MATERNAL ALLOCATION DECISIONS IMPACTED BY VARIABLE ECOLOGICAL CONDITIONS IN A SOLITARY BEE: BEHAVIOURAL ECOLOGY PERSPECTIVES

by

Jason Harvey Peterson
B.Sc. Augustana University College, 2000
B.A. Augustana University College, 2000
M.Sc. Simon Fraser University, 2005

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Name:	**Jason Harvey Peterson**
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Examining Committee:

Chair:

Dr. Rick Routledge
Department of Statistics and Actuarial Science, S.F.U.

Dr. Bernard D. Roitberg
Senior Supervisor
Department of Biological Sciences, S.F.U.

Dr. Elizabeth Elle
Supervisor
Department of Biological Sciences, S.F.U.

Dr. David B. Lank
Supervisor
Department of Biological Sciences, S.F.U.

Dr. David J. Green
Public Examiner
Department of Biological Sciences, S.F.U.

Dr. Jay A. Rosenheim
External Examiner
Department of Entomology
University of California, Berkeley

Date Defended/Approved: December 1, 2010

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Abstract

Parents can invest in offspring through a variety of behaviours. I use a combination of theoretical and empirical studies to examine how resource and sex allocation decisions toward offspring were altered in response to changing ecological conditions. My empirical work was done using the haplodiploid alfalfa leafcutter bee (*Megachile rotundata*), where sex of offspring can be controlled and mothers do all offspring provisioning.

My theoretical research demonstrated no single factor determining sex allocation; instead, there is some ‘optimal balance’ between factors. Empirical work results suggest a similar situation. All three experiments demonstrated different factors that impacted sex allocation decisions: flight distance to resources, resource levels, and local population density.

Longer flight distances resulted in fewer offspring produced throughout the season, but a greater proportion of daughters produced in the first half of the season and a lower proportion of daughters in the second half of the season compared to mothers with short distance to resources. Lower resource-level treatments had similar effects during the first half of the season as with long flight distance; however, during the second half mothers continued to produce a greater proportion of daughters under low resource conditions compared to high. Lower local population density resulted in both a greater proportion of daughters being produced as well as a greater number of offspring per individual nest compared with high-density conditions.

We also addressed two other allocation decisions. We used flight distance to address the question of what foraging currency mothers maximize when collecting resources for offspring. We found mothers increased load size with increased flight distance, suggesting that they are behaving in a manner that maximizes efficiency as opposed to net rate of energy intake. In regards to nest defence, mothers slightly increased nest defense as the nest size increased. However, unlike traditionally studied organisms, this increase in defense continued more steeply until the nest was basically completed and sealed, after which defense dropped suddenly.

In combination, these studies contribute to our basic understanding of offspring allocation decisions in solitary organisms.

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Keywords: nest provisioning decisions; offspring sex ratio; optimizing multiple resources; dynamic state variable model; Hymenoptera; nest defence; nest state; net rate of energy intake; efficiency; resource allocation; sex allocation; flight distance; resource availability; population concentration; solitary organism; *Megachile rotundata*; leafcutter bee.

Dedication

I would like to dedicate this thesis to my wife Teri who experienced a wild variety of emotions in response to what was happening to my body during my Ph.D. and was always my rock. As well as to parents, whom I could not have done this without and Monty who was always up for an argument, regardless of the subject. I also want to truly acknowledge the support of the countless people who did so much for me, in so many different ways. Last but not least I want to thank Ezri, for inspiring me with her joy of discovery during the writing of this entire thesis. Thank you.

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1: Introduction to Offspring Resource and Sex Allocation

Jason H. Peterson[1]

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[1] Evolutionary and Behavioural Ecology Research Group, Department of Biological Sciences, Simon Fraser University, Burnaby, BC, V5A lS6 Canada

1.1 Literature Review

All organisms hold the potential for exponential growth of descendants, however various factors limit survival and reproduction, the key elements of growth. Ecologists study these limiting factors, often within a behavioural ecology context that seeks to understand the evolutionary basis of the responding behaviours. Some species inhabit diverse environments, resulting in them expressing behaviours that have evolved to be flexible and influenced by local ecological conditions. Although allocation theory has generally assumed that a single limiting factor controls allocation decisions, in reality the production of offspring often requires the investment of qualitatively different types of resources (Frank 1990; Rosenheim *et al.* 2010). A population where fitness is consistently limited by a single factor will respond predictably to alterations of this factor, while populations whose fitness is limited by various factors at different times or locations will respond less predictably to perturbations (Rosenheim *et al.* 2010). Here, I consider two key issues with regard to offspring allocation: 1) Multiple resources are required for the successful production of offspring; and 2) Ecological conditions can alter the costs and benefits of various factors that impact success such as resource and sex allocation decisions.

Numerous organisms inhabit stochastic environments where a variety of allocation decisions must be made to maximize lifetime reproduction (Hengeveld *et al.* 2009). Allocation of resources to mitigate the impact of potentially limiting factors is possible; however, the finite nature of resources means an increased allocation to deal with one limiting factor will be traded off with reduced allocation to cope with another limiting factor (Rosenheim *et al.* 2010). In particular, offspring resource and sex allocation decisions are multidimensional, where the magnitude and importance of each dimension is dynamic.

Parental allocation to offspring has been of interest to researchers for some time (Klug and Bonsall 2010). These decisions along with the choice of mate determine the fitness benefits received by the parent. The classically studied offspring-allocation trade-off is between the number and quality of offspring produced in a single reproductive event (Campbell 1996). Research has generally shown that large clutch sizes and small young are found in organisms with high offspring mortality rates and vice versa. However, parents of nearly all organisms make allocations decisions beyond this question of size and number, for example, how much parents should allocate to each individual offspring (Carlisle 1982; Winkler 1987; Clark and Ydenberg 1990; Westneat and Sherman 1993; Gross 2005). Allocation research helps us to better understand not only the decisions that parents make, but also the extent to which parental control can be exerted over decisions such as the sex of their offspring.

To understand the optimal sex, size and resources to allocate towards each offspring we must determine a given species' ability to control these decisions (e.g. primary, secondary or tertiary control of offspring sex). Unless stated otherwise, sex ratio refers to primary sex ratio, though I acknowledge that, in most studies, secondary sex ratio is usually the point when the sex ratio can be determined. The focus of this thesis is on allocation at the individual parent level although the background population is considered. The goal is to understand how individual mothers alter resource and sex allocation decisions under different sets of conditions.

With regard to sex allocation, Darwin (1871) realized in the first edition of "The Descent of Man and Selection in Relation to Sex" that the occurrence of unbiased sex ratio posed a problem to his theory of natural selection. However, in the second edition of the book (1874) he decided that his previous attempts at an answer were unsatisfactory and that the question should be left for future generations.

The history of sex allocation theory tends to start with Fisher (1930). Although this concept had previously been discussed by Düsing (1883; 1884a; 1884b) it was Fisher's writing that seemed to inspire future research. Prior to this, the focus was on the 50:50 sex ratio. However, Fisher (1930) realized the key was not equal numbers of each sex but equal investment in each sex. Therefore, if a given sex is twice as expensive to produce, the mother will only produce half as many of that sex, resulting in equal investment in the two sexes, but not an equal sex ratio. This theory has been expanded to situations where fitness of sons and daughters is affected differently by, for example, environmental factors, where theory predicts that parents will bias the offspring sex ratio toward the sex with the highest fitness benefits (Hamilton 1967; Trivers and Willard 1973; Clark 1978; Charnov 1982).

Modern sex allocation theory started around the publication of Hamilton's (1967) work that made numerous pivotal contributions to sex allocation and evolutionary biology in general (West 2009). Trivers and Williard (1973) then demonstrated how individuals can be selected to adjust the sex of their offspring in response to environmental conditions. Charnov (1982) took this work and expanded it into an encompassing theory that made predictions that were clearly open to empirical testing. The result was a surge in sex allocation research that continues to this day (Frank 2002;

West, 2009). It is these two major publications (Hamilton 1967; Trivers and Williard 1973) that inspire the focus of the sex allocation portion of this thesis.

The scope of organisms involved in offspring sex allocation manipulation was previously thought to be limited as a result of the common chromosomal (genetic) sex determination system in vertebrates (Williams 1979). However, studies on numerous groups have demonstrated this is clearly untrue and sex ratio adjustments of broods are made pre-egg laying, depending upon factors such as environmental conditions (Komdeur 1996; Komdeur and Pen 2002). Pen and Weissing (2000) also demonstrated that the nature of an Evolutionary Stable Strategy means sex ratio prediction depends on whether all females produce similar or varied sex ratios.

Clutton-Brock *et al.*'s (1984; 1986) work on red deer females producing different sexed offspring depending on their condition provided empirical support for Trivers and Williard's (1973) theoretical assumptions and predictions. This differential sex allocation has now been observed in numerous taxa (Hamilton 1967; Clark 1978; West 2009). Variation in sex ratio has also been shown to respond to numerous factors, with birds being the most well studied taxon. Avian sex ratio adjustments have been reported to correlate with helpers (West 2002), mate quality (Sheldon *et al.* 1999), social status of females (Westerdahl *et al.* 2000), females' size-related weight (e.g., Nager *et al.* 1999; Thuman *et al.* 2003), attractiveness of fathers (e.g., Ellegren *et al.* 1996; Sheldon *et al.* 1999; Griffith *et al.* 2003), and maternal age (Heg *et al.* 2000). These are all traits that could influence the differential relative fitness benefit from sons and daughters. Other factors that have been found to affect the sex ratio of offspring in various organisms include: time in breeding season (e.g., Dijkstra *et al.* 1990; Lessells *et al.* 1996;

Andersson *et al.* 2003), egg-laying order (e.g., Heinsohn *et al.* 1997), distance to resources (Peterson and Roitberg 2006), resource levels (Kim 1999), and territory and habitat quality (Komdeur 1996; Komdeur *et al.* 1997; Byholm *et al.* 2002; Kasumovic *et al.* 2002; Ewen *et al.* 2003; Forsman *et al.* 2008).

Frank (1987; 1990) demonstrated how Trivers and Williard's (1973) sex adjustments may result in biased population sex ratio investment. He also realized that the direction and magnitude of these adjustments might be difficult to predict depending on biological details that are extremely complex or nearly impossible to know. An important implication of this, which West (2009) states is rarely appreciated, is that analysis of population level sex ratio at large will often be useless in demonstrating whether allocation is being adjusted in response to local conditions.

The sex ratio impact of various patch qualities in terms of parental fitness consequences was demonstrated in the Seychelles warbler by Komdeur (1998). He showed the advantage of producing a specific sex based on patch quality. Sons were produced under low quality conditions and daughters under high quality conditions. Komdeur also found that breeding success of offspring increased more rapidly for daughters than sons with higher quality territory. This environmental influence on offspring quality may be a major factor impacting parental sex allocation decisions. This work provided a clear example of sex ratio adjustment in vertebrates, suggested a preovulation control of sex (as there was no gap between the first and second eggs, Komdeur *et al.* 2002), and also illustrated how multiple selection factors can work simultaneously on sex ratio decisions in vertebrates. Environmental influence has been shown to have a key influence on offspring quality (i.e. allocation) (Komdeur 1998;

Doutrelant *et al.* 2004). Komdeur (1998) also addresses common sex ratio theory assumptions, in particular showing how selection acts on sex ratio variation providing explicit evidence for the adaptive nature of this variation.

A good deal of work has also been conducted on organisms that manipulate secondary sex allocation. Female zebra finches (*Taeniopygia guttata*) were found to alter allocation to reared offspring based on the mothers' manipulated wing quality (Foster and Burley 2007). Mothers with trimmed flight wings focused efforts on rearing sons, while control females allocation was focused on daughters. In this species, males also provide substantial care to the altricial young (Delesalle 1986; Burley 1988). Males mating with control females did not show a consistent preference towards one sex (Foster and Burley 2007). However, males mating with manipulated females demonstrated the opposite behaviour of their mates and focused effort on rearing daughters. Not only do mothers allocate preferentially to a given sex based on condition, in species with paternal investment, male behaviour may not simply mimic that of the mother. In this thesis I concentrate on Hymenoptera where the female perspective will be the focus as males are not involved in allocation to offspring.

When sex allocation has been addressed in hymenoptera the focus is generally on the social impact of these decisions, whether it is daughters helping mothers or the complex eusocial benefits of each sex to the various classes within the colony. There have been many interesting discoveries such as the differential optimal sex allocation between the queen and her workers (West 2009). However, in this thesis I focus on the less-studied solitary bees; these bees can be used to address resource and sex allocation questions without the confounding factors of relatedness, multiple queens, and differing optimal sex allocation for members of a hive.

Parental investment in the production of offspring includes the cost of locating and obtaining resources to provision those offspring. Central place foragers are a prime example of forgers that must repeatedly pay such costs through multiple “return trips” from a single location (Schoener 1979; Orians and Pearson 1979; Stephens and Krebs 1986). Under such conditions, resource exploitation rates having been found to decrease as distance from the “home” increases (Rosenberg and McKelvey 1999). Therefore, in situations where maternal fitness returns change differentially for each sex, changes in the cost of investing in each offspring has the potential to impact optimal offspring allocation and offspring sex ratio.

Hymenoptera are particularly useful in addressing questions regarding offspring allocation because their haplodiploid sex determination means mothers directly control the sex of their offspring through fertilization decisions (Cook 1993). This allows mothers to adjust to more subtle benefits of a given sex as a result of the low cost. The alfalfa leafcutter bee (*Megachile rotundata*), a protandrous species, will be used in this research. Nesting hymenopterans are an excellent system for studying resource allocation, as the size of a female is expected to be related to that individual’s lifetime fitness, which is typical of most insects. In addition, adult size is strongly correlated to the amount of food provisioned by the mother to the progeny in the brood cell (Klostermeyer *et al.* 1973; Freeman 1981a; Tepedino *et al.* 1984; Bosch and Vicens 2002). The nests produced by numerous solitary bees and wasps are linear nests where discrete brood cells are provisioned and laid sequentially, eliminating direct competition among offspring for food and unitizing investment along an easily followed time-line (Freeman 1981b).

1.2 Life History of Leafcutter Bees

Megachile rotundata (Fabricius), also known as the alfalfa leafcutter bee, is a solitary, gregarious, cavity-nesting, leaf-cutting bee native to southeastern Europe and southwestern Asia (Kemp and Bosch 2000). The leafcutting bee was accidently introduced to the eastern United States in the 1930s and then intentionally introduced into Canada in 1962 for the purpose of alfalfa (*Medicago sativa*) pollination (Hobbs 1972).

The leafcutter bee is a central place forager (Orians and Pearson 1979; Schoener, 1979) and, at most North American latitudes, emerges and mates during June and July (Stephen 1981; Rank and Goerzen 1982). Females then start building nests in pre-existing cavities such as beetle burrows in trees (Richards 1984). Mating generally only occurs once (Gerber and Klostermeyer 1970; Krunic 1971) and females retain enough sperm to fertilize their complete complement of eggs (Richards 1994). Linear nests are constructed with a number of brood cells in preformed cavities, allowing for the use of standardized artificial nests (McCorquodale and Owen 1997). Females complete all work, males are only involved in mating. *Megachile rotundata* is polylectic, with females preferring flowers of the legume genera *Medicago* and *Melilotus* (Kemp and Bosch 2000).

Each mother constructs brood cells by lining the walls of the nest cavity with leaf pieces and filling these ‘cells’ with provisions of pollen and nectar. Single eggs are then laid on this pollen and nectar provision and the brood cell is sealed with leaf pieces. New cells are initiated in front of just-completed cells. When a nest is completed, the mother uses 10 - 40 leaf pieces to form a cap that seals the entrance to the nest. Offspring overwinter as pre-pupa (this species is univoltine in our study area) and emerge from brood cells as adults. All food consumed before adulthood is provided by the mother. This species is protandrous in that males emerge first. A wide range of sex ratios (proportion female ranges from 0.20 to 0.45) have previously been reported for this species (Maki and Moffett 1986; Jay and Mohr 1987; McCorquodale and Owen 1997; Tepedino et al. 1994), suggesting that females vary the sex ratio of offspring in response to changing conditions.

This bee is sexually dimorphic, with females 1.2-1.3 times larger than males (Klostermeyer and Gerber 1969; Klostermeyer et al. 1973). Adult size is controlled by the amount of food provided by the mother in the brood cell (a small egg provisioned with pollen and nectar, wrapped in leaves) (Klostermeyer et al. 1973), and the heritability of body size seems to be minimal (Tepedino et al. 1984).

1.3 Overview of Thesis Chapters

Resource-and-sex allocation theory has developed as an integral part of evolutionary biology. Fisher's (1930) equal investment and Trivers and Willard's (1973) maternal condition facultative sex allocation hypotheses are two of the most important works in sex ratio theory and have fuelled much further research on the subject. My current work elaborates on the work of others and myself, here using the alfalfa leafcutter bee (*Megachile rotundata*) to demonstrate the impact of changing environmental and ecological conditions on resource and sex allocation. I also examine how nest defence is impacted by nest state and what foraging currency mothers maximize. In Chapter 2 a dynamic state variable model is used to address the question of whether mothers optimize a single factor (nest state or mature egg state) related to the investment in offspring, or do mothers find an optimal balance between these two states in relation to maximizing lifetime reproduction. Chapter 3 addresses the question of how nest defence changes in relation to the developmental state of the nest. Chapter 4 aims to determine the foraging currency that leafcutter bees maximize by examining behaviours when nests are located various distances from resources. Chapter 5 is a combination of three separate experiments that examine how changing ecological conditions and differing population densities impact sex allocation decisions. Finally, in Chapter 6, I summarize the findings in a unified framework, especially comparing the theoretical work and how those finds compare to field sex allocation experiment results.

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2: Egg Maturation, Nest State, and Sex Ratios: a Dynamic State Variable Model

Jason H. Peterson[2] & Bernard D. Roitberg[2]

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[2] Evolutionary and Behavioural Ecology Research Group, Department of Biological Sciences, Simon Fraser University, Burnaby, BC, V5A lS6 Canada

2.1 Abstract

Background: Parents can invest in offspring through a variety of behaviours. Optimization models of these behaviours are usually based on determination of the single 'factor' parents optimize for a given set of conditions. Interactions between factors are rarely considered.

Question: Do mothers optimize a single factor related to the investment in offspring (e.g. current nest state or mature egg state), or do mothers find an optimal balance between these two in relation to maximizing lifetime reproduction?

Methods and key assumptions: A dynamic state variable model. We develop a 'hybrid' model that examines mothers' allocation decisions to offspring by considering mature egg and nest state as well as other environmental/ecological factors. We assume that mothers alter reproductive decisions based on their perception of costs and benefits of brood cell and nest construction. Some of these construction behaviours determine investment in one or a few offspring within a brood but others affect the entire brood. Egg maturation rate is a constant.

Conclusions: Our results demonstrate that there is no single limiting factor; instead, there is some 'optimal balance' between mature egg and nest state that determines the optimal reproductive decision.

Keywords: dynamic state variable model, Hymenoptera, nest provisioning decisions, offspring sex ratio, optimizing multiple resources.

2.2 Introduction

Numerous allocation problems occur in a variety of organisms when disparate resources must be optimized to maximize lifetime reproduction (Hengeveld *et al.*, 2009). In a similar fashion, when state dependence impacts payoffs, there will be occasions when the state of two different resources, often requiring disparate inputs, must be co-optimized. For example, one state could impact survival whereas another could determine reproductive rate, but the resources needed to satisfy these state dependencies could vary independently. Here we examine the relationship and interrelationship between current nest state and mature egg state in relation to maximizing lifetime reproduction.

Deducing the evolutionarily stable Fisherian sex ratio depends on the limiting factor in parental reproduction (Rosenheim *et al.*, 1996). This limiting factor becomes the 'currency' for the optimization of parental investment. Parents that optimize this limiting factor will gain the greatest lifetime fitness. For example, when food is the limiting factor, parents should maximize their fitness per unit of food allocated to offspring. Many such factors have been shown to impact reproductive fitness across a wide variety of species (Freeman *et al.*, 1980; Lloyd and Venable, 1992; Wrensch and Ebbert, 1993; Brunet and Charlesworth, 1995). Unfortunately, as noted above, there is frequently more than one limiting factor and such factors may not trade off in a simple 'either/or' fashion. When this is the case, there will be some optimal balance between reproductive factors that determine offspring allocation decisions.

In many species, this optimal determination will involve the central life-history decision on when to terminate investment in the current reproductive event. The basic assumption is that parental investment benefits show diminishing returns while at the same time investment in the current brood reduces future reproductive potential (Williams, 1966a; 1966b). The assumption is that the investment is terminated when the long-term benefit (measured as the marginal fitness gain from the current brood) and cost (measured as the marginal fitness loss due to foregone future reproduction) of further investment are equal.

Two key factors that impact the lifetime reproductive success of a mother are the rate at which she can mature eggs and the value of her current brood. In simple terms, all else being equal, the faster a mother can produce eggs, the more eggs she will be able to deposit during her lifetime. The value of her current (and possibly only) brood is key, as the size, number, health, protection, and so on of these offspring will determine how many of the mother's genes are passed on to the next generation. Here we develop a unifying theory using the common currency of fitness to address these two disparate but related resources, eggs and nest.

Although the 'present–future trade-off ' is conceptually simple when idealized as a single, discrete event, in reality parental investment in offspring occurs through a vast array of activities. Parents obtain various types of food, build nests, defend young from predators, and keep offspring warm. These investments are often difficult to quantify, as some are directed at a single or a few offspring while others are directed at an entire brood. This complex problem has received some attention, theoretically exploring mature egg state (Rosenheim *et al.*, 1996) and nest state (Peterson *et al.*, 2007). In this paper, we develop a hybrid model that examines mothers' allocation decisions to offspring by considering the state variables: mature eggs state, nest state and time state; as well as environmental/ecological factors, including mortality, food accessibility, and availability of nesting sites.

We develop a dynamic state variable model (Mangel and Clark, 1988; Clark and Butler, 1999; Clark and Mangel, 2000; Alonzo, 2002) to elucidate reproductive decisions in a solitary bee where both mature egg and nest state are primary state variables in the model. Mothers make the decision on the sex of their offspring and also when to terminate one form of investment by stopping one type of investment (provisioning) and engaging in another (sealing the nest). This nest-termination decision is based on the number of mature eggs, risk of mortality, nest state, provisioning state of the current brood cell, and the availability/ease of obtaining resources. The value of sealing the nest is a result of increased protection for offspring in the nest from predators, parasites, and other threats. We find that the different nesting behaviours are easily incorporated into the above framework and considering them expands the range of interesting phenomena explained (e.g. sex order of offspring).

Mortality can be the result of numerous factors such as predators, food shortage or parasites. In this model, mortality has been simplified with all of these different factors being combined into a background mortality risk. This risk of mortality is then based on the activity in which the mother is engaged (e.g. collecting pollen).

Oocyte production limits reproductive success in two ways over an individual's lifetime (Rosenheim *et al.*, 1996). There is both the physiological cost of egg production and the 'opportunity cost' of depleting a finite resource. The physiological cost of egg production in other insects has been shown to be high, resulting in reduced survivorship of the mother (Roitberg, 1989; Lessells, 1991; Tatar *et al.*, 1993). The result is that eggs are produced at considerable cost (Rosenheim *et al.*, 1996; Nager, 2006). The opportunity cost results from already-deposited eggs being unavailable for any future reproductive event. Egg maturation rate may be a further limiting factor in reproductive decisions (e.g. completing the nest, sex allocation) and thus has been integrated into our model. This allows us to better understand the effect of the interaction between mature egg and nest state on sex allocation decisions.

The construction of nests in solitary bees involves mothers building nests comprised of a number of brood cells, built and provisioned sequentially, with one cell being completed before the next is initiated (Freeman, 1981). The adult size of offspring is strongly correlated with the amount of pollen (we refer to pollen and nectar collectively simply as pollen) that is provisioned to the brood cell (Klostermeyer *et al.*, 1973; Bosch and Vicens, 2002). All food consumed by the developing progeny prior to adulthood is provided solely by the mother during production of that individual brood cell. Furthermore, the haplodiploid sex determination of hymenoptera allows the mother bee to completely control the primary sex ratio of her offspring, without the high costs found in other species [e.g. killing offspring of a certain sex (Burley, 1982)]. Sons and daughters are usually provisioned with different amounts of pollen, with daughters generally emerging approximately 20% larger than sons (Klostermeyer and Gerber, 1969; Klostermeyer *et al.*, 1973; Alcock, 1979; Cowan, 1981). Sealing (‘capping’) of the nest entrance provides extra protection from enemies, mostly parasitoids, and therefore likely increases the value of all offspring when sealed in the nest. However, once the nest is sealed, time and energy must be spent searching for a new nest construction site to harbour future offspring.

We used the alfalfa leafcutter bee, *Megachile rotundata*, as our model species and parameter values were either known or determined through experimental work reported elsewhere (Peterson, 2004; Peterson and Roitberg, 2006a, 2006b). We have observed that *M. rotundata* nests are often sealed early in the season before the nest cavity is completely full (J.H. Peterson and B.D. Roitberg, unpublished data). We also found that the size of the sealing 'cap' and even the number of 'caps' in a nest varied among individual females (Peterson, 2004). Caps varied in size from 1 to 30 mm and the number of caps from zero to three (J.H. Peterson and B.D. Roitberg, unpublished data). The timing of these activities is also likely to be affected by the availability of mature oocytes (Rosenheim *et al.*, 1996).

2.3 Methods

We combined two previous models that examined allocation decisions based on egg maturation (Rosenheim *et al.*, 1996) and nest state (Peterson *et al.*, 2007) to develop a more comprehensive investment termination behavioural model for solitary bees. Like our previous model (Peterson *et al.*, 2007), this new model examines when a bee should terminate investment in current offspring, how many offspring to produce in a nest, the sex of the offspring, and when to seal the nest. However, this model also considers the impact of egg maturation rate and its interaction with other factors. We assume that the mechanisms underlying these decisions evolved to maximize expected lifetime reproductive success.

A basic assumption of the model is the shape of the function relating the amount of pollen provisioned to each offspring to maternal fitness (Figure 2-1). Here, fitness is defined as the number of copies of alleles passed on to future generations wherein parents who invest more in an offspring generally accrue greater fitness returns from that offspring.

Our model is heuristic. However, wherever possible, we use parameter values that closely represent natural systems. We make three basic assumptions in drawing the maternal fitness functions. First, there are diminishing returns, so that the mother's fitness gain from successive pollen deliveries (i.e. the marginal fitness) falls. Second, each daughter requires more initial investment (they are larger), but eventually gives higher fitness returns (e.g. large females are able to produce more eggs while large males in a non-aggressive species have less benefit from increased size). Third, we have ignored potential population sex ratio effects on these functions (e.g. if males were rare, their value would be higher) (for more details, see Peterson *et al.*, 2007).

These assumptions in combination with previously proposed fitness curves for species such as *M. rotundata*, which have larger females than males (Frank, 1995; Rosenheim *et al.*, 1996), were used to develop our maternal fitness return curves:

$$f_m = -4 + (a_m(1 - e^{-(p\gamma_m)})) \tag{1a}$$

$$f_f = -6 + (a_f(1 - e^{-(p\gamma_f)})) \tag{1b}$$

where f_m is the fitness for a son (male) and f_f is the fitness for a daughter (female). The variables a_m and a_f control the asymptote of the curve of males (m) and females (f) respectively, and γ controls the slope based on the number of pollen loads (p) and the sex.

2.3.1 State dynamics

The three state variables in our model (Table 2-1) are: (1) pollen state (P) – the amount of provision in the current brood cell; (2) mature egg state (E) – the state of mature oocyctes; and (3) nest state (V) – the total value of completed brood cells (based on size, sex, and number) in the current nest. Mothers gain fitness both from the completion of an offspring brood cell [based on the amount of pollen provision (size) and sex of that offspring] and when a nest is sealed (based on the total value of the offspring in the nest increasing by a given percentage as a result of all cells being better protected).

Two of the state variables – pollen state (P) and nest state (V) – can change independently during each time step (t) depending on the decision made by the mother. In terms of mature egg state, regardless of the decision made by the mother she matures eggs at a constant rate (E). Time is considered in discrete units (t). Within each time unit, the mother bee faces a background probability of death based on which of the following decisions she chooses:

1. Rest, the safest choice. During rest (and all other activities), eggs mature at a constant rate, *e*, per time unit:

$$P(t + t_r) = P(t) \tag{2a}$$

$$V(t + t_r) = V(t) \tag{2b}$$

$$E(t + t_r) = E(t) + e(t_r) \tag{2c}$$

where t_r is the time spent resting.

2. Add more pollen to the current brood cell:

$$P(t + t_p) = P(t) + 1 \tag{3a}$$

$$V(t + t_p) = V(t) \tag{3b}$$

$$E(t + t_p) = E(t) + e(t_p) \tag{3c}$$

where t_p is the time spent collecting pollen.

3. Lay a male egg (son) and complete the brood cell:

$$P(t + t_m) = 0 \tag{4a}$$

$$V(t + t_m) = V_t + O_{mp} \tag{4b}$$

$$E(t + t_m) = E_t + e(t_m) \tag{4c}$$

where O_{mp} is the increment in nest value from adding a son of pollen value (p) and t_m is the time required to deposit a male egg and close the brood cell.

4. Lay a female egg (daughter) and complete the cell:

$$P(t + t_f) = 0 \tag{5a}$$

$$V(t + t_f) = V_t + O_{fp} \tag{5b}$$

$$E(t + t_f) = E_t + e(t_f) \tag{5c}$$

where O_{fp} is the increment in nest value from adding a daughter of pollen value (p) and t_f is the time required to deposit a female egg and close the brood cell.

5. Seal the nest entrance and search for a new nesting site. The value of sealing the nest is determined by the nest state at the time of sealing.

$$P(t + t_s + t_n) = 0 \tag{6a}$$

$$V(t + t_s + t_n) = 0 \tag{6b}$$

$$E(t + t_s + t_n) = E(t) + e(t_s + t_n) \tag{6c}$$

where t_s is sealing time and t_n is time to find a new nest. The act of sealing the nest results in the final fitness benefit the mother will receive from this nest. The benefit is based on the value of offspring sealed in the nest, as each offspring has increased protection. No further fitness can be gained from this nest and a new nest site must be found. Therefore, P and V take values of 0.

2.3.2 Terminal fitness

All fitness is accrued through offspring production, provisioning, and sealing. Thus when $t = T$, $F(p, e, v, T) = 0$ for all values of P, E, and V.

2.3.3 Dynamic programming equation

For this dynamic programming equation (7), Line 1, right-hand side, calculates the expected fitness from resting and amount of eggs that mature during this time. The e values in each of the following lines takes the current values of mature eggs plus the eggs that mature during the time required to complete this decision, discounted by survival while resting $(1 - \mu_r)$. Line 2, right-hand side, is expected fitness from adding pollen and eggs matured during this time, discounted by survival while collecting pollen; Line 3, right-hand side, is current plus expected fitness from producing a son (f_m) and matured eggs, discounted by survivorship during the son production process; Line 4, right-hand side, the same as above but for a daughter (f_f); and Line 5, right-hand side, is fitness from sealing the nest and maturing eggs discounted by survival while sealing plus expected fitness discounted by survival while seeking a new nest. The 'max' term refers to the decision to commit to one of the five mutually exclusive behaviours, which yields the maximum fitness value.

$$F_{max}(p, e, v, t) = \left[\begin{array}{l} (1 - \mu_r)\ F(p, e + e_r, v, t + t_r) \\ (1 - \mu_p)\ F(p + 1, e + e_p, v, t + t_p) \\ f_m + (1 - \mu_m)^{t_m}\ F(0, e + e_m, v + 0_{m_p}, t + t_m) \\ f_f + (1 - \mu_f)^{t_f}\ F(0, e + e_f, v + 0_{f_p}, t + t_f) \\ (1 - \mu_s)^{t_s}\ vk\ (1 - \mu_n)^{t_n}\ F(0, e + e_s, 0, t + t_s + t_n) \end{array} \right] \tag{7}$$

The basic parameter values were estimated when data were not available. A given value was chosen that would produce biologically meaningful results (e.g. reasonable number of offspring produced in a lifetime), making sensitivity analysis more informative. We varied the time required to provision a brood cell, time required to mature oocyctes, risk of adult mortality (μ), value of sealing the nest (k), time required to locate a new nest site, and the shape of the maternal fitness return curves. The many mortality factors (e.g. predators and parasitoids) were generalized into a background mortality rate that assumes there is a given chance of death for every time unit, allowing the model to be more generally applicable. As we increase this background mortality, the less likely a mother is to survive each time unit. There are no reliable data available on the value (fitness benefit) of sealing a nest (k) for solitary hymenopterans. Therefore, we considered a wide range of values from zero to a five-fold increase in the value of the nest.

We employed our model in two different ways. First, we used backward induction starting from $t = T$ (where all states are known) and moving back in time to determine the optimal decision for every possible set of circumstances. This process yields an optimal decision matrix. Then we employed this matrix in a forward iteration (i.e. simulation) starting at $t = 1$ with a given set of conditions and then moving forward through time before drawing the appropriate decision from the matrix.

2.4 **Results**

In our current model, as well as in Peterson *et al.* (2007), the results showed that the greater the mortality risk (probability of death per unit time = μ), the more likely mothers are to produce sons; here we examine the point where production switches to a majority of sons. First, we examine the relationship between death rate and egg maturation rate (Table 2-2). Then we examine how this relationship is impacted by several other factors: pollen collection rates, time to lay an egg and complete the cell, value of sealing, sealing time, time to find a new nest site, and length of season. When the chance of death per time unit is extremely high ($\mu > 0.04$), these factors have very little impact on maternal offspring-related decisions. Therefore, we focus on μ values less than 0.04 unless otherwise stated.

2.4.1 Death rate and egg maturation

Considering sex allocation decisions, the general relationship between the impact of egg maturation rate and death rate (μ) on sex allocation is that as the maturation rate increases, the lower the μ value before mothers switch to producing more sons than daughters (Figure 2-2). Using our benchmark values, there is an almost 30% increase in μ values where mothers switch to producing a majority of sons between high egg maturation (e = 0.10 eggs matured per time unit) and low egg maturation (e = 0.05 eggs matured per time unit). This trend continues as egg maturation rates increase, until females mature eggs at a rate where they are constantly available.

Egg maturation rate impacted nest size (sealing decisions) across all variables, except when μ was extremely high (above 0.4). The lower the egg maturation rate, the more frequently the nests were sealed (i.e. fewer offspring were produced before the current nest was sealed and a search began for a new nesting site). Figure 2-3 shows higher egg maturation rates result in a greater number of offspring produced before the nest is sealed and also how this effect declines as μ increases.

Total cell production was double with very low μ values when comparing high (0.10) and low (0.05) egg maturation rates. This difference decreased with increasing μ values to the point where the number of cells produced was similar with very high μ values (0.04).

We now use these baseline results to examine the impact of other factors on individual variables that most impact mothers' allocation decisions. For a complete table of generalized results, see Table 2-3.

2.4.2 Pollen collection rates

Sex ratio was only impacted when pollen collection rates and egg maturation were both very low, resulting in basically only sons, even with very low μ values. Total cell production was consistently 30% greater with high compared with low egg maturation rates.

2.4.3 Time to lay an egg and complete the cell

The size of nests doubled with low compared with high egg maturation rates at very low μ values.

2.4.4 Value of nest sealing

Nest size was consistent across sealing values with low egg maturation, but with high egg maturation the size of nests at very low μ values was many times greater. This difference disappeared with even moderate μ values.

2.4.5 Length of season

All season lengths demonstrated a gradual increase in nest size as the egg maturation rate increased. This increase was most significant between egg maturation rates of 0.1 and 0.2 (unlimited eggs) where the size of nest before sealing doubled. When the season is shorter, mothers tolerate a much higher μ value before switching to the production of males.

2.4.6 Sealing time

High egg maturation rates and very low μ values resulted in mothers not sealing the nest until later, resulting in very large nests. Mothers sealed the nest earlier (i.e. with fewer offspring) when time required to seal the nest was short and the egg maturation rate was high, while nest size remained relatively constant when the egg maturation rate was low.

2.4.7 Time to locate a new nest

Increasing the time required to find a new nesting site, after the previous nest had been sealed, dramatically increased nest size to the point where mothers produced only one nest with very high search time to find a new nest site.

2.5 Discussion

Our model deals with animals that must allocate disparate but related resources towards their offspring. The goal of previous theoretical models was to identify factors that limit lifetime reproductive success. In those models, a single limiting factor determined how mothers maximized investment. These models generally considered factors such as availability of different resources, with Rosenheim *et al.* (1996) examining egg maturation and Peterson *et al.* (2007) nest state. Depending on the conditions employed, different factors were limiting and controlled the optimal decision. Our current model takes another step towards broad applicability by considering the possibility that no single limiting ‘resource’ is optimized. In reality, mothers need to balance the use of multiple resources. Our current model considers all of the above factors and asks whether changes in more than one resource under a given set of conditions alter optimal decisions. Our results demonstrate that, in general, there is no single limiting factor but some ‘optimal balance’ between mature egg and nest state to determine the optimal reproductive decision.

Maternal investment models traditionally have the benefits from decisions incremented once. However, there are circumstances in which the investment is incremented a second (or more) time (Peterson *et al.*, 2007); such is the case for many solitary bees. A mother is able to accrue fitness first by producing offspring and then add on fitness by further protecting that offspring through sealing the entrance to the nest. This is a form of Clark’s (1994) asset-protection principle. The theoretical bees in both our current and previous model appear to follow this principle, as the greater the benefit of sealing the nest the more frequently this terminal activity occurred. Accepting a guaranteed smaller reward from sealing now, as opposed to waiting for a potential larger reward in the future, indicates mothers benefit more by ensuring some sealing benefit is received. In addition to these theoretical results, we have experimental evidence to support the asset-protection principle in leafcutter bees (J.H. Peterson and B.D. Roitberg, unpublished results; Chapter 5).

While single-factor optimization models for reproduction decisions have been useful, in reality decisions are rarely controlled by a single factor. Instead, it is the interrelationship between factors that controls decisions made by organisms across a variety of situations. Considering both egg maturation rate and nest state (asset protection) allows us to examine the more realistic world where the choice may not be ‘either/or’ but some optimal balance. In our unifying model, using the common currency of maternal fitness to address the impact of various states we found that a balance between factors determined optimization. Comparing the results of our model for an egg maturation rate of 0.05 and the time to collect a unit of pollen (t_p) is 1 versus 2, the female sex ratio drops below 50% at $\mu = 0.046$ and $\mu = 0.019$ respectively, suggesting that pollen collection is the limiting factor. However, when we compare the difference between these pollen collection rates with a higher egg maturation rate (0.10), these same μ values are 0.036 and 0 respectively. Examining the four vastly different μ values means that both the egg maturation rate and the pollen collection time alter the optimal decisions in a non-linear fashion. There is no single limiting resource; there is an optimal trade-off between various resources.

Our results demonstrate that the lower the egg maturation rate, the fewer offspring are produced before the nest is sealed. Asset protection is employed with smaller nests when the time to mature an oocyte is greater. In this context, time spent sealing the nest is not so much ‘lost time’ as eggs are matured during this time, and the mother’s only other option may be to rest when she has no mature eggs ready.

In general, high death rates ($\mu > 0.4$) tend to override all other factors in influencing maternal decisions (e.g. Figure 2-3). At lower μ values, many factors impact nest size as we observed previously: time required to provision a brood cell, risk of adult mortality, value of sealing the nest, time required to locate a new nest site, and the shape of the maternal fitness return curves (Peterson *et al.*, 2007). When we considered egg maturation rates and μ, we found that lower egg maturation rates generally muted or even eliminated the impact of these factors. At high egg maturation rates, the size of the nests increased as μ decreased, as we found previously. Low egg maturation rates masked other effects and the size of nests remained consistent across μ values.

Considering sex allocation decisions specifically, our theoretical model demonstrates complex interactions between egg maturation rates and death rates. However, in general, the more dangerous the environment, the more likely mothers are to produce the cheaper sex, in this case sons. However, the lower the maturation rate, the greater the risk of death before production shifted to sons. In other words, as egg maturation becomes a more limiting factor, the probability of producing a daughter increases. In extremely low egg maturation systems, the amount of time spent provisioning the cell is less important, as the mother will have to wait even after the cell is provisioned for an oocyte to mature so that she can deposit the egg and complete the brood cell. This waiting behaviour is frequently seen after provisioning is completed on one cell and before a provisioning a new cell begins (J.H. Peterson and B.D. Roitberg, unpublished data).

There is an upper maximum above which further increases in the maturation rate do not impact decisions. Above this point the mother essentially always has a mature egg ready to deposit and therefore egg maturation is no longer a factor in allocation decisions. At the other extreme, where mature oocyctes are extremely limited, eggs may become the only factor determining allocation decisions. Given our proposed maternal fitness curves (Figure 2-1), mothers who must spend time waiting for oocyctes to mature will gain more fitness per unit time by producing a large daughter than by either producing a larger son or a small son and spending the remainder of the time resting in the nest. The exception to the latter would be when the environment is extremely dangerous and resting in the nest is very safe. Under these conditions, the mother may choose to produce a small male. Notwithstanding these specific circumstances, the production of females when maturation rates are slow returns the greatest maternal fitness per unit time. However, a single factor does not control the best decision, as in the 'multifaceted parental investment model' (Rosenheim *et al.*, 1996). There is an interaction between multiple factors, which determines the most profitable decision.

Comparing decisions across a variety of μ values for scenarios where the total time (T) is 200, versus T = 50, results in a dramatic shift when the chance of death was low. When egg maturation rate was low, the μ value at which females switched to the production of sons when T = 50 was extremely high (μ = 0.090) compared with μ = 0.044 when T = 200. To put this into perspective, at a μ value of 0.09 a mother stands only a 50% chance of surviving to produce a single daughter. Increased egg maturation rates decreased this difference between long and short seasons such that when eggs were basically unlimited, the μ value at which mothers switched to the production of sons was similar.

Although we considered time as a variable, it is also important to remember that our model is aseasonal, and as such, we do not address the question of changing conditions over the course of the season (i.e. fall approaches). It is possible that capping a nest at the end of the season does not have the same benefits as capping a nest half way through the season. Carrying out experiments as described in Chapter 4, at various times throughout the year may help to elicit an answer to this question of the importance of typical changing conditions as the season progresses.

When pollen can be collected quickly, there is almost no impact of egg maturation rate on offspring sex ratio. However, as the pollen collection time increases, the μ values at which females switch to the production of sons become much lower with a high egg maturation rate. Increased pollen collection time is likely to occur later in the season when available resources are further from the nest; it is also later in the season when mothers are far more likely to produce sons (Rothschild, 1979). Doubling the time required to obtain pollen from the baseline value resulted in a switch to sons at an even lower μ value, and with a high egg maturation rate mothers never produced more than 30% females and quickly switched to all males as μ increased above zero. Our results at extreme values were congruent with results for a single limiting resource, although at many intermediate values there was an interaction between factors that impacted the optimal decision in these situations.

Although we have focused on solitary bees as our example species, the issue of dealing with multiple states and optimal balance is important across numerous groups. Blood-feeding mosquito mothers do not choose a single resource to optimize but make foraging decisions that explicitly consider the trade-offs between blood feeding, sugar feeding, and oviposition (Ma and Roitberg, 2008). In *Aphytis melinus*, there is a strong interaction between the influences of host feeding and honey feeding on lifetime reproductive success (Heimpel *et al.*, 1997). For parasitoids, the host organism supplies both an egg-laying site and the egg-laying nutrients; however, sugar sources such as floral nectaries are spatially separate (Bernstein and Jervis, 2008). Belovsky *et al.* (1989) considered predator–prey interactions such as bats hunting insects and snakes foraging on frogs. This work demonstrated that when considering foraging in more realistic (i.e.

complex) environments, simple predictions of single-diet preferences disappeared in favour of multifaceted wide-ranging diets. These examples demonstrate that the interaction between factors, as opposed to simply determining a single factor to optimize, is key to understanding numerous behaviours.

Our unifying model facilitates examination of optimal decisions among a variety of disparate resources. Using the common currency of fitness in this model to address the impact of various states demonstrates that optimal decisions were not simply a matter of finding the limiting factor, but a trade-off between multiple limiting factors. Many examples where two different variables were modified demonstrate that each of the four combinations resulted in different optimal decisions, such as altering pollen collection time and egg maturation rates. Not only is more than one factor being optimized, as conditions become more extreme one factor can become more influential. Lower egg maturation rates muted the impact of other factors and nest size varied little, whereas with high egg maturation rates nest size increased with decreased risk. We also found that mothers who must spend time waiting for oocyctes to mature or have shorter maximum life spans gain the most fitness by producing daughters. A single factor does not control the best decision; an interaction between multiple factors determines the most profitable decision.

2.6 Figures

Figure 2-1. Assumed cumulative maternal fitness gain is a function of the amount of time spent provisioning a single son (---) or daughter (—) for a given environment. Cumulative maternal fitness is considered the number of copies of alleles passed on to future generations wherein parents who invest more in an offspring generally accrue greater fitness returns from that offspring. [Both curves would reach the upper limit earlier (curves shifted left) if resources were easy to obtain, and the opposite would occur if resources were more difficult to obtain.] Daughters require greater initial investment [daughters are 20% larger than sons (Klostermeyer and Gerber, 1969; Klostermeyer *et al.*, 1973)], but increased investment yields greater fitness returns (i.e. large females are able to produce more eggs, while large males in a non-aggressive species have less benefit from increased size). These curves subsume survival and fecundity.

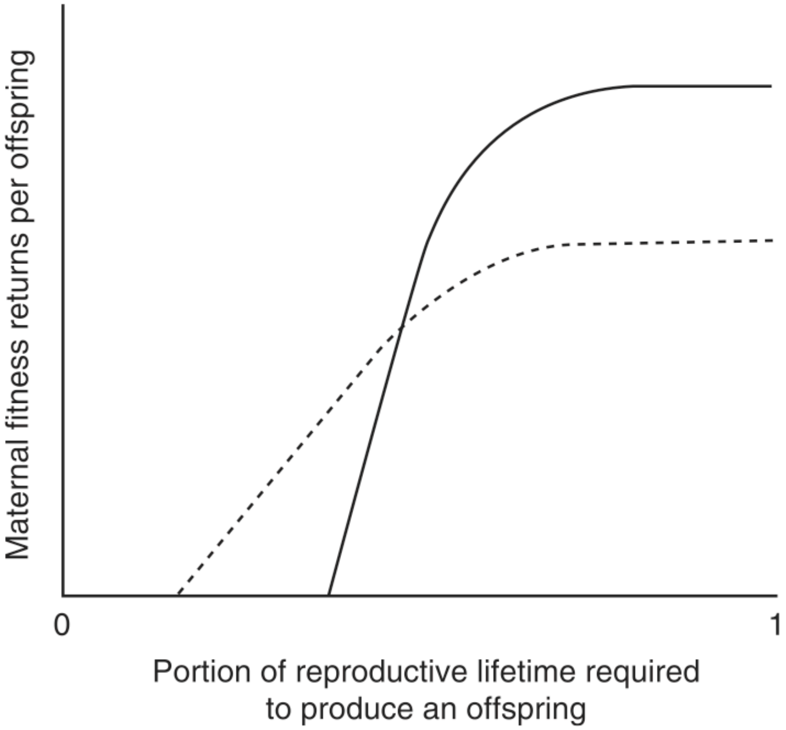


Figure 2-2. Sex allocation decisions by mothers over various egg maturation rates. The slower that eggs are matured, the greater the chance of death (μ) before mothers switch to producing sons.

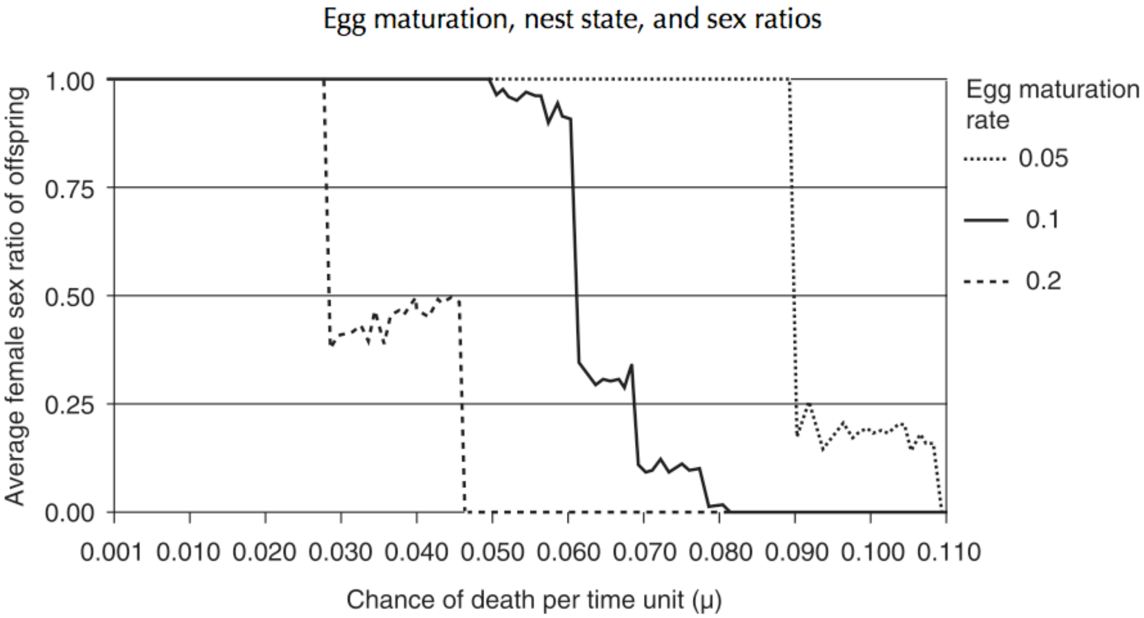
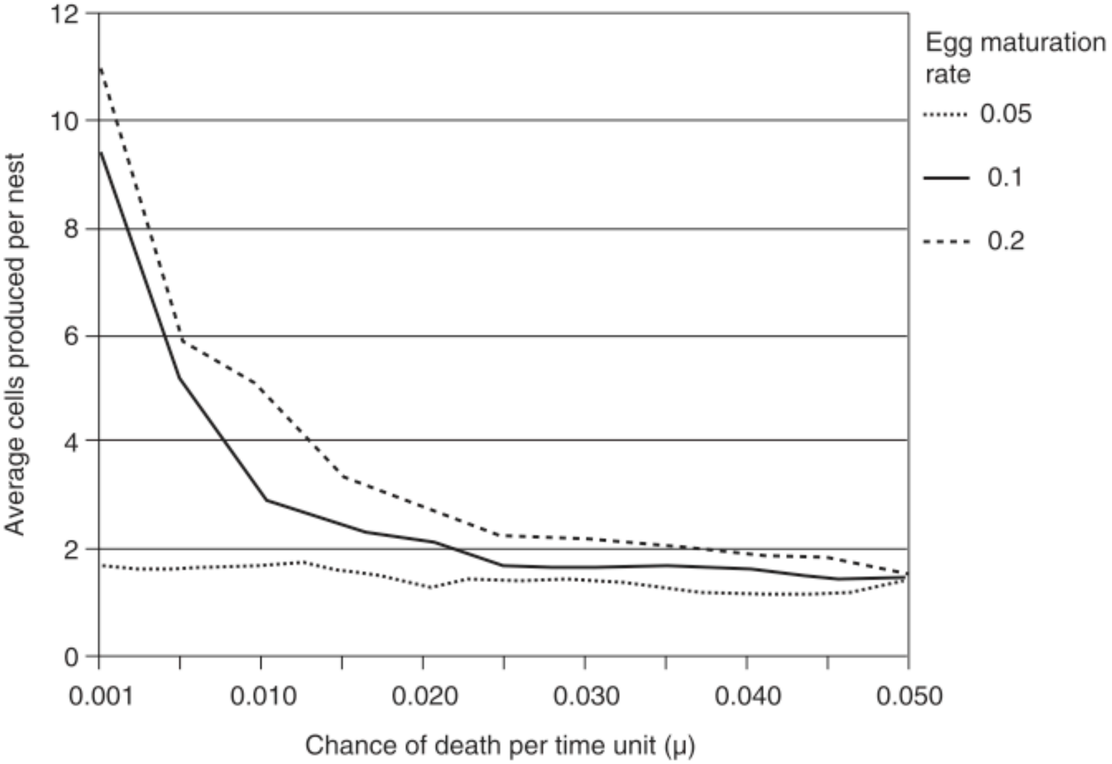


Figure 2-3. Number of cells produced per nest over a variety of egg maturation rates as the chance of death (μ) increases. When eggs are slow to mature (0.05), mothers cap small nests frequently, essentially waiting for eggs to mature, resulting in smaller nests. This behaviour decreases as μ increases, to the point where all egg maturation rates produce nests of similar size.



2.7 Tables

Table 2-1. Summary of state variables and other parameters used in our dynamic state variable model, and forward simulation of offspring allocation decisions in solitary bees.

Variable or parameter	Description	Range of values tested
Time		
T	Total number of time units	200
t	Time units	(1, 200)
State variables		
$P(t)$	Current pollen value collected	(0, 9)
$V(t)$	Current nest value	(0, 49)
$E(t)$	Mature egg state	
Parameters		
μ	Chance of death per time unit	(0, 0.1)
k	Multiplier of nest value from sealing	(1, 5)
t_p	Time required to add pollen	(0.5, 2)
t_m	Time required to produce a son	(1, 5)
t_f	Time required to produce a daughter	(1, 5)
t_s	Time required to seal the nest	(1, 30)
t_n	Time required to find new nest site	(1, 10)
O_{mp}	Fitness benefit for producing a son (m) as a function of the amount of pollen in the brood cell (p)	Based on equation (1a)
O_{fp}	Fitness benefit for producing a daughter (f) as a function of the amount of pollen in the brood cell (p)	Based on equation (1b)

Table 2-2. Optimal decisions (sex ratio, nest size, offspring size) made under high and low egg maturation rates with low, medium, and high death rates of hypothetical mother leafcutter bees.

	High egg maturation			Low egg maturation		
Chance of death:	Low	Medium	High	Low	Medium	High
Sex ratio (female)	1.0	0.5	0.0	0.5	0.0	0.0
Nest size (# offspring)	18	5	2	8	4	2
Offspring size, male	4	4	4	4	4	4
Offspring size, female	6	6	6	6	6	6

Table 2-3. Summary of how sex ratio, nest size, and cell production change with high and low egg maturation rates for various factors in a state-dependent foraging model for hypothetical leafcutter bees.

Note: The sex ratio column considers the chance of death required for mothers to switch to producing a majority of sons.

	Sex ratio: chance of death where offspring sex switches		Nest size		Cell production	
	High egg maturation	Low egg maturation	High egg maturation	Low egg maturation	High egg maturation	Low egg maturation
Default model values (increasing chance of death)	*Lower switch to sons*	*Higher switch to sons*	*Decreasing*	*Constant*	*Greater*	*Lower*
Increasing pollen collection time	Constant	Increases subtly	Increases	Constant	Increases dramatically	Increases dramatically
Increasing time to lay egg	Increases greatly	Increases	Decreases subtly	Decreases subtly	Decreases subtly	Decreases subtly
Increasing value of nest sealing	No change	No change	Decreases	Constant	Decreases subtly	Decreases subtly
Increasing length of season from $T = 50$ to $T = 200$	Decreases greatly	Decreases greatly	Increases greatly	Increases greatly	Increases	Increases
Increasing sealing time	No change	No change	Increases	Becomes parabolic	No change	Decreases subtly
Increasing time to locate new nest	No change	No change	Increases	Increases greatly	Decreases	Decreases

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3: Variation in Maternal Nest Defense Related to Nest State

Jason H. Peterson[1], Thomas S. Hoffmeister[2], & Bernard D. Roitberg[1]

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[1] Evolutionary and Behavioural Ecology Research Group, Department of Biological Sciences, Simon Fraser University, Burnaby, BC, V5A 1S6 Canada

[2] Institute of Ecology, FB 2, University of Bremen, 28359 Bremen, Germany

3.1 Abstract

Parental protection of offspring is found in numerous animal species. Protection provides offspring with a greater chance of surviving to be able to reproduce themselves, while at the same time posing a cost to the parent. Therefore the value of defense for the parent can vary depending on the developmental stage (i.e. value to the parent) of the offspring and their ability to defend themselves. For example, in commonly studied organisms (e.g. birds), defense level increases until offspring are able to leave the nest and from there, parental defense wanes. We examine these nest defence decisions in the solitary bee system that poses an interesting variation to traditionally studied organisms. Nest value does not simply increase as offspring are added to the nest. Here, individualized investments are allocated to each offspring, then as a final act, the nest entrance is sealed. This sealing action provides increased protection for all developing offspring in the nest, therefore strongly increasing their value. Our observational experiment found that mothers slightly increased nest defense as nest size increased. However, unlike traditionally studied organisms, this increase in defense continued more steeply until the nest was basically completed and sealed after which defense dropped suddenly.

3.2 Introduction

Parental protection is required for offspring survival in numerous animal species (Archer 1988; Clutton-Brock and Godfray 1991). This protection is generally risky for parents, resulting in a tradeoff between investment in current and future offspring (Trivers 1972; Dawkins and Carlisle 1976; Blancher and Roberstson 1982; Nealen and Breitwisch 1997; Olendorf and Robinson 2000; Hendry et al. 2004). Current offspring value is therefore likely to influence parental decisions on risky or energetically-costly offspring defense (reviewed in Montgomerie and Weatherhead 1988; Redondo 1989; Rytkönen et al. 1995). In fact, nest defense has been shown to be plastic and sensitive to variation in value of offspring (Sergio and Bogliani 2001).

Parents in a wide range of species have been shown to alter defense decisions based on offspring age (Montgomerie and Weatherhead 1988), brood size (Olendorf and Robinson 2000), renesting potential (Barash 1975), potential nest value (Patterson *et al.* 1980) and also the current state of the nest (Carlisle 1985; Curio 1987; Winkler and Wilkinson 1988). In general, parental defense increases over time as the offspring develop and where there is an increasing likelihood that progeny will survive to produce offspring of their own (Redondo and Carranza 1989). Thus, parents should be increasingly willing to defend their offspring asset as their investment and thus the asset value of the offspring increases over time (see Clark's Asset Protection Principle – Clark (1994)). An offspring's chance of unassisted survival increases later in development and therefore parental investment in defense decreases (Montgomerie and Weatherhead 1988). For example, in birds, newly laid eggs are less valuable, more vulnerable, and more cheaply replaced than nearly-fledged offspring and therefore less worth protecting.

Similarly, the marginal returns from investing in defense of offspring that are fully developed and capable of self-defense are not nearly so high as returns from defending offspring that are close to fledging. Figure 3-1A demonstrates how parents will increase defense with offspring age early in the offspring's life, but then decrease defense later when parental fitness benefit from defense decreases, as offspring are less vulnerable. Brood and nest defense follow patterns similar to defense of individual offspring and this general trend can be seen across numerous groups such as mammals, birds, and insects (Caro 2005).

Nesting birds are one of the most well researched organisms regarding nest defense behavior (Goubault *et al.* 2007). By contrast, the much less studied solitary bees offer unique opportunities to evaluate nest defense hypotheses. Here, parental fitness is gained from individually allocating pollen and nectar resources to each offspring sequentially, but then further increasing the value of all offspring at nest completion by plugging the entrance to the nest, protecting all the offspring therein. These temporally isolated offspring are helpless until given protection from a sudden single nest plugging in a clear-cut, easily quantifiable manner. Situations where organisms complete parental investment with some type of protective shield are found in a number of taxa (e.g. Stokes and Boersma 1998; Bourgeois and Vidal 2007). By contrast, in many other systems and in particular avian taxa, nestling vulnerability varies slowly and continuously with age and between offspring making interpretation of nest defense difficult because multiple factors may co-vary during that time.

Nest defense traditionally increases as the size and value of the nest rises then slowly decreases as offspring increase their ability to self-defend and/or avoid predator attacks (Figure 3-1A). With solitary bees that seal their nest, however, we hypothesized that the mother continues increasing defense until the nest cap (seal) is completed or near completed at which point nest defense is expected to cease. This maternal fitness benefit in solitary bees that nest capping provides is expected to alter the shape of Figure 3-1A to give the asymmetric defense curve Figure 3-1B. Mothers defend their nest through both encounters with attackers via "active defense" and protect the nest with a cap via "capping defense". Once the capping has occurred, the marginal returns from active defense are very low (analogous to mature nestlings) so active defense should rapidly fall off. We tested our hypothesis by simulating attacks on bees at nests at various stages of completion.

Using a simulated attack with a dried bee, means that we cannot be certain whether the mother is responding to a conspecific attack or a foreign predator. However, what we are interested is the defense activities of the mother based on this general attack of her nest.

3.3 Methods

We worked with alfalfa leafcutter bees (*Megachile rotundata*) at large, communal nest sites to conduct an observational experiment. Simulated nest attacks were conducted between July 21st and 27th 2009 near Tilley, Alberta, Canada (50°22' North, 111°40' West) at times when the temperature was above 24°C and bees were active. *Megachile rotundata* mothers nesting in commonly used commercial laminate wood domiciles (8cm deep and 5mm diameter) were "attacked" using a dried *M. rotunda* female attached to a flexible wire. The simulated bee intruder was "flown" into the randomly selected nesting cavity and manipulated to make repeated physical contact with the nesting female, in the same manner as natural attacks observed and analyzed via videotape (Peterson and Roitberg, unpublished data). We recorded the time from initial contact with the simulated bee intruder to the point at which the mother bee abandoned the nest or until 60 seconds had elapsed; we chose this termination criterion because attack durations longer than 60 seconds were extremely rare during our hundreds of hours of videoed observations. After each attack we moved to a new nesting site (at least 100 metres away) and haphazardly selected a new nest to attack. This avoided a cumulative impact of attacking nests of neighbouring mothers of this gregarious species.

Once the simulated attack ended as described above, the nest was immediately dissected. We measured the current length of the nest, the number of completed brood cells, the length of any uncompleted cells and/or the length of any cap that was under construction.

Since defense times of bees were right-censored in 114 of 419 cases wherein bees did not abandon the nest within the 60 seconds observation time, we used survival analysis in R 2.10.0 (R Development Core Team 2009) to test nest state dependent differences in the defense times of bees. We compared tests with an exponential data distribution (assuming a constant probability per unit time to leave the patch) and a Weibull distribution (assuming an increasing leaving tendency across time) and finally used a Weibull distribution because of its better fit to the data.

At the end of the season, 40 fully capped nests, which had not been subjected to simulated attack, were randomly chosen and dissected to determine the average length (mm) of caps in completed nests. This allowed us to determine the cap sizes that were in early versus late stages of construction during our simulated attack. We chose nests that were in close proximity to the attacked nests to remove any possible micro-site effects.

To determine the relationship between nest cap construction time and nest size, 13 nests were videotaped during the entire nest capping process. Mothers were videotaped entering and leaving the nest to collect each leaf piece that was used in cap construction. This time spent collecting leaves and using them to build the cap was recorded. Upon completion of the cap, the nest was dissected to measure the size of each cap.

We also investigated the relationship between the cap size and the number of brood cells in the nest. Therefore, at the end of the season 490 nests in the same domicile boxes as those in our simulated attack were dissected and the number of brood cells counted and the depth of the cap measured in each nest.

Each experimental attack is a specific data point, which was categorized in the above work. We also wanted to evaluate the results looking at each group along a continuum to examine the shape of the defense curve as nest size (i.e. amount completed) increases. We compare our results to a number of possible generalized linear models as a test of these competing hypotheses (Figure 3-2):

(i) A flat line (defense level is a constant), as we might expect in a situation where offspring susceptibility is invariant with age and so the value of the mother defense does not change.

(ii) There is greater investment into defense with offspring age. Offspring value alone determines level of defense. Here, the mother is willing to provide higher and higher levels of defense in order to protect her increasingly valuable offspring.

(iii) Parabolic models based upon returns from defense. Here, offspring become increasingly valuable yet defenseless as they age, but at some point there is a sudden change in value or susceptibility (e.g. offspring develop a trait after which they are able to defend themselves or become invulnerable to attacks).

As our experimental data contained censored values and can thus not be directly used for the analysis with generalized linear models, we drew 10 randomly Gamma distributed data sets with 100 replicates for each of the four nest states, with shape parameter and scale based upon the standard deviation and mean, respectively, of the original data sets. We thus created 10 simulated data sets that are conservative, i.e. they underestimate the mean of nest state 3, and compared generalized linear models for each of the data sets fitting just the intercept, linear models, quadratic models, and cubic models using Akaike values.

3.4 Results

The dissection of the 40 fully-capped nests at the end of the season resulted in only one cap that was less than 5mm in depth and 36 that where equal to or greater than 10mm. Therefore we defined nests with caps less than 5mm as Under Cap Construction and caps that are equal to or greater than 10mm as Completed Cap.

We divided the mothers that were attacked into four categories based on the state of their nest's construction at the time of attack (Figure 3-3): (1) New Nest (≤3.5cm of brood cells), (2) Advanced Nest (>3.5cm of brood cells produced, but no cap), (3) Under Cap Construction (<5mm deep cap) and (4) Completed Cap (≥10mm deep cap). Only after the nest was dissected were we able to place the mother into one of these categories; the size of the nest was not known during the simulated attack.

Nest defense continued to increase as more brood cells were constructed and as the cap construction began, before dropping dramatically near the end of cap construction. Defense times increased significantly from nest state 1 (new nests) to nest state 2 and nest state 2 to nest state 3 (advanced nests and cap construction initiated; Figure 3-4). Defense times were significantly lower for nest state 4 (completed cap) than nest state 1 (survival analysis with Weibull data distribution, χ^2=503.07, df=3, n=419, P<0.0001, all contrasts to nest state 1 P<0.001).

The relationship between the cap size and time spent capping was highly significant for the 13 capping events observed (Capping Size = 0.541 + 1.49 x Cap Time) ($F_{1,12}$=74.35, *P*<0.0001) with a R^2 of 0.87. The mean cap size was 10.8mm ± 1.0mm SE. The cap size to nest size (number of cells) relationship was also significant, however it generated an extremely low R^2 value of 0.07 from the 474 nests examined (Nest Size=6.10+0.15 x Cap Size) ($F_{1,472}$=35.7, *P*<0.0001).

A visual examination of our data and trend analysis of 10 randomly re-sampled data sets using generalized linear models with Gamma distribution of data and inverse link function suggests a rising parabolic curve with a strong drop near the end (Figure 3-2). Models for a linear increase of defense behavior (Figure 3-2B) are not significant (P>0.25 for all replicates) and do not explain the data better than a model assuming no effect of nest state on defense (Figure 3-2A); Akaike values consistently and on average for the 10 replications are lower for the latter, AIC=3445 ± 17.8 SD vs. 3443 ± 17.7 SD. In contrast, parabolic models (Figure 3-2C and 3-2D) are significant (P<0.0001) and always have lower Akaike values. A comparison of quadratic (Figure 3-2C) and cubic models (Figure 3-2D) suggests that cubic models with their slower increase and more rapid decline explain the data better than quadratic models; AIC=3061 ± 43.9 SD vs. 3145 ± 43.8 SD; 58.7 ± 3.1 % of the deviance explained by cubic models vs. 49.5 ± 4.2% for quadratic models.

3.5 Discussion

Nest defense research has generally focussed on social insects, birds, and mammals (Breed et al. 2004; Goubault et al. 2007). The parental defense curve has mostly been shown to increase as the value of offspring increases and then decrease (Figure 3-1A) as offspring begin leaving the nest and are able to fend for themselves or are less likely to suffer harm (Montgomerie and Weatherhead 1988; Clutton-Brock 1991). Solitary bees pose an interesting variation to traditionally studied organisms, because nest value does not increase in a simple monotonic fashion over time. Instead, the one-time sealing (capping) of the nest entrance just before the mother leaves the nest provides increased protection for all developing offspring in the nest. This act increases each offspring’s value, independent of any offspring behaviours as in other organisms.

Mothers must readily defend their nests from conspecific attacks, conspecific nest parasites, parasitoids (eg. *pteromalus* spp.) and other predators. All of these cases are likely to result in the loss of offspring. Prior to sealing behavior, we found that nest defense increased as nest size increased as generally expected. However, as the capping process begins nest defense increases dramatically and then drops off rapidly when the cap is of a significant size. The survival analysis in Figure 3-4 demonstrates that soon after capping is completed, the defense drops to levels even lower than at early nest states. The statistical analysis of generalized linear models suggests that a cubic model explains the data best. Thus, the first half of the defense curve is similar to the traditional curve, but there is no gradual and symmetrical drop off in nest/offspring defense like in quadratic models, but the drop off in defense intensity is much more pronounced. In our trend analyses, nest states have been spaced out evenly; however, it should be noted that the construction of a nest can take from one week to an entire lifetime, thus the time intervals between nest states 1 and 2, and 2 and 3 are much wider than the time interval between nest states 3 and 4. Consequently, the curve only declines during the last hours of nest construction. Taking this into account would only influence model selection such that the relative explanatory power of cubic models over quadratic models would increase.

In traditionally studied species, the parental decision benefits are incremented once (e.g. when the offspring has been fledged). However, solitary bees accrue fitness first by producing offspring and later by further protecting those offspring via sealing the nest (Peterson et al. 2007). This second fitness benefit is possibly a reason for the dramatic shift in the shape of the parental defense curve.

It is also important to remember that our observations were aseasonal, with the experiment taking place over a few days and so the impact of changing seasons was unlikely to be a factor. This begs the question, for future research, of how defence behaviour may differ based on whether the nest is capped half way through the season or near the end of the season.

There are numerous hypotheses for factors that control nest defense. These include parental investment being determined by: 1) parental predation risk; 2) reproductive value of offspring (Parent investment theory); and 3) the harm that unprotected offspring would suffer (Dale et al. 1996; Riou and Hamera 2008). In reality all of these hypotheses likely apply to any species, but their relative importance varies with breeding conditions, the type of predator/attacker, and current versus future reproductive value. The results from our solitary bee research support a combined version of the latter two hypotheses i.e. reproductive value and mortality risk to offspring.

We have approached the nest defense trade-off as an optimization problem despite the fact that interactions between conspecifics at nest sites are more like an owner-intruder game than a simple response to background predation (e.g. Bentley et al. 2009). However, note that the result of loss to an intruder is loss of brood and as such is analogous to encounters with predators at the nest. In addition, since we fixed the behavior of the simulated bee, we controlled for interactions between players and allowed for owner behavior to be expressed with regard to brood value and mortality risk at a single intruder value. Should intruders escalate their attempts at nest usurpation the outcome might change in a manner not predicted by our non-game competing hypotheses.

Research on a wide variety of organisms has shown that defense of offspring varies depending on conditions. Here we examined a less-well-studied situation where the value of nest defense does not taper off as offspring begin to fend for themselves, but drops off suddenly once the entrance to the nest has been sealed. Both birds and bees have this parabolic relationship (Figure 3-1), however in bees the curve is highly asymmetric compared to the slow drop over time in animals where offspring mature and gain defensive attributes. Further study of “nest” defense in a wider array of organisms outside of the bird and mammal taxa is likely to reveal other organisms displaying a similar defense strategy.

3.6 Figures

Figure 3-1. Theoretical values of defense for a mother at incubation or nest provisioning: A. Traditionally studied species such as nesting birds and mammals (key developmental points for birds included, i.e. L: egg-laying; H: hatching; D: nest departure; and I: age of independence) (Montgomerie and Weatherhead 1988). B. Species such as many solitary bees with capping/extra production.

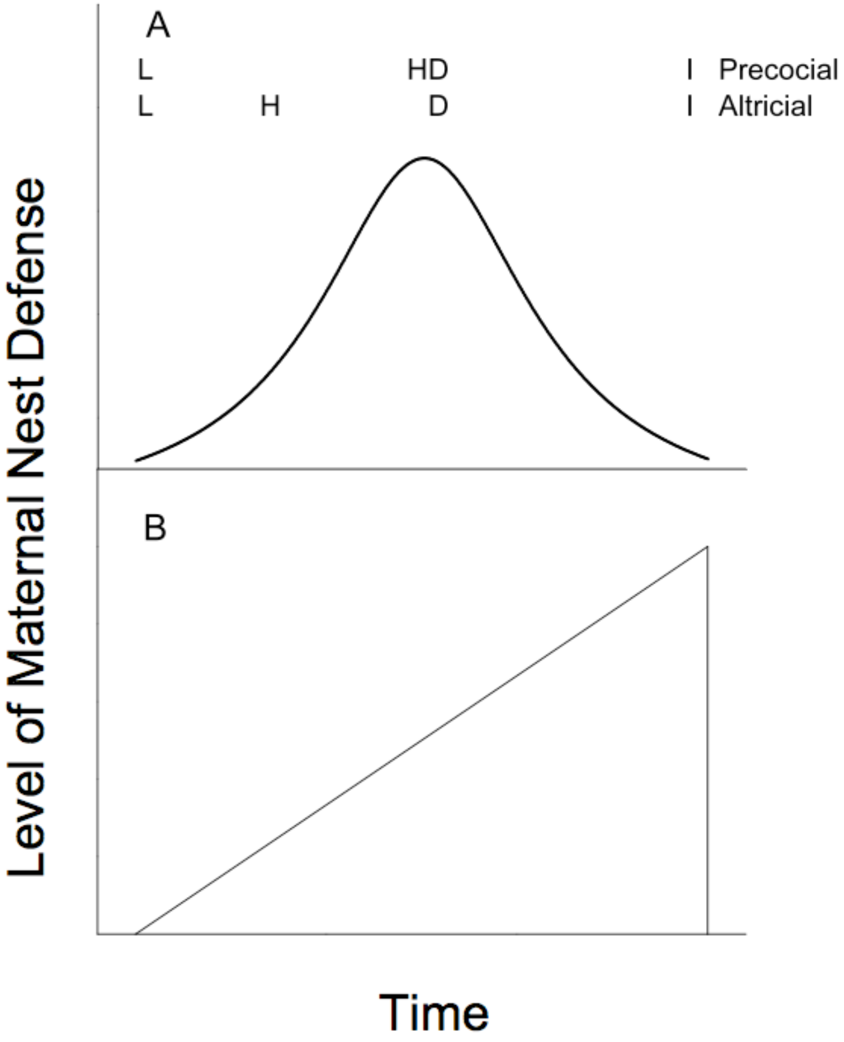


Figure 3-2. Nest defense intensity of solitary leafcutter bees across the four nest states as defined in Figure 3-3. Nest state 3 and 4 both occur near the end of nest construction. Solid circles represent arithmetic means for experimental data. The solid lines represent trend analyses for 10 randomly drawn Gamma distributed data sets with 100 data points for each nest state using generalized linear models with Gamma distribution and inverse link function. Panel A: Nest defense that is insensitive to nest state, estimating the intercept; Panel B: Linear models for nest defense as a function of nest state; Panel C: Quadratic models; and Panel D: Cubic models. (See text for the fit of the respective models to the data sets; Cubic is the best fit statistically)

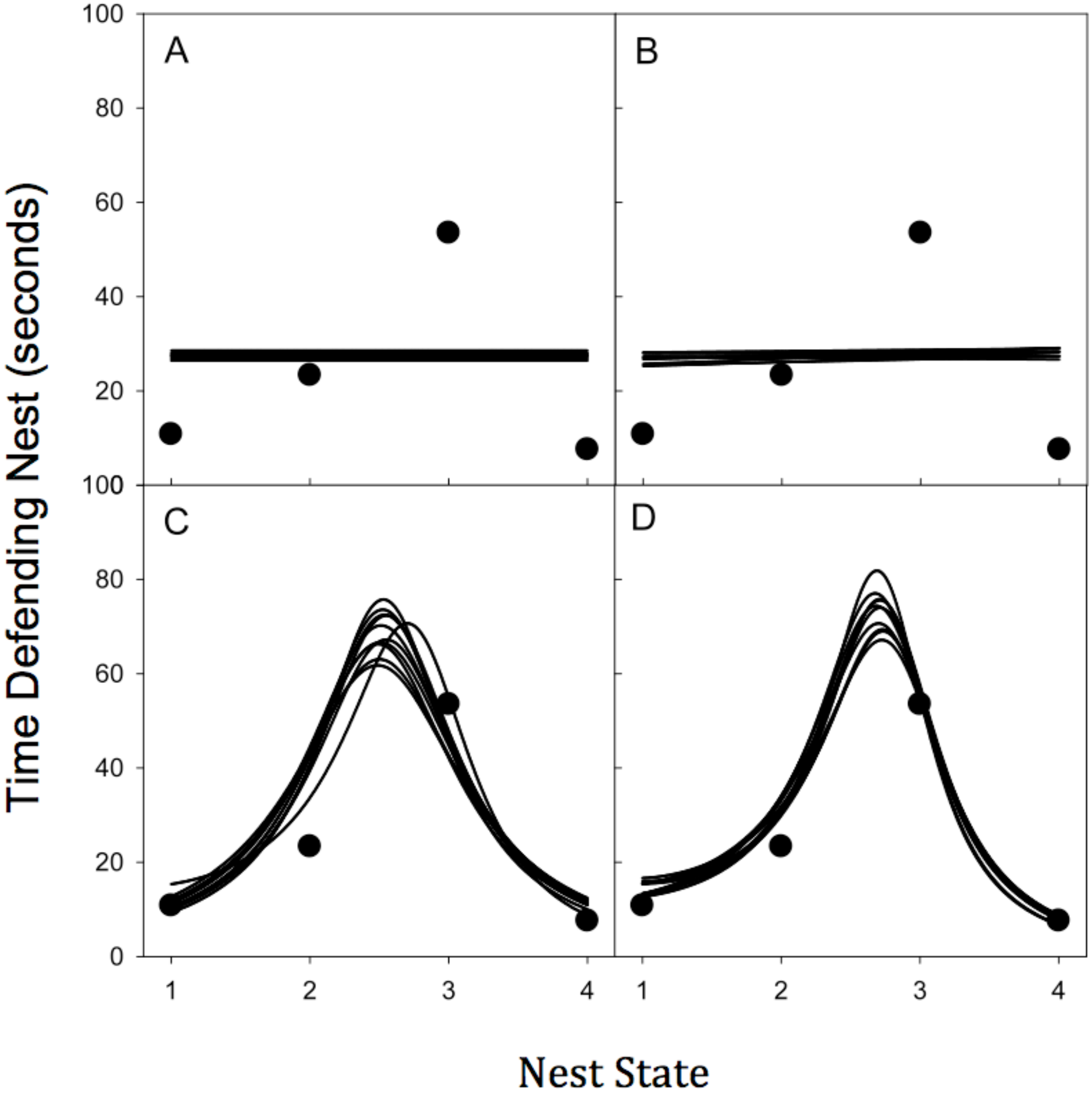


Figure 3-3. We divided the nests of the mothers that were attacked into four categories based on the state of their nest's construction at the time of attack: (1) New Nest (≤3.5cm of brood cells); (2) Advanced Nest (>3.5cm of brood cells produced, but no cap); (3) Under Cap Construction initiated (cap <5mm deep); and (4) Completing Cap (cap ≥10mm deep). The parts of the nest considered are:

i. Completed Brood Cell
ii. Brood Cell Under Construction
iii. Cap Under Construction
iv. Cap Completed

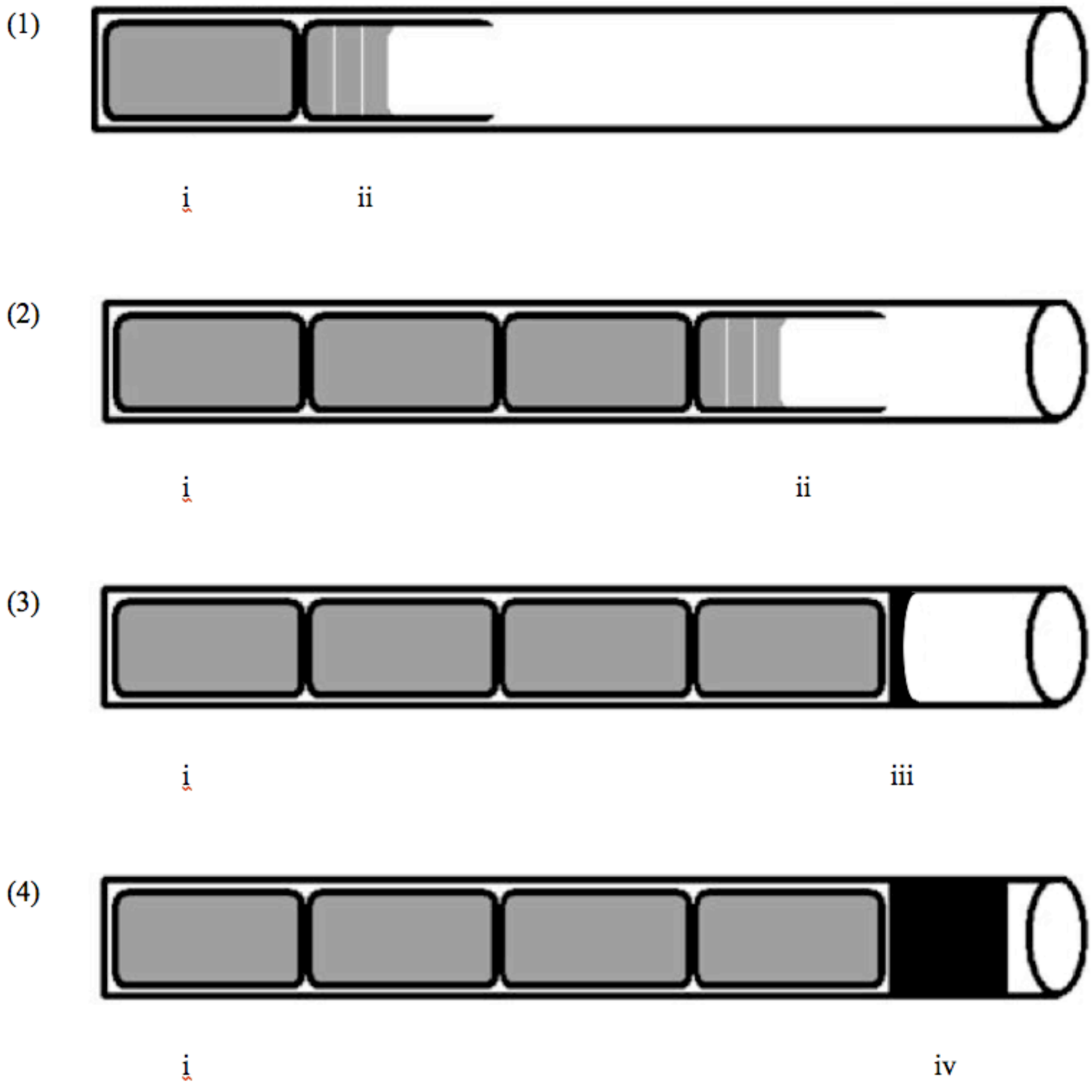
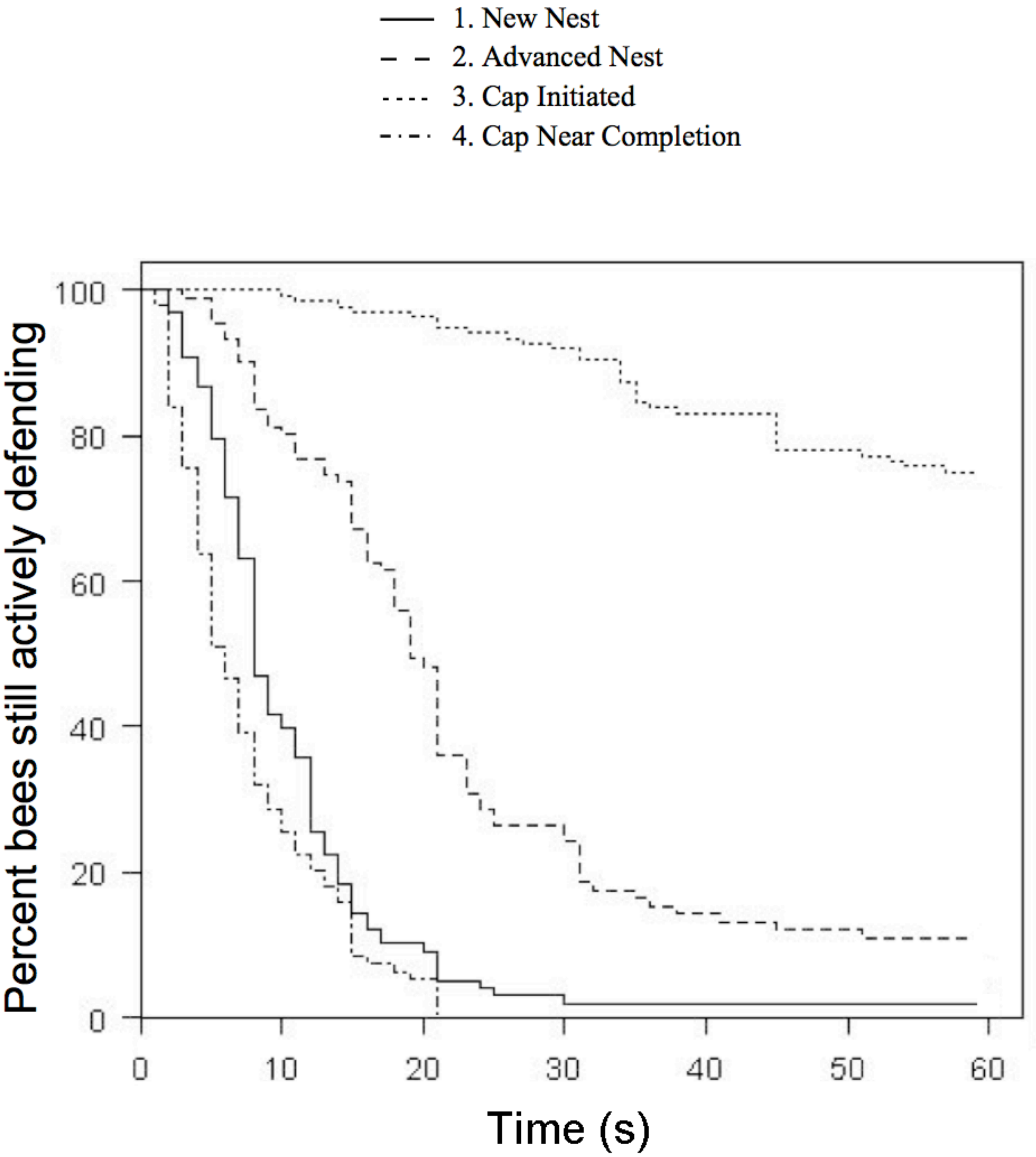


Figure 3-4. Plot of the time (mean + SE) mothers actively defended their nest from a simulated attack using a dried *Megachile rotunda* female attached to a flexible wire. The simulated bee was "flown" into the nesting cavity and made repeated physical contact with the nesting female. Nest states: (1) new nest (≤3.5cm of brood cells) solid line; (2) advanced nest (>3.5cm of brood cells produced, but no cap), dashed line; (3) cap construction initiated (<5mm deep), dotted line; and (4) where the cap is near completion (≥10mm deep), dash-dotted line.



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4: Foraging Currency in Reproductive Leafcutter Bees

Jason H. Peterson[1] & Ralph V. Cartar[2]

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[1] Evolutionary and Behavioural Ecology Research Group, Department of Biological Sciences, Simon Fraser University, Burnaby, BC, V5A 1S6 Canada

[2] Department of Biological Sciences, University of Calgary, Calgary, AB, T2N 1N4 Canada

4.1 Abstract

Foraging allows organisms to obtain needed resources. In order to understand this behaviour we seek to determine what foraging currency a given organism maximizes in order to maximize fitness (i.e. lifetime reproductive success). Optimal foraging theory traditionally assumes foragers maximize long-term net rate of energy intake (net energy/time). More recent research has suggested that other currencies such as efficiency (ratio of benefits to costs), are being maximized in numerous situations. We conducted field experiments to determine the amount of time a central place foraging female bee (*Megachile rotundata*) spent involved in the various activities required for nest construction. These included time out of the nest to collect a leaf or a load of pollen and nectar and time in the nest after collecting a leaf or load of pollen and nectar. We found that mothers when flying longer distances to obtain pollen and nectar increased the amount of pollen and nectar collected per trip compared to their short distance counterparts. However, fewer collection trips occurred when flying longer distances, resulting in smaller offspring under long flight distance conditions. If mothers were maximizing the net rate of energy intake, we would always expect full loads of pollen and nectar to be collected during foraging trips when pollen and nectar availability are high, regardless of the flight distance from the nesting site. The increase in load size with increased flight distance suggests mothers are behaving more in a manner that maximizes efficiency.

4.2 Introduction

Organisms forage to obtain resources. To understand this behaviour we seek to determine what foraging currency a given organism maximizes in order to maximize fitness (i.e. lifetime reproductive success). Classical optimal foraging theory assumes forager fitness is maximized through maximizing long-term net rate of energy intake (NREI) (i.e., net Energy/time) (e.g. Pyke, 1978; Waddington and Holden, 1979; Hodges, 1981). Maximizing the net gain per time unit makes sense, in that this will produce the greatest average foraging gain. This apparently simple and obvious theory of NREI has therefore been used in the vast majority of models as the currency organisms maximize (Stephens and Krebs, 1986). In regards to the application of this theory, a key prediction is that parents always collect a full load of resources unless intake rates are decreasing over time (i.e. resources become very scarce) (Orians and Pearson, 1979). However, this pattern has not always been observed in nature (Schmid-Hempel *et al.*, 1985; Welham and Ydenberg, 1988; Ydenberg, 1998).

The ratio of benefits to costs (i.e. efficiency) was initially rejected as a foraging currency, both because small cost-trivial benefits and larger cost-substantial benefits are not distinguished and because maximizing efficiency might often lead to low rates of energy intake (Stephens and Krebs, 1986). However, more recent empirical studies have found behaviours consistent with efficiency maximization and divergent from NREI (Schmid-Hempel *et al.*, 1985; Welham and Ydenberg, 1988; Welham and Ydenberg, 1993; Ydenberg *et al.*, 1994; Houston, 1995; Ydenberg, 1998). Efficiency has been shown to be a valid optimal foraging currency when the energy a forager can assimilate is limited and a forager is seeking to meet an energy requirement (Ydenberg *et al.*, 1994;

Nolet, 2002). Rasheed and Harder (1997) demonstrated that maximization of efficiency best predicted behaviour of bumblebees collecting non-energetic resources (pollen) for offspring.

Maximizing energetic efficiency per foraging trip predicts changing resource loads when foraging costs increase in relation to load mass (Schmid-Hempel *et al.*, 1985). Instances of foragers leaving non-depleting patches without full resource loads have been documented in numerous groups including honeybees and hummingbirds (Núñez, 1982; De Benedictis *et al.*, 1978). These organisms may be limited by a fixed foraging life and/or a limited flight budget (Schmid-Hempel *et al.*, 1985). In the case of the latter constraint, flight metabolism degenerates as the flight budget is used and bees become unable to forage (Neukirch, 1982). This is akin to a honeybee's wings wearing out (Higginson and Gilbert, 2004) or beak wear in true bugs (Roitberg *et al.*, 2005) effectively ending the "foraging life" of an organism.

Studies on central place foragers allow us to differentiate between the two aforementioned foraging currencies (NREI and efficiency) as these organisms are required to continually return resources to a single location, usually the nest (Schoener 1979; Orians and Pearson 1979; Stephens and Krebs 1986). The further resources are from this central location, the greater the cost of obtaining those resources, resulting in a lower net benefit. However, the impact of this distance-based cost on optimal load size differs between the currencies (see below), allowing for the potential to distinguish between them. That is, whether the currency the parent is maximizing is NREI or efficiency can be determined by examining parental decisions related to the amount of resources obtained per foraging trip and the cost of obtaining those resources.

Nesting hymenopterans provide an excellent system to study resource allocation. There is a direct correlation between the amount of food provisioned to the progeny in the brood cell and the subsequent size of the progeny as an adult (Klostermeyer *et al.* 1973; Freeman 1981; Johnson 1988; Bosch and Vicens 2002). Therefore, measuring offspring size and counting the number of pollen and nectar collection trips (referred to below simply as pollen trips) allows us to determine the average amount of pollen collected per trip. The leafcutter bee, *Megachile rotundata* (Fabricius), is a central place forager that makes numerous trips to collect both the leaves that cover and cap brood cells and the pollen and nectar that is provisioned for each egg laid (McCorquodale and Owen 1997). Each brood cell is complete before the mother begins work on the next cell; the mother does all provisioning. When unlimited resources are available maximizing NREI generally predicts a full load of pollen being collected. In contrast, maximizing Efficiency predicts smaller load size for nearby compared with distant resources. We hypothesized that the cost of increased flight distance to resources from the nesting site will result in mothers collecting a greater (full) load of pollen in order to decrease the required number of costly resource collection flights.

4.3 Material and Methods

4.3.1 Leafcutter bee life history

The alfalfa leafcutter bee, *M. rotundata*, is commonly used in the pollination of many seed crops, including alfalfa (*Medicago sativa*). Leafcutter bees nest gregariously (nest cavities are adjacent), and females use preformed cavities to construct linear nests with a number of brood cells, allowing for the use of standardized artificial nests (McCorquodale and Owen 1997). A mother constructs a brood cell by lining the nest cavity with leaf pieces and then filling this “cell” with provisions of pollen (Figure 4-1). The result is a wet foraging mass comprised of pollen and nectar that weighs about 35mg (Klostermeyer *et al.* 1973). An egg is laid onto this mass; covering the exposed front of the brood cell with leaves completes the brood cell. New cells are initiated in front of just-completed cells. Our previous work has shown that *M. rotundata* build nests with 1-15 brood cells where each brood cell requires an average of 12-13 pollen trips and 15 trips for leaves (Peterson and Roitberg, unpublished data). Offspring overwinter as prepupa (this species is univoltine in our study area) and emerge from brood cells as adults in late spring. The mother provides all food consumed prior to adulthood in the brood cell. Observations and measurements can easily be made to determine the number of pollen collection trips, realized fecundity, flight time, and investment per offspring. Mother’s must defend their nest from conspecific attacks, conspecific nest parasites, parasitoids (eg. *pteromalus* spp.) and other predators.

4.3.2 Field experiment

Flight times for various nest-building activities, as a function of distance to resources, are not well known for the megachilids. Thus, we carried out a field experiment to determine the amount of time and number of trips required to construct a brood cell. The experiment was conducted on a set of 5th year alfalfa (*Medicago sativa*) plots located in Tilley, Alberta, Canada (50°22' North, 111°40' West).

Bees were released in a bee domicile on the morning of July 6th, 2003. Bees were given 20 days before videotaping started to allow them to become established in the environment. Bee activity at the domiciles for both the short and long distance treatments was simultaneously videotaped during all periods of activity (7:30-20:00) on July 26 and

27, 2003, using digital camcorders (Canon^{TM} GL-2 and Sony^{TM} DVC-TRV) with 720x480 resolution. Cameras were placed one metre below and 0.5m in front of the nests with the cameras positioned at a 70º upward angle to observe the type of material being carried into the nest by the mother. This allowed for determination of the exact time bees exited and entered the nest and the transported material (leaves or pollen). This information yielded the amount of time the mothers spent in and out of the nest when carrying out these various collection activities and the number of trips required for each of these activities to produce a complete brood cell.

We experimentally controlled the distance mothers had to fly to collect pollen by using two flight distance treatments: bee domicile directly beside (distance = 5m) unlimited pollen (i.e., a large alfalfa plot) or 150 metres from unlimited pollen. In both situations leaf material was available directly beside the mothers' nesting domicile. At the end of the season, all videoed nests were dissected, hatched out and the mass and head width of the dried adult offspring was determined. For further details on the field experiment see Peterson and Roitberg (2006), run concurrently and set up under the same conditions as this work. The difference in distance to pollen resources between the treatments allowed us to determine the extra time required when bees must obtain resources from 150m away. We used the number of provisioning trips and the offspring's adult dry weight, which is determined by the amount of food in the brood cell (Klostermeyer *et al.* 1973), to establish maternal investment in each offspring.

To solve for optimal maternal behaviours and determine what currency was being maximized, field values for several parameters are required: time to collect each type of resource (pollen and leaves), time in nest after collecting resources, number of pollen collection trips to provision a brood cell and amount of pollen invested in each brood cell (which determines offspring birth weight). The latter two allowed us to estimate average individual pollen load size for each offspring. Currencies that may be maximized are net rate of energy intake ([B-C]/t) and efficiency (B/C), with B being the gross rate of gain (Joules/second), C the rate of energy expenditure (J/s) and t is time (s).

The construction of a brood cell involves several activities that differ in energy costs (Heinrich 1975). These behaviours are time spent foraging, activities within the nest and resting. Heinrich (1975) found that the cost of flight in bumblebees was more than 10 times that of resting, with flight costing 0.426 $Jg^{-1}s^{-1}$ compared to 0.034 $Jg^{-1}s^{-1}$ for walking (Cartar 1991). Therefore, the time and distance involved in collecting pollen and nectar for offspring is a significant portion of the energy budgeted to brood cell construction. These values were used to estimate bee-energy expenditures and the consequences of returning to the nest with different-sized loads (equating pollen with nectar). Foraging currency requires estimates for the maternal investment per offspring. These estimates were based on the offspring dry weight at emergence and number of maternal pollen trips for each individual offspring.

4.4 Results

Table 4-1 shows the complete results including values and statistics for each behaviour that was examined.

The time the mother was away from the nest collecting pollen and the mass of daughters and sons varied significantly between the near and far flight treatments; mothers foraging farther from the nest came back with larger loads (Table 4-1). Time away from the nest collecting leaves, time at the nest after collecting leaves, and time at the nest after collecting pollen were not significantly different between the near and far treatments.

The total number of pollen-collection trips per offspring decreased significantly with greater flight distance to resources, from 16.4 to 13.0 trips (t=-2.14, df=20, P=0.023). When the pollen collection trip values were analyzed with the average dry mass of offspring within each treatment, the results showed 7.74mg per trip in the short treatment compared to 8.85mg per trip in the long treatment (t=-1.99, df=21, P=0.030). Therefore, the far treatments produced smaller offspring but carried a larger load per individual trip. The change in load size with distance was closer to that expected for maximizing efficiency (which predicted greater load size, and longer foraging trips, with increased distance). NREI predicted no shift in load with foraging distance.

4.5 Discussion

Central place foragers are faced with the issue of collecting multiple resources at varying distances from their nest site. These distances impact the cost of obtaining resources (Zurbuchen, 2010) and raise the question of what currency such organisms maximize in order to maximize lifetime reproductive success. Mothers flying longer distances to obtain pollen increased the amount of pollen collected per trip compared to their short distance counterparts; however, fewer collection trips occurred, resulting in smaller offspring. If mothers were maximizing the net rate of energy intake (NREI) we would always expect full loads of pollen being collected during foraging trips when pollen availability is high, regardless of the flight distance from the nesting site (Orians and Pearson, 1979). We found the mass and head capsule width of the offspring produced decreased significantly between the near and far treatments. Therefore, mothers collected larger loads of pollen with each trip when forced to fly longer distances to those resources, but completed fewer total trips per offspring.

Increased flight distance to resources amplified the amount of time required by mothers to obtain a load of pollen by more than 50%. In the near treatment, the flight time to the resource patch was less than 60 seconds as resources were only a few metres from the nesting site. Wolf *et al.* (1999) found that bees spend at least as much time feeding on flowers as in flight and usually significantly more time feeding. With the flight speeds we observed, mothers were likely not spending a significant portion of the extra time flying in the long treatment; however, mothers were still required to spend a significantly greater proportion of time flying 150m to resources in the long treatment compared to 5m in the short treatment. Given Heinrich's (1975) costs of flight being an order of magnitude greater than that for walking, this increase in time-spent-flying is likely to have a large impact on energy expenditure. Some of the extra flight time was also spent flying with the load of pollen, which is more energetically costly than flight during foraging where the pollen load is lower. This increased flight distance may require increased self-feeding or resting time in the field resulting in greater time to collect a load of pollen. As our Dynamic Programming Model (Chapter 2) demonstrated, it is also possible that the optimal maximization decisions and currency may shift as foraging parameters vary over time.

If the relationship between the cost of flying and the amount (discretized on a per unit basis) of pollen were linear, we would have expected mothers to collect a maximum load of pollen every trip regardless of the flight distance. The pollen loads of females flying greater distance were larger per trip, suggesting the impact of flight distance on decision-making. The efficiency model predicts larger load sizes when flying longer distance as a result of the increased cost of each trip. In line with this prediction, our results showed an increase in load size with longer flight distance.

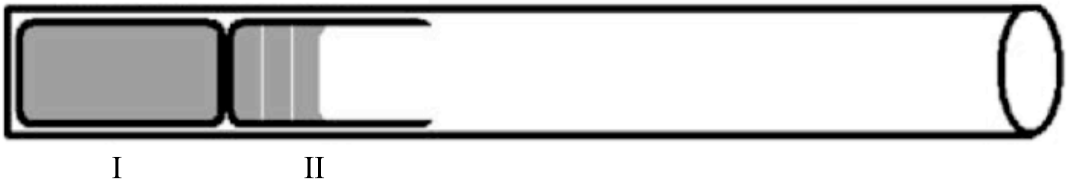
Larger load sizes also may be beneficial when flying long distances as this decreases the amount of time the mother is away from the nest (i.e. in flight). The greater the time the nest is left unprotected, the greater the chance of parasitism (Neff, 2008). If mothers were to take a greater number of trips and collect less pollen each trip, the total time away from the nest would be great, increasing the potential for parasitism.

Insect flight is among the most expensive activities found in the animal kingdom (Wolf *et al.*, 1999). Although foraging currency has been extensively studied in bees, this work has focused on the eusocial honeybees and bumblebees (eg. Cartar and Dill, 1990; Ydenberg *et al.*, 1994; Goulson, 2000; Cakmak *et al.*, 2009). This previous work has demonstrated that NREI is certainly not the sole currency being maximized and that efficiency is often a key currency. Our work expands these findings, demonstrating that efficiency can be the currency being maximized in solitary bees as well. This is important as solitary bee behaviour is reflected more closely in other groups we are interested in studying such as other insects, birds and mammals rather than the highly specialized eusocial bees.

Female bees maximize efficiency by increasing the amount of pollen collected per trip when flight distance to this resource is increased. Therefore, mothers are not maximizing net energy intake over the long-terms, suggesting factors beyond those traditionally measured need to be considered. The increased pollen load per trip and decreased number of trips may be a trade-off dealing with the significant costs of increased flight distance to the nest. This is a cost that central place foraging mothers are required to pay repeatedly as a result of repeated resource collection trips. There might also be costs such as wing breakage/wear, exposure to predators or the degradation of other body parts/functions. Our results demonstrate that when flight distance to resources is considered, mother's behaviour is more similar to maximizing efficiency rather than maximizing long-term NREI.

4.6 Figures

Figure 4-1. Representation of a linear nest created by the solitary bee *Megachile rotundata* (cross-section view). **I** Completed brood cell: a leaf-wrapped cell provisioned with pollen and nectar, with a tiny egg. **II** Brood cell in progress: a leaf-wrapped cell with three provisions of pollen and nectar, but no egg has been laid and the cell has not been sealed.



4.7 Tables

Table 4-1. Results from an experiment where leafcutter bees, *Megachile rotundata*, were given foraging resources adjacent to nesting sites (near) or 150m away (far). The experiment was conducted 20 days after the bees were released in the field and had established. Behaviours were recorded between the first flight on the morning of July 26 and the last flight the evening of July 27, 2003. In the short treatment 38 individual bees were observed over the two days and 55 individual bees in the long treatment. Not all bees displayed both behaviours (collecting leaves and pollen). Pollen refers to both the collection of pollen and nectar. Dry weight added per collection trip is calculated by dividing the mass of the offspring by the number of collection trips.

	Near flight distance treatments		Far flight distance treatments		*t*	*df*	P
	Mean	SE	Mean	SE			
Out of nest collecting leaf piece (Minutes)	**10.3**	1.5	**10.7**	1.1	0.18	87	**0.42**
In nest after collecting leaf piece (Minutes)	**3.5**	0.3	**3.9**	0.2	1.12	87	**0.24**
Out of nest collecting pollen & nectar (Minutes)	**37.0**	4.0	**58.0**	4.0	-3.99	73	**<0.001**
In nest after collecting pollen (Minutes)	**5.2**	1.3	**4.5**	1.2	-0.45	72	**0.66**
Number of trips to collect pollen per offspring	**16.4**	1.1	**13.0**	1.5	-2.14	20	**0.023**
Dry weight added per collection trip (mg)	**7.7**	0.3	**8.9**	0.5	-1.99	21	**0.030**
Dry weight of daughters (mg)*	**127**	1	**115**	4	3.53	6	**0.006**
Dry weight of sons (mg)*	**89**	1	**81**	4	2.70	6	**0.009**
Head capsule width of daughters (mm)*	**3.07**	0.027	**2.89**	0.019	4.65	6	**0.002**
Head capsule width of sons (mm)*	**2.88**	0.026	**2.78**	0.033	2.43	6	**0.025**

*Peterson and Roitberg (2006)

4.8 References

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5: Changing Resource Conditions and Population Levels Alter Sex Allocation Decisions

Jason H. Peterson[1] & Bernard D. Roitberg[1]

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[1] Evolutionary and Behavioural Ecology Research Group, Department of Biological Sciences, Simon Fraser University, Burnaby, BC, V5A lS6 Canada

5.1 Abstract

The evolution of sexual reproduction introduced the possibility of parental decisions regarding sex allocation. The sex of offspring produced and allocation of resources to each sex became a potential factor impacting parental fitness. Hymenoptera control the sex of offspring through the low-cost method of either fertilizing or not fertilizing an egg. Previous work has demonstrated that solitary bees will alter sex allocation decisions based on factors such as ecological conditions. To address the question of response to changing ecological conditions and differing local population concentrations we conducted field experiments using the central-place-foraging alfalfa leafcutter bee (*Megachile rotundata*). Three separate experiments were run, addressing the question of sex allocation decisions in response to: 1) variable flight distance to resources during the year (i.e. lifetime), 2) variable level of resources available during the year, and 3) local population concentration. All three experiments produced changes to allocation decisions. Mothers experiencing long flight distance early in the season and short flight distance later in the season produced a greater proportion of female offspring than mothers experiencing the opposite. Under high resource conditions early in the season and low resources later in the season mothers actually increased the proportion of female offspring later in the season. Under all other resource conditions the proportion of female offspring remained constant. In the population concentration experiment, having more females in a given area resulted in a lower proportion of daughters produced.

5.2 Introduction

Sexual reproduction has been an extremely successful evolutionary development that also introduced the interesting possibility of parental decisions regarding sex allocation. In species that are capable of controlling the sex of their offspring or altering the allocation of resources based on sex, these decisions can alter parental fitness (Trivers and Willard 1973; Charnov 1982; Clutton-Brock 1991). The methods used to control the sex ratio of offspring vary widely from the costly method of killing young of a certain sex to the much cheaper method of the mother either fertilizing or not fertilizing the egg, as in haplodiploid species such as Hymenoptera (Williams 1979; Burley 1986; Davison and Ward 1998). In haplodiploid species males develop from unfertilized eggs and females from fertilized eggs (Cook 1993).

Parental decisions regarding offspring sex and investment have important consequences. Maternal fitness returns from a given allocation have been shown to differ, depending on the sex of the offspring, across numerous organisms (Rosenheim 1996). A mother's ability to invest in offspring and the cost of increasing investment to offspring vary depending on the conditions she experiences. The benefits depend on conditions likely to be experienced by offspring of each sex. Thus, the most beneficial size and sex of the offspring will be condition dependant and may change during the parent's lifetime.

The investigation of sex ratios in animals has led to the acknowledgement of two remarkable facts: (1) nearly equal numbers of males and females are found in the vast majority of populations, and (2) a significant number of exceptions to this rule exist. Explaining this paradox was aided by Fisher's (1930) theory of offspring investment, which stated that natural selection favours not those parents that produce equal numbers of sons and daughters, but those who invest equally in each sex. If, for example, daughters are twice as expensive to produce as sons, we expect the production of twice as many sons, resulting in equal investment in each sex (Fisher 1930; Charnov 1982) but unequal sex ratios. The sex allocation decision is therefore condition dependant; all else being equal, if the cost of producing females rises to three times that of males, we would expect three times as many sons to be produced compared to daughters.

Investments that deviate from equal (Fisherian) sex allocation are also expected in situations where the fitness return on maternal investment is determined by gain functions that differ in shape between sons and daughters (Trivers and Willard 1973). Life-history theory predicts reproductive effort to be concentrated on the sex that generates comparatively larger fitness payoffs (Charnov 1982). In ungulates, for example, only a large investment in sons returns a payoff because only large males can secure mates (Trivers and Willard 1973; Clutton-Brock 1984); in contrast, daughters of almost any size generally produce offspring. Optimal sex investment can be further complicated when fitness-return curves change temporally and/or spatially. Examples of variable curves include situations where one sex matures faster, which would affect selection on the timing of production of the sexes or situations where increasing difficulty in securing resources changes relative offspring production costs over time. Here, natural selection should favour the evolution of facultative sex allocation in response to particular life history or environmental situations (Charnov 1982).

Parental investment in the production of offspring includes the cost of locating and obtaining resources to provision those offspring. Central place foragers must continually pay this cost wherein resources are repeatedly returned to a single location

(Schoener 1979; Orians and Pearson 1979; Stephens and Krebs 1986). For example, parents in many bird species perform numerous trips to obtain nest construction materials, followed by foraging trips to collect food for altricial young. Metrics such as the distance to all necessary resources, availability of resources and con-specific competition are likely to be important factors in determining an optimal nesting site. This has been shown in central place foragers where resource exploitation rates decrease as distance from the "home" increases (Rosenberg and McKelvey 1999). Therefore, in situations where maternal fitness returns change differentially for each sex, changes in the cost of investing in each offspring have the potential to impact optimal offspring resource and sex allocation decisions.

Hymenoptera are particularly useful in addressing these questions because their haplodiploid sex determination means mothers directly control the sex of their offspring (Cook 1993). In addition, adult size is strongly correlated to the amount of food provisioned by the mother to her progeny in the brood cell (Klostermeyer *et al.* 1973; Freeman 1981a; Tepedino *et al.* 1984; Bosch and Vicens 2002), and the size of an individual is expected to be related to lifetime fitness (Figure 5-1). The nests produced by numerous solitary bees and wasps are linear nests where discrete brood cells are provisioned and laid sequentially, eliminating direct competition among offspring for food and unitizing investment along an easily followed time-line (Freeman 1981b). The central-place-foraging leafcutter bee, *Megachile rotundata* (Fabricius), requires numerous trips to obtain the resources necessary to build a single brood cell (6-20 pollen/nectar trips and 10-25 leaf piece trips (Peterson and Roitberg, unpublished data)). Therefore, any changes in the cost of obtaining resources over time (e.g. more search time required to obtain resources when resources become scarce) will be amplified by the many trips required per offspring. Finally, a difference in the required investment and developmental time between the sexes affects costs and benefits for producing males or females.

When the amount of food that young receive is correlated to their adult size, and when the smaller sex becomes the more profitable as adult size decreases (Figure 5-1), the smaller sex will be favoured as resource availability decreases (Torchio and Tepedino 1980). In *M. rotundata*, the smaller sex, males, becomes more profitable to the mother at lower investment because a viable son can be produced with less investment than a viable daughter (Phillips and Klostermeyer 1978) and the lack of male conflict/territoriality (Paxton 2005) means little added benefit for larger sons. Previous work has demonstrated that flight distance (Peterson and Roitberg 2006a) and resource levels (Frank 1995; Rosenheim *et al.* 1996) can alter sex allocation decisions. The more costly/rare resources become, the greater the proportion of offspring produced that are the smaller sex and thus the cheaper sex.

Environmental and ecological conditions that can impact allocation decisions can change both within and between seasons. Previous work focused on fixed environmental conditions over the entire season (Frank 1995; Rosenheim *et al.* 1996; Peterson and Roitberg 2006a). Here we examine allocation responses with changing ecological conditions and population densities within a season. To understand the condition dependence of sex allocation decisions we ran three separate experiments addressing (1) changes in flight distance to resources, (2) changes in resource levels and (3) local population concentration. The background for each of these three experiments is explained in the following sections.

5.2.1 Flight Distance

Mothers decrease the proportion of daughters in the nest, the size of offspring, and the number of offspring in the nest when constantly exposed to longer flight distance to resources (Peterson and Roitberg 2006a). As flight distance to resources is likely to vary during the year/lifetime for central place foragers, we tested for mother's responsiveness to altered resource availability in sex allocation decisions. We hypothesized that sex allocation decisions under changing resource cost conditions (flight distance) will have less impact earlier than later in the season. Early on, the mothers are young and healthy (i.e. wings in good condition), and there is likely sufficient time for nest construction and provisioning, lessening the impact of increased flight distance to resources. As the likelihood of the season ending approaches, however, we predicted there would be a dramatic drop in the relative allocation towards the size of offspring and therefore the proportion of daughters. This is because as the odds of life ending increase (summer ending/mother dying/wings wearing out) mothers are more likely to be able to complete a viable son, which requires less investment (i.e. time), than a daughter (Figure 5-1).

To partly separate effects of body wear versus season and age *per se*, we used treatments that allowed us to compare the performance of more and less flight-worn individuals during the second half of the season. These two groups, those that flew long distances and those that flew little, during the first half of the season, were then exposed to the same flight conditions during the second half of the season.

5.2.2 Resource Levels

Previous research demonstrated an increase in the number and size of offspring produced as available resources increased. However, there was no difference in sex allocation (Peterson and Roitberg, 2006b). Here we examine how changing resource availability during the season impacts food provisioning and sex allocation decisions. We predicted that early in the season mothers would produce more daughters under low resource availability. Mothers will be spending time in the nest simply waiting for flowers to develop and the cost of going out and obtaining pollen is low. Later in the season, as the end-of-life approaches, mothers with low resource availability are likely to reduce the number of daughters produced as they are more likely to be able to produce a completed son brood cell before the end of their life. Here, the past resource level conditions are not expected to alter the general sex allocation decisions later in life because of the low maternal energy expenditures under both resource conditions (i.e. little flight-searching time required).

5.2.3 Local Population Concentration

Central place foragers are required to obtain a variety of specific nesting resources, which are generally limited in their availability. These resources, as well as forager population density are likely to vary both spatially and temporally. We hypothesize that under higher population concentrations (females per given area), mothers will produce a smaller portion of daughters and produce fewer offspring overall. This is expected as mothers respond to the risk of competition/loss of nest site to conspecifics. This decision may be size dependent, with smaller females more likely to be more strongly affected. Thus, for the experiments described below, mothers were selected so they were all of similar size.

5.3 Materials and Methods

5.3.1 The Study system

The alfalfa leafcutter bee, *M. rotundata*, is sexually dimorphic, with females 1.2-1.3 times larger than males and adult size controlled by the amount of food in the brood cell (Klostermeyer *et al.* 1973). As a non-territorial species, the advantage of increased size in males is expected to be limited (Alcock 1979). Larger females can accrue greater reproductive fitness due to their enhanced foraging abilities (Alcock 1979), greater ability to usurp nests from other females (Larsson 1990), and consequently a greater longevity and higher fecundity (Sugiura and Maeta 1989; but see Tepedino and Torchio 1982). Although the Fisherian theory (Fisher 1930) assumes linear maternal fitness returns, there are good reasons to expect curvilinear curves in most hymenopterans that differ between sons and daughters (Frank 1995; Rosenheim *et al.* 1996). The curves of the two sexes likely intersect, such that as resource allocation to a single offspring increases there will be a point where the most profitable sex for the mother to produce switches. For these reasons we assume: (1) lower minimum investment for viable *M. rotundata* sons versus daughters and (2) lower male asymptotes for fitness return curves. This situation creates discrete regions of investment where one sex always gives higher returns (Figure 5-1). Numerous sex ratios (female proportion range from 0.20 to 0.80) for this species have been observed (Maki and Moffett 1986; Jay and Mohr 1987; Tepedino *et al.* 1994; Pitts-Singer and Bosch 2010), suggesting that optimal sex ratio regions may be condition dependant. Increasing resource cost conditions as described below means less return per unit effort and thus increasing the size of the son-as-optimum region.

5.3.2 Experiments

Leafcutter bee behaviour experiments were carried out using a set of alfalfa plots southwest of Tilley, Alberta, Canada (50°22' North, 111°40' West) in the summers of 2007, 2008 and 2009. Bees were kept in artificial nests consisting of Bidwell^{TM} wood laminate blocks with a diameter of 6 mm and a depth of 127 mm. Blocks were strapped against a 20cm x 30cm plywood backing with a sheet of fibre fill in between. Nest blocks were placed into domiciles with nest holes facing due east. The area one metre out from the domiciles was sprayed with Roundup^{TM} to remove ground cover. The alfalfa plots were sprayed three times with a 5% Malathion^{TM} solution (one, two and four weeks prior to bee release - avoiding any impact on the bees) to prevent alfalfa weevils and plant bugs from destroying the crop's bloom.

As described by Peterson *et al.* (1992), loose-cell, prepupal bees were obtained from Richard Braul Farms (Rosemary, AB), placed in hatching trays and incubated for 21 days. One night prior to release, adult bees of equal size, compared to bees of the same sex, were divided into trays with two males to every female. Bees were released when the alfalfa field had sufficient bloom available to support all the released bees (i.e. mothers would not run out of pollen and nectar early in the season) and when the forecast was for a warm, sunny day.

When nest blocks were removed from the field, the blocks were stored at 20°C for two weeks to allow all larvae to reach the prepupal overwintering stage and were then cooled to 8°C until April to break diapause. At the end of the season, the production of alfalfa seed in each plot was estimated to the nearest 50 kilograms per hectare using visual inspection of alfalfa plants with the assistance of an alfalfa seed expert. In April, the nest blocks were taken apart and the numbers of brood cells and whether the nest entrance was sealed (capped) were recorded. If the nest was sealed, the size of the nest cap was also recorded. Each brood cell was removed from the nest block and placed into trays with individual compartments so that each bee could hatch separately. To enable identification of the offspring in each compartment, the brood cell's position within the nest and within the entire nest block was recorded. The cells were incubated at 30°C for 30 days, at which time all viable offspring had emerged. The sex of each offspring was determined by visually assessing the emerged adult. Each bee was then dried for 1 h at 90°C, and weighed on a Sartorius^{TM} scale accurate to the nearest 0.0001g. This adult-emergence dry weight was used as a metric for the mother's resource allocation to each individual offspring. This information allowed for the reconstruction of each nest with the number of offspring produced as well as the size and sex of each individual offspring.

When necessary, unhatched bees were sexed according to their position in the nest sequence, as described by Frohlich and Tepedino (1986). Female bees are produced in the innermost cells and there is most often a single switch to the production of males in the outer cells. In the rare case where an unhatched brood cell was in the position between the switch from females to males, these cells were discarded from the sex ratio analysis. The sex ratio for each replicate was calculated as the mean proportion of daughters per nest in the replicate.

5.3.3 Statistical Analysis

A MANOVA was used to analyze all the response variables together, for each of the three experiments, to estimate the overall effect of treatments. A least-squared means, one-way analysis of variance (ANOVA) was then used to interpret which variables contributed to significant results. We analyzed the effect of treatment on the nest sex ratio (mean of number of females in a nest/total number of offspring in a nest), the mean number of cells per nest, the number of nests per treatment, number of nests initiated per treatment, the adult weight of sons and daughters, portion of nests capped and offspring survival. For the first two experiments we compared (1) the first half of the season for each treatment, (2) the second half of the season for each treatment and (3) changes between the first and the second half for both treatments. For the third experiment we compared results from the three different population concentrations (1, 4 and 16 females)

5.3.3.1 Impacts of changing costs

To investigate the impact of changing costs of reproduction on resource and sex allocation decisions, we manipulated flight distances using four distance treatments (two experimental [replicates of each: 8 (2007) and 5 (2008)] and two controls [replicates of each: 3 (2007) and 4 (2008)]) in the summer of 2007 and 2008. The experimental design was similar to our previous work (Peterson and Roitberg 2006a). Each treatment consisted of a plot of 3rd/4th year alfalfa (12×12 m) and a three sided corrugated metal domicile (2m x 2m x 1m) with a wooden block containing 169 individual nesting sites/holes. These artificial nests comprised a matrix of 13 by 13 holes, providing more than enough nesting sites for all 50 females. The nesting site was placed either directly next to the plot of alfalfa (Near) or 150 metres away (Far). The 150m distance was chosen because female leafcutter bees generally forage within 100m of their nest (Pankiw and Siemens 1974; Tasei and Delaude 1984). The size of the plot allowed the bees to feed *ad libitum* for the entire season: that is, only distance to the resource was manipulated. Each shelter and alfalfa plot was isolated from other sources of resources by at least five hectares of a non-food and non-nest building resource (i.e. wheat fields). In the Far treatment, a 2-m-wide strip was mowed out of the wheat from the shelter to the alfalfa plot, providing a flight path for the bees. The area both beneath and one metre out from the domicile was sprayed with Roundup^{TM} to remove ground cover.

In two experimental treatments the distance from the nesting site to resources was changed half way through the season (half way = 14 flying days [temperature > 23°C]). In the first experimental treatment, the nesting site started far from resources (150m) and after 14 flying days, shelters were moved near to resources (0m). The switch was done between midnight and 3am when all bees were in their nests and dormant. The second experimental treatment was the reverse situation with nest sites near to resources for the first half of the season and then switched to far. Having reciprocal treatments allowed us to separate to some extent differences due to time in the season *per se* from those due to effects of aging. The two controls replicated Peterson and Roitberg (2006a), with the bees either being placed 0m or 150m from the resources for the entire season (28 flight days). The 50 females and 100 males were placed in each shelter in the early morning of July 6th (2007) and July 3rd (2008).

To assist the establishment of the bees at the shelter site, 10 potted alfalfa plants were placed in front of each shelter to provide food during the first three days after release. On the 2nd day, the pots were evenly spaced over a 150-m distance east of the shelter, and by the 3rd day the potted alfalfa plants were ignored by the bees, which flew directly to the alfalfa plot.

The experiment started July 6th/3rd (2007/2008 dates), treatments were switched on July 22nd/21st, and nesting sites were removed from the field on August 12th/10th. Behavioural observations on bee activity and nests that had been capped (sealed) were recorded 3 to 4 times per week thereby providing the date during the season when each nest was completed. Nest blocks were removed from the field (August 12th 2007; July 21st and August 10th 2008). The reason for two removals in 2008 is explained below.

In 2007, straw inserts were placed in each nesting hole and after 14 flying days, the portion of the straw that had been filled with completed brood cells was marked on each straw. Therefore, at the end of the season it was determined which offspring were produced during each of the two time periods. In 2008, after 14 flying days, the shelters were moved (where applicable) and all nesting blocks were placed in the sun the next morning to encourage the bees to vacate the blocks, and new nest blocks were placed in the shelter. When exposed to direct sun, all nesting sites were vacated and females began nesting in the new blocks. The removed blocks were stored as described above.

The number of brood cells produced uses data from 2007 and 2008, and the sex ratio results are from the summer of 2008. In 2007, extreme summer temperatures >37ºC and the use of plastic straw in the nest cavities combined to result in the death of the vast majority of developing larva.

5.3.3.2 Impacts of changing resource availability

Experiments to investigate the impact of changing resource availability were carried out in an additional alfalfa field, also in 2007 and 2008. Sixteen steel frame tents (3m by 2m by 1m; 1.8m high in the centre) with mesh covers (1.5 mm) were used, one per replicate. The crop under each tent was sprayed with Malathion^{TM} six days before bee release to prevent aphid outbreaks and to standardize tents with regards to eliminating other potential pollinators.

We used two resource conditions: all the alfalfa plants under the tent were left standing (high resources), or the plants in the eastern (front) half of the tent were cut at ground level (low resources). These resource conditions were changed halfway through the season, again considered 14 flying days (temperature above 23°C). The experimental tents were randomly assigned to one of four treatments (2 experimental; 2 control), using a Latin square with the roll of a die: (Experimental 1) High resource level for the first half of the season, low resource level for the second half of the season; (Experimental 2) the reverse with low resources first and high resource second half of season; (Control 1) High resources the entire season; (Control 2) Low resources the entire season. In both years, four replicates of each tent type were completed (i.e. the tent was the unit of replication).

A plywood roof (30cm by 15cm) was nailed at a 30° angle to the top of the nesting structure to prevent rain from entering the nests. The entire nesting structure was positioned against the west end of the tent with the open end of the nest holes facing due east. The release of 15 females and 30 males into the tents occurred in the early morning of June 25th/July 2nd in 2007 and 2008, respectively. Resource conditions were changed July 12th/24th and nests were removed August 2nd/12th.

5.3.3.3 Impacts of population density on sex allocation

This research was carried out in the same alfalfa field as Experiment #2 using the same research tents, bee hatching method and initial setup, except that here, no alfalfa manipulations were made. This experiment was carried out in 2007, 2008 and then on a larger scale with replication in 2009. In 2007 and 2008 six tents were used, with female bee concentrations of 1, 2, 4, 10, 16, and 32, with double that number of males, respectively. Based on these results, a replicated experiment was performed in 2009, using female concentrations of 1, 4, and 16 with seven replicates of each. The artificial nests (7 holes by 13 holes) provided more than enough nesting sites for all females. The tents housing the bees were 3m by 2m by 1m (1.8m high in the centre) with mesh covers (1.5 mm).

The bee release days for the three seasons were June 26, 2007; July 3, 2008; and July 11, 2009. Nests were removed on August 25th when all the adults had died.

5.4 Results

5.4.1 Impacts of changing flight distances

Overall differences between the distance treatments were found with a MANOVA: $F_{3,14}$=6.0, P=0.008. There was greater total brood production in the near treatments than in the far treatments (Table 5-1/Figure 5-2) during the first half of the season (F=15.6, df=17, P<0.001). In the second half of the season, the greatest brood production remained in the near-control treatment, and the lowest in the far-control treatment (Table 5-1). Production from the two experimental treatments where distances were switched were intermediate; the treatment that was near for the second half did not differ from treatment that was far. This same pattern held for the number of brood cells per individual nest in each treatment (F=7.4, df=17, P=0.003) and nests per replicate (F=12.4, df=17, P<0.001) during the second half of the season.

All nest sex ratios were significantly different when comparing sex allocation during the 1st and 2nd halves of the season with an ANOVA (F=16.1, df=17, P<0.001) (Figure 5-3). The greater proportion of daughters produced during the first half of the season in the far treatment was not significantly different than the near treatment (F=1.79, df=17, P=0.20). In the second half of the season, the near treatment had a significantly higher proportion of daughters produced (F=6.6, df=17, P=0.005). Considering the offspring nest sex ratio for the entire season, the Far-Near treatment had a significantly higher sex ratio than the Near-Far treatment (F=5.5, df=17, P=0.01).

Comparing the dry mass of offspring between the treatments and between the first and second half of the season did not result in any significant differences for daughters (F=0.32, df=15, P=0.93) or sons (F=2.37, df=15, P=0.12). Daughters always had significantly higher masses than sons (F=75, df=31, P<0.001).

5.4.1.1 Impacts of Body Condition

To explore worn versus fresher females behaviour during the latter part of season we compared Far-Far versus Near-Far, and Near-Near versus Far-Near groups during the second half of the season. In both comparisons the production of offspring was more than double when the females were fresher (i.e. first half of season was spent near to resources), even though conditions during the second half were the same (Figure 5-2).

The same comparison looking at offspring sex ratio reveals no significant difference during the second half of the season for either comparison (Figure 5-3).

Looking at the second half of the season, when comparing the female sex ratio of offspring for all mothers in the Far treatment (0.32 ± 0.03) to the Near treatment (0.48 ± 0.03), the latter is significantly more female biased (F=18.9, df=17, P<0.001).

5.4.2 Impacts of changing resource levels

Overall differences between the resource level treatments were found with a MANOVA: $F_{3,9}$=6.6, P=0.012. Brood cell production was significantly greater in the first half of the season under high resource conditions compared to low in both 2007 and 2008 (2007: F=109, df=13, P<0.001; 2008: F=8.4, df=15, P=0.003) (Table 5-2). This was also true for the second half of the season in 2007 regardless of the starting resource levels (F=7.0, df=11, P=0.007). In 2008, the results were the same except that there was a drop in production under the high/high control treatment in the second half (F=25.0, df=15, P<0.001).

In the first half of the season (all results, 2008) there was a significantly higher proportion of daughters per nest in the low treatment (F=3.8, df=15, P=0.04). The proportion of daughters in the low/low treatment was also higher than the high/high treatment in the second half of the season (F=4.2, df=14, P=0.03) (Table 5-3).

When comparing the first and second half of the season (all results 2008), dry mass of sons was constant while daughter dry mass decreased significantly in all treatments, except in the Low to High treatment, where the mass of offspring actually increased slightly in the second half of the season under high resources (F=5.48, df=3, P=0.021) (Table 5-4). Daughters always had significantly higher masses than sons (F=59, df=32, P<0.001).

5.4.3 Impacts of population density

Data were analysed using each tent and bee density as a replicate; therefore, there was a single replicate in 2007 and 2008, and 7 replicates in 2009. The data were also analysed with each year weighted equally and the results did not significantly vary. Overall differences between the population density treatments were found with a MANOVA: $F_{2,11}=6.1$, $P=0.016$. The proportion of female offspring was significantly higher in the 4-female treatment compared with the 16 (F=3.94, df=23, P=0.035) (Table 5-5). In 2007 (the year for which information on five different concentration levels is available), when five different treatments (no replicates) were run, the female sex ratio of offspring produced with 2, 4, 10, 16, and 32 mothers were: 0.80, 0.56, 0.46, 0.38, and 0.21, respectively.

The number of brood cells per nest was significantly higher in the one and four females treatments compared with the 16-female treatment (F=8.26, df=17, P=0.004). The trend was for decreasing cells per nest as the population increased (Table 5-5).

When comparing the number of offspring per nest in the 4-female treatment for capped (9.6±1.6) and uncapped (5.1±1.6) nests differed significantly (t=-1.97, df=10, P=0.038) (Table 5-6, Figure 5-6). In contrast, there was no significant difference between number of capped vs. uncapped nests with a single female (Number of brood cells per nests always high) or with 16 females (Number of brood cells per nests always low). There was no significant difference in the number of unhatched brood cells per nest between the treatments (F=0.71, df=20, P=0.51) or the portion of nests capped (F=0.27, df=17, P=0.77).

There were no significant differences among the three treatments in the mean mass of sons (F=0.87, df=16, P=0.44) or daughters (F=1.6, df=17, P=0.23). Daughters always had a significantly higher mass than sons (F=29.9, df=34, P<0.001).

5.5 Discussion

By examining offspring allocation decisions under a variety of changing conditions, we were able to demonstrate that parents adjust allocation decisions in response to numerous alterations in resource conditions. We had previously demonstrated that some allocation decisions varied based on whether parents were near to or far from resources (Peterson and Roitberg 2006a) or whether resources were in high or low abundance (Peterson and Roitberg 2006b). Under a more realistic situation, we would expect conditions to vary within the year, in response to both weather and resource use by the local community of organisms. Here we were able to examine allocation decisions when these conditions changed half way through the season as well as the response to local population concentration. We can now confirm that sex allocation decisions are affected by flight distance to resources, resource levels and local population density. A key element of these allocation decisions in our focal animal, the leafcutter bee, *M. rotundata*, is the fact that females are larger than males and thus require greater investment per viable individual (Klostermeyer *et al.* 1973).

5.5.1 Changing flight distances

Looking at each of our three experiments independently, mothers who had to fly longer distances during the first half of the season produced fewer offspring and a more female biased offspring sex ratio compared to similar mothers who nested near resources during that time. During the second half of the season (i.e. second half of their lives) there was a dramatic shift in this behaviour, wherein mothers with a long flight distance still produced fewer offspring, but now biased their production more towards sons compared with mothers in the near resource treatment. This general trend held regardless of what distance to resources the mother had experienced during the first half of the season.

This experiment also allowed us to explore the behaviour of "worn" versus "fresher" females during the latter part of season by comparing treatments that were Far during the second half (Far-Far versus Near-Far) and also by comparing treatments that were Near during the second half (Near-Near versus Far-Near). 'Worn' mothers are those that had to fly a long distance to resources during the first half of the season while 'fresh' mothers are those that were nesting next to resources for the first half. Worn females produced far fewer offspring than fresher females under both comparisons; however, the sex ratio of those offspring was not significantly different for either comparison. Worn females likely had factors such as much greater wing wear impacting the time and energy required to produce each offspring, resulting in fewer offspring being produced. Sex allocation decisions, on the other hand, were not impacted by past conditions, only present. When only considering the second half of the season and compare the sex ratio of offspring from mothers with Near and Far distances to resources, there is more than a fifty percent increase in the proportion of daughters produced. Mothers appear to be impacted by past conditions when it comes to the number of offspring they produce, but it is current conditions that impact sex allocation decisions.

5.5.2 Changing resource levels

When looking at the resource availability experiment, mothers under low resource conditions for the first half of the season produced a smaller number of offspring and more female biased nests compared to those with high resources. However, during the second half of the season, mothers under low resource conditions continued to produce a smaller number of offspring, which remained significantly female biased, compared to the high resource treatment. These overall results are in line with those found by Pitts-Singer and Bosch (2010) in *M. rotundata*.

5.5.3 Comparing changing flight distance and resource levels

Comparing the results of these two experiments, offspring production and sex ratio of offspring under low resources levels was similar to long flight distance during the first half of the season. However, sex allocation was opposite during the second half of the season; mothers maintained a bias towards daughters under low resource conditions but switched to very male-biased sex ratio under long flight distance conditions.

Mothers under low resource conditions were in an isolated environment where there was nowhere to search for new resources. Therefore, these mothers were required to spend little energy to obtain those resources when they were available. Time was spent “waiting” in the nest for resources to become available (and therefore also protecting the nest), as opposed to energetically costly flight in the long flight distance condition. The result was that during the second half of the season, mothers behaved very differently under low resource conditions compared to mothers who had unlimited resources, but who had to expend a large amount of energy in order to obtain those resources. As the end of the season/life approached, mothers who where expending large amounts of energy to fly and obtain resources switched to smaller, cheaper sons, while mothers living in a very 'small world' i.e. limited resources continued to produce a greater proportion of daughters.

5.5.4 Impacts of population density

Allocation decisions by female bees were not only impacted by resources, but also by conspecifics nesting and collecting resources in the same area. Despite the fact that food was available *ad lib* and ample nesting sites were provided for each female, there was still a significant change in nesting and sex allocation decisions when the concentration of females in a given area increased. The proportion of daughters produced under low female concentrations (4 females) was much greater than at high concentrations (16 females); the female offspring sex ratios were 0.69 and 0.43 respectively. This pattern held for the size of nests and number of offspring per nest, which decreased continuously from concentrations of 1 to 16 females (Table 5-5). The portions of nests that were capped did not vary significantly; however, the number of brood cells in capped nests under low concentrations was more than double that under high concentrations. Interestingly, this difference did not hold when comparing uncapped nests, where the size of the nests was not significantly different between the concentrations of 4 and 16 mothers. Despite the availability of both resources and nesting sites, mothers in high concentrations appear to be employing Clark's (1994) asset-protection principle. Mothers at high concentrations accept smaller “guaranteed” rewards from either producing males, which require fewer resources and less input, and from sealing the nest with far fewer offspring inside. This is interesting because the cost of sealing the nest is the same regardless of the nest size. It is the benefit that increases the greater the nest size. If the mother is killed or her nest usurped by another female prior to sealing, the mother would not receive the aforementioned increased fitness and possibly no benefit at all, if a nest-usurping conspecific removed her offspring. The later situation is most common when nesting sites are scarce; however, usurpation also occurs when nesting sites are readily available (personal observations).

5.5.5 Integration of results

Sex allocation decisions are traditionally considered to be controlled by the most limiting factor (Rosenheim *et al.* 1996). As we have shown here, several factors can impact sex allocation decisions. Our theoretical model of this type of situation (Chapter 2; Peterson and Roitberg 2010) suggests there may be an optimal balance between various factors impacting sex allocation. Organisms may not simply optimize the most limiting factor but may make allocation decisions based on an optimal balance between various conditions (e.g. resource availability, competition for nesting sites, sex ratio of local population). Further, field work altering multiple factors simultaneously (i.e. in a factorial manner) will be required to address this questions as to whether bees optimize a single limiting resource or whether limiting resources vary over time and possibly interact resulting in an optimal balance.

When allocation decisions are considered in general there are at least two mechanisms that impact sex allocation: (1) differential immediate costs and (2) future prospects for each sex. Differential immediate costs to production and threat from conspecifics are consistent with our female density result and the lower female sex ratio in the latter part of the season for the flight distance experiment. At higher local breeding density, females are increasing the completion rate of cells by producing more males and sealing the nest sooner (i.e. a smaller nest). This reduces the chance of losing a nest and provides the mother some fitness benefit from sealing. Having more competition is akin to running out of time: essentially, the goal is to complete offspring, and this can be done more quickly by changing the sex balance towards the smaller sons that require less time and energy to provision. The second mechanism is future prospects for male and female offspring. Differential future opportunities for grandchild production by one's male versus female offspring are the focus here. This helps explain why sons are smaller than daughters in general (e.g. differential size provides greater marginal returns to future fecundity for daughters than sons). Future prospects appear to be most strongly impacting mothers in the resource level experiment, where the most valuable offspring continues to be produced throughout the year under limited resource conditions, and the early season bias is towards females under all conditions, when the offspring with the greatest maternal fitness benefit (large daughters) are produced. When mothers have sufficient time, a low threat of losing their nest and/or cheap resources, the marginal returns of offspring become more important for sex allocation decisions and the production of daughters increases.

Sex allocation decision research has focused on hymenoptera (Hardy and Godfray 1990), likely a result of the apparent low cost and easy control over sex allocation decisions in this haplodiploid group. However, in all sexually reproducing organisms, individuals are faced with the decision of what investment in each sex results in the greatest returns. Such decisions must be made by most sexual species that are dioecious, sequential hermaphrodites, simultaneous hermaphrodites and even possibly sex changers (West 2009).

In any situation where one sex may be slightly more beneficial, many organisms are limited by the cost of controlling sex allocation, particularly if they only have control over their secondary or tertiary sex ratio. Primary sex ratio is at fertilization, secondary at birth and tertiary at offspring sexual maturity (West 2009). Therefore, organisms without primary sex ratio control may have to kill offspring of a given sex, resulting in a situation where the costs will usually outweigh the benefit. However, sex allocation decisions are not limited to hymenoptera or organisms with control of the primary sex ratio. Species as varied as red deer (Clutton-Brock 1984), blue tit (Sheldon *et al.* 1999), zebra finch (Burley 1981) and the Japanese frog (Sakisaka *et al.* 2000) have been shown to alter sex allocation. Here, the driving factors include resource availability (i.e. health) and parental attractiveness. We are just beginning to understand the control that various other non-hymenopterans have over the sex of their offspring and when and how they are able to exert that control (West 2009). Mothers of various species appear to be able to determine the sex before birth, for example through resorption of embryos prior to birth (Blanco *et al.* 2003).

The number of offspring produced per season and/or lifetime is also a key consideration when applying sex allocation theory across a wide variety of organisms. Solitary bees produce offspring every few days and therefore can make sex allocation decisions based on current conditions. This is because the decision of when to switch to the production of sons can be re-evaluated at least every few days, and even after switching to the production of sons, mothers have been observed switching back to the production of daughters (Peterson and Roitberg, unpublished data). In non-hymenopteran species that may produce only a single offspring every few years and have only secondary sex ratio control, current conditions are likely to be much less influential. In order for an offspring in this situation to be terminated based on the sex, there must be a massive benefit to producing the other sex as mothers may have to wait a significant period of time to produce another offspring, on top of the investment in the terminated offspring which has now been lost.

Between those organisms that can make sex allocation decisions cheaply and those to which sex allocation alteration is expensive, we can imagine a spectrum across which organisms would fall in terms of the cost in making sex allocation decisions (West and Sheldon 2002). All else being equal and assuming a difference in the value of the two sexes in a given set of conditions, sex allocation alterations would be most common in species where the cost of doing so is low (or the benefits very large). Organisms may also have the option of simply investing more resources into provisioning the offspring of the preferred sex, instead of killing the less beneficial sex, a situation that is likely to be less costly and therefore apply to a larger group of organisms.

All three of our experiments can also be viewed from the perspective of decisions made by young versus old females (i.e. first half versus second half of their lives) (Roitberg *et al.* 1992; 1993; Chapter 2). Deteriorating physiological condition resulting from aging processes (see Williams 1957) may limit the capabilities of the older females competing with younger ones (i.e. ability to invest in the costly sex). This important factor of offspring sex ratio that affects reproductive performance may vary with parental age (Daunt *et al.* 2001). Veiga *et al.* (2008) examined birds (spotless starling) and found young and middle-aged females, mated with high quality attractive males, biased their offspring production toward the more expensive sex, males. However, old females under these same conditions produced significantly greater proportion of daughters. In this situation, mating with the high quality male means the mother is likely to experience competition with other females for the limited resources offered by polygamous males, such as nesting sites or paternal investment. Older females are therefore at a disadvantage because of the physiological drawbacks imposed by aging. These older females minimize costs by producing a greater proportion of (cheap) daughters as well as producing smaller broods, leaving more energy to allocate towards competition with their male's other mates. In our flight distance experiments, females in the second half of the season, when they were flying a long distance to resources, were in worse physiological condition due to wing wear, and produced a greater proportion of the cheaper sex. These mothers were 'older' because the greater time and energy to produce offspring means only a limited number can be produced compared to females near to resources during the second half of the season.

Comparing these results to mothers under long flight distance conditions at the beginning of the season (i.e. young mothers), these mothers produced a high percentage of the expensive sex. Then during the second half of the season, mother's sex allocation decisions appeared to be based solely on the current distance to resources and were not impacted by past conditions. These same results were not seen in the resource level experiments. In these experiments mothers under low resource conditions were not nearly as physiologically ‘spent’ as females in the flight distance experiment as these females simply rested in their nests when resources were not available, instead of expending large amounts of energy to fly to the resources. As a result, a similar switch to the production a greater proportion of the cheaper sex was not seen.

In our theoretical model (Chapter 2; Peterson and Roitberg 2010) we assumed no senescence, which is clearly an unrealistic assumption for animals whose limbs wear with use. We might expect that senescence in our system will also cause a shift to earlier production of the smaller and cheaper sons than would occur if mothers remained in a constant physiological condition throughout their lives. Wing wear is likely to be a major factor contributing to senescence (Cartar 1992; Higginson and Barnard 2004). Considering the far flight distance to demonstrate greater ‘senescence’ than the near flight distance, increased senescence decreased the production of offspring per nest in the second half of the season but did not impact the sex ratio of those offspring.

Veiga *et al.*’s (2008) results on the spotless starling can also be compared to our population-level experiment where females living in denser populations produced fewer offspring. These mothers under high population conditions can be considered ‘older’. At the beginning of the season, females with no competition were approximately nine offspring away from the end of their lives, while females in high concentrations produced less than four offspring from the end of their life. The latter also likely had to spend a greater percentage of their energy defending their nest the same as in Veiga *et al.*’s (2008). The result in our population-level experiments was that mothers in these higher population concentrations (i.e. ‘older’) produced more of the cheaper sex and produced fewer offspring in total, the same as older spotless starling.

Although this research was inspired by Trivers and Williard (1973), this system appears to be somewhat unique from the more typical situation where mothers have a set amount of resources to draw from. In our work the female can choose how much to invest in each individual offspring and the cost constants are not for the individual offspring but over the mother's lifetime. The result is that bees have more flexibility in decision making for each individual offspring and are not faced with the 'all or nothing' risk of producing a specific sex as the red deer mothers are when producing a male (Clutton-Brock, 1984).

A key discrepancy between Trivers and Williard's (1973) theory and our observations is the high proportion of the more expensive sex, daughters, under low resource conditions. This is the opposite of what Trivers and Williard's theory predicts, although they are assuming that when resources are scarce the female is constrained to providing fewer resources per offspring. In the solitary bee system, this assumption may not be satisfied. When resources are scarce, mothers can produce fewer daughters, but continue to provide each one with the same amount of pollen. Further research is needed to understand and perhaps develop a theory that is applicable in situations where females are not constrained to provide fewer resources per offspring, but can simply produce fewer offspring.

In our bees, mothers alter sex allocations decisions in response to numerous ecological and environmental conditions. Not only do such mothers alter sex allocation decisions in response to general conditions, but they also respond to changing conditions and update their sex allocation decisions as conditions change. The conditions that they respond to also appear to be extensive and variable. Although sex allocation is traditionally considered to be controlled by the single most limiting factor, it is possible that an optimal balance between numerous factors is indeed being employed. Here we have demonstrated that maternal allocation decisions are not simply static, but indeed plastic. As natural conditions are unlikely to be static, these bees appear to be adapted to alter sex allocation decisions in response to changing conditions. As we begin to better understand the control various organisms have over sex allocation we will be better able to investigate and predict resource and sex allocation responses to changing conditions in a wider variety of organisms.

5.6 Figures

Figure 5-1. Assumed cumulative maternal fitness gain is a function of the amount of time spent provisioning a single son (---) or daughter (—) for a given environment. Cumulative maternal fitness is considered the number of copies of alleles passed on to future generations wherein parents who invest more in an offspring generally accrue greater fitness returns from that offspring. [Both curves would reach the upper limit earlier (curves shifted left) if resources were easy to obtain, and the opposite would occur if resources were more difficult to obtain.] Daughters require greater initial investment [daughters are 20% larger than sons (Klostermeyer and Gerber, 1969; Klostermeyer *et al.*, 1973)], but increased investment yields greater fitness returns (i.e. large females are able to produce more eggs, while large males in a non-aggressive species have less benefit from increased size).

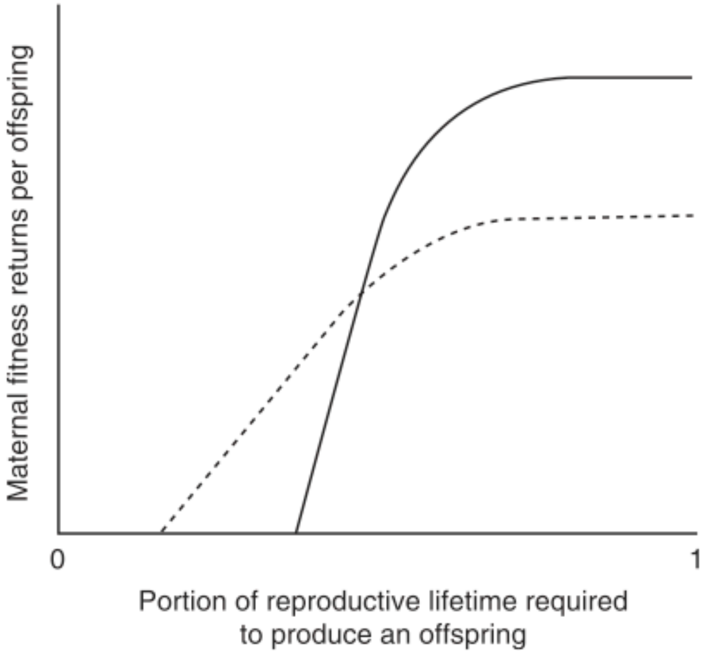


Figure 5-2. Flight Distance Experiment: Brood cell production (+SE) per treatment (distance from nesting site to resources). Distances were switched for two treatments halfway through the season, so data are presented separately for the first and second halves of the season (half season=14 flying days).

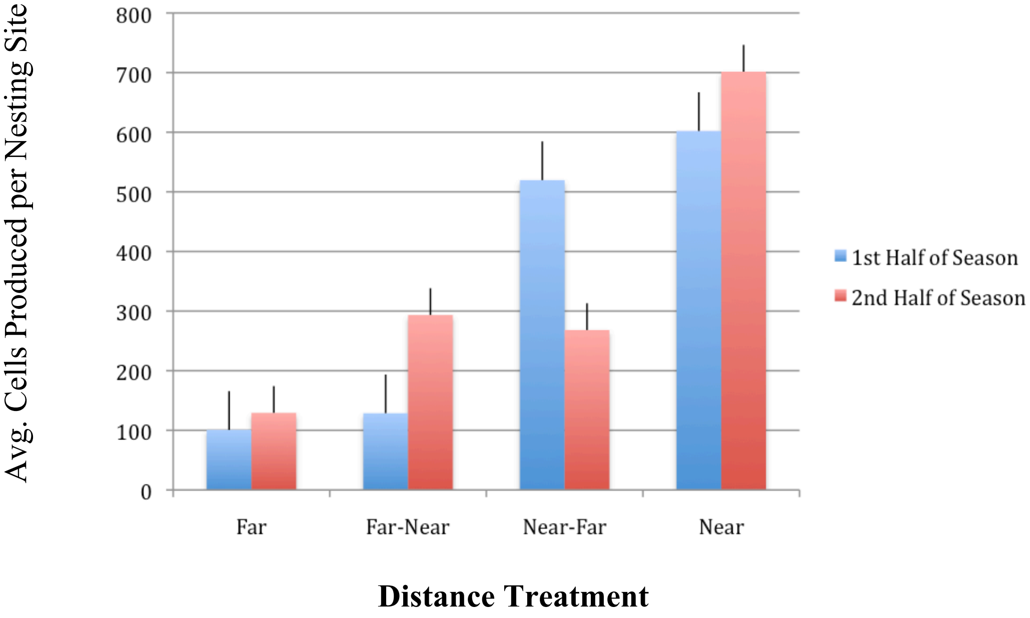


Figure 5-3. Flight Distance Experiment: The average proportion of daughters (+SE) produced per nest at each replicate for the four different treatments (distance from nesting site to resources) during the first and second half of the season (Half Season=14 flying days) as well as the average season-long nest sex ratio for each treatment. ANOVA compared the first and second half of the season (F=16.1, df=17, P<0.001).

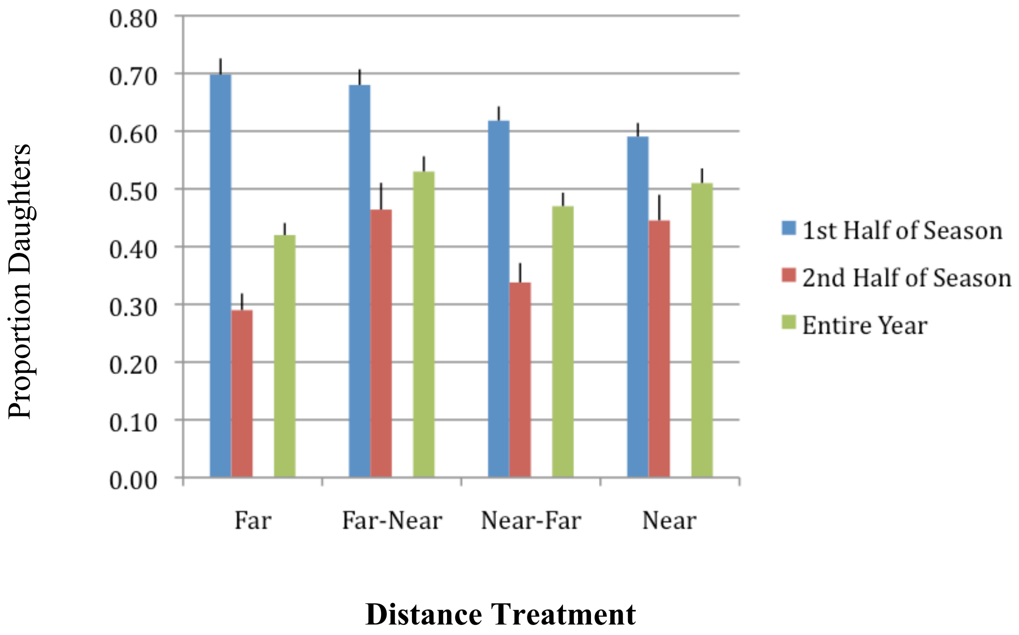


Figure 5-4. Resource Level Experiment: Comparing the proportion (+SE) of daughters in the nest for the 1st compared to the 2nd half of the season using an ANOVA showed that there was a significant difference between the High/Low treatment compared with the other three treatments (F=35, df=7, P>0.001). The sex ratio increased significantly when the resource level was dropped from high to low for the 2nd half of the season.

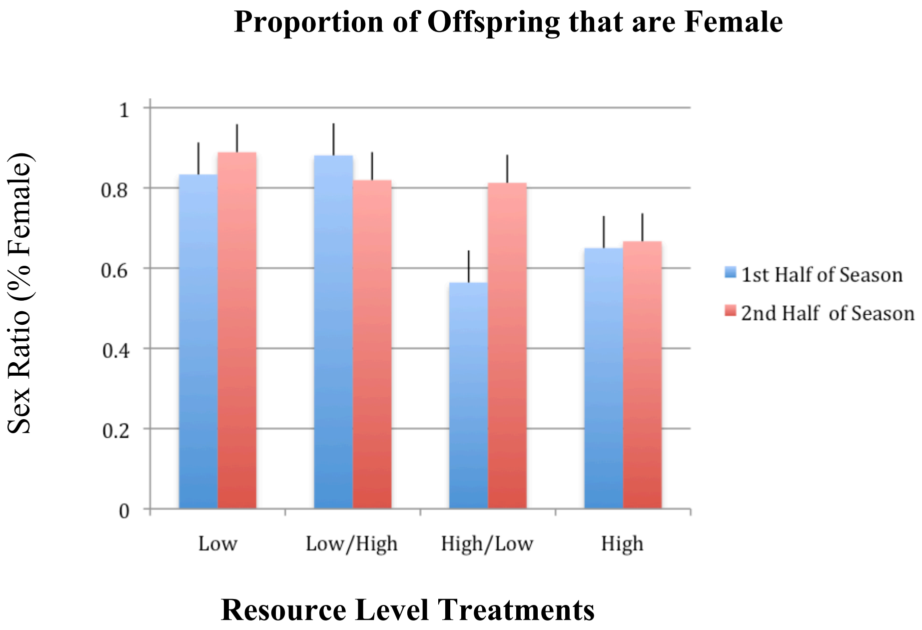


Figure 5-5. Resource Level Experiment: The number (+SE) of offspring produced per replicate site (tent) for each treatment during the 1st and 2nd half of the season over the four different resource level treatments.

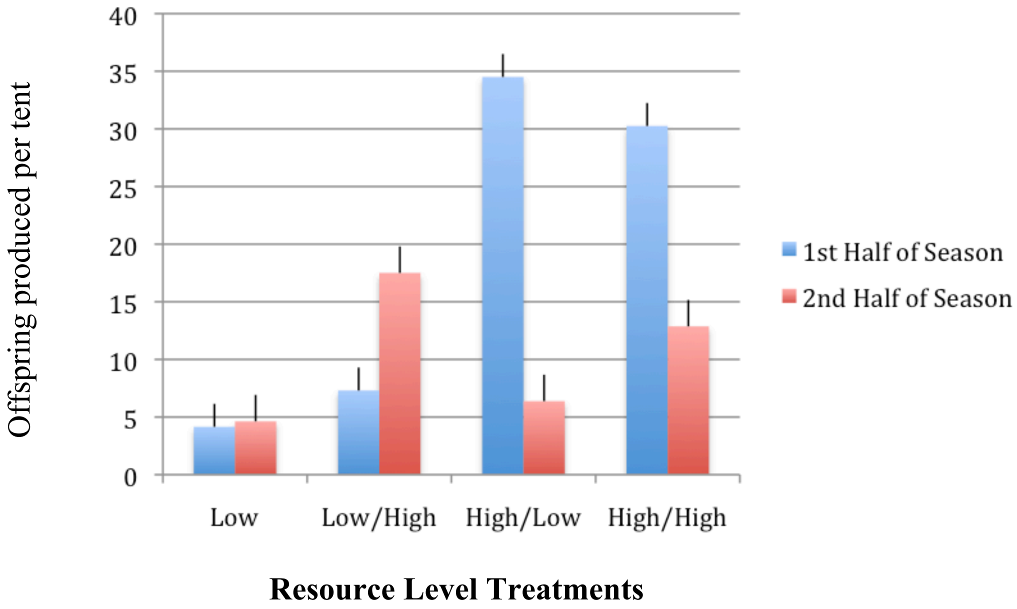
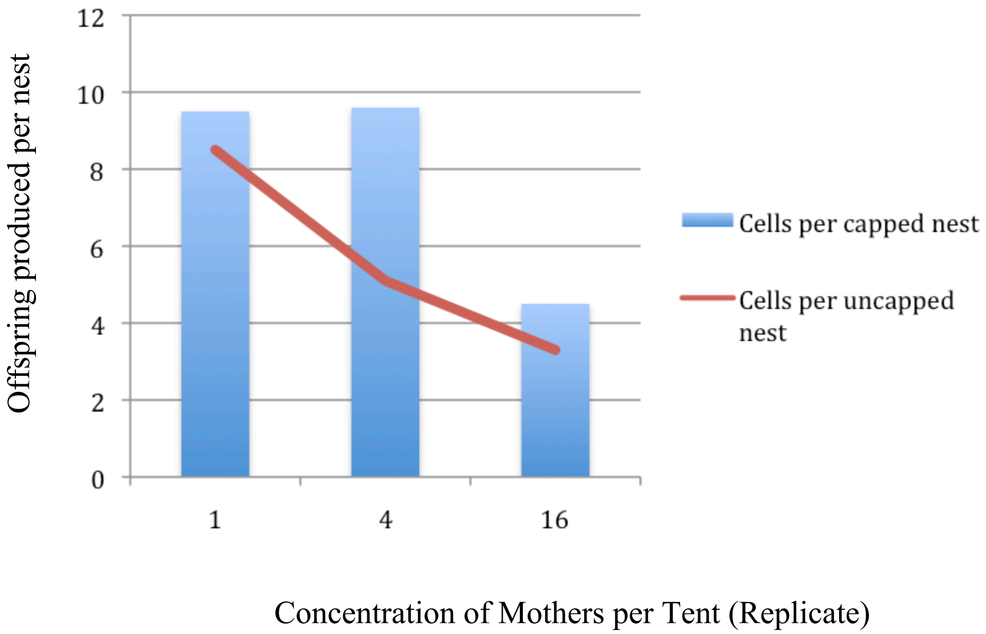


Figure 5-6. Population Concentration Experiment: Brood cells produced per nest from female concentration experiment with 1, 4, and 16 females in a confined tent with a set number of nesting sites (91), for capped and uncapped nests. Only the latter was significantly different between the three treatments. This work was replicated once in 2007 and 2008 and 6 times in 2009. ANOVA for capped cells (F=3.7, df=15, P=0.054) and uncapped cells (F=5.6, df=16, P=0.017)



5.7 Tables

Table 5-1. Flight Distance Experiment: The average number of brood cells produced per replicate for each of the four flight treatments during the first and second half of the season (Half Season=14 flying days) in 2007 (n=22) and 2008 (n=20), and results of ANOVA and post hoc treatment assignments within each half of the season. The treatment for the portion of the season being analyzed is bolded.

First Half of Season			**Second Half of Season**		
Treatment	Sig. Diff.	Brood Cell Production (Mean ± SE)	Treatment	Sig. Diff.	Brood Cell Production (Mean ± SE)
Far-Far	A	101±69	Far-**Far**	A	129±48
Far-Near	A	129±62	Far-**Near**	B	293±43
Near-Far	B	519±62	Near-**Far**	B	268±43
Near-Near	B	602±69	Near-**Near**	C	702±48
F=15.6, df=17, P<0.001			F=29.7, df=17, P<0.001		

Table 5-2. Resource Level Experiment: The number of offspring produced per replicate site (tent) for each treatment (±SE) during the 1st and second half of the season when the available resources varied during the season (Four different treatments). The ANOVA model includes year, treatment, and time in season (F=3.3, df=56, P<0.0016). Significant different "letters" are comparing within that column.

Treatment		2007-2008			
		Number of Offspring			
1st Half	2nd Half	1st Half		2nd Half	
Low	Low	4.2±3.1	A	4.6 ± 1.6	A
Low	High	7.3±3.4	A	17.5 ± 1.7	B
High	Low	34.5±3.4	B	6.4 ± 1.7	A
High	High	30.3±3.6	B	12.9 ± 2.4	B

Table 5-3. Resource Level Experiment: The available resources varied during each half of the season were compared using four different treatments (±SE). The average proportion of offspring that are daughters produced in each nest per replicate site (tent) for each treatment during the 1st and 2nd half of the season, as well as an average the two halves (full season/live time) are displayed. An ANOVA was conducted that includes treatment and time in season (F=3.2, df=29, P=0.003). Significant different “letters” are comparing within that column.

Treatment		2008					
		Sex Ratio					
1st Half	2nd Half	1st Half		2nd Half		Full Life Time - Average	
Low	Low	0.84 ± 0.1	A	0.89 ± 0.1	A	0.87 ± 0.1	A
Low	High	0.89 ± 0.1	A	0.82 ± 0.1	A,B	0.86 ± 0.1	A
High	Low	0.56 ± 0.1	B	0.81 ± 0.1	A,B	0.69 ± 0.1	A,B
High	High	0.63 ± 0.1	B	0.65 ± 0.1	B	0.64 ± 0.1	B

Table 5-4. Resource Level Experiment: The dry weight of each offspring was measured and compared between treatments (±SE). There was no significant difference within the first half or within the second half of the season for either males or females. When comparing the first and second half of the season, male weight did not change significantly while female weight decreased significantly in a number of the treatments, except in the Low/High treatment where the weight of offspring actually increased slightly in the second half of the season under high resources (F=5.48, df=1, P=0.021). The low/low treatment did not produce a significant portion of males in the second half of the season to allow for a statistical analysis.

A. Females

Treatment		**Female Offspring Weight (g)**			
		1st Half		2nd Half	
1st Half	2nd Half	Weight	SE	Weight	S.E.
Low	Low	0.0134	0.0006	0.0101	0.0010
Low	High	0.0124	0.0006	0.0126	0.0005
High	Low	0.0139	0.0004	0.0117	0.0008
High	High	0.0126	0.0004	0.0111	0.0010

B. Males

Treatment		**Male Offspring Weight (g)**			
		1st Half		2nd Half	
1st Half	2nd Half	Weight	S.E.	Weight	S.E.
Low	Low	0.0099	0.0013	-	-
Low	High	0.0092	0.0013	0.0098	0.0011
High	Low	0.0100	0.0005	0.0097	0.0013
High	High	0.0107	0.0008	0.0089	0.0009

Table 5-5. Population Concentration Experiment: Offspring production from female concentration experiment of 1, 4, and 16 females in a confined tent with a set number of nesting sites (91) using an ANOVA. The second column demonstrates significant differences. This work was replicated once in 2007 and 2008 and 6 times in 2009. All values are ±SE.

Females/ tent	Offspring Sex Ratio (Proportion female)		Cells per Nest		Unhatched (dead cells/nest)		Portion of nests capped	
1	0.57 ± 0.08	A,B	10.0 ± 0.8	A	2.1 ± 0.5	A	0.33 ± 0.14	A
4	0.69 ± 0.07	A	7.4 ± 0.7	B	2.0 ± 0.4	A	0.46 ± 0.11	A
16	0.43 ± 0.07	B	3.7 ± 0.7	C	1.4 ± 0.4	A	0.40 ± 0.11	A
	F=3.94, df=23, P=0.035		F=19.1, df=23, P=<0.001		F=0.71, df=20, P=0.51		F=0.27, df=17, P=0.768	

Table 5-6. Population Concentration Experiment: Number of brood cells produced (±SE) in capped and uncapped nests with female concentrations of 1, 4, and 16 per tent with a set number of nesting sites (91). The second column demonstrates significant differences (1 and/or 2)) from the ANOVA. This work was replicated once in 2007 and 2008 and 6 times in 2009. Significant different "letters" are comparing within that column.

	Cells per capped nest		Cells per uncapped nest	
1	9.5 ± 1.8	A,B	8.5 ± 1.2	A
4	9.6 ± 1.5	A	5.1 ± 0.9	A,B
16	4.5 ± 1.5	B	3.3 ± 1.0	B
	F=3.68, df=15, p=0.054		F=5.57, df=16, p=0.017	

5.8 References

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6: Conclusions and Future Work

Jason H. Peterson[1]

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[1]Evolutionary and Behavioural Ecology Research Group, Department of Biological Sciences, Simon Fraser University, Burnaby, BC, V5A lS6 Canada

6.1 Overview

Although 45 years have passed since Williams (1966 pp. 273) stated, “I would regard the problem of sex ratio as solved”, allocation between the sexes continues to be an expanding and successful field of research. The explosion of sex allocation research following Charnov’s (1982) book has resulted in a situation today where empirical and theoretical literature have become disjointed (West, 2009). West states numerous cases where, for example, local mate competition studies do not take into account the extensions that have been added to the theory over the years; he also finds frequent mistakes in the direction of sex allocation impacts and population-level consequences when studying Trivers and Willard (1973) related hypotheses. Therefore, a unifying framework is important both for the field in general and, on a smaller scale, within the variety of chapters within this thesis.

My co-authors and I have attempted to integrate the experimental and theoretical in a way that allows us to improve our empirical predictions through our theoretical work and vice versa. Our empirical work helped us to realize that oocyte production was not a factor we were easily able to measure or manipulate in the field, while our theoretical model demonstrated how multiple factors may impact sex allocation decisions and that single factor approaches could be misleading.

One of the resounding implications of our research is that numerous factors have the potential to impact resource and sex allocation decisions. Where traditionally we consider these decisions to be controlled by the most limiting factor, these decisions may involve trade-offs between factors. As such, organisms might make allocation decisions based on an optimal balance between the various conditions. Our results suggest that a future step is to see how widely applicable this conclusion is for other organisms, beyond leafcutter bees.

6.2 Thesis Summary

Our theoretical chapter (chapter 2) allowed us to address the key question of whether optimal investment in offspring (to maximize lifetime reproduction) is controlled by a single factor or is an optimal balance between multiple factors. Our results demonstrate that there is no single limiting factor; instead, there is some ‘optimal balance’ between mature egg and nest state that determines the optimal reproductive decision.

Maternal allocation in the form of defending the nest from conspecifics (chapter 3) appears to vary from the pattern of gradually increasing and then gradually decreasing over time that is found in traditionally studied organisms (Redondo and Carranza, 1989). We found that mothers did slightly increase nest defense as the nest size increased. However, unlike traditionally studied organisms, this increase in defense continued more steeply until the nest was basically completed and sealed, after which defense dropped suddenly.

Reproduction by its very nature requires individuals to invest some type of resource to produce progeny. When organisms must forage for resources to reproduce, we seek to determine what foraging currency a given organism maximizes in order to maximize fitness (Chapter 4). We found an increase in foraging load size per resource collection trip with increased flight distance to resources suggesting mothers are behaving more in a manner that maximizes efficiency rather than the more traditional currency, net rate of energy intake.

Following from our previous theoretical and empirical resource allocation research, we used field experiments to address the question of how changing ecological conditions impact sex allocation (Chapter 5). As mentioned previously, a wide variety of factors impacted sex allocation decisions, further suggesting the potential for organisms to find an ‘optimal balance’ between various resources. We also found opposite results to situations that appear to be similar (e.g. results for flight distance to resources compared to resource levels), suggesting the need for further research.

6.3 Unification

Our theoretical model was not explicitly developed to test hypotheses, but was designed to explore how our solitary bee’s offspring allocation decisions might respond to a wide variety of conditions. We are therefore interested in discussing commonalities and differences between our theoretical and empirical findings, not in testing the results of our model.

When we compare, in general terms, the results of our model (Chapter 2) to our flight distance experiments the mothers behaved as expected in terms of sex ratio of offspring (Chapter 5). When mothers had to fly longer to obtain pollen and nectar the proportion of the small sex, males that were produced increased. This was true regardless of the conditions mothers had experienced earlier in the season. However, when we compare the results of our resource level experiment (Chapter 5) to the model (Chapter 2) we find the model completely failed to predict the behaviour of mothers under low and high resource conditions.

We predicted similar results to those in the flight distance experiment; in the field we basically saw the opposite result. In general, mothers under low resource conditions produced a greater proportion of the more expensive sex, females. Unlike what is traditionally observed and what we found in our flight distance experiment, the production of daughters did not decrease in the second half of the season but instead remained constant. The one exception to this was when resources decreased in the second half of the season; here we actually observed an increase in the proportion of daughters. Both our model and Trivers and Williard (1973) predicted a decrease in proportion of daughters produced when resource become scarce. However, females are obviously making decisions based on some presumably optimal allocation 'system' that is not represented in these models.

This mismatch between theory and empirical data is not limited to our study system. Research in amniotes has raised doubts as to whether traditional adaptive sex allocation theory is valid in these taxa (Schwanz et al., 2010). Despite the wide spread use of the Trivers and Willard's theory, it is important to go back to the assumptions of the theory and see if they are met for any given organism. The assumptions are: (1) Condition of offspring at the end parental investment correlates with mother's condition during parental investment; (2) Variations in offspring body mass at weaning is maintained into adulthood; and (3) Differences in body condition have a greater impact on reproductive success of one sex. Various organisms have been suggested to violate one or more of these assumptions (Sikes, 1996). Our solitary bee appears to violate the first assumption.

Although this research was inspired by Trivers and Williard (1973), this solitary bee system appears to be somewhat unique from their situation where mothers have a set amount of resources from which to draw. In our work the female can choose how much to invest in each individual offspring and the cost constraints are not for the individual offspring but over the mother's lifetime. The result is that bees have more flexibility in decision making for each individual offspring and are not faced with the 'all or nothing' risk of producing a specific sex as the red deer mothers are when producing a male (Clutton-Brock, 1984).

A key discrepancy between Trivers and Williard's (1973) theory and our observations is this high proportion of the more expensive sex, daughters, under low resource conditions. This is the opposite of what Trivers and Williard's theory predicts, although they assumed that when resources are scarce the female is constrained to providing fewer resources per offspring. In the solitary bee system, this assumption may not be satisfied. When resources are scarce, mothers can produce fewer daughters, but continue to provide each one with the same amount of pollen. In other words, these bees have more flexibility in their offspring allocation decisions in comparison to the more traditional Trivers and Williard examples species, such as red deer. Mother bees can also work harder to collect the needed resources to produce a large daughter under poor conditions. Although there will be a cost to this decision, mothers do have the ability to make this decisions and consequently are not as constrained by conditions in regard to individual offspring as species such as the red deer. Therefore, the traditional Trivers and

Williard's (1973) theory does not appear to apply to our study system and we are currently in need of an applicable sex allocation theory. Further research is needed to better understand solitary bees and similar systems, the goal being to develop theory that is applicable to situations where females are not constrained to provide fewer resources per offspring, but can simply produce fewer offspring.

6.4 The Future

The results of our research suggest future work in two directions. The first is to delve deeper into the questions we addressed to understand allocation decisions and not only that they change, but when, how and exactly why such changes occur. The second is that there is also the opportunity to look at the issue more broadly.

Perhaps the most exciting aspect of the success the sex allocation research community has had as a whole is the opportunity for its use in examining more general questions of widespread importance in biology. Sex allocation research has played a pivotal role in social evolution, parent-offspring conflict, genomic conflict, and impacts on the development and testing of evolutionary models (West, 2009). Although the broader implications of sex allocation research have often been unrealized both within and outside the field of sex allocation, tremendous potential exists for future work to address similarly big issues. This unification may help lead to discoveries such as a better understanding of the relative importance of various potential evolutionary constraints.

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