.01$; 0 times vs 3 times: $P < 0.01$).

Discussion

Larger females had greater longevity, indicating that body mass influences a key component of lifetime reproductive success. It is important to note that wasps in this experimental design were provided with water but no food. Thus, longevity here may reflect the size-dependence of energy (e.g. fat stores) possessed by the wasp at emergence, or the efficiency of energy use, which can influence survival early in the adult stage (Blanckenhorn *et al.*, 2007). On the other hand, there was no relationship between wasp body mass and longevity in males. Thus in terms of viability at and shortly after emergence, larger body size in male *C. bakeri* may not be as advantageous as in females, perhaps helping to account for the female-biased sexual size dimorphism documented in previous work (Saeki *et al.*, 2009).

I did not find significant relationships between female body size and potential fecundity, despite the typically positive relationship detected in many other wasp species (Shine, 1988; Honěk, 1993), including other *Copidosoma* species (Ode & Strand, 1995). Yet brood identity of adult females accounted for 71% of the variance in the number of eggs, indicating that the number of eggs may reflect variable genetics associated with a mixture of reproductive strategies, or nontogenetic maternal effects associated with different broods and thus different hosts, making size relationships that may be present difficult to detect. Alternatively, perhaps oviposition opportunities for smaller females are much more variable and unpredictable in terms of locating hosts than for larger females, making it advantageous for smaller females to contain more eggs per unit mass and thus similar total numbers to those of larger females. This unexpected size-independence of egg number requires attention in future work.

I found *C. bakeri* to be weakly synovigenic, in which a modest proportion of the total eggs are produced after emergence. Since *Copidosoma floridanum* is completely proovigenic (Grbić,

2003; Jervis *et al.*, 2003), and smaller koinobiont parasitoid species like *C. bakeri* are typically pro-ovigenic (Jervis *et al.*, 2003; Jervis *et al.*, 2008), the possibility that some eggs already present were too small to be counted at emergence remains a plausible explanation for my results. Yet synovigeny is common overall (> 98%) in parasitoid wasps (Jervis *et al.*, 2001). In addition, when probabilities of finding and parasitizing hosts are low (e.g. when hosts are patchily distributed), parasitoid wasps tend to be synovigenic, perhaps to invest more in survival until hosts are available (Ellers *et al.*, 2000; Ellers & Jervis, 2003). Thus, my results may suggest relatively low encounter rate with hosts in *C. bakeri*, with synovigeny providing greater realized fecundity (actual number of eggs oviposited) to larger females that live longer. Larger females may have a greater tendency to be synovigenic, using their larger size to retain greater flexibility over investment in eggs over a longer life, a possibility worth testing.

In addition, females matured their eggs by increasing egg volume at the 7th day after emergence. In other species, larger eggs often correlate positively with larval size hatching from the egg, and hatchling's survival to adulthood and growth rate (McLain & Mallard, 1991; García-Barros, 2000; Fischer *et al.*, 2002; Boivin & Gauvin, 2009, but see Lalonde, 2005). Whether these advantages apply to *C. bakeri*, which develops polyembryonically from a yolkless egg and obtains resources from the host, needs further investigation.

In addition, evaluating the performance of larger eggs laid by older females in terms of brood size, brood mass, and wasp mass might help us to understand the relationship between wasp egg maturation and reproductive success. It should be noted that there was a significant increase in mortality with time after emergence. Thus, non-random mortality effects could account for the increase in the number of eggs and egg volume, in which that females with more and larger eggs may have survived longer, skewing my results. Finally, increasing egg volume with age may

simply reflect a gradual increase in egg hydration through time. This explanation seems consistent with generally low contributions of brood identity to egg volume. Dry weights of individual eggs removed at different times since emergence could help evaluate this possibility in future work.

The tendency of mated females to produce more eggs than virgin females at the 7th day after emergence was also unexpected. Synovigenic species may acquire extrinsic resources for egg production after emergence (Ellers & Jervis, 2003), perhaps including the seminal fluid transferred with sperm (Boggs & Gilbert, 1979; Heifetz *et al.*, 2001; Liana, 2005; Zhao & Zhu, 2011). Alternatively, females may not start maturing new eggs until mating. Facilitated egg production or maturing after mating may allow females to maximize opportunities to lay female eggs, which may confer higher fitness at low population densities (Crowley *et al.*, 2009).

In contrast to females, male body mass may not affect fitness in terms of mating success. *C. bakeri* females do not seem to choose males based on size. It has been documented that both large and small males can have relative advantages in sexual selection (larger males: van den Assem *et al.*, 1989; Fairbairn & Preziosi, 1994; smaller males: Blanckenhorn, 2000). For *C. bakeri*, the scramble mating system without courtship (Y.S. personal observation) and possible nutritional contributions from sperm (see above) may reduce female choosiness for males.

The mating-and-longevity experiment showed that mating reduced male longevity, indicating a survival cost of mating. Mating costs for males have been reported (Wang *et al.*, 2005; Oliver & Cordero, 2009), and copulation, courtship, and production of ejaculate can be physiologically demanding (Cordero, 2000; Burton-Chellew *et al.*, 2007). Since the male *C. bakeri* immediately mounts the female without apparent courtship (Y.S. personal observation), physiological costs may derive from copulation and ejaculate production. If males transfer nutritious substances with sperm to enhance egg production of the female, the investment by males

may be substantial, and the investment per mating could be size dependent (Burton-Chellew *et al.*, 2007). In addition, the experimental setting in which males were provided only water might have reduced longevity of mating males because the *replenishing* of sperm is energetically demanding (Härdling *et al.*, 2008).

Overall, larger body size increased longevity of females but not males. Although I did not detect a positive relationship between body size and potential fecundity, larger females may gain advantages through increased mating, host finding, and ovipositing opportunities by living longer. The difference in the effects of body mass on longevity between males and females implies sex-specific selection pressures on body mass. I observed effects of brood identity on fecundity, suggesting a role for genetic or host-associated maternal differences.

I have attempted to evaluate some of the implications for reproductive success of the sex-specific trade-off between brood size and body mass observed in *C. bakeri*. My previous work showed flexible shifts in both brood size and body mass depending on host quality, while female-biased sexual size dimorphism was robust. The mechanisms underlying these determinations of brood size and body size have been examined during the pre-emergence interval (Saeki *et al.*, 2009; Saeki and Crowley, submitted manuscripts). In the current study, I found that body size has different effects on males and females following emergence, in accord with the female-biased size dimorphism at emergence. Three main selection pressures have been proposed to account for sexual dimorphism: fecundity of the female, sexual selection on the male, and viability selection on both. These must counter-balance each other to stabilize the body size of each sex (Blanckenhorn, 2000, 2005). For parasitic species, host and mate searching efficiency and oviposition success in relation to body size are also major influences on adult female fitness (Mackauer, 1996; Crowley & Saeki, 2009; Crowley *et al.*, 2009). The size effects on these

components, particularly efficiency of host location and successful oviposition, need further attention.

Finally, in the case of polyembryonic broods derived from a single egg, fitness is maximized at the level of the whole brood of genetically identical individuals (Godfray, 1994). Thus, the optimal body mass and brood size in such cases should be considered the best packaging of the brood mass into a certain total number of individual body masses; this maximizes the fitness of the whole brood by achieving the best allocation pattern of body size and number of offspring.

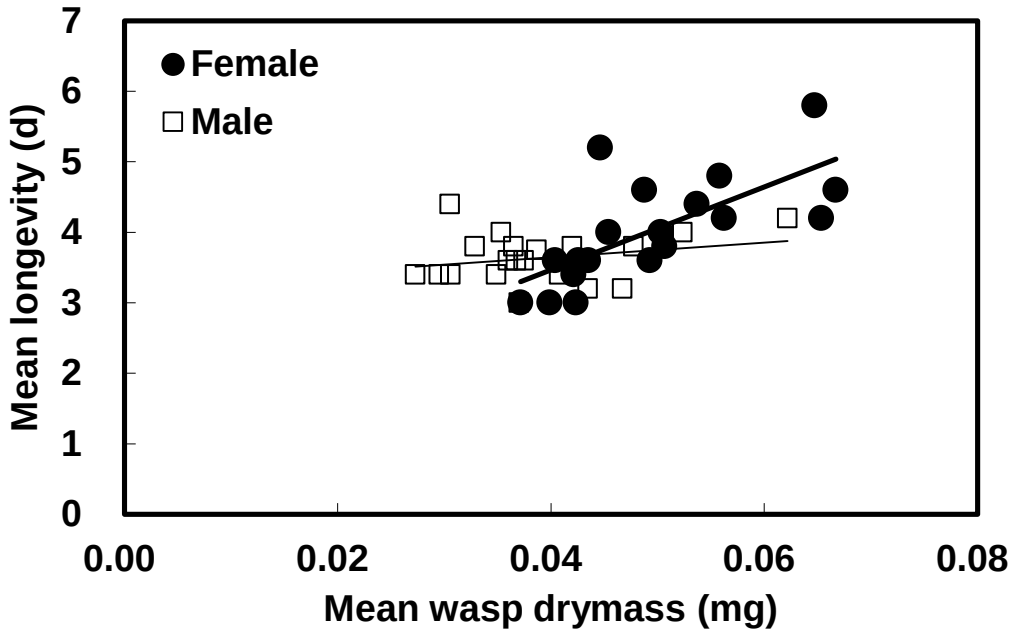
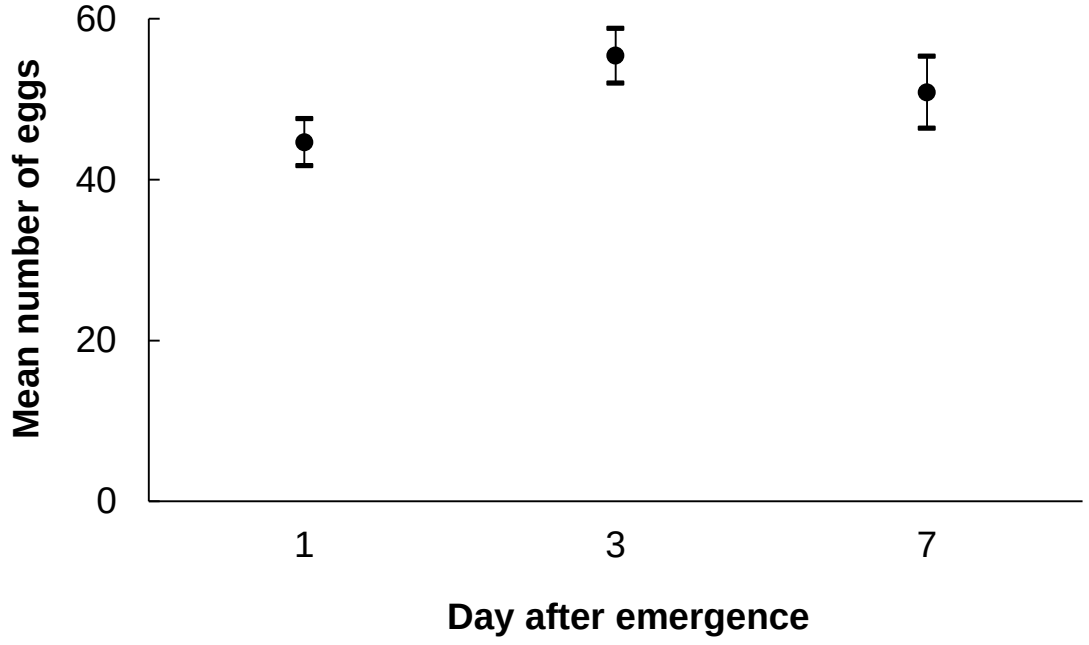
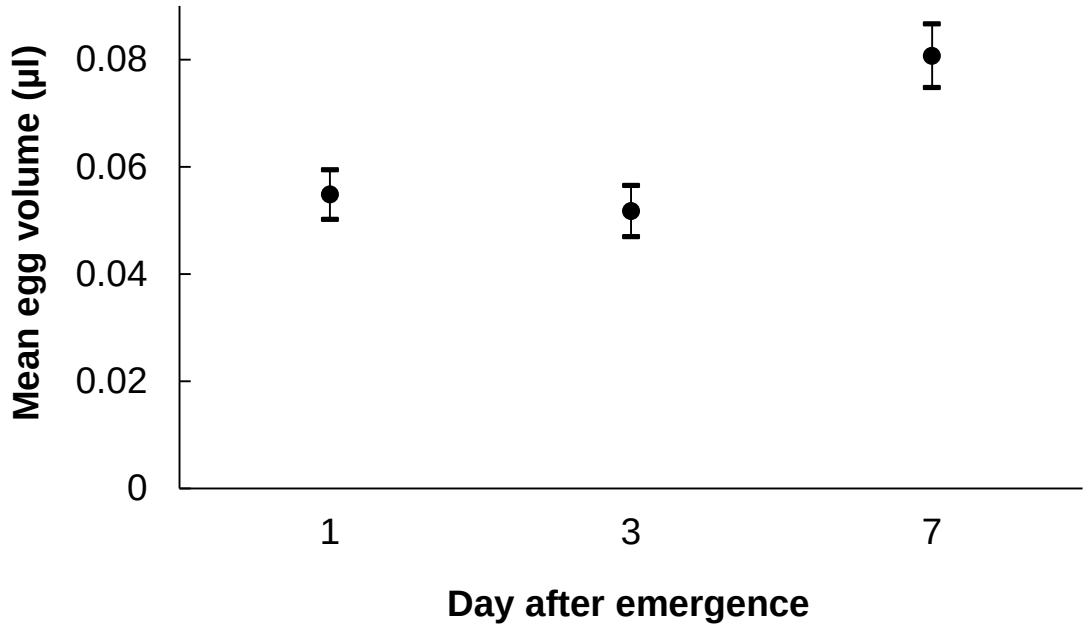


Figure 6.1
Relationships between wasp dry mass and longevity for females and males, where each point indicated brood means. The relationship was significant for females, but not for males. The best fitted line for the female is Y = a X + b (where a = 58.9 ± 14.7 days and b = 1.11 ± 0.74 days/mg).

(a)


60
40
20
0
Mean number of eggs
1
3
7
Day after emergence

(b)


0.08
0.06
0.04
0.02
0
Mean egg volume (µl)
1
3
7
Day after emergence

(c)

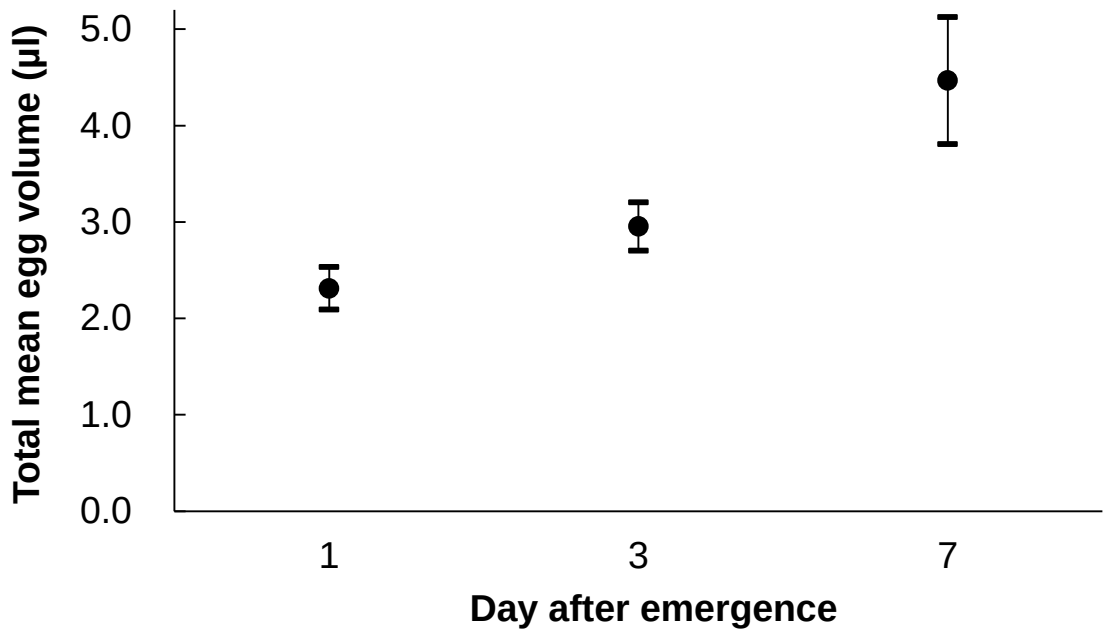


Figure 6.2
(a) Mean number of eggs (± 1 SE) at 1st, 3rd, and 7th days after emergence. (b) Mean egg volume in µl (± 1 SE) at age 1st, 3rd, and 7th days after emergence. (c) Mean total egg volume in µl (± 1 SE) at age 1st, 3rd, and 7th days after emergence.

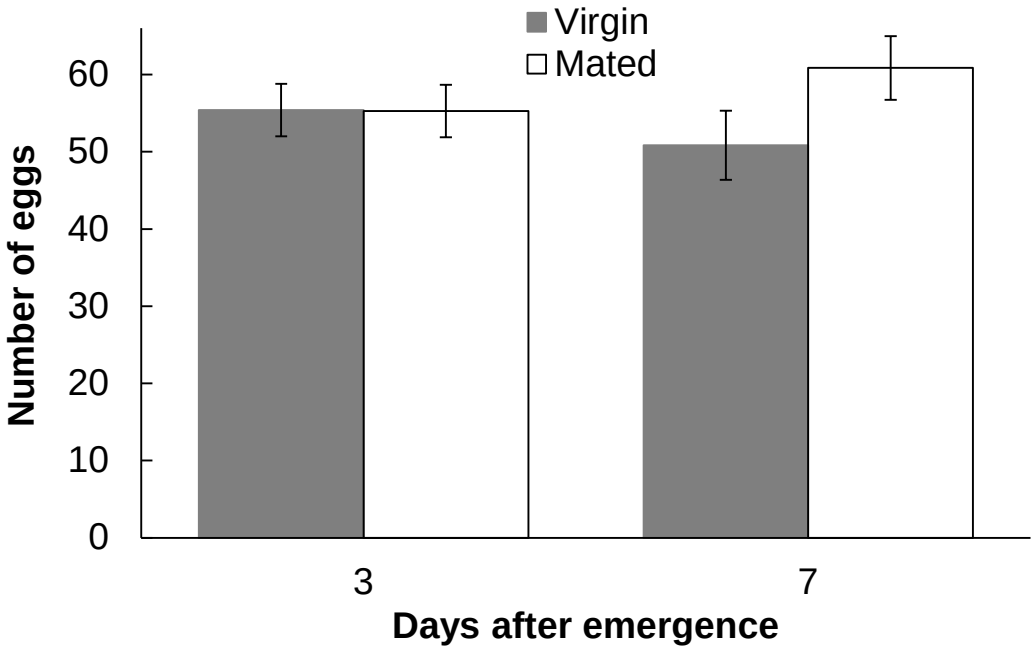


Figure 6.3
Effects of mating on mean (± 1 SE) egg maturation or production.

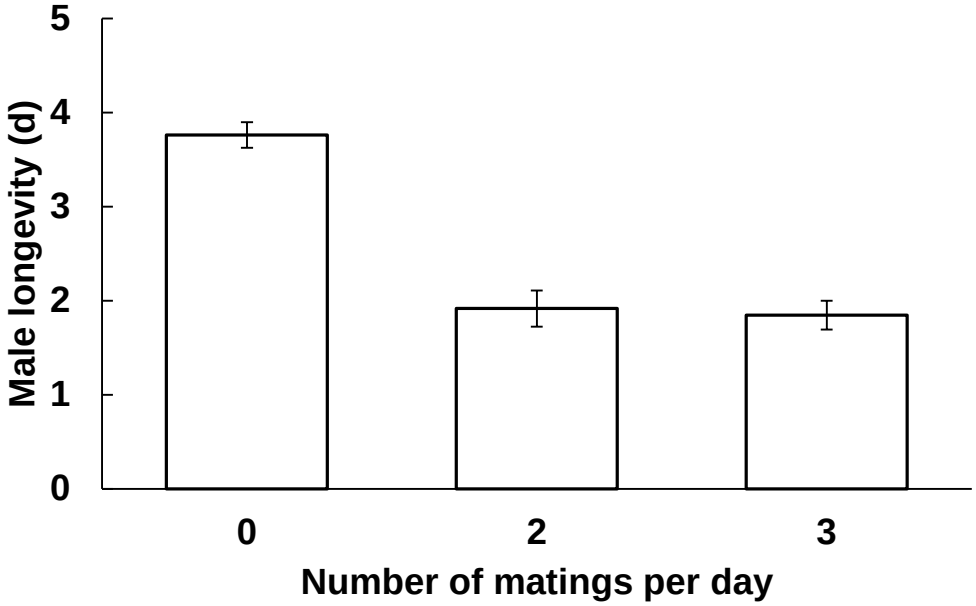


Figure 6.4
Effects of mating on male longevity in days (means ± 1 SE).

Copyright © Yoriko Saeki 2012

Chapter 7 Key Findings and Future Directions

In this chapter, I summarize findings from the series of studies that I have conducted and indicate the main issues raised and opportunities for follow-up work.

I reviewed empirical and conceptual studies focusing on the types and shapes of trade-off relationships, classified as linear, convex (usually inverse), and concave (chapter 2). These distinctions reflect the constraint responsible for the trade-off and the relationships between components of the trade-off. Models have predicted curve shapes, curve locations, and the positions of allocation patterns on the curve, depending on the environment. Surprisingly, empirical documentation of curves representing trade-off relationships seems uncommon.

I then investigated a sex-specific size-number trade-off in *Copidosoma bakeri* and its interaction with the host by observing the growth of the parasitized and unparasitized host in chapter 3. The parasitoid body mass and brood size were traded-off in an inverse relationship, because the brood mass constraint imposed by maximum mass of the host limits the multiplicative product of body mass and brood size. Female and male broods differed in their allocation patterns between body mass and brood size: female body mass was larger than male body mass, but male brood size was larger than female brood size. The difference in the allocation patterns between females and males suggests different selection pressures on each sex requiring future investigation.

In chapters 4 and 5, I investigated responses to different environments of wasp brood size and body mass in the size-number trade-off. I first examined effects of resource availability in different developmental stages on the sex-specific trade-off between brood size and body mass in chapter 4. In accord with some models, greater maximum host mass resulting from more resources shifted the trade-off relationship between wasp body mass and brood size for both sexes toward

both greater wasp brood size and greater individual body mass. On the other hand, when the host developed faster, the trait combinations shifted along the *same* trade-off curve toward greater wasp body mass but smaller brood size. In Chapter 5, my objective was to examine responses of wasp brood size and body mass to different temperatures. When parasitized *A.ipsilon* was exposed to high temperature only late in their development, the usual enlargement of the host by the parasitoid was reduced. *C. bakeri* brood size significantly increased when high temperature was applied early in host development, even though host final size was unchanged from the low temperature. There was no shift in the allocation pattern of the size-number trade-off with temperature, suggesting strong selection pressure to maintain body mass and brood mass of *C. bakeri* across a range of environmental conditions.

In Chapter 6, I tested effects of body mass on male and female reproductive success of *C. bakeri*. For females, I found that larger body size increased the longevity of females. In addition, I found that *C. bakeri* was slightly synovigenic, meaning that eggs keep being produced after maturation, and mated females produced more eggs than unmated females. However, I did not detect a relationship between body mass and fecundity. Thus larger females may increase egg production and opportunities to find mates and hosts by living longer. For males, there were no significant relationships between body mass and longevity or between mating competitiveness and body mass. Independent of body mass, mating reduced male longevity, suggesting a cost of mating. Females seem to gain more advantages from larger body mass than do males, suggesting sex-specific selection pressures on body mass resulting in female-biased sexual size dimorphism. The implications of larger egg size with greater female age, with possible implications for subsequent development, also deserve further study. Finally, body size effects on efficiency of host location and successful oviposition in cages, greenhouses, and the field are potentially important

follow-up studies.

In conclusion, I emphasize two major directions for advancing our understanding of the size-number trade-off using this polyembryonic parasitoid system: experimental analyses of effects of body mass and brood size on adult performance under natural (or near-natural) conditions, and more concrete, mechanistic physiological and developmental studies of parasitoid-host interactions. Finally, examinations of the potential role of bet hedging (Crowley & Saeki 2010) are essential to achieve a deeper understanding of the size-number trade-off in this fascinating system.

Throughout the dissertation, I looked at a subset of possible factors that would affect the size-number trade-off of the parasitoid wasp. Allocation patterns in size-number trade-offs are based on both genetics and plastic responses to different environments. Selection pressures in both mature and immature life stages play a role in shaping the genetic and plasticity-based allocation patterns. For example, development rate of immature larvae interacting with the host and searching ability of adult wasps for mates and hosts are both likely to be under strong selection. However, selection pressures at different life stages may imply different directions and magnitudes of selection for each trait. I assume that what I observed throughout my dissertation was the integration of selection from different life stages. Fully accounting for allocation patterns in the size-number trade-off thus requires examining the implications of selection for each life stage, a challenging goal for the future.

Copyright © Yoriko Saeki 2012

Appendix Standardizing brood size and body mass of parasitoids

Let n be brood size and y be body mass of parasitoids, and let h be maximum body mass of the host caterpillar. I assume that the total parasitoid brood mass ny is proportional to h (see Fig. 3.2), and thus $ny = kh$, where k is a constant of proportionality (independent of n, y, and h) representing the fraction of the maximum host mass attributable to the parasitoid brood. Let $\bar{h}$ be the mean maximum host mass over the set of hosts and parasitoid broods to be analyzed, and let the subscript i identify a particular host and brood, for which $n_i y_i = kh_i$.

For the ith host and brood, I define the standardized parasitoid brood size to be $N_i = n_i \sqrt{\frac{\bar{h}}{h_i}}$, and the standardized parasitoid body mass to be $Y_i = y_i \sqrt{\frac{\bar{h}}{h_i}}$, where standardization removes the effect of host mass from the magnitude of brood size and body mass. To see this, substitute for n_i and y_i in $n_i y_i = kh_i$, resulting in $\left[\frac{N_i}{\sqrt{\frac{\bar{h}}{h_i}}}\right]\left[\frac{Y_i}{\sqrt{\frac{\bar{h}}{h_i}}}\right] = kh_i$, or $N_i Y_i = k\bar{h}$. This means that N_i and Y_i will be inversely related to each other across all i independently of host mass.

Copyright © Yoriko Saeki 2012

References

Agnew, P., Hide, M., Sidobre, C., & Michalakis, Y. 2002. A minimalist approach to the effects of density-dependent competition on insect life-history traits. *Ecological Entomology* 27: 396–402.

Agrawal, A. A., Conner, J. K., & Rasmann, S. 2010. Trade-offs and negative correlations in evolutionary ecology in: Bell, Eanes, Futuyma, and Levinton (Eds.), *Evolution After Darwin: the First 150 Years*, Sinauer Associates. 243–268.

Allen, R. M., Buckley, Y. M., & Marshall, D. J. 2008. Offspring size plasticity in response to intraspecific competition: an adaptive maternal effect across life-history stages. *American Naturalist* 171: 225–237.

Angilleta, M. J. Jr., Niewiarowski, P. H., & Navas, C. A. 2002. The evolution of thermal physiology in ectotherms. *Journal of Thermal Biology* 27: 249–268.

Angilletta, M. J. Jr. & Dunham, A. E. 2003. The temperature-size rule in ectotherm: Simple evolutionary explanations may not be general. *American Naturalist* 162: 332–342.

Angilletta, M. J. Jr. 2009. *Thermal Adaptation: A theoretical and Empirical Synthesis*. Oxford University Press, Oxford.

Angilletta, M. J. Jr., Steury, T. D., & Sears, M. W. 2004. Temperature, growth rate, and body size in ectotherms: fitting pieces of a life-history puzzle. *Integrative and Comparative Biology* 44: 498–509.

Angilletta, M. J. Jr., Wilson, R. S., Navas, C. A., & James, R. S. 2003. Tradeoffs and the evolution of thermal reaction norms. *Trends in Ecology and Evolution* 18: 234–240.

Arendt, J. D. 1997. Adaptive intrinsic growth rates: an integration across taxa. *Quarterly Review of Biology* 72: 149–174.

Arnold, S. J. 1992. Constraints on phonotypic evolution. *American Naturalist* 140: S85–S107.

Arnott, S. A., Chiba, S., & Conover, D. O. 2006. Evolution of intrinsic growth rate: metabolic costs drive trade-offs between growth and swimming performance in *Menidia menidia*. *Evolution* 60: 1269–1278.

Atkinson, D. & Sibly, R. M. 1997. Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. *Trends in Ecology and Evolution* 12: 235–239.

Atkinson, D. 1994. Temperature and organism size – a biological law for ectotherms? *Advances in Ecological Research* 25: 1–58.

Atkinson, D. 1996. Ectotherm life-history responses to developmental temperature. In: Johnston, I.A., Bennett, A.F. (Eds.), *Animals and Temperature: Phenotypic and Evolutionary Adaptation. Cambridge University Press*, Cambridge, pp. 183–204.

Baehrecke, E. H. & Strand, M. R. 1990. Embryonic morphology and growth of the polyembryonic parasitoid *Copidosoma floridanum* (Ashmead) (Hymenoptera: Encyrtidae). *International Journal of Insect Morphology & Embryology* 19: 165–175.

Beckage, N. E. & Riddiford, L. M. 1982. Effects of parasitism by *Apanteles congregatus* on the endocrine physiology of the tobacco hornworm, *Manduca sexta*. *General and Comparative Endocrinology* 47: 308–322.

Beckage, N. E. 1997. The parasitic wasp's secret weapon. *Scientific American* 277: 50–55.
Bell, G. 1980. The costs of reproduction and their consequences. *American Naturalist* 116: 45–76.
Bennett, D. M. & Hoffmann, A. A. 1998. Effects of size and fluctuating asymmetry on field fitness of the parasitoid *Trichogramma carverae* (Hymenoptera: Trichogrammatidae). *Journal of Animal Ecology* 67: 580–591.
Berger, D. & Gotthard, K. 2008. Time stress, predation risk and diurnal-nocturnal foraging trade-offs in larval prey. *Behavioral Ecology and Sociobiology* 62: 1655–1663.
Berger, D., Friberg, M., & Gotthard, K. 2011. Divergence and ontogenetic coupling of larval behavior and thermal reaction norms in three closely related butterflies. *Proceedings of the Royal Society B: Biological Sciences* 278: 313–320.
Berner, D. & Blanckenhorn, W. U. 2007. An ontogenetic perspective on the relationship between age and size at maturity. *Functional Ecology* 21: 505–512.
Berrigan, D. & Charnov, E. L. 1994. Reaction norms for age and size at maturity in response to temperature: A puzzle for life historians. *Oikos* 70: 474–478.
Billerbeck, J. M., Lankford, T. E. Jr. & Conover, D. O. 2001. Evolution of intrinsic growth and energy acquisition rates. I. trade-offs with swimming performance in *Menidia menidia*. *Evolution* 55: 1863–1872.
Biro, P. A., Abrahams, M. V., Post, J. R. & Parkinson, E. A. 2006. Behavioural trade-offs between growth and mortality explain evolution of submaximal growth rates. *Journal of Animal Ecology* 75: 1165–1171.
Blanckenhorn, W. U. 2000. The evolution of body size: what keeps organisms small? *Quarterly Review of Biology* 75: 385–407.
Blanckenhorn, W. U. 2005. Behavioral causes and consequences of sexual size dimorphism. *Ethology* 111: 977–1016.
Blanckenhorn, W. U., Fanti, J. & Reim, C. 2007. Size-dependent energy reserves, energy utilization and longevity in the yellow dung fly. *Physiological Entomology* 32: 372–381.
Blanckenhorn, W. U., Hoeck, P. E. A., Reim, C., & Teuschl, Y. 2011. A cost of being large: genetically large yellow dung flies lose out in intra-specific food competition. *Evolutionary Ecology* 25: 875–884.
Boggs, C. L. & Gilbert, L. E. 1979. Male contribution to egg production in butterflies: evidence for transfer of nutrients at mating. *Science* 206: 83–84.
Boggs, C. L. 2009. Understanding insect life histories and senescence through a resource allocation lens. *Functional Ecology* 23: 27–37.
Boivin, G. & Gauvin, M. 2009. Egg size affects larval performance in a coleopteran parasitoid. *Ecological Entomology* 34: 240–245.
Boivin, G. 2010. Phenotypic plasticity and fitness in egg parasitoids. *Neotropical Entomology* 39: 457–463.
Bolmgren, K. & Cowan, P. D. 2008. Time-size trade-offs: a phylogenetic comparative study of flowering time, plant height and seed mass in a north-temperate flora. *Oikos* 117: 424–429.
Burton,-Chellew, M. N., Sykes, E. M., Patterson, S., Shuker, D. M. & West, S. A. 2007. The cost of mating and the relationship between body size and fitness in males of the parasitoid wasp *Nasonia vitripennis*. *Evolutionary Ecology Research* 9: 921–934.

Busching, M. K. & Turpin, F. T. 1977. Survival and development of black cutworm (*Agrotis ipsilon*) larvae on various species of crop plants and weeds. *Environmental Entomology* 6: 63–65.

Byers, J. R., Yu, D. S., & Jones, J. W. 1993. Parasitism of the army cutworm, *Euxoa auxiliaris* (GRT) (Lepidoptera: Noctuidae), by *Copidosoma bakeri* (Howard) (Hymenoptera: Encyrtidae) and effect on crop damage. *Canadian Entomologist* 125: 329–335.

Carrière, Y. & Boivin, G. 1997. Evolution of thermal sensitivity of parasitization capacity in egg parasitoids. *Evolution* 51: 2028–2032.

Charnov, E. L. & Skinner, S. W. 1984. Evolution of host selection and clutch size in parasitoid wasps. *Florida Entomologist* 67: 5–20.

Charnov, E. L. & Skinner, S. W. 1985. Complementary approaches to the understanding of parasitoid oviposition decisions. *Environmental Entomology* 14: 383–391.

Charnov, E. L. 1979. The genetical evolution of patterns of sexuality: Darwinian Fitness. *American Naturalist* 113: 465–480.

Charnov, E. L. 1986. Life history evolution in a “recruitment population”: why are adult mortality rates constant? *Oikos* 47: 129–134.

Charnov, E. L., Los-den Hartogh, R. L., Jones, W. T. & van den Assem, J. 1981. Sex ratio evolution in a variable environment. *Nature* 289: 27–33.

Cheng, H. H. 1977. Insect parasites of the darksided cutworm, *Euxoa messoria* (Lepidoptera: Noctuidae), in Ontario. *Canadian Entomologist* 109: 137–142.

Chown, S. L. & Gaston, K. J. 2010. Body size variation in insects: a macroecological perspective. *Biological Reviews* 85: 139–169.

Christians, J. K. 2000. Trade-offs between egg size and number in waterfowl: an interspecific test of the van Noordwijk and de Jong model. *Functional Ecology* 14: 497–501.

Clauss, M., Frey, R., Kiefer, B., Lechner-Doll, M., Loehlein, W., Polster, C., Rössner, G. E. & Streich, W. J. 2003. The maximum attainable body size of herbivorous mammals: morphophysiological constraints on foregut, and adaptations of hindgut fermenters. *Oecologia* 136: 14–27.

Cody, M. 1966. A general theory of clutch size. *Evolution* 20: 174–184.

Colinet, H., Boivin, G., & Hance, Th. 2007. Manipulation of parasitoid size using the temperature-size rule: fitness consequences. *Oecologia* 152: 425–433.

Cordero, C. 2000. Trade-off between fitness components in males of the polygynous butterfly *Callophrys xami* (Lycaenidae): the effect of multiple mating on longevity. *Behavioral Ecology and Sociobiology* 48: 458–462.

Corley, L. S., White, M. A., & Strand, M. R. 2005. Both endogenous and environmental factors affect embryo proliferation in the polyembryonic wasp *Copidosoma floridanum*. *Evolution and Development* 7: 115–121.

Creel, S. & Christianson, D. 2008. Relationships between direct predation and risk effects. *Trends in Ecology and Evolution* 23: 194–201.

Crowley, P. H. & Saeki, Y. 2009. Balancing the size-number tradeoff in clonal broods. *Open Ecology Journal* 2: 100–111.

Crowley, P. H., Saeki, Y., & Switzer, P. V. 2009. Evolutionarily stable oviposition and sex ratio in parasitoid wasps with single-sex broods. *Ecological Entomology* 34: 163–175.

Crumb, S. E. 1929. Tobacco Cutworms. U.S. Dep. Agric. *Technical Bulletin* 88.
Cruz, Y. P. 1981. A sterile defender morph in a polyembryonic hymenopterous parasite. *Nature* 294: 446–447.
Cushing, J. M. 1992. A size-structured model for cannibalism. *Theoretical Population Biology* 42: 347–361.
De Block, M. & Stoks, R. 2003. Adaptive sex-specific life history plasticity to temperature and photoperiod in a damselfly. *Journal of Evolutionary Biology* 16: 986–995.
Dell, A. I., Pawar, S., & Savage, V. M. 2011. Systematic variation in the temperature dependence of physiological and ecological traits. *Proceedings of the National Academy of Science of the United States of America* 108: 10591–10596.
DeWitt, T. J., Robinson, B. W., & Wilson, D. S. 2000. Functional diversity among predators of a freshwater snail imposes an adaptive trade-off for shell morphology. *Evolutionary Ecology Research* 2: 129–148.
Diamond, S. E. & Kingsolver, J. G. 2010. Environmental dependence of thermal reaction norms: Host plant quality can reverse the temperature-size rule. *American Naturalist* 175: 1–10.
Dijkstra, L. J. 1986. Optimal selection and exploitation of hosts in the parasitic wasp *Colpoclypeus florus* (Hym.: Euophidae). *Netherlands Journal of Zoology* 36: 177–301.
Dmitriew, C. M. 2011. The evolution of growth trajectories: what limits growth rate? *Biological Reviews* 86: 97–116.
Ellers, J, Driessen, G., & Sevenster J. G. 2000. The shape of the trade-off function between egg production and life span in the parasitoid *Asobara tavida*. *Netherlands Journal of Zoology* 50: 29–36.
Ellers, J. & Jervis, M. 2003. Body size and the timing of egg production in parasitoid wasps. *Oikos* 102: 164–172.
Ellers, J., Sevenster, J. G. & Driessen, G. 2000. Egg load evolution in parasitoids. *American Naturalist* 156: 650–665.
Emlen, D. J. 2001. Cost and the diversification of exaggerated animal structures. *Science* 291: 1534–1536.
Fairbairn, D. J. & Preziosi, R. F. 1994. Sexual selection and the evolution of allometry for sexual size dimorphism in the water strider, *Aquarius remigis*. *American Naturalist* 144: 101–118.
Fama, E. F. & French, K. R. 2002. Testing Trade-off and pecking order predictions about devidends and debt. *Review of Financial Studies* 15: 1–33.
Fischer, B., Taborsky, B., & Kokko, H. 2011. How to balance the offspring quality-quantity trade-off when environmental cues are unreliable. *Oikos* 120:258–270.
Fischer, K. & Fiedler, K. 2000. Sex-related differences in reaction norms in the butterfly *Lycaena tityrus* (Lepidoptera: Lycaenidae). *Oikos* 90: 372–380.
Fischer, K. & Fiedler, K. 2002. Reaction norms for age and size at maturity in response to temperature: a test of the compound interest hypothesis. *Evolutionary Ecology* 16: 333–349.
Fischer, K., Zwaan, B. J., & Brakefield, P. M. 2002. How does egg size relate to body size in butterflies? *Oecologia* 131: 375–379.

Folguera, G., Mensch, J., Muñoz, J. L., Ceballos, S. G., Hasson, E., & Bozinovic, F. 2010. Ontogenetic stage-dependent effect of temperature on developmental and metabolic rates in a holometabolous insect. *Journal of Insect Physiology* 56: 1679–1684.

Forbes, S. A. 1905. *Noxious and Beneficial Insects*, R. R. Donnelly & Sons Co., Chicago.

Fox, C. W. & Czesak, M. E. 2000. Evolutionary ecology of progeny size in arthropods. *Annual Review of Entomology* 45: 341–369.

García-Barros, E. 2000. Body size, egg size, and their interspecific relationships with ecological and life history traits in butterflies (Lepidoptera: Papilionoidea, Hesperioidea). *Biological Journal of the Linnean Society* 70: 251–284.

Ghalambor, C. K., Reznick, D. N., & Walker, J. A. 2004. Constraints on adaptive evolution: The functional trade-off between reproduction and fast swimming performance in the Trinidadian guppy (*Poecilia reticulate*). *American Naturalist* 164: 38–50.

Gilchrist, G. W. 1995. Specialists and generalists in changing environments. I. Fitness landscapes of thermal sensitivity. *American Naturalist* 146: 254–270.

Giron, D., Dunn, D. W., Hardy, I. C. W. & Strand, M. R. 2004. Aggression by polyembryonic wasp soldiers correlates with kinship but not resource competition. *Nature* 430: 676–679.

Giron, D., Ross, K. G. & Strand, M. R. 2007. The presence of soldier larvae determines the outcome of competition in a polyembryonic wasp. *Journal of Evolutionary Biology* 20: 165–172.

Glazier, D. S. 2000. Smaller amphipod mothers show stronger trade-offs between offspring size and number. *Ecology Letters* 3: 142–149.

Godfray, H. C. J. & Parker, G. A. 1991. Clutch size, fecundity and parent-offspring conflict. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* 332: 67–79.

Godfray, H. C. J. & Parker, G. A. 1992. Sibling competition, parent-offspring conflict and clutch size. *Animal Behaviour* 43: 473–490.

Godfray, H. C. J. 1986. Models for clutch size and sex ratio with sibling interaction. *Theoretical Population Biology* 30: 215–231.

Godfray, H. C. J. 1987. The evolution of clutch size in parasitic wasps. *American Naturalist* 129: 221–233.

Godfray, H. C. J. 1994. *Parasitoids: Behavioral and evolutionary ecology*. Princeton University Press, New Jersey.

Gotthard, K. Nylin, S., & Wiklund, C. 2000. Individual state controls temperature dependence in a butterfly (*Lasiommata maera*). *Philosophical Transactions of the Royal Society of London* 267: 589–593.

Grbić, M. 2003. Polyembryony in parasitic wasps: evolution of a novel mode of development. *International Journal of Developmental Biology*, 47, 633–642.

Grbić, M., Ode, P. J. & Strand, M. R. 1992. Sibling rivalry and brood sex ratios in polyembryonic wasps. *Nature* 360: 254–256.

Guisande, C., Sánchez, J., Maneiro, I., & Miranda, A. 1996. Trade-off between offspring number and offspring size in the marine copepod *Euterpina acutifrons* at different food concentrations. *Marine Ecology-Progress Series* 143: 37–44.

Hardy, I. C. W. 1992. Non-binomial sex allocation in the parasitoid Hymenoptera. *Oikos* 65: 143–150.

Harper, J. L. 1977. *Population Biology of Plants*. Academic Press, London.
Harris, C. R., Mazurek, J. H. & White, G. V. 1962. The life history of the black cutworm, *Agrotis ipsilon* (Hufnagel), under controlled conditions. *Canadian Entomologist* 94: 1183–1187.
Harvey, J. A. 1996. *Venturia canescens* parasitizing *Galleria mellonella* and *Anagasta kuehniella*: Is the parasitoid a conformer or regulator? *Journal of Insect Physiology* 42: 1017–1025.
Harvey, J. A., Bezemer, T. M., Elzinga, J. A., & Strand, M. R. 2004. Development of the solitary endoparasitoid *Microplitis demolitor*: host quality does not increase with host age and size. *Ecological Entomology* 29: 35–43.
Harvey, J. A., Corley, L. S., & Strand, M. R. 2000. Competition induces adaptive shifts in caste ratios of a polyembryonic wasp. *Nature* 406: 183–186.
Harvey, J. A., Kadash, K., & Strand, M. R. 2000. Differences in larval feeding behavior correlate with altered developmental strategies in two parasitic wasps: implications for the size-fitness hypothesis. *Oikos* 88: 621–629.
Hedrick, A. V. & Temeles, E. J. 1989. The evolution of sexual dimorphism in animals: hypotheses and tests. *Trends in Ecology and Evolution* 4: 136–138.
Heifetz, Y., Tram, U. & Wolfner, M. F. 2001. Male contributions to egg production: the role of accessory gland products and sperm in *Drosophila melanogaster*. *Proceedings of the Royal Society of London series B. Biological Sciences* 268: 175–180.
Hendrix, W. H., Gunnarson, D. F., & Showers, W. B. 1991. Modification of a meridic diet for rearing black cutworm (Lepidoptera, Noctuidae) larvae. *Journal of the Kansas Entomological Society* 64: 45–50.
Honěk, A. 1993. Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos* 66: 483–492.
Hu, Y., Yuan, X., & Lei, C. 2011. Sexual size dimorphism decreases with temperature in a blowfly, *Chrysomya megacephala* (Fabricius)(Diptera: Calliphoridae). *Ecological Entomology* 36: 111–115.
Huey, R. B. & Kingsolver, J. G. 2011. Variation in universal temperature dependence of biological rates. *Proceedings of the National Academy of Sciences of the United States of America* 108: 10377–10378.
Hughes, R. N. 1987. The functional ecology of clonal animals. *Functional Ecology* 1: 63–69.
Hughes, R. N., D'Amato, M. E., Bishop, J. D. D., Carvalho, G. R., Craig, S. F., Hansson, L. J., Harley, M. A., & Pemberton, A. J. 2005. Paradoxical polyembryony? Embryonic cloning in an ancient order of marine bryozoans. *Biology Letters* 1: 178–180.
Hulsey, C. D., Mims, M. C., & Streelman, J. T. 2007. Do constructional constraints influence cichlid craniofacial diversification? *Proceedings of the Royal Society B-Biological Sciences* 274: 1867–1875.
Hunter, K. W. Jr. & Stoner, A. 1975. *Copidosoma truncatellum*: effect of parasitization of food consumption of larval *Trichoplusia ni*. *Environmental Entomology* 4: 381–382.
Ivanova-Kasas, O. M. 1972. Polyembryony in insects. In: S.J. Counce, S. J. and Waddington, C. H. (eds.), *Developmental Systems*, Vol. 2: Insects. Academic Press, pp. 243–271.

Jervis, M. A., Ellers, J., & Harvey, J. A. 2008. Resource acquisition, allocation, and utilization in parasitoid reproductive strategies. *Annual Review of Entomology* 53: 361–385.

Jervis, M. A., Ferns, P. N. & Heimpel, G. E. 2003. Body size and the timing of egg production in parasitoid wasps: a comparative analysis. *Functional Ecology* 17: 375–385.

Jervis, M. A., Heimpel, G. E., Ferns, P. N., Harvey, J. A., & Kidd, N. A. 2001. Life-history strategies in parasitoid wasps: a comparative analysis of 'ovigeny'. *Journal of Animal Ecology* 70: 442–458.

Jessup, C. M. & Bohannan, B. J. M. 2008. The shape of an ecological trade-off varies with environment. *Ecology Letters* 11: 947–959.

Johansson, F., Stoks, R., Rowe, L., & De Block, M. 2001. Life history plasticity in a damselfly: effects of combined time and biotic constraints. *Ecology* 82: 1857–1869.

Jones, D., Jones, G., van Steenwyk, R. A., & Hammock, B. D. 1982. Effect of the parasite *Copidosoma truncatellum* on development of its host *Trichoplusia ni*. *Annals of the Entomological Society of America* 75: 7–11.

Jørgensen, C. & Fiksen, Ø. 2006. State-dependent energy allocation in cod (Gadus morhua). *Canadian Journal of Fisheries and Aquatic Sciences* 63: 186–199.

Juliano, S. A., Olson, J. R., Murrell, E. G., & Hatle, J. D. 2004. Plasticity and canalization of insect reproduction: testing alternative models of life history transitions. *Ecology* 85: 2986–2996.

Karlsson, B., Stjernholm, F., & Wiklund, C. 2008. Test of a developmental trade-off in a polyphenic butterfly: direct development favours reproductive output. *Functional Ecology* 22: 121–126.

Ketterson, E. D. & Nolan, V. Jr. 1999. Adaptation, exaptation, and constraint: a hormonal perspective. *American Naturalist* 154: S4–S25.

King, E. G., Roff, D. A., & Fairbairn, D. J. 2010. Trade-off acquisition and allocation in *Gryllus firmus*: a test of the Y model. *Journal of Evolutionary Biology* 24: 256–264.

King, E., Roff, D. A., & Fairbairn, D. J. 2011. The evolutionary genetics of acquisition and allocation in the wing dimorphic cricket, *Gryllus firmus*. *Evolution* 65: 2273–2285.

Kingsolver, J. G. & Pfennig, D. W. 2004. Individual-level selection as a cause of Cope's rule of phyletic size increase. *Evolution* 58: 1608–1612.

Kingsolver, J. G. 2009. The well-temperatured biologist. *American Naturalist* 174: 755–768.

Kingsolver, J. G., Shlichta, J. G., Ragland, G. J., & Massie, K. R. 2006. Thermal reaction norms for caterpillar growth depend on diet. *Evolutionary Ecology Research* 8: 703–715.

Klingenberg, C. P. & Spence, J. R. 1997. On the role of body size for life-history evolution. *Ecological Entomology* 22: 55–68.

Klingenberg, C. P. & Zimmermann, M. 1992. Dyar's rule and multivariate allometric growth in nine species of waterstriders (Heteroptera: Gerridae). *Journal of Zoology* 227: 453–464.

Kolm, K., Goodwin, N. B., Balshine, S., & Reynolds, J. D. 2005. Life history evolution in cichlids 2: directional evolution of the trade-off between egg number and egg size. *Journal of Evolutionary Biology* 19: 76–84.

Kolm, K., Goodwin, N. B., Balshine, S., & Reynolds, J. D. 2006. Life history evolution in cichlids 2: directional evolution of the trade-off between egg number and egg size. *Journal of Evolutionary Biology* 19: 76–84.

Lack, D. 1947. The significance of clutch-size. *Ibis* 89: 302–352.

Lacoume, S., Bressac, C., & Chevrier, C. 2006. Effect of host size on male fitness in the parasitoid wasp *Dinarmus basalis*. *Journal of Insect Physiology* 52: 249–254.

Lalonde, R. G. 2005. Egg size variation does not affect offspring performance under intraspecific competition in *Nasonia vitripennis*, a gregarious parasitoid. *Journal of Animal Ecology* 74: 630–635.

Lawrence, P. O. 1986. Host-parasite hormonal interactions: an overview. *Journal of Insect Physiology* 32: 295–298.

Le Lann, C., Wardziak, T., van Baaren, J., & van Alphen, J. J. M. 2011. Thermal plasticity of metabolic rates linked to life-history and foraging behaviour in a parasitic wasp. *Functional Ecology* 25: 641–651.

le Masurier, A. D. 1987. A comparative studyof the relationship between host size and brood size in *Apanteles* spp. (Hymenoptera: Braconidae). *Ecological Entomology* 12: 383–393.

Leips, J., Richardson, J. M., Rodd, F. H., & Travis, J. 2009. Adaptive maternal adjustments of offspring size in response to conspecific density in two populations of the least killifish, *Heterandria formosa*. *Evolution* 63: 1341–1347.

Levins, R. 1962. Theory of fitness in a heterogeneous environment. I. The fitness set and adaptive function. *American Naturalist* 891: 361– 373.

Liana, M. 2005. First copulation increases longevity and fecundity of *Histiostoma feroniarum* (Acari: Astigmata: Acaridida) females. *Experimental and Applied Acarology* 35: 173–181.

Mackauer, M. & Sequeira, R. 1993. Patterns of development in insect parasites. In: Beckage, N. E., Thompson, S. N. and Federici, B. A. (eds.), *Parasites and Pathogens of Insects, Vol. 1: Parasites*. Academic Press, pp. 1–23.

Mackauer, M. 1996. Sexual size dimorphism in solitary parasitoid wasps: influence of host quality. *Oikos* 76: 265–373.

Malausa, T., Guillemaud, T., & Lapchin, L. 2005. Combining genetic variation and phenotypic plasticity in trade-off modeling. *Oikos* 110: 330–338.

Mangel, M. & Stamps, J. 2001. Trade-offs between growth and mortality and the maintenance of individual variation in growth. *Evolutionary Ecology Research* 3: 583–593.

Mappes, T. & Koskela, E. 2004. Genetic basis of the trade-off between offspring number and quality in the bank vole. *Evolution* 58: 645–650.

Mayhew, P. J. & Glaizot, O. 2001. Integrating theory of clutch size and body size evolution for parasitoids. *Oikos* 92: 372–376.

Mayhew, P. J. 1998. Offspring size-number strategy in the bethylid parasitoid *Laelius pedatus*. *Behavioral Ecology* 9: 54–59.

Maynard Smith, J., Burian, R., Kauffman, S., Alberch, P., Campbell, J., Goodwin, B., Lande, R., Raup, D., & Wolpert, L. 1985. Developmental constraints and evolution. *Quarterly Review of Biology* 60: 265–287.

McLain, D. K. & Mallard, S. D. 1991. Sources and adaptive consequences of egg size variation in *Nezara viridula* (Hemiptera: Pentatomidae). *Psyche* 98: 135–164.

McMillan, E. 1930. A preliminary study of the Polyembryonic Cutworm parasite, *Berecyntus bakeri var. gemma* Girault. M. Sc. Thesis, University of Saskatchewan, Saskatoon, Sask.

McNamara, J. M. & Houston, A. I. 1996. State-dependent life histories. *Nature* 380: 215–221.

Messina, F. J. & C. W. Fox. 2001. Offspring size and number. In: C.W. Fox, D.A. Roff, and D.J. Fairbairn (eds.), *Evolutionary Ecology: Concepts and Case Studies*. pp. 113–127. Oxford Univ. Press, New York.

Metcalfe, N. B. & Monaghan, P. 2001. Compensation for a bad start: grow now, pay later? *Trends in Ecology and Evolution* 16: 254–260.

Michod, R. E., Viossat, Y., Solari, C. A., Hurand, M., & Nedelcu, A. M. 2006. Life-history evolution and the origin of multicellularity. *Journal of theoretical Biology* 239: 257–272.

Moczek, A. P. & Nijhout, H. F. 2004. Trade-offs during the development of primary and secondary sexual traits in a horned beetle. *American Naturalist* 163: 184–191.

Moczek, A. P., Sultan, S., Foster, S., Ledón-Rettig, C., Dworkin, I., Nijhout, H. F., Abouheif, E., & Pfennig, D. W. 2011. The role of developmental plasticity in evolutionary innovation. *Proceedings of the Royal Society B-Biological Sciences* 278: 2705–2713.

Mole, S. 1994. Trade-offs and constraints in pant-herbivore defense theory: a life-history perspective. *Oikos* 71: 3–12.

Nadel, H. 1987. Male swarms discovered in Chalcidoidea (Hymenoptera: Encyrtidae, Pteromalidae). *Pan-Pacific Entomologist* 63: 242–246.

Nager, R. G., Monaghan, P., & Houston, D. C. 2000. Within-clutch trade-offs between the number and quality of eggs: experimental manipulations in gulls. *Ecology* 81: 1339–1350.

Nealis, V. G., Jones, R. E., & Wellington, W. G. 1984. Temperature and development in host-parasite relationships. *Oecologia* 61: 224–229.

Nespolo, R. F., Roff, D. A., & Fairbairn, D. J. 2008. Energetic trade-off between maintenance costs and flight capacity in the sand cricket (*Gryllus firmus*). *Functional Ecology* 22: 624–631.

Niewiarowski, P. H. & Angilletta, M. J. Jr. 2008. Countergradient variation in embryonic growth and development: do embryonic and juvenile performances trade off? *Functional Ecology* 22: 895–901.

Nijhout, H. F. & Emlen, D. J. 1998. Competition among body parts in the development and evolution of insect morphology. *Proceedings of the National Academy of Sciences of the United States of America* 95: 3685–3689.

Nijhout, H. F. & Williams, C. M. 1974. Control of moulting and metamorphosis in the tobacco hornworm, *Manduca sexta* (L.): Growth of the last-instar larva and the decision to pupate. *Journal of Experimental Biology* 61: 481–491.

Nijhout, H. F. 1981. Physiological control of molting in insects. *American Zoologist* 21: 631–640.

Nijhout, H. F., Roff, D. A., & Davidowitz, G. 2010. Conflicting processes in the evolution of body size and development time. *Proceedings of the Royal Society of London, Series B* 365: 567–575.

Nylin, S. & Gotthard, K. 1998. Plasticity in life-history traits. *Annual Review of Entomology* 43: 63–83.
Ode, P. & Strand, M. R. 1995. Progeny and sex allocation decisions of the polyembryonic wasp *Copidosoma floridanum*. *Journal of Animal Ecology* 64: 213–224.
Oliver, C. & Cordero, C. 2009. Multiple mating reduces male survivorship but not ejaculate size in the polygamous insect *Stenomacra marginella* (Heteroptera: Largidae). *Evolutionary Ecology* 23: 417–424.
Olofsson, H., Ripa, J., & Jenzén, N. 2009. Bet-hedging as an evolutionary game: the trade-off between egg size and number. *Proceedings of the Royal Society of London, Series B* 276: 2963–2969.
Partridge, L. & French, V. 1996. Thermal evolution of ectotherm body size: why get big in the cold? In: Johnston, I.A., Bennett, A.F. (Eds.), *Animals and Temperature: Phenotypic and Evolutionary Adaptation*. Cambridge University Press, Cambridge, pp. 265–292.
Partridge, L. & Sibly, R. 1991. Constraints in the evolution of life histories. *Philosophical Transactions of the Royal Society B: Biological Sciences* 332: 3–13.
Paul-Victor, C. & Turnbull, L. A. 2009. The effect of growth conditions on the seed size/number trade-off. *PLos ONE* 4: 1–10.
Pearse, J. S., Pearse, V. B., & Newberry, A. T. 1989. Telling sex from growth: Dissolving Maynard Smith's paradox. *Bulletin of Marine Science* 45: 433–446.
Pease, C. M. & Bull, J. J. 1988. A critique of methods for measuring life history trade-offs. *Journal of Evolutionary Biology* 1: 293–303.
Petersen, C., Woods, H. A., & Kingsolver, J. G. 2000. Stage-specific effects of temperature and dietary protein on growth and survival of *Manduca sexta* caterpillars. *Physiological Entomology* 25: 35–40.
Pfenning, B., Poethke, H. J., & Hovestadt, T. 2007. Dealing with time constraints on development: the effect of food availability. *Ecological Entomology* 32: 273–278.
Polis, G. A. 1980. The effect of cannibalism on the demography and activity of a natural population of desert scorpions. *Behavioral Ecology and Sociobiology* 7: 25–35.
Potter, K. A., Davidowitz, G., & Woods, H. A. 2011. Cross-stage consequences of egg temperature in the insect *Manduca sexta*. *Functional Ecology* 25: 548–556.
Preston, F. W. 1974. The volume of an egg. *Auk* 91: 132–138.
Qualls, C. P. & Shine, R. 1995. Maternal body-volume as a constraint on reproductive output in lizards: evidence from the evolution of viviparity. *Oecologia* 103: 73–78.
Rahman, M. 1970. Effect of parasitism on food consumption of *Pieris rapae* larvae. *Journal Economic Entomology* 63: 820–821.
Reudler Talsma, J. H., Elzinga, J. A., Harvey, J. A., & Biere, A. 2007. Optimum and maximum host sizes at parasitism for the endoparasitoid *Hyposoter didymator* (Hymenoptera: Ichneumonidae) differ greatly between two host species. *Environmental Entomology* 36: 1048–1053.
Roff, D. A. & Fairbairn, D. J. 2007. The evolution of trade-offs: where are we? *Journal of Evolutionary Biology* 20: 433–447.
Roff, D. A. & Gélinas, M. B. 2003. Phenotypic plasticity and the evolution of trade-offs: the quantitative genetics of resource allocation in the wing dimorphic cricket, *Gryllus firmus*. *Journal of Evolutionary Biology* 2003: 55–63.
Roff, D. A. 1981. On being the right size. *American Naturalist* 118: 405–422.

Roff, D. A. 1992. *The evolution of life histories*. Chapman and Hall, New York.
Roff, D. A. 2000. Trade-offs between growth and reproduction: an analysis of the quantitative genetic evidence. *Journal of Evolutionary Biology* 13: 434–445.
Roff, D. A. 2002. *Life History Evolution*. Sinauer Associates, Massachusetts, USA.
Rosenheim, J. A. 1996. An evolutionary argument for egg limitation. *Evolution* 50: 2089–2094.
Rueffler, C., van Dooren, T. J. M., & Metz, J. A. J. 2004. Adaptive walks on changing landscapes: Levin's approach extended. *Theoretical Population Biology* 65: 165–178.
Sadras, V. O. 2007. Evolutionary aspects of the trade-off between seed size and number in crops. *Field Crops Research* 100: 125–138.
Saeki, Y., Crowley, P. H., Fox, C. W., & Potter, D. A. 2009. A sex-specific size-number trade-off in clonal broods. *Oikos* 118: 1552–1560.
Sagarra, L. A., Vincent, C., & Stewart, R. K. 2001. Body size as an indicator of parasitoid quality in male and female *Anagyrus Kamali* (Hymenoptera: Encyrtidae). *Bulletin of Entomological Research* 91: 363–367.
Saglam, I. K., Roff, D. A., & Fairbairn, D. J. 2008. Male sand crickets trade-off flight capability for reproductive potential. *Journal of Evolutionary Biology* 21: 997–1004.
Schaaf, A. C. 1972. The parasitoid complex of *Euxoa ochrogaster* (Guenee) (Lepidoptera: Noctuidae). *Quaestiones entomologicae* 8: 81–120.
Schaffer, W. M. 1974. Selection for optimal life histories: the effects of age structure. *Ecology* 55: 291–303.
Sevenster, J. G., Ellers, J., & Driessen, G. 1998. An evolutionary argument for time limitation. *Evolution* 52: 1241–1244.
Shine, R. 1988. The evolution of large body size in females: a critique of Darwin's "fecundity advantage" model. *American Naturalist* 131: 124–131.
Showers, W. B. 1997. Migratory ecology of the black cutworm. *Annual Review of Entomology* 42: 393–425.
Sibly R. M. & Calow, P. 1989. A life-cycle theory of responses to stress. *Biological Journal of the Linnean Society* 37: 101–116.
Sibly, R. M. & Calow, P. 1986. *Physiological Ecology of Animals: An Evolutionary Approach*. Blackwell Scientific Publications.
Sibly, R. M. 1991. The life-history approach to physiological ecology. *Functional Ecology* 6: 184–191.
Sinervo, B. & Licht, P. 1991. Proximate constraints on the evolution of egg size, number, and total clutch mass in lizards. *Science* 252: 1300–1302.
Sinervo, B. & Svensson, E. 1998. Mechanistic and selective causes of life history trade-offs and plasticity. *Oikos* 83: 432–442.
Sinervo, B. 1999. Mechanistic analysis of natural selection and a refinement of Lack's and William's principles. *American Naturalist* 154: S26–S42.
Slansky, F. Jr. 1978. Utilization of energy and nitrogen by larvae of the imported cabbageworm, *Pieris rapae*, as affected by parasitism by *Apanteles glomeratus*. *Environmental Entomology* 7: 179–185.
Slansky, F.Jr. 1986. Nutritional ecology of endoparasitic insects and their hosts: An overview. *Journal Insect Physiology* 32: 255–261.

Smith, C. C. & Fretwell, S. D. 1974. The optimal balance between size and number of offspring. *American Naturalist* 108: 499–506.
Snow, S. J. 1925. Observations on the cutworm, *Euxoa auxiliaris* Grote, and its principal parasites. *Journal of Economic Entomology* 18: 602–609.
Stearns, S. C. & Hoekstra, R. F. 2005. *Evolution: an introduction*. Oxford University Press, New York.
Stearns, S. C. & Koella, J. C. 1986. The evolution of phenotypic plasticity in life-history traits: predictions of reaction norms for age and size at maturity. *Evolution* 40: 893–913.
Stearns, S. C. & Koella, J. C. 1986. The evolution of phenotypic plasticity in life-history traits: predictions of reaction norms for age and size at maturity. *Evolution* 40: 893–913.
Stearns, S. C. 1989. Trade-offs in life-history evolution. *Functional Ecology* 3: 259–268.
Stearns, S. C. 1992. *The Evolution of Life Histories*, Oxford University Press, New York.
Stearns, S. C. 2000. Life history evolution: successes, limitations, and prospects. *Naturwissenschaften* 87: 476–486.
Stevens, D. J., Hansell, M .H., Freel, J. A., & Monaghan, P. 1999. Developmental trade-offs in caddis flies: increased investment in larval defense alters adult resource allocation. *Proceedings of the Royal Society B-Biological Sciences* 266: 1049–1054.
Stillwell, R. C., Blanckenhorn, W. U., Teder, T., Davidowitz, G., & Fox, C. W. 2010. Sex differences in phenotypic plasticity affect variation in sexual size dimorphism in insects: From physiology to Evolution. *Annual Review of Entomology* 55: 227–245.
Stirling, G., Fairbairn, D. J., Jensen, S., & Roff, D. A. 2001. Does a negative genetic correlation between wing morph and early fecundity imply a functional constraint in *Gryllus firmus*? *Evolutionary Ecology Research* 3: 157–177.
Stoks, R., De Block, M., Van de Meutter, F., & Johansson, F. 2005. Predation cost of rapid growth: behavioural coupling and physiological decoupling. *Journal of Animal Ecology* 74: 708–715.
Strand, M. R. & Grbić, M. 1997. The development and evolution of polyembryonic insects. *Current Topics in Developmental Biology* 35: 121–159.
Strand, M. R. 1989a. Development of the polyembryonic parasitoid *Copidosoma floridanum* in *Trichoplusia ni*. *Entomologia Experimentalis et Applicata* 50: 37–46.
Strand, M. R. 1989b. Oviposition behavior and progeny allocation of the polyembryonic wasp *Copidosoma floridanum* (Hymenoptera: Encyrtidae). *Journal or Insect Behavior* 2: 355–369.
Strand, M. R. 1989c. Clutch size, sex ratio and mating by the polyembryonic encyrtid *Copidosoma floridanum* (Hymenoptera: Encyrtidae). *Florida Entomologist* 72: 32–42.
Strand, M. R., Dover, B. A., & Johnson, J. A. 1990. Alterations in the ecdysteroid and juvenile hormone esterase profiles of *Trichoplusia ni* parasitized by the polyembryonic wasp *Copidosoma floridanum*. *Archives of Insect Biochemistry and Physiology* 13: 41–51.
Stuefer, J. F., van Hulzen, J. B., & During, H. J. 2002. A genotypic trade-off between the number and size of clonal offspring in the stoloniferous herb *Potentilla reptans*. *Journal of Evolutionary Biology* 15: 880–884.

Suzuki, Y., Miyake, T., & Yamahira, K. 2010. An acquisition trade-off with fast growth in a fish, the medaka *Oryzias latipes*: why do low-latitude ectotherms grow more slowly? *Evolutionary Ecology* 24: 749–759.

Tatar, M. & Carey J. R. 1995. Nutrition mediates reproductive trade-offs with age-specific mortality in the beetle *Callosobruchus maculatus*. *Ecology* 76: 2066–2073.

Teder, T. & Tammaru, T. 2005. Sexual size dimorphism within species increases with body size in insects. *Oikos* 108: 321–334.

Thornhill, R. & Alcock, J. 1983. *The Evolution of Insect Mating System*. iUniverse.com, Inc., New York.

Torregrossa, A-M., Azzara, A. V., & Dearing, M. D. 2012. Testing the diet-breadth trade-off hypothesis: differential regulation of novel plant secondary compounds by a specialist and a generalist herbivore. *Oecologia* 168: 711–718.

Uhl, G., Schmitt, S., Schäfer, M. A. & Blanckenhorn, W. 2004. Food and sex-specific growth strategies in a spider. *Evolutionary Ecology Research* 6: 523–540.

Uller, T. & Olsson, M. 2005. Trade-offs between offspring size and number in the lizard *Lacerta vivipara*: a comparison between field and laboratory conditions. *Journal of Zoology, London* 265: 295–299.

Van Baaren, J., Outreman, Y., Boivin, G., 2005. Effect of low temperature exposure on oviposition behavior and patch exploitation strategy in parasitic wasps. *Animal Behaviour* 70: 153–163.

van den Assem, J., van Iersel, J. J. A., & Los-den Hartogh, R. L. 1989. Is being large more important for female than for male parasitic wasps? *Behaviour* 108: 160–95.

Van der Have, T. M., & de Jong, G. 1996. Adult size in ectotherms: Temperature effects on growth and differentiation. *Journal Theoretical Biology* 183: 329–340.

van Noordwijk, A. J. & de Jong, G. 1986. Acquisition and allocation of resources: their influence on variation in life history tactics. *American Naturalist* 128: 137–142.

Vertainen, L., Alatalo, R. V., Mappes, J., & Parri, S. 2000. Sexual differences in growth strategies of the wolf spider *Hygrolycosa rubrofasciata*. *Evolutionary Ecology* 14: 595–610.

Via, S., Gomulkiewicz, R., De Jong, G., Scheiner, S. M., Schlichting, C. D., & Van Tienderen, P. H. 1995. Adaptive phenotypic plasticity: consensus and controversy. *Trends in Ecology & Evolution* 10: 212–217.

Vinson, S. B. & Iwantsch, G. F. 1980. Host regulation by insect parasitoids. *Quarterly Review of Biology* 55: 143–165.

Visser, M. E. 1994. The importance of being large: the relationship between size and fitness in females of the parasitoid *Aphaereta minuta* (Hymenoptera: Braconidae). *Journal of Animal Ecology* 63: 963–978.

Vitt, L. J. & Congdon, J. D. 1978. Body shape, reproductive effort, and relative clutch mass in lizards: resolution of a paradox. *American Naturalist* 112: 595–608.

Walters, R. J. & Hassall, M. 2006. The temperature-size rule in ectotherms: May a general explanation exist after all? *American Naturalist* 167: 510–523.

Wang, X. P., Fang, Y. L., & Zhang, Z. N. 2005. Effect of male and female multiple mating on the fecundity fertility, and longevity of diamondback moth, *Plutella xylostella* (L.). *Journal of Applied Entomology* 129: 39–42.

Warton, D. I., Wright, I. J., Falster, D. S., & Westoby, M. 2006. Bivariate line-fitting methods for allometry. *Biological Reviews* 81: 259–291.

Werner, E. E. & Anholt, B. R. 1993. Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. *American Naturalist* 142: 242–272.

West, S. A. & Sheldon, B. C. 2002. Constraints in the evolution of sex ratio adjustment. *Science* 295: 1685–1688.

West, S. A., Flanagan, K. E., & Godfray, H. C. J. 2001. Variable host quality, life-history invariants, and the reproductive strategy of a parasitoid wasp that produces single sex clutches. *Behavioral Ecology* 12: 577–583.

Williamson, R. C. & Potter, D. A. 1997a. Turfgrass species and endophyte effects on survival, development, and feeding preference of black cutworms (Lepidoptera: Noctuidae). *Journal of Economic Entomology* 90: 1290–1299.

Williamson, R. C. & Potter, D. A. 1997b. Nocturnal activity and movement of black cutworms (Lepidoptera: Noctuidae) and response to cultural manipulations on golf course putting greens. *Journal of Economic Entomology* 90: 1283–1289.

Worley, A. C., Houle, D., & Barrett, S. C. H. 2003. Consequences of hierarchical allocation for the evolution of life-history traits. *American Naturalist* 161: 153–167.

Yoshida, T. 2006. Ecological stoichiometry and the shape of resource-based trade-offs. *Oikos* 112: 406–411.

Young, R. L. & Badyaev, A. V. 2004. Evolution of sex-biased maternal effects in birds: I. Sex-specific resource allocation among simultaneously growing oocytes. *Journal Evolutionary Biology* 17: 1355–1366.

Zera, A. J. & Harshman, L. G. 2001. The physiology of life history trade-offs in animals. *Annual Review of Ecology and Systematics* 32: 95–126.

Zhao, L. & Zhu, D. 2011. Effect of mating status on the fecundity of a cricket, *Teleogryllus emma*. *Insect Science* 18: 320–324.

Zhao, W. & Wang, Q. 2006. Asymmetric size effect of sexes on reproductive fitness in an aphid parasitoid *Aphidius ervi* (Hymenoptera: Aphidiidae). *Biological Control* 36: 293–298.

Vita

Date of birth: March 7, 1974
Date of place: Tokushima, Japan

Education:
Bachelor of Science
Eastern Illinois University, Charleston, IL 61920 (Fall 1999- Spring 2002)
Major: Biological Sciences; Minor: Chemistry

Master of Science
Eastern Illinois University, Charleston, IL 61920 (August 2002- July 2004)
Major: Biological Sciences

Honors and Awards:
University of Kentucky
Teaching Assistantship, Department of Biology, 2006-2008
Ribble fund, Department of Biology, 2007, $500.00
Sigma Xi Grants-in-Aid of Research, 2006, $700.00
University of Kentucky Graduate School Academic Fellowship, 2005-2006
Research Assistantship, Department of Entomology, 2004-2005

Eastern Illinois University
Phi Sigma Graduate Student Award, 2004
Graduate Student Investigator Award, 2004
Graduate teaching assistantship, 2002-2004
Errett/Mazie Warner award in Zoology, 2002
Baccalaureate with honors, 2002
International Student Scholarship, 2000-2002
Hugh C. Rawls Award, 2001
Outstanding International Student Award: Biological Science, 2000

Presentations:
Poster
Saeki, Y. and McGilliard, K. L. 2002. Effect of caffeine on the ventilatory response to CO_2 in newborn rats (2002)
Saeki, Y., Kruse, K. C., and Switzer, P. V. Physiological costs of mate guarding in Japanese beetle (*Popillia japonica* Newman).
Entomological Society of America Annual Meeting (2004)

Saeki, Y., Crowley, P. H., Fox, C. W., and Potter, D. A. Fitting the parasitoids to the host: the polyembryonic wasp, *Copidosoma bakeri*, parasitizing Black Cutworms, *Agrotis ipsilon*.
Animal Behaviour Society Annual Meeting (2007)

Oral

Saeki, Y., Kruse, K. C., and Switzer, P. V. Physiological costs of mate guarding in Japanese beetle (*Popillia japonica* Newman).
Phi Sigma Annual Research Symposium (2004)
Eastern Illinois University Science Festival (2004)

Saeki, Y. Growth rate of black cutworm larvae parasitized by the polyembryonic wasp, *Copidosoma bakeri*.
Annual Symposium, Center for Ecology, Evolution, and Behavior, University of Kentucky (2006)

Publications

Crowley, P. H. & Saeki, Y. 2009. Balancing the size-number tradeoff in clonal broods. *Open Ecology Journal* 2: 100–111.

Saeki, Y., Crowley, P. H., Fox, C. W., and Potter, D. A. 2009. A Sex-Specific Size-Number Trade-off in Clonal Broods. *Oikos* 118: 1552–1560.

Crowley, P. H., Saeki, Y., and Switzer, P. V. 2008. Evolutionarily stable oviposition and sex ratio in parasitoid wasps with single-sex broods. *Ecological Entomology* 34: 163–175.

Saeki, Y., Kruse, K. C., and Switzer, P. V. 2005. Male preference for large females and female reproductive condition in the Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae). *Journal of the Kansas Entomological Society* 78(1):13-19.

Saeki, Y., Kruse, K. C., and Switzer, P. V. 2005. The social environment affects mate guarding behavior in Japanese beetles, *Popillia japonica* Newman (Coleoptera: Scarabaeidae). *Journal of Insect Science* 5:18.

Saeki, Y., Kruse, K. C., and Switzer, P. V. 2005. Physiological costs of mate guarding in Japanese beetle (*Popillia japonica* Newman). *Ethology* 111(9): 863- 877.