SEX ALLOCATION IN A SOLITARY BEE:
A BEHAVIOURAL ECOLOGY APPROACH

by

Jason Harvey Peterson
B.Sc. Augustana University College, 2000
B.A. Augustana University College, 2000

THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

In the
Department
of
Biological Sciences

SIMON FRASER UNIVERSITY

Fall 2004

APPROVAL

Name: Jason Harvey Peterson

Degree: Master of Science

Title of Thesis:

Sex allocation in a solitary bee: A behavioural ecology approach

Examining Committee:

Chair: Dr. E. Palsson

Dr. B.D. Roitberg, Professor
Department of Biological Sciences, S.F.U.

Dr. L.M. Dill, Professor
Department of Biological Sciences, S.F.U.

Dr. M.L. Winston, Professor
Department of Biological Sciences, S.F.U.
Public Examiner

December 13 / 04
Date Approved

SIMON FRASER UNIVERSITY

PARTIAL COPYRIGHT LICENCE

The author, whose copyright is declared on the title page of this work, has granted to Simon Fraser University the right to lend this thesis, project or extended essay to users of the Simon Fraser University Library, and to make partial or single copies only for such users or in response to a request from the library of any other university, or other educational institution, on its own behalf or for one of its users.

The author has further granted permission to Simon Fraser University to keep or make a digital copy for use in its circulating collection.

The author has further agreed that permission for multiple copying of this work for scholarly purposes may be granted by either the author or the Dean of Graduate Studies.

It is understood that copying or publication of this work for financial gain shall not be allowed without the author's written permission.\

Permission for public performance, or limited permission for private scholarly use, of any multimedia materials forming part of this work, may have been granted by the author. This information may be found on the separately catalogued multimedia material and in the signed Partial Copyright Licence.

The original Partial Copyright Licence attesting to these terms, and signed by this author, may be found in the original bound copy of this work, retained in the Simon Fraser University Archive.

W. A. C. Bennett Library
Simon Fraser University
Burnaby, BC, Canada

ABSTRACT

Organisms reproducing sexually face the question of how to allocate investment to offspring between the sexes. My research objective was to evaluate the impact of ecological factors on maternal allocation decisions related to size, sex and number of offspring produced. Empirical and theoretical work was carried out using the alfalfa leafcutter bee, *Megachile rotundata*.

Decreased resource levels resulted in lower maternal investment per daughter and fewer offspring produced; investment per son and sex ratio were unaffected. Increased flight distance required to obtain resources resulted in decreased investment per individual daughter and son, overall production of offspring and proportion of daughters produced. Using a dynamic state variable model to determine optimal maternal investment, I found optimal offspring size was controlled by the shape of maternal fitness return curves while sex ratio was influenced by numerous ecological factors.

In conclusion, ecological factors appear to be important determinants of maternal investment in offspring.

Dedication

I would like to dedicate this thesis to my parents who have given me so much and helped me in so many ways throughout my entire life. As well as to Teri, whom I could not have done this without and Monty who was always up for an argument, regardless of the subject. Thanc you.

Acknowledgements

First and foremost, I would like to thank my senior supervisor, Bernie Roitberg, for his guidance and mentorship throughout this entire project. I would also like to thank my supervisory committee – Dr. Bernie Roitberg and Dr. Larry Dill – for their carefully considered advice as I prepared for and conducted this thesis research.

I am grateful to Dr. Ron Ydenberg for his help developing the dynamic state variable model, and Graeme Cox who helped write the computer program.

I also owe a debt of gratitude to those who supplied the land and materials to conduct my research: North Shore Farms, M.r. Pollination, BnR Farms, Scott Burton (who wasn't sure why I wanted to do it, but let me mow down parts of his field for my bees), Agriculture and Agri-Food Canada Lethbridge Research Centre and Undone Films.

The manuscripts were improved by thoughtful and appreciated comments from Larry Dill, Bob Lalonde, Amanda Cardinal Ludwig, Brian Ma, Jen Perry, Bernie Roitberg and Monty Simo (who also gave me the ability to travel between Vancouver and Alberta freely).

I am obliged to my laboratory assistant Angela Mah, for her assistance. Gordon Peterson was also of great help as a field assistant and a sounding board for my experimental designs, even if he thought what I was doing was sometimes a little crazy.

The research was supported by the National Science and Engineering Research Council (NSERC) in graduate support and grants to Bernie Roitberg. I also would like to acknowledge the graduate support from the department.

Finally, I must express my appreciation for the Roitberg lab group. My numerous discussions with Roitbergers improved countless aspects of this work, not to mention that I probably would not have survived without them. My mom also gets a special thank you, not only for imputing copious amounts of data, but for instilling in me, seemingly every day since 1984, the importance of backing up computer files. This came in handy when my hard drive self destructed six days before my thesis was due and then my laptop crashed five days before my thesis defence.

Table of Contents

List of Figures

List of Tables

"I heard of a man
who says words so beautifully
that if he only speaks their name
women give themselves to him."
- Leonard Cohen

“You told me again, you preferred handsome men
but for me you would make an exception.”
- Leonard Cohen

Chapter 1
An Introduction to Sex Ratio Theory

1.1 AN INTRODUCTION

Behavioural ecology seeks to understand the evolutionary basis for animal behaviour. Members of a species are frequently found in diverse environments, therefore their behaviours are often flexible and influenced by ecological conditions. One such behaviour is that of sex ratio allocation. When producing offspring, numerous organisms must decide which sex to produce and the amount to invest per individual progeny.

All reproduction was asexual, when life first developed on earth. The emergence of sexually reproducing organisms meant two individuals were required for reproduction, a male and female (Ricklefs, 1990). Populations tended to remain half males and half females, seemingly producing an utopian situation where there is one male for every female and vice versa.

In the first edition of "The Descent of Man", Charles Darwin (1871) argued that if one of the sexes was overproduced, that sex would have a more difficult time finding a mate and therefore natural selection would favour mothers who produced more of the rarer sex. This apparently straightforward evolution of an even sex ratio has resulted in a number of interesting questions. Why are there only two sexes per species? What forces maintain a near 1:1 ratio in a large number of species? Why do a substantial minority of species vary from the 1:1 ratio? Of those with skewed ratios, why are some male-biased and others female-biased, even among closely related species?

In all sexually reproducing "higher" animals and plants the gametes are of different size, i.e., anisogamous. Females are the individuals with the larger gametes, while males are the individuals with the smaller gametes. This asymmetry has important implications for sex ratio theory because of the differences in investment required to produce these gametes. In the most basic sense, females are making the larger per gamete investment. Males on the other hand, have a greater number of cheaply produced gametes and so have the greater reproductive potential. With a lower lifetime reproductive potential in number of offspring, the quality of each offspring is likely of greater importance to females. This higher reproductive ceiling in males also means there will be much more reproductive variance in output between males than females.

Given that males can fertilize a large number of females, a species would seem to benefit from producing a female biased population that would be able to produce a greater number of offspring. Unless males participate in important defence or parental care activities, a female bias would avoid the waste of producing unnecessary males. However, natural selection acts first and foremost on the individual not the population or the species. For example, if there are two individuals in a population and one produces two daughters while the other produces one son and one daughter, the latter will pass more copies of alleles onto the next generation by way of her son who will mate with all the females. Although a population that has a female biased sex ratio will produce more offspring, natural selection will favour the female that produced the 1:1 ratio because relatively more copies of her alleles will be passed on to the next generation. The result is a population made up of individuals which overall produces an even sex ratio.

Fisher (1930) further developed the argument that natural selection favours the rarer sex. The key to Fisher's verbal argument is that selection does not favour equal production of sons and daughters, but equal investment in sons and daughters. His theory predicts an even sex ratio when sons and daughters require the same investment. When one sex is more expense to produce, a bias towards the cheaper sex is expected. However, numerous factors may alter allocation from this simple equal investment formula. In certain cases the same factor can cause opposite results in different species. In spider monkeys and barbary macaques high-ranked females produce more sons than daughters (McFarland Symington, 1987; Paul and Kuester, 1987), while in bonnet macaques and rhesus macaques the bias is reversed (Silk, 2001; Maestripieri, 2001). These results suggest that the relative reproductive success of sons and daughters can vary among parents. Therefore, if mothers are capable of facultative sex ratio allocation, ecological conditions may affect these decisions. Note that sex ratios may be adjusted at the primary (fertilization) or higher (e.g., post birth) levels.

Sex ratio may be determined by maternal condition. This notion is not new (Schenk, 1898; Dawson, 1917). Trivers and Willard (1973), developed a successful theoretical argument using a natural selection approach to facultative sex ratio. They argued that in situations where males compete for females only those males in the best condition will mate. On the other hand all females are likely to be able to produce some offspring. Therefore only mothers in good conditions will be able to invest enough to produce a son of sufficient size and condition to successfully compete for mates. This hypothesis was supported by work on the red deer, *Cervus elaphus*, where only healthy males are able to acquire and maintain a harem (Clutton-Brock et al., 1984). Mothers in poor condition gain greater rewards from the producing a daughter of poor condition who will be able to produce some progeny, while a son in poor condition will likely produce no offspring. The reverse may be the case in many non-territorial insect species where larger size (i.e., investment) is more important for females, allowing for benefits such as greater egg production and increased foraging rates whereas benefits to males of increased size is limited. Therefore, we need to examine these situations to determine what decisions will allow mothers to maximize fitness.

Studies on invertebrates have provided a wealth of evidence suggesting that breeding adults maximize their fitness by manipulating the sex ratio of their offspring (Charnov, 1982). Many of these studies have examined haplo-diploid organisms such as Hymenoptera because the mechanism that determines sex ratio is well understood (daughters-fertilized egg; males-unfertilized egg) (Cook, 1993). Although social hymenopterans have revealed important discoveries such as how relatedness conflicts can affect sex ratio (Hamilton, 1964), the majority of bees and wasps are actually solitary breeders. In solitary species the entire maternal investment usually occurs before the offspring emerges, therefore there is no conflict between mothers and daughters (Freeman, 1981). Females provision progeny entirely on their own, limiting conflict with the fathers whose genes are only passed on through daughters. The female investment in offspring includes the time and energy necessary to produce an ova and provision the brood cell. Measuring this investment allows us to test Fisher's (1930) hypothesis and variations thereof.

Studies of sex-investment patterns have revealed a variety of factors that potentially cause deviation from Fisher's (1930) prediction. These include local resource competition (Clark, 1978; Silk, 1983), local mate competition (Hamilton, 1967), conflict between mates (Brockmann and Grafen, 1989), maternal size (Sugiura and Maeta, 1989), partially overlapping generations (Tepedino and Parker, 1988) and local resource enhancement (Schwarz, 1988; Stark, 1992). In addition to these factors, resource availability has been suggested as another important factor affecting sex allocation (Rosenheim et al., 1996; Kim, 1999). Several authors have developed theories to explain a shift in sex allocation in response to changing resource levels (e.g., Frank, 1995; Rosenheim et al., 1996). Individual females have been suggested to use facultative sex allocation based on the availability of resources and oocyctes to maximize reproductive success (Rosenheim et al., 1996).

Deviation from Fisherian (equal) sex allocation is expected under ecological conditions where the function describing fitness returns on maternal investment differs in shape between sons and daughters (Charnov, 1982; Frank, 1990; Campbell, 2000). A shift is expected towards the sex function showing relatively larger reproductive gains (Charnov & Bull, 1977; Charnov, 1982; Frank, 1990). Ecological conditions may also impact maternal investment in progeny. For example, in a solitary bee, years with high pollen availability were correlated to an increase in the average number of progeny per nest (Minckley et al., 1994). If ecological conditions, such as resource levels, can impact both sex ratio and resource allocation, the manner in which resources are obtained by the female is likely to be key.

An important characteristic of solitary bees is that they are central place foragers (Orians and Pearson, 1979; Schoener, 1979). Solitary bees choose a central place where a nesting site is found or built and all the needed resources (e.g., nest building materials, provisions) must be transported to this location. This is important when there is more than one category of provisioning resources and when these resources may be spatially disparate. Many parasitic wasps on the other hand seek out hosts and deposit an egg when a suitable host is located. They have no "home" or central location that they need to return to. Work on beavers has demonstrated a trade-off in terms of choosing a location for a lodge based on the supply of trees and suitable water location (Fryxell and Doucet, 1990). This type of trade-off is important to consider in species that exhibit central place foraging. The cost of obtaining resources mothers require while building a nest, both for themselves and their offspring, will be impacted by proximity of the nest to required resources. As with the beavers, when resources are spatially separate, and building a nest close to one resource requires greater travel distance to obtain a different resource a trade-off is likely to occur. As the method of provisioning offspring is quite different in central place foragers compared to other species, such as many solitary wasps, changing ecological conditions are likely to impact the costs of collecting resources differently. With the importance of ecological conditions on a species which is rooted in a single location, we expect facultative sex allocation to alter the sex ratio and resource allocation to optimal levels based on local conditions.

Sex ratio 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 in sex ratio theory and have fuelled further research on the subject. My work evaluated the impact of ecological factors such as resource availability and flight distance on sex ratio and resource allocation. I examine the impact on sex allocation of resource levels in Chapter 2 and of flight distance in Chapter 3. In Chapter 4 I develop a dynamic programming model to evaluate how different ecological conditions interact and how this may affect optimal maternal sex allocation decisions. Finally, in Chapter 5, I summarize the findings in a unified framework.

1.2 REFERENCES

Brockmann, H.J. and A. Grafen. 1989. Mate conflict and male behavior in a solitary wasp *Trypoxylon politum* (Hymenoptera: Sphecidae). Animal Behaviour 37:232-255.

Campbell, D.R. 2000. Experimental tests of sex-allocation theory in plants. Trends in Ecology and Evolution 15:227-232.

Charnov, E.L. 1982. The theory of sex allocation. Princeton University Press, Princeton.

Charnov, E.L. and J. Bull. 1977. When is sex environmentally determined. Nature 266:828-830.

Clark, A.B. 1978. Sex ratio and local resource competition in a prosimian primate. Science 201:163-165.

Clutton-Brock, T.H., S.D. Albon, and F.E. Guinness. 1984. Maternal dominance, breeding success and birth sex ratios in red deer. Nature 308:358-360.

Cook, J.M. 1993. Sex determination in the hymenoptera, a review of models and evidence. Heredity 71:421-435.

Darwin, C.R. 1871. The descent of man and selection in relation to sex. John Murray, London.

Dawson, E.R. 1917. The causation of sex in man. Paul B. Hoeber, New York.

Fisher, R.A. 1930. The genetical theory of natural selection. Clarendon, Oxford.

Frank, S.A. 1990. Sex allocation theory for birds and mammals. Annual Review of Ecology and Systematics 21:13-55.

Frank, S.A. 1995. Sex allocation in solitary bees and wasps. American Naturalist 146:316-323.

Freeman, B.E. 1981. Parental investment, maternal size and population dynamics of a solitary wasp. American Naturalist 117:357-362.

Fryxell, J.M. and C.M. Doucet. 1990. Provisioning time and central-place foraging in beavers. Canadian Journal of Zoology 69:1308-1313.

Hamilton, W.D. 1964. The genetical evolution of social behaviour. Journal of Theoretical Biology 7:1-52.

Kim, J-Y. 1999. Influence of resource level on maternal investment in a leaf-cutter bee (Hymenoptera: Megachilidae). Behavioral Ecology 10:552-556.

Maestripieri, D. 2001. Female-biased maternal investment in rhesus macaques. Folia Primatologica 72:44-47.

McFarland Symington, M. 1987. Sex ratio and maternal rank in wild spider monkeys when daughters disperse. Behavioral Ecology and Sociobiology 20:421-426.

Minckley, R.L., W.T. Wcislo, D. Yanega, and S.L. Buchmann. 1994. Behavior and phenology of a specialist bee (*Dieunomia*) and sunflower (*Helianthas*) pollen availability. Ecology 75:1406-1419.

Orians, G.H. and N.E. Pearson. 1979. On the theory of central place foraging. Pp. 155-177, *in* Analysis of ecological systems. Ed. D.J. Horn, G.R. Stairs and R.D. Mitchell. Ohio State University Press, Columbus, Ohio.

Paul, A. and J. Kuester. 1987. Dominance, kinship and reproductive value in female barbary macaques (*Macaca sylvanus*) at Affenberg Salem. Behavioral Ecology and Sociobiology 21:323-331.

Ricklefs, R.E. 1990. Ecology. 3rd edition. W.H. Freeman, New York.

Rosenheim. J.A., P. Nonacs and M. Mangel. 1996. Sex ratios and multifaceted parental investment. American Naturalist 148:501-535.

Schenk, L. 1898. Schenk's theory: the determination of sex. The Werner Company, New York.

Schoener, T.W. 1979. Generality of the size-distance relation in models of optimal foraging. American Naturalist 114:902-914.

Schwarz, M.P. 1988. Local resource enhancement and sex ratios in a primitively social bee. Nature 331:346-348.

Silk, J.B. 1983. Local resource competition and facultative adjustment of sex ratios in relation to competitive abilities. American Naturalist 121:56-66.

Silk, J.B. 2001. Bonnet Macaques: Evolutionary Perspectives on Females' Lives. Pp. 433-452, *in* Model systems in behavioral ecology. Ed. L.A. Dugatkin. Princeton University Press, Princeton.

Stark, R.E. 1992. Sex ratio and maternal investment in the multivoltine large carpenter bee *Xylocopa sulcatipes* (Apoidea: Anthophoridae). Ecological Entomology 17:160-166.

Sugiura, N. and Y. Maeta. 1989. Parental investment and offspring sex ratio in a solitary Mason bee, *Osmia cornifrons* (Radoszkowski) (Hymenoptera, Megachilidae). Japan Journal of Entomology 57:861-875.

Tepedino, V.J. and F.D. Parker. 1988. Alternation of sex ratio in a partially bivoltine bee, *Megachile rotundata* (Hymenoptera: Megachilidae). Annals of the Entomological Society of America 81:467-476.

Trivers, R.T. and D.E. Willard. 1973. Natural selection of parental ability to vary the sex ratio of offspring. Science 179:90-92.

Chapter 2

Impact of Resource Levels on Sex Ratio and Resource Allocation in the Solitary Bee, *Megachile rotundata*

2.1 ABSTRACT

Maternal investment is strongly influenced by the availability of resources. A mother is expected to trade-off allocation to size, number and sex of offspring in order to maximize her lifetime reproductive success. Optimal allocation is likely affected by ecological conditions which can alter the costs and benefits of different allocation decisions. When resources are rare, mothers have fewer resources to invest in offspring, likely altering optimal resource allocation. I examined maternal resource allocation to progeny under three different resource levels in the solitary bee *Megachile rotundata* (Fabricius). I found that females with access to low resource levels invested less per individual daughter and produced fewer total offspring compared to females with access to greater resource levels. Resource limitation had no impact on sex ratio.

2.2 INTRODUCTION

Animals usually time reproduction to avoid periods when resources are limited, avoiding constraints on resource acquisition that can limit reproductive success (Sedinger and Raveling, 1986; Verhulst et al., 1995). Once resources have been obtained, parents not only make decisions on allocation among individual offspring, but also between sons and daughters (Charnov, 1982). Fisher's (1930) theory of parental investment and sex allocation predicts equal investment in each sex. In other words, if both sexes require the same amount of parental investment we expect an equal production of sons and daughters. However, if for example females are twice as expensive to produce as males, we expect twice as many sons as daughters to be produced (Fisher, 1930; Charnov, 1982).

Parental investment refers specifically to activities that decrease future potential to produce offspring (Williams, 1966a,b). The current body of sex ratio theory identifies numerous factors that can cause sex investment to deviate from Fisher's (1930) theory: local resource competition (Clark, 1978; Silk, 1983), local resource enhancement (Schwarz, 1988), relatedness issues (Hamilton, 1964, 1979), local mate competition (Hamilton, 1967) and sexual asymmetry regarding minimal quality (Trivers and Willard, 1973). More recently, changing resource levels and ova production have been hypothesized as factors that may alter investment costs and therefore sex ratio (Frank, 1990; Rosenheim et al., 1996). As multiple factors likely influence any particular system, determining the most influential factor under a given set of conditions is a key consideration (Rosenheim et al., 1996). Further, it is important to consider ecological contexts that can influence the costs of investment in offspring.

Where fitness is defined as the number of copies of alleles passed on to future generations, parents who invest more in an offspring generally accrue greater fitness returns from that offspring. Numerous studies have documented a positive relationship between size and lifetime reproductive success (Southwood, 1966; Alcock, 1979; Hendry et al., 2001; Boltnev and York, 2001; Skow and Jakob, 2003), and this size/fitness relationship frequently varies with respect to gender. Trivers and Willard (1973) proposed that parental condition could influence sex investment decisions when such differences occur. For example, in a population where only the healthiest males are able to reproduce, as in many territorial species, investing heavily in each son is necessary if any parental fitness is to be obtained from producing a son. When only those males who can outcompete conspecifics for territory are able to mate, parents should either produce a son vigorous enough to defend a territory or produce a daughter who should produce a non-zero number of offspring. In contrast, the benefits to a larger son are limited in non-territorial species, whereas a larger daughter is generally able to produce more offspring at a faster rate and is less vulnerable to egg limitations (Alcock, 1979; Cowan, 1981; Mangel et al., 1994; Rosenheim et al, 1996). Therefore, the maternal fitness return curves, per unit of investment (Fig. 2-1), will likely vary across species and also between the sexes within a species. This relationship between progeny sex, parental investment and fitness plays an important role in maternal sex allocation and investment decisions.

Solitary hymenoptera have been used to test numerous resource allocation and sex ratio hypotheses (Tepedino and Torchio, 1982; Kim, 1999). As haplodiploid organisms, mothers are able to control the sex of each zygote by whether the ovum is fertilized (daughters-fertilized egg; males-unfertilized egg) (Cook, 1993). In nest-building solitary bees, all the food resources a developing offspring will receive, which in turn control adult progeny size, are collected by the mother and sealed into the brood cell (Klostermeyer et al., 1973). Foraging to provision offspring takes place over a relatively short period of time and is carried out exclusively by the mother. Ecological conditions experienced by the mother during that time should factor heavily in investment decisions. Returning to my notion of fitness, it is often assumed that reproductive success in solitary bees is limited by the availability of resources used to provision offspring, however few studies have combined independent measures of resource availability with realized lifetime reproductive success (Rosenheim et al., 1996).

In order to understand the investment in offspring made by the mother, it is important to consider that nest-building solitary bees are central place foragers (Orians and Pearson, 1979; Schoener, 1979). In such systems, a number of disparate resources must be collected and brought to a single location in order to build a nest. For many hymenopteran species, these resources include materials to line the nest, pollen, and nectar. The costs of obtaining these resources are expected to vary temporally and/or spatially (Parker and Frohlich, 1985; Minckley et al., 1994). Tepedino and Torchio (1982) demonstrated increased production of female offspring when maternal bees had access to super-abundant resources throughout the year. Subsequent theoretical work has been carried out attempting to explain the alteration in resource allocation and sex ratio in response to changing resource levels (Frank, 1995; Rosenheim et al., 1996). This work suggests that as food becomes more scarce, time and energy spent obtaining food will become a greater proportion of the overall investment in offspring.

I examined sex ratio and resource allocation in the solitary alfalfa leafcutter bee *Megachile rotundata* (Fabricius). This species is non-territorial and sexually dimorphic with females being 1.2 - 1.3 times larger than males (Klostermeyer and Gerber, 1969; Klostermeyer et al., 1973), suggesting that increased size may be most important in females (Alcock, 1979; Cowan, 1981). The 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 appears to be minimal (Tepedino et al., 1984). Based on this, I predicted that resource availability would have a large impact on offspring body size.

I tested sex ratio and resource allocation decisions under three different resource levels (high, medium and low - each level has half the resources of the previous level). As the amount of resources available decreased, the amount of maternal investment required to produce a daughter is expected to increase more than for a son. This is because daughters are generally larger and so require greater investment to produce a viable offspring. The greater the amount of investment required to produce a daughter, the less likely a mother will be able to invest that amount in a single offspring. I hypothesized that decreases in available resources would result in a decrease in the maternal benefit per unit of investment. Assuming that mothers have a fixed energy budget, mothers should: 1) increase the proportional production of males (the smaller sex), and 2) decrease the amount of resources allocated to each offspring, because of a decreased optimal size.

2.3 MATERIAL AND METHODS

Megachile rotundata is a leaf-cutting bee commonly used in the pollination of many seed crops, including alfalfa (*Medicago sativa*). A wide range of sex ratios has previously been reported for this species (e.g., Maki and Moffett, 1986; Jay and Mohr, 1987; Tepedino et al., 1994 ; McCorquodale and Owen, 1997), suggesting that females may vary the sex ratio of offspring in response to changing optima. Previous work in these systems that tested hypotheses generated from sex allocation and resource availability theory have focused on either observational data or laboratory experiments (Rosenheim et al., 1996). Here I conducted field experiments in large tents where I manipulated the amount of available resources and measured the effect of these manipulations on the size, sex and number of offspring produced.

2.3.1 Leafcutter bee life history

The alfalfa leafcutter bee, *M. rotundata*, has a number of characteristics that make it an excellent subject to address questions of sex allocation and maternal investment. Female leafcutter bees mate only once (Gerber and Klostermeyer, 1970; Krunic, 1971) and retain enough sperm to fertilize their complete complement of eggs (Richards, 1994). These bees nest gregariously and females build linear twig nests (Fig. 2-2) composed of a number of brood cells in pre-formed cavities. This allows for the use of standardized artificial experimental nests (McCorquodale and Owen, 1997). Thimble-shaped brood cells are initiated and completed one at-a-time over a period from one day to a few days (Klostermeyer and Gerber, 1969). The mother lines the walls of the nest cavity with masticated leaves and then fills this cell with provisions of pollen and nectar. An egg is laid on this pollen and nectar (below referred to collectively as pollen) "loaf", the cell is sealed with leaf pieces and a new cell is started in front of the completed cell. When a nest, made up of a number of brood cells, is completed, the mother uses 10-40 leaf pieces to seal the entrance to the nest with a plug or "cap." Although *M. rotundata* is facultatively multivoltine, in southern Alberta, where this work was carried out, they are univoltine, with adults emerging in early summer and living approximately six weeks (Tirgari, 1963). This allows resource levels to be controlled during the entire adult life.

Offspring over-winter as prepupa and emerge from brood cells as adults, therefore all of the food consumed prior to adulthood is provided by the mother (Johansen et al., 1969). Measurements can easily be performed to determine putative factors contributing to maternal fitness such as realized fecundity, offspring sex and investment (using emergence weight and head capsule width) per offspring.

2.3.2 Field experiment

Experiments were carried out in Tilley, Alberta, Canada (2003) on a third year alfalfa seed field (54°24' North, 111°41' West). Aluminium frame tents (3m x 2m x 1m; 1.8m high in the centre) with a mesh cover were used for each replicate. The crop under each tent was sprayed with malathion six days prior to bee release to prevent aphid outbreaks while ensuring no impact on the bees.

The 21 experimental tents were randomly assigned to one of three treatments, using the roll of a die: (1) all the alfalfa plants under the tent were left standing (high treatment); (2) the plants in the eastern (front) half of the tent were cut at ground level (medium treatment); (3) the plants in the front three quarters of the tent were removed (low treatment). This resulted in seven replicates for each of the three resource level treatments.

Nesting sites were provided using a single polystyrene Beaver Plastics Mega Block^{TM} hive (585x300x76mm with 1710 6mm diameter nesting holes) strapped to a plywood backing (30cmx120cm) with a sheet of fibrefill sandwiched in between them. A small plywood roof (15cm x 12cm) was nailed at an angle to the top of the hive to prevent rain from entering the nests. The hive was hung against the west end of the tent with the open end of the nest holes facing due east.

Loose-cell bee larvae were obtained from North Shore Farms (Tilley, Alberta) and placed in hatching trays for 21 days of incubation as described by Peterson et al. (1992). The night before the bees were released, 21 groups of 25 females and 50 males were separated out and one group was placed in each tent on the morning of July 14th 2003. This sex ratio ensured there were sufficient males to mate with the females. Starting population sex ratio does not appear to affect the sex ratio of the next generation, as long as access to males is not limiting (Mayer, 1994). Twice during the season tents were opened during the night, when the bees are not active, to re-cut the plants in those cages where a portion of the alfalfa had been removed.

During the season, bi-weekly observations were also made on seed set and bloom availability with the help of an experienced alfalfa seed farmer. This gave me information on (1) the number of flowers that had been visited by female bees, and (2) how availability changed during the season. One tent from each treatment was also outfitted with a max-min thermometer to ensure the air temperature did not vary among the treatments (the temperature did not vary).

On August 28th, 2003 after all the adult bees had died, the hives were removed and stored for two weeks at 20°C to allow the larvae to complete development to the prepupal stage. After this time, they were cooled to 8°C for five months to break diapause. In February, each brood cell was individually removed from the hive and placed into trays with individual compartments so that each bee could hatch separately. The brood cell's location within the nest and the nest's location within the hive were recorded so that after the bees hatched I would know what sex of bee was produced in each brood cell of every nest. Brood cells were incubated at 30°C for 30 days until all viable larvae had developed into adults and could be sexed. To complete the drying process, the brood cells were dried for one hour at 90°C. The sex, head capsule width (using an ocular micrometer) and weight (using a Sartorius™ scale accurate to the nearest 0.0001g) of each offspring were recorded. Where possible, larvae that did not hatch were sexed according to their position in the linear nest as described by Frohlich and Tepedino (1986). This is possible because daughters are laid in the innermost cells generally followed by a single switch to the production of sons (Rothschild, 1979). When brood cells that were within this switch zone failed to hatch, they could not be sexed and were discarded from the sex ratio analysis. Adult emergence dry weight and head capsule width were measured in order to determine the mother's resource allocation response to the resource availability.

At the end of the season, the number of flowers utilized per m^2 in each plot was determined by visual analysis. This was again done with the aid of an experienced alfalfa seed farmer by examining the size and number of pods on each plant in the plot.

2.3.3 Statistical analysis

The tent was the unit of replication. A least squared means one-way ANOVA was used to analyse 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, the adult weight and head capsule width of sons and daughters, and alfalfa seed yield.

The following data were transformed by arcsine square root prior to statistical analysis using a least squared means ANOVA: proportion of nests capped (sealed), proportion of brood cells where adults failed to emerge, proportion of nests with only females, proportion of nests with only males, and proportion of nests with more than a single female.

2.4 RESULTS

The flowers in one of the medium resource level tents did not bloom and no offspring were produced in that tent; as a result it was removed from the analysis. Therefore, there were seven replicates of the low resource level, six of the medium and seven of the high.

There was no significant difference in proportion of daughters (female sex ratio) produced between treatments: low 0.31 ± 0.06 (mean +/- SE), medium 0.39 ± 0.06 and high 0.36 ± 0.06 (F=0.44, *d.f.*=2, P=0.33, β=0.11). However there was a significant difference in the number of brood cells-per-nest and the number of nests-per-treatment. The average numbers of cells-per-nest in the treatments were: low 2.5 ± 0.4, medium 3.1 ± 0.4 and high 4.0 ± 0.4 (F=4.60, *d.f.*=2, P=0.025). The average number of nests-per-treatment were low 5.1 ± 1.0, medium 8.5 ± 1.1 and high 16.0 ± 1.0, respectively (F=32.01, *d.f.*=2, P<0.001) (Fig. 2-3). There was also a significant difference between treatments in the mean production of both daughters (F=16.73, *d.f.*=2, P<0.001) and sons

(F=22.06, *d.f.*=2, P<0.001) per nest (Fig. 2-4). The increase in the proportion of nests which were capped with respect to the increased availability of resources was marginally non-significant (F=3.62, *d.f.*=2, P=0.051, β=0.58). The proportion of brood cells from which adults failed to emerge was not significantly different between treatments: low 0.26 ± 0.07, medium 0.26 ± 0.05 and high 0.30 ± 0.03 (F=0.42, *d.f.*=2, P=0.66, β=0.11). Overall, with increasing resource levels, there was a significant increase in the production of nests, sons and daughters; however, there was no effect on sex ratio, proportion of capped nests or offspring that died during development.

The proportion of nests containing only male offspring was 0.58 ± 0.08 in the low treatment, 0.36 ± 0.09 in the medium and 0.36 ± 0.08 in the high (F=2.41, *d.f.*=2, P=0.12, β=0.42). The proportion of nests containing only females was 0.23 ± 0.06, 0.19 ± 0.06 and 0.19 ± 0.06 for the low, medium and high treatments respectively (F=0.02, *d.f.*=2, P=0.98, β=0.05). The proportion of nests where more than one female was produced was 0.000 + 0.04 in the low, 0.195 ± 0.05 in the medium and 0.204 ± 0.04 in the high treatments (F=16.95, *d.f.*=2, P<0.001).

The number of flowers that were visited per m^2 was uniform, 30 ± 1g of seed produced per m^2 in each treatment, and was used as a proxy for the number of flowers visited by the females to collect pollen and nectar (F=0.11, *d.f.*=2, P=0.90, β=0.06).

The average weight for female offspring was 0.0109 ± 0.0004g, 0.0120 ± 0.0004g and 0.0123 ± 0.0004g for the low, medium and high treatments respectively (F=3.62, *d.f.*=2, P=0.025) (Fig. 2-5A). The average weight of males was 0.0091 ± 0.0002g, 0.0091 ± 0.0003g and 0.0093 ± 0.0002g for low, medium and high treatments, respectively (F=0.21, *d.f.*=2, P=0.21, β=0.08) (Fig. 2-5A). Analysis of the head capsule width revealed the same trend as weight, with female head width increasing significantly with increasing resources: 2.73 ± 0.04mm, 2.82 ± 0.04mm, 2.90 ± 0.04mm (F=4.45, *d.f.*=2, P=0.015) (Fig. 2-5B). Male head width did not follow this same pattern: 2.73 ± 0.05mm, 2.80 ± 0.05mm, 2.76 ± 0.05mm (F=0.54, *d.f.*=2, P=0.30, β=0.13) (Fig. 2-5B). There were significant differences between the weights of males and females in all treatments: low (t=3.73, *d.f.*=11, P=0.003), medium (t=6.52, *d.f.*=10, P<0.001) and high (t=7.09, *d.f.*=12,

$P<0.001$). Overall weight and head capsule width increased with increasing resource for daughters but not for sons.

2.5 DISCUSSION

Follow up studies to Trivers and Willard's (1973) work on sex allocation have revealed that ecological conditions have the potential to influence sex allocation decisions (Kruuk et al., 1999; Koskela et al., 2004). Using the solitary bee *M. rotundata*, I tested the prediction that, under semi-field conditions, decreasing resource availability would increase the proportional production of the cheaper sex (males) and decrease the amount of resources invested per individual. I show here that varying resource levels had an impact on brood cell production and the amount of resources allocated to daughters, however no impact on sex ratio was found. I suggest reasons for this last result may be that resource levels are not a factor that affects sex ratio or because all three treatments represented limiting food resource conditions.

The multifaceted parental investment model (Rosenheim et al., 1996) predicts that when multiple resources are required to perform a single task, changing conditions will result in changes in the proportional influence of each factor. The more effort (i.e., investment) required to obtain a resource, the greater the impact that resource has on the cost of offspring production. I demonstrated a significant proportional increase in the production of brood cells per nest and in nests produced as food resource levels (pollen and nectar) increased. Since nest construction materials (i.e., alfalfa leaves) were readily available in all treatments, they were unlikely to be a limiting factor. This increase in production of progeny suggests that under my experimental conditions, food resource levels limited offspring production. With access to greater food resources, mothers were able to invest in larger offspring (discussed later) and in a greater number of offspring. The production of offspring more than doubled with each doubling of available resources. It is important to realize that the amount of resources available to invest in offspring is not the total a mother can collect, but the net total after she consumes some of the resources to maintain somatic functions. In simple terms, if the mother requires one unit of resources to sustain herself and two units to provision an offspring, then a 66%

increase in available resources from three units to five units will result in a 100% increase in the production of offspring. As well, increasing abundance of resources is likely to result in less time spent per load of resources collected. This increase in offspring production, which is proportionally greater than the increase in resource levels, is likely a result of these factors. However, these factors did not appear to impact the sex ratio in the same manner.

While no significant difference was found between the sex ratios in each of the treatments, the sex ratio was always significantly different from 1:1. This difference is predicted under Fisher's (1930) sex ratio theory, as sons require fewer resources than daughters during the period of parental care. However, the proportion of daughters produced in all treatments was low compared to other sites in Alberta. The average adult-emergence sex ratio, based on total male and female production in each treatment (the standard for commercial sex ratio tests), was 0.29. In this same year, the average female sex ratio in commercial fields in southern Alberta was 0.37, with 0.45 and 0.28 being the most extreme cases (Canadian Leafcutter Bee Cocoon Testing Centre, unpublished data).

In non-territorial bees where large females are able to produce more offspring than smaller females, decreasing adult size results in sons returning more maternal fitness than daughters (Torchio and Tepedino, 1980). Therefore, the production of sons may be favoured as resource availability decreases. In my experiments, uniformly low female sex ratio suggests that all three resource levels were at the low end of the resource spectrum where male production is favoured. In addition to this, visual observations allowed us to determine that after the first two weeks flowers were utilized nearly as soon as they opened in all treatments, suggesting resource availability was limited. This also suggests that, in general, ovum production did not limit flower foraging. If females had to wait for eggs to develop we would have expected unexploited flowers to be available, but it appears that the maximum amount of foraging was occurring as all flowers were being utilized upon opening. Nevertheless, without data on ova maturation times I can not exclude the possibility that some mothers were less able to compete for the limited resources because of the time required to mature ova. Overall, it is possible the resource levels were different enough to result in changes to the number of offspring produced but not to alter the value of producing one sex versus the other.

Although sex ratios were not significantly different in the three treatments, the mothers did appear to employ different sex ratio tactics under different conditions. When resources were the most limiting there were significantly fewer nests (0) that produced more than one daughter. There also was a trend towards proportionally more nests with only sons. Under these very limiting resource conditions mothers appeared to be faced with either producing multiple sons or a single daughter. This behaviour is predicted by Trivers and Willard (1973): parents in the best condition are expected to produce the sex in which being large and healthy is most important, while parents in poor condition are expected to produce the other sex. Under the limiting conditions of the low food treatment, it is possible that only the mothers in the best condition were able to collect enough resources to both maintain their own somatic function and produce a large, healthy daughter. By contrast, the smaller amount of pollen other females were capable of collecting was best utilized to produce a son. The other possibility is that all females were of similar vigour and the low resource levels presented them with a choice; mothers could only produce a small number of offspring so they could either put all their resources into a single daughter or produce a nest with several sons. In order to support either of these hypotheses, we need quantitative information on the condition of each mother to compare with the mother's sex ratio and investment decisions.

When resources are rare at a given location, parents are predicted to adjust not only the sex ratio of their offspring but also the size, decreasing the amount of resources given to each offspring (Rosenheim et al., 1996). As I demonstrated earlier, the number of offspring-per-nest and the number of nests increased with greater resources, however the average resource investment per female offspring increased as well. I used both emergence dry weight and head capsule width as proxies for resource investment. Daughter adult weight and head capsule width increased significantly with greater resources. These increases did not occur for males where average weights and head capsule width were similar across treatments. Less variation has previously been found in male hymenoptera, since differences in size have little fitness impact in males (Freeman, 1981). This situation of limited variation in the size of males is likely to be exacerbated in limiting resource conditions when there are high costs to increasing the size of a male but little fitness benefit for the mother. My hypothesized maternal fitness return curves for investment in sons and daughters (Fig. 2-1) demonstrate a possible explanation for these findings.

Males are generally smaller than females in *M. rotundata*, thus I expect that the minimum amount of resources required to produce a viable son will be less than needed to produce a daughter (Torchio and Tepedino, 1980; Rosenheim et al., 1996). On the other hand females gain more benefits from being large because larger females produce larger eggs and forage at a faster rate. Therefore the female maternal fitness return curve rises faster, passing the male curve with increased investment. If my hypothetical curves are qualitatively accurate then the optimal son investment will be determined by either the point where a line extending from the origin is tangent to the curve (indicating the highest rate of return per unit investment) (Smith and Fretwell, 1974) or the point where the son curve intersects the daughter curve, whichever occurs first (Fig. 2-1). In contrast, optimal daughter investment is solely determined by the corresponding tangent point on the curve and is not affected by the intersection between the son and daughter curves. One possible implication of this difference might be that optimal investment in daughters will fluctuate more under changing ecological conditions. This is because with access to greater resources, females would be able to provide more resources at a lower cost per unit of resources. This would increase the optimal female investment, whereas optimal male investment might increase but may be truncated when the female curve rises above the male curve. In effect, by increasing resource treatment levels, the maternal fitness return curves were shifted to the left, assuming that mothers did not alter their total (i.e., lifetime) investment. Mothers would not be expected to produce a son when they could produce a daughter with the same amount of investment and gain a higher fitness return. This hypothesis will not apply if the male curve saturates before intersecting the female curve. Therefore, the theory is most likely to hold in species where sexual size dimorphism is not extreme. Here I have focused on size as it applies to mating success.

However, there are other advantages to larger size, including the possibility of improved survival.

I demonstrated decreased resource allocation to daughters as resource levels declined. Fewer resources decrease the ability of developing larvae to survive adverse conditions during the development period (Tepedino and Torchio, 1982; Sugiura and Maeta, 1989). However, I did not find an increase in offspring which died during development as resource levels decreased. This may be the result of the overriding limiting resource levels or that brood cells were not exposed to the adverse conditions more typically found in nature. My over wintering system was designed to minimize stress and maximize emergence. It is possible that because altering of investment appears to be limited to daughters, differences in survival are limited to female offspring. More detailed, sex specific studies on over-wintering survival are needed to resolve this issue.

I have proposed a possible explanation for the differences in optimal resource investment between sons and daughters as resource levels change. Previous work on solitary bees has revealed a similar trend, with lower resource levels only the weight of daughters decreased significantly (Kim, 1999). The theory that optimal investment can be controlled by different factors for sons and daughters may be applicable to other organisms which have similar life history traits. In the lesser black-backed gull, *Larus fuscus*, where males are the larger sex, changes in maternal condition (through decreased food and egg removal) resulted in changes to the survival of sons but not daughters (Nager et al., 1999). In the subantarctic fur seal, *Arctocephalus tropicalis*, male harem size, and thus breeding success, depends on adult body size; thus there is more variation in reproductive success of males than females (Georges and Guinet, 2001). In the fur seal, mothers in good condition allocate more resources to sons (the larger sex) than mothers in poor condition, but there is no difference in resource allocation to daughters. If these species have qualitatively similar parental fitness returns per unit of investment as proposed here, the size of the smaller sex may be limited at some point by the higher returns of investing in the other sex at larger sizes. Analysis of fitness returns per unit of investment in sons and daughters across species will help us to better understand optimal investment levels and the factors that influence them.

Fisher's (1930) theory of sex allocation has proved a fruitful jumping off point for research over the past 75 years. In those species where individuals do not invest equally in sons and daughters, numerous theories have been developed to help explain these findings. I have shown that resource levels can have a dramatic impact on the number and size of offspring produced. Further work needs to be carried out to help better understand other important factors such as ova production and perhaps even predation pressure. Detailed work also needs to be done to obtain quantitative information on the maternal fitness benefits for producing sons and daughters and to learn how they are altered with changing ecological conditions.

2.6 REFERENCES

Alcock, J. 1979. The relation between female body size and provisioning behavior in the bee *Centris pallida* Fox (Hymenoptera: Anthophoridae). Journal of the Kansas Entomological Society 52:623-632.

Boltnev, A.I. and A.E. York. 2001. Maternal investment in northern fur seals (*Callorhinus ursinus*): Interrelationships among mothers' age, size, parturition date, offspring size and sex ratios. Journal of Zoology 254:219-228.

Charnov, E.L. 1982. The theory of sex allocation. Princeton University Press, Princeton.

Clark, A.B. 1978. Sex ratio and local resource competition in a prosimian primate. Science. 201:163-165.

Cook, J.M. 1993. Sex determination in the Hymenoptera, a review of models and evidence. Heredity 71:421-435.

Cowan, D.P. 1981. Parental investment in two solitary wasps *Ancistrocerus adiabatus* and *Euodynerus foraminatus* (Eumenidae: Hymenoptera). Behavioral Ecology and Sociobiology 9:95-102.

Fisher, R.A. 1930. The genetical theory of natural selection. Clarendon Press, Oxford.

Frank, S.A. 1990. Sex allocation theory for birds and mammals. Annual Review of Ecology and Systematics 21:13-55.

Frank, S.A. 1995. Sex allocation in solitary bees and wasps. American Naturalist 146:316-323.

Freeman, B.E. 1981. Parental investment, maternal size and population dynamics of a solitary wasp. American Naturalist 117:357-362.

Frohlich, D.R. and V.J. Tepedino. 1986. Sex ratio, parental investment, and interparent variability in nesting success in a solitary bee. Evolution 40:142-151.

Georges, J-Y and C. Guinet. 2001. Investment in the subantarctic fur seal, *Arctocephalus tropicalis.* Canadian Journal of Zoology 79:601-609.

Gerber, H.S. and E.C. Klostermeyer. 1972. Factors affecting the sex ratio and nesting behaviour of the alfalfa leafcutter bee. Technical Bulletin No. 73: Washington Agricultural Experiment Station, Washington, DC.

Hamilton, W.D. 1964. The genetical evolution of social behaviour. Journal of Theoretical Biology 7:1-52.

Hamilton, W.D. 1979. Wingless and flightless males in fig wasps and other insects. *In* Sexual selection and reproductive competition in insects. Ed. M.S. Blum and N.A. Blum. Academic Press, New York.

Hendry, A.P., T. Day and A.B. Cooper. 2001. Optimal size and number of propagules: Allowance for discrete stages and effects of maternal size on reproductive output and offspring fitness. American Naturalist 157:387-407.

Jay, S.C. and N. Mohr. 1987. The effect of nest replacement on the production of females of the alfalfa leaf-cutter bee *Megachile rotundata.* Journal of Apicultural Research 26:69-72.

Johansen, C.A., E.C. Klostermeyer and H.S. Gerber. 1969. Suggestions for alfalfa leafcutter bee management. Washington Agricultural Extension Service. E.N. 2777.

Kim, J-Y. 1999. Influence of resource level on maternal investment in a leaf-cutter bee (Hymenoptera: Megachilidae). Behavioral Ecology 10:552-556.

Klostermeyer, E.C. and H.S. Gerber. 1969. Nesting behaviour of *Megachile rotundata* (Hymenoptera: Megachilidae) monitored with an event recorder. Annals of the Entomological Society of America 62:1321-1325.

Klostermeyer, E.C., S.J. Mech, Jr. and W.B. Rasmussen. 1973. Sex and weight of *Megachile rotundata* (Hymenoptera: Megachilidae) progeny associated with provision weights. Journal of the Kansas Entomological Society 46:536-548.

Koskela, E., O. Huitu, M. Koivula, E. Korpimaki and T. Mappes. 2004. Sex-biased maternal investment in voles: importance of environmental conditions. Proceedings of the Royal Society Biological Sciences Series B 271:1385-1391.

Krunic, M.D. 1971. Mating of the alfalfa leafcutter bee, *Megachile rotundata* (F.), in the laboratory. Canadian Journal of Zoology 49:778-779.

Kruuk, L.E.B., T.H. Clutton-Brock, S.D. Albon, J.M. Pemberton and F.E. Guinness. 1999. Population density affects sex ratio variation in red deer. Nature 399:459-461.

Maki, D.L. and J.O. Moffett. 1986. Nesting cell position, sex ratios, and emergence times of the alfalfa leafcutting bee, *Megachile rotundata* (F) in northwest Oklahoma. Southwest Entomologist 11:107-112.

Mangel, M., J.A. Rosenheim and F.R. Adler. 1994. Clutch size, offspring performance and intergenerational fitness. Behavioral Ecology 5:412-417.

Mayer, D.F. 1994. Effects of male to female ratio and number of females per nesting tunnel on sex ratio and number of progeny of the alfalfa leafcutter bee *Megachile rotundata* (Hymenoptera: Megachilidae). Journal of the Entomological Society of British Columbia 91:43-46.

McCorquodale, D.B. and R.E. Owen. 1997. Allozyme variation, relatedness among progeny in a nest, and sex ratio in the leafcutter cell, *Megachile rotundata* (Fabricius)(Hymenoptera: Megachilidae). The Canadian Entomologist 129:211-219.

Minckley, R.L., W.T. Wcislo, D. Yanega and S.L. Buchmann. 1994. Behavior and phenology of a specialist bee (*Dieunomia*) and sunflower (*Helianthus*) pollen availability. Ecology 75:1406-1419.

Nager, R.G., P. Monaghan, R. Griffiths, D.C. Houston and R. Dawson. 1999. Experimental demonstration that offspring sex ratio varies with maternal condition. Proceeding of the National Academy of Science of the United States of America 96:570-573.

Orians, G.H. and N.E. Pearson. 1979. On the theory of central place foraging. *In* Analysis of ecological systems. Ed. D.J. Horn, G.R. Stairs and R.D. Mitchell. Ohio State University Press, Columbus, Ohio.

Parker, F.D. and D.R. Frohlich. 1985. Studies on management of the sunflower leafcutter bee *Eumegachile pugnata* (Say) (Hymenoptera: Megachilidae). Journal of Apicultural Research 24:125-131.

Peterson, S.S., C.R. Baird, and R.M. Bitner. 1992. Current status of the alfalfa leafcutting bee, *Megachile rotundata*, as a pollinator of alfalfa seed. Bee Science 2:135-142.

Richards, K.W. 1994. Ovarian development in the alfalfa leafcutter bee, *Megachile rotundata*. Journal of Apicultural Research 33:199-203.

Rosenheim. J.A., P. Nonacs and M. Mangel. 1996. Sex ratios and multifaceted parental investment. American Naturalist 148:501-535.

Rothschild, M. 1979. Factors influencing size and sex ratio in *Megachile rotundata*. Journal of the Kansas Entomological Society 53:392-401.

Schoener, T.W. 1979. Generality of the size-distance relation in models of optimal foraging. American Naturalist 114:902-914.

Sedinger, J.S. and D.G. Raveling. 1986. The timing of nesting of Canada Geese in relation to the quality and availability of their food plants. Journal of Animal Ecology 55:1083-1102.

Silk, J.B. 1983. Local resource competition and facultative adjustment of sex ratios in relation to competitive abilities. American Naturalist 121:56-66.

Skow, C.D. and E.M. Jakob. 2003. Effects of maternal body size on clutch size and egg weight in a pholcid spider (*Holocnemus pluchei*). The Journal of Arachnology 31:305-308.

Smith, C.C. and S.D. Fretwell. 1974. The Optimal Balance between Size and Number of Offspring. American Naturalist 108:499-506.

Southwood, T.R.E. 1966. Ecological methods. Methuen, London.

Strohm, E. and K. E. Linsenmair. 1999. Measurement of parental investment and sex allocation in the European beewolf *Philanthus triangulum* F. (Hymenoptera: Sphecidae). Behavioral Ecology and Sociobiology 47:76-88.

Sugiura, N. and Y. Maeta. 1989. Parental investment and offspring sex ratio in a solitary Mason bee, *Osmia cornifrons* (Radoszkowski) (Hymenoptera, Megachilidae). Japan Journal of Entomology 57:861-875.

Schwarz, M.P. 1988. Local resource enhancement and sex ratios in a primitively social bee. Nature 331:346-348.

Tepedino, V.J., D.R. Frohlich and C.R. Baird. 1994. Effect of intertunnel distance and nest-surface aspect on progeny production rate and sex ratio in the alfalfa leafcutting bee (Hymenoptera: Megachilidae). Journal of Economic Entomology 87:27-30.

Tepedino, V.J. and P.F. Torchio. 1982. Phenotypic variability in nesting success among *Osmia lignaria propinqua* females in a glasshouse environment (Hymenoptera: Megachilidae). Ecological Entomology 7:453-462.

Tepedino, V.J., R. Thompson and P.F. Torchio. 1984. Heritability for size in the megachilid bee *Osmia lignaria propinqua* Cresson. Apidologie 15:83-88.

Tirgari, S. 1963. The biology and management of the alfalfa leafcutter bee, *Megachile rotundata*. PhD thesis, Utah State University, Logan.

Torchio, P.F. and V.J. Tepedino 1980. Sex ratio, body size and seasonality on a solitary bee, *Osmia lignaria propinqua* Cresson (Hymenoptera: Megachilidae). Evolution 34:993-1003.

Trivers, R.T. and H. Hare. 1976. Haplodiploidy and the evolution of the social insects. Science 191:249-263.

Trivers, R.T. and D.E. Willard. 1973. Natural selection of parental ability to vary the sex ratio of offspring. Science 179:90-92.

Verhulst, S., J.H. van Balen, and J.M. Timbergen. 1995. Seasonal decline in reproduction success of the great tit: Variation in time or quality? Ecology 76:2392-2403.

Williams, G.C. 1966a. Adaptation and natural selection. Princeton University Press. Princeton.

Williams, G.C. 1966b. Natural selection, the costs of reproduction and a refinement of Lack's principle. American Naturalist 100:687-690.

2.7 FIGURES

Figure 2-1. Theoretical parental fitness returns with increasing resource investment in a single son and daughter (cumulative fitness of a single offspring). The solid line represents the sex where large investment provides the greatest payoff. In *Megachile rotundata* the solid curve would be for daughters and the dashed curve for sons. The optimal investment is determined using Smith and Fretwell (1974) (steepest slope). Using this method the optimal investment for sons is A, and for daughters B. However, depending on the position of the son and daughter curves in relation to one another, it is possible to have the optimal investment for a son decrease to C (As is the case for the curves drawn here). This will be the case when the daughter curve rises above the son curve, wherein any investment greater than that point makes it more beneficial to produce a daughter. The result is that C becomes the optimal investment in a son.

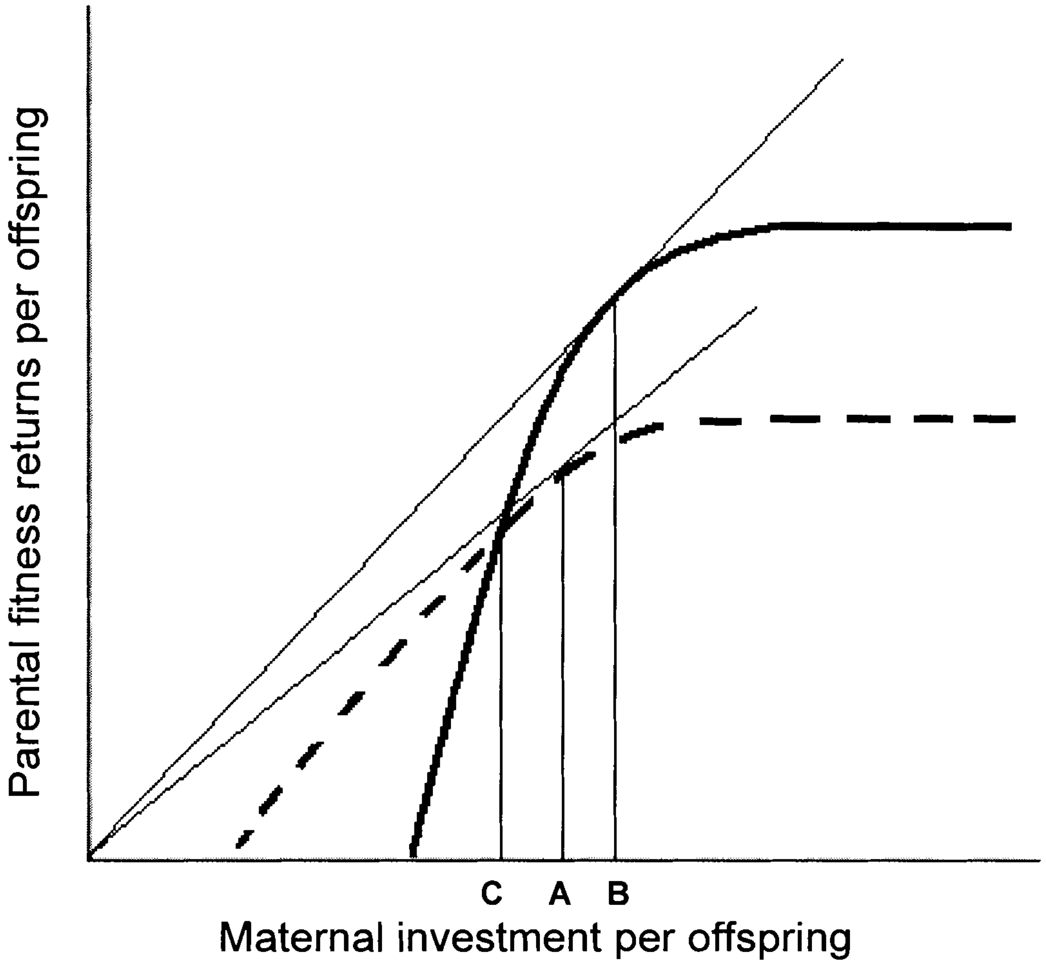

Parental fitness returns per offspring
C
A
B
Maternal investment per offspring

Figure 2-2. Representation of a linear nest created by the solitary bee *Megachile rotundata* (cross-section view).

- A: Completed brood cell - A leaf-wrapped cell provisioned with pollen and nectar, and with a tiny egg.
- B: 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.
- C: Nest cap - 10-40 leaf pieces are plastered together to “cap” the outside of the nest and prevent access to the nest or further use.

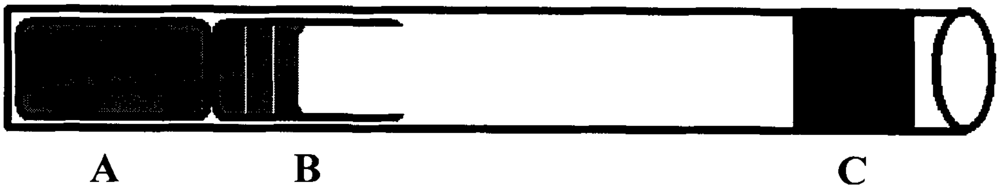
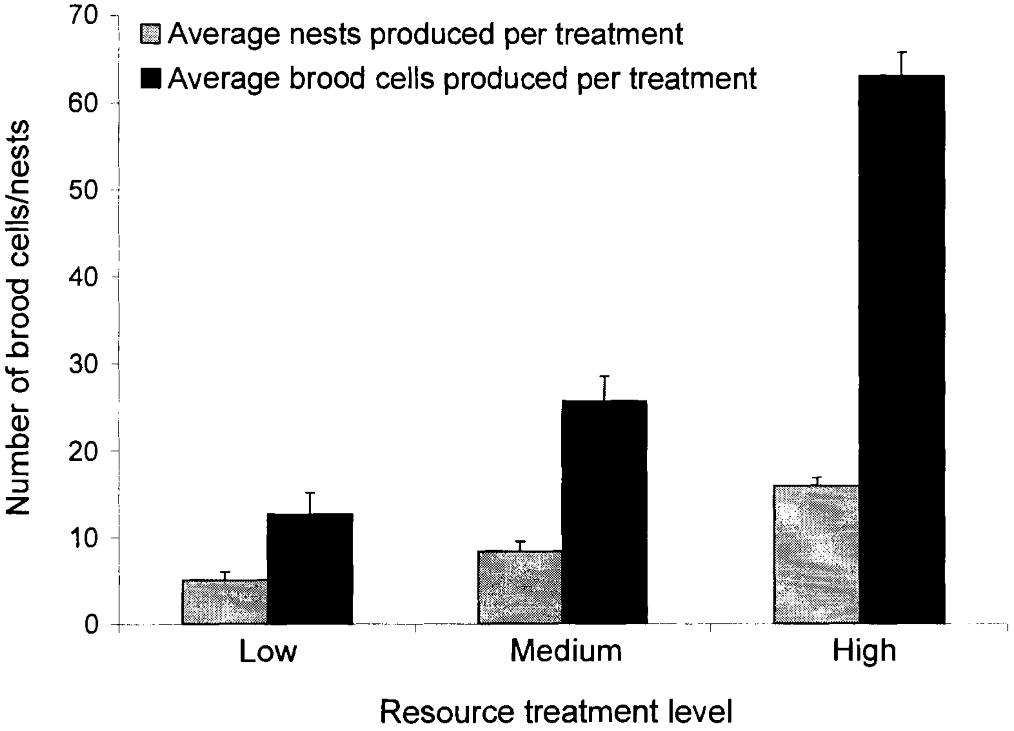
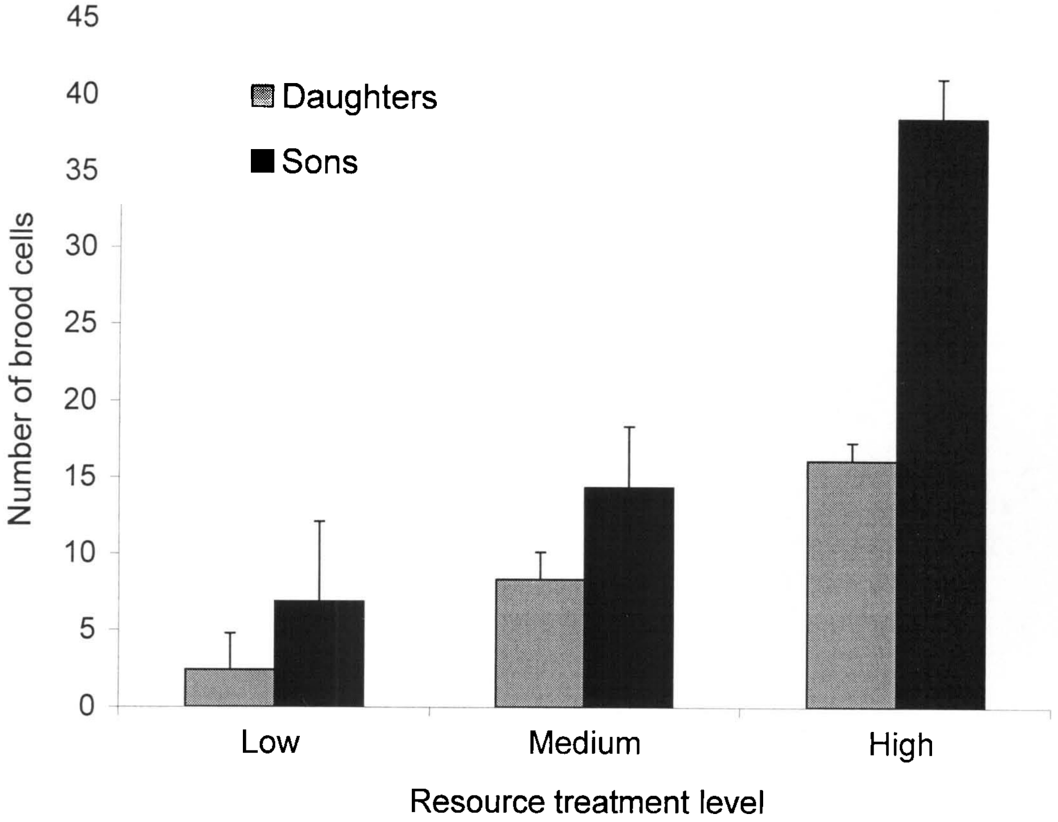

A
B
C

Figure 2-3. The average production of brood cells and nests per treatment in the solitary bee *Megachile rotundata* under three resource (food - alfalfa plants) treatment levels (Low n=7, Medium n=6, High n=7). Each replicate consists of a tented enclosure with 25 female and 50 male bees. The increase in production is significant using a LSM one-way ANOVA for both the average brood cells per treatment (F=97.8, *d.f.*=2, P<0.0001) and nests per treatment (F=32.01, *d.f.*=2, P<0.0001).



▨ Average nests produced per treatment
■ Average brood cells produced per treatment
Number of brood cells/nests
70
60
50
40
30
20
10
0
Low
Medium
High
Resource treatment level

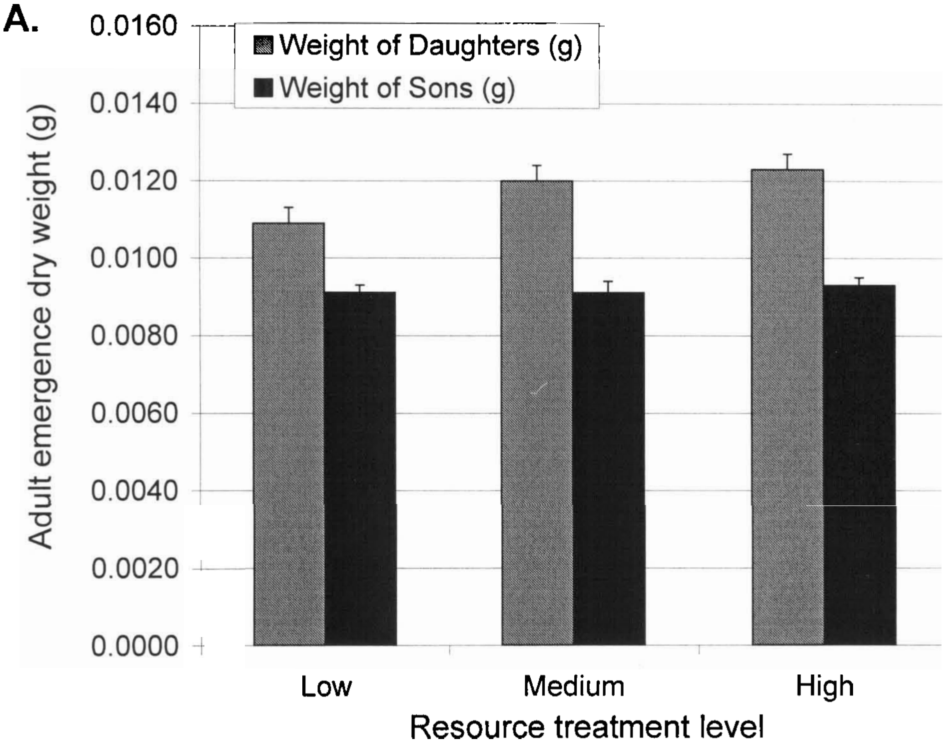
Figure 2-4. The production of sons and daughters per nest in the solitary bee *Megachile rotundata* under three resource (food - alfalfa plants) treatment levels (Low n=7, Medium n=6, High n=7). Each replicate consists of a tented enclosure with 25 female and 50 male bees. The increase in production is significant using a LSM one-way ANOVA for both daughters (F=16.73, *d.f.*=2, P<0.0001) and sons (F=22.06, *d.f.*=2, P<0.0001).



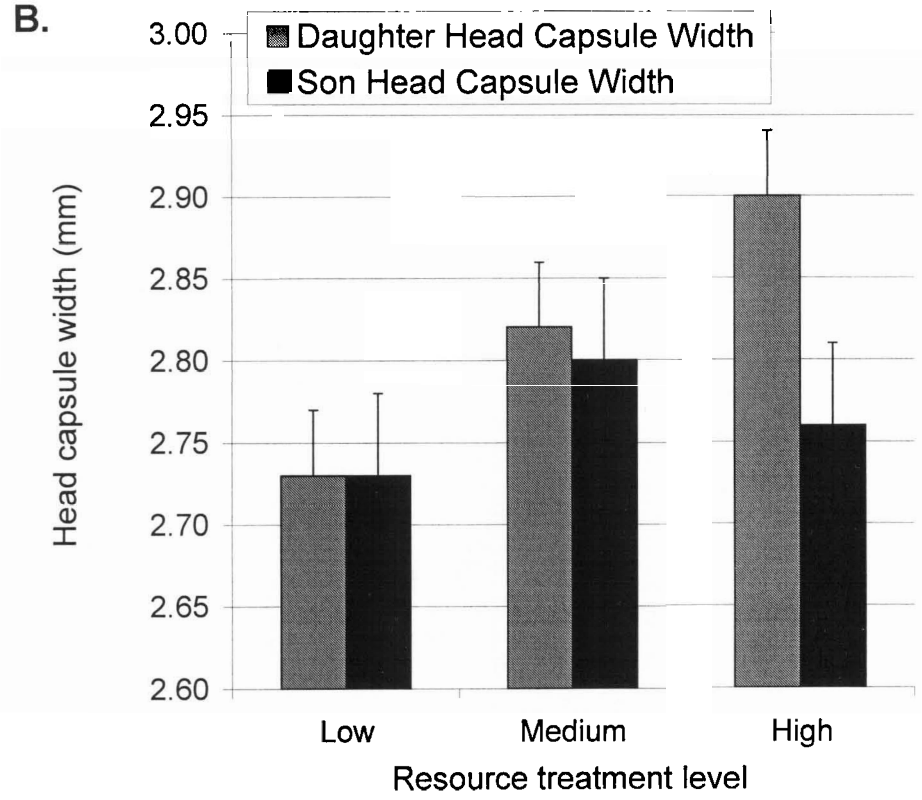
45
40
35
30
25
20
15
10
5
0
▨ Daughters
■ Sons
Number of brood cells
Low
Medium
High
Resource treatment level

Figure 2-5. Comparative investment into sons and daughters by *Megachile rotundata* at three different resource (alfalfa) treatment levels (Low n=7, Medium n=6, High n=7). Investment is indexed by adult dry weight (A) and head capsule width (B) of emerged offspring across resource levels (note: in the low treatment only 6 of the replicates produced females). The weight (g) and head width (mm) for each replicate was determined by averaging the weights of all bees of the particular sex produced in that replicate. Dry bees were weighed using a Sartorius^{TM} scale accurate to the nearest 0.0001g. Head width was measured as the largest lateral distance of the head using an ocular micrometer.

A.


0.0160
0.0140
0.0120
0.0100
0.0080
0.0060
0.0040
0.0020
0.0000
Adult emergence dry weight (g)
▨ Weight of Daughters (g)
■ Weight of Sons (g)
Low
Medium
High
Resource treatment level

B.


3.00
2.95
2.90
2.85
2.80
2.75
2.70
2.65
2.60
Head capsule width (mm)
▨ Daughter Head Capsule Width
■ Son Head Capsule Width
Low
Medium
High
Resource treatment level

Chapter 3
Impacts of Flight Distance on Sex Ratio and Resource Allocation to Offspring in the Leafcutter Bee, *Megachile rotundata*

3.1 ABSTRACT

Fisher's (1930) theoretical prediction of equal investment in each sex for a panmictic population can be altered by a number of factors. For example, sex ratio theory predicts variation from equal investment in each sex when the maternal fitness gains from increased investment differ between sexes. Changing sex allocation because of changing payoffs may result from different ecological conditions, such as foraging conditions. I investigated the impact of foraging travel cost on relative investment in sons *versus* daughters. Field studies were carried out with the central place foraging leafcutter bee *Megachile rotundata* (Fabricius), which has smaller males than females. Therefore, less investment is required to produce a viable son compared to a daughter. I found that with increased flight distance to resources, females produced a greater proportion of sons. Females also invested fewer resources in individual sons and daughters and produced fewer offspring with increased flight distance.

3.2 INTRODUCTION

The investigation of sex ratios in animals has led to the acknowledgement of two remarkable facts: (1) the vast majority of populations have near equal numbers of males and females, and (2) there are a significant number of exceptions to this rule. Fisher's (1930) theory of offspring investment helped explain this by stating that natural selection favours not those parents that produce equal numbers of sons and daughters, but those who invest equally in each sex. Investment is defined as any activity involved in the production of offspring that decreases the parents' future ability to invest in offspring (Williams, 1966a,b). When both sexes require the same amount of parental investment, we expect equal production of sons and daughters. However, if, for example, females are twice as expensive to produce as males, we expect twice as many sons to be produced, resulting in equal investment in each sex (Fisher, 1930; Charnov, 1982). The current body of sex ratio theory identifies numerous factors that can cause sex investment to deviate from Fisher's (1930) prediction: local mate competition (Hamilton, 1967, 1979), local resource competition (Clark, 1978), local resource enhancements (Schwarz, 1988), relatedness (Hamilton, 1964) and sexual asymmetry regarding minimal quality (Trivers and Willard, 1973). More recently, changing resource levels and ova production have been hypothesized as factors that may alter investment costs and therefore sex ratio (Frank, 1990; Rosenheim et al., 1996).

Skewed investments that deviate from Fisherian (equal) sex allocation are expected in situations where the fitness return on maternal investment is determined by functions that differ in shape between sons and daughters (Trivers and Willard, 1973; Charnov, 1982; Frank, 1990; Campbell, 2000). Life-history theory predicts that reproductive effort should be concentrated on the sex that generates comparatively larger fitness payoffs (Charnov and Bull, 1977; Charnov, 1982; Bortolotti, 1986; Frank, 1990; Stearns, 1992). Ungulates provide an example of this, where the most beneficial sex to produce shifts from female to male as maternal condition improves (Trivers and Willard, 1973; Clutton-Brock et al., 1984). Here, only a large investment in sons returns any payoff, because only large males can secure mates. In contrast, daughters of almost any size can produce offspring. Optimal sex investment can be further complicated when fitness return curves change temporally and/or spatially. In this situation, natural selection should favour the evolution of facultative sex allocation in response to particular life history or environmental situations (Charnov, 1982).

The investments required to produce an offspring include the cost of locating and obtaining resources to provision the offspring. This is especially true for central-place-foragers that must continually return resources to a single location (Schoener, 1979; Orians and Pearson 1979; Stephens and Krebs 1986). For example, parents in many bird species make numerous trips to collect materials for nest construction, followed by foraging trips to collect food for altricial young. The distance to all necessary resources is an important metric in determining an optimal nesting site. Work on central place foragers has shown that 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 travel distance may impact optimal resource allocation and sex ratio.

Hymenopterans have proven to be excellent systems for testing Fisher's (1930) theory on sex ratio and resource allocation. Haplodiploid sex determination, whereby females are diploid and males are haploid, allows maternal control over offspring sex by controlling whether or not the ovum is fertilized (daughters-fertilized egg; males-unfertilized egg) (Cook, 1993). In addition, the amount of food provisioned to the progeny in the brood cell is correlated to the subsequent size of the progeny as an adult (Klostermeyer et al., 1973; Freeman, 1981a; Johnson, 1988; Bosch and Vicens, 2002) and the heritability of body size appears low (Tepedino et al., 1984). Therefore, the size of progeny will be controlled by the amount of provisions supplied by the mother and not by genetic variation. Finally, solitary nesting bees build linear nests where discrete brood cells are provisioned and laid sequentially, eliminating competition among offspring for food and unitizing investment along an easily followed timeline (Freeman, 1981b). I take advantage of these features of solitary nesting bees in order to study sex allocation decisions.

I examined sex allocation in the solitary alfalfa leafcutter bee *Megachile rotundata* (Fabricius). As central place foragers, *M. rotundata* mothers, who carry out all foraging and nesting activities, must find a nesting location within flying proximity of all the resources needed to feed themselves and provision a nest. With the need for multiple trips, the distance between resources and the nest location has the potential to greatly influence the costs of collecting resources for offspring, and therefore the differential benefits of producing a son or daughter.

Although Fisherian (1930) theory assumes linear maternal fitness returns for each sex, there are reasons to believe that such curves are curvilinear in many hymenopterans and likely differ between sons and daughters (Frank, 1995, Rosenheim et al., 1996). When the amount of food young receive is correlated to their adult size, and when one sex becomes the more profitable of the two as adult size decreases, that sex will be favoured as resource availability decreases (Torchio and Tepedino, 1980). Increased male production with decreased resource levels has been predicted (Frank, 1995; Rosenheim et al., 1996) and demonstrated (Minckley et al., 1994; Kim, 1999) in solitary bees. However, here I consider resources in a different manner - not the absolute amount available but the amount of effort required to obtain them. Thus, I focus on the relationship between flight distance to resources (i.e., foraging effort) and maternal investment in the size and sex of offspring.

Based on the predicted general shape of maternal fitness curves (Fig. 3-1), I hypothesized that increased flight distance would result in a decrease in the maternal benefit per unit of investment. Assuming that mothers have a fixed energy budget, as the flight distance required to obtain resources increases, there will be: 1) an increase in the proportional production of males, the smaller sex; 2) a decrease in the amount of resources allocated to each offspring; and 3) a decrease in the total number of offspring produced. Changes in flight distance are expected to alter the maternal fitness returns, as greater investment is required per progeny, resulting in a decrease in optimal size of offspring. The increase in effort required to obtain resources also means that fewer offspring are likely to be produced. This will be the case when the amount of resources required per offspring decreases (because of decreased optimal size) such that the total investment required to obtain this decreased amount of resources is similar or greater than when flight distances are shorter. These hypotheses were tested in the field using manipulation experiments.

3.3 MATERIAL AND METHODS

Megachile rotundata is a leafcutter bee commonly used in the pollination of many seed crops, including alfalfa (*Medicago sativa*). Numerous sex ratios have previously been reported for this species (e.g., Maki and Moffett, 1986; Jay and Mohr, 1987; Tepedino et al., 1994; McCorquodale and Owen, 1997), suggesting that the optimal sex ratio may change depending on the conditions. I define sex ratio as the number of daughters produced divided by the total number of offspring produced in a nest.

3.3.1 Leafcutter bee life history

The alfalfa leafcutter bee *M. rotundata* is an excellent subject to address questions of sex allocation and maternal investment for a number of reasons. Female leafcutter bees mate only once (Gerber and Klostermeyer, 1970; Krunic, 1971) and retain enough sperm to fertilize their complete complement of eggs (Richards, 1994). These bees nest gregariously and females build linear twig nests with a number of brood cells in preformed cavities, allowing for the use of standardized artificial nests (McCorquodale and Owen, 1997). A mother constructs a brood cell by lining the walls of the nest cavity with masticated leaves and then filling this "cell" with provisions of pollen and nectar (Fig. 3-2). An egg is then laid on this pollen and nectar (referred to below collectively as pollen) "loaf" 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 over-winter as prepupa (this species is univoltine in Alberta) and emerge from brood cells as adults, such that all the food consumed prior to adulthood is provided by the mother. Measurements can easily be taken to determine key factors contributing to maternal fitness, including: realized fecundity, offspring sex and investment (using emergence weight and head capsule width) per offspring.

3.3.2 Proposed maternal fitness curves

Based on the available data, I estimated the general shape of the maternal fitness functions from producing sons and daughters. *Megachile rotundata* is sexually dimorphic, females being 1.2 - 1.3 times larger than males, with adult size being controlled by the amount of food in the brood cell (Klostermeyer et al., 1973). Males can therefore be produced with fewer pollen loads than females (Phillips and Klostermeyer, 1978). Based on these differences, I predict that the curves will start from different positions. The shape of these curves will be dictated by the fitness benefits of increased investment (i.e., producing larger offspring) for sons and daughters.

As a non-territorial species, the advantage of increased size in males is limited (Alcock, 1979; Cowan, 1981). The fact that many bee species have larger females than males has been suggested as evidence that female fitness rises more rapidly with size compared to that of males (Charnov et al., 1981). Larger females can accrue greater reproductive fitness due to their greater longevity and higher fecundity (Sugiura and Maeta, 1989), enhanced foraging abilities (Alcock, 1979; Cowan, 1981; Visscher and Danforth, 1993; Kim, 1997) and greater ability to usurp nests from other females (Larsson, 1990; Barthell and Thorp, 1995). Therefore, the returns with increased investment in daughters are predicted to increase faster than sons and to asymptote at higher levels.

3.3.3 Field experiment

Field work was carried out in the summer of 2003 on a set of 5th year alfalfa plots in Tilley, Alberta, Canada (54°23' North, 111°41' West). In order to test the effect of flight distance on the resource allocation and sex ratio decisions of female *M. rotundata,* I used two distance treatments. Each treatment consisted of a plot of alfalfa (14x12m) and a shelter (large covered domicile) with a wood hive containing nest sites. The near treatment utilized a plot of alfalfa directly in front of the shelter (5 replicates); the far treatment employed an alfalfa plot 150 metres due east of the shelter (4 replicates). The 150m distance was chosen as female leafcutter bees generally forage within 100 metres of their nest (Pankiw and Siemens, 1974; Tasei and Delaude, 1984). The size of plot allowed the bees to feed *ad lib* for the entire season. Each shelter and alfalfa plot was isolated from other sources of resources by at least five hectares of wheat, a non-food resource. In the far treatment, a two metre wide strip was mowed out of the wheat from the shelter to the alfalfa plot, providing a flight path for the bees.

The bees were housed in shelters covered with corrugated sheet metal (2m x 2m x 1m). Each shelter had three covered sides, a roof and one large, open side. These shelters were placed with the open side facing due east. The area beneath and one metre out from the shelter was sprayed with Roundup™ to remove ground cover. The alfalfa plots were sprayed twice with a 5% malathion solution (two and three weeks prior to bee release - avoiding any impact on the bees) to prevent alfalfa weevils and plant bugs from destroying the crop's bloom. The artificial nests comprised two Bidwell™ wood laminate hives - 48 holes by 13 holes, 12.7cm deep, with each hole having a diameter of 6mm, providing more than enough nesting sites for all females. These two hives were strapped against a piece of plywood (0.33m x 1m) with a sheet of fibre fill sandwiched between the back of the hives and the plywood. The entire structure (referred to as a hive) was positioned horizontally in the upper left hand corner of the shelter.

Loose-cell, pre-pupal bees were obtained from North Shore Farms (Tilley, Alberta), placed in hatching trays and incubated for 21 days as described by Peterson et al. (1992). The night prior to release, bees were divided into trays of 50 females and 100 males and one tray was placed in each shelter in the early morning of July 6th.

In order to help establish the bees in the shelter, 20 potted alfalfa plants were placed in front of each shelter to provide food during the first three days after release. These plotted plants were grown in a greenhouse from roots obtained in the same field as the experimental plots. Each day the potted plants were moved further from the shelters of both treatments. By the third day they were evenly spaced over a 150-metre distance east of the shelter and by this time the potted alfalfa plants were ignored by the bees, which flew directly to the alfalfa plot.

The experiment ran from July 6th to August 28th 2003. Behavioural observations on bee activity were made daily and each afternoon nests that had been capped (sealed) within the past 24 hours were recorded. This gave me the date during the season when each nest was completed. Hives were collected on August 28th, after all the adult bees had died. The hives were stored at 20°C for two weeks to allow all larvae to reach the prepupal over-wintering stage and were then cooled to 8°C for six months to break diapause. At the end of the season the production of alfalfa seed in each plot was estimated using visual inspection of alfalfa plants with the help of an alfalfa seed farmer.

In March the hives were taken apart and the dimensions of each nest measured (length of nests composed of: bee cells, empty space and caps). Each brood cell was placed into an individual tray according to it's position in the nest. These trays were incubated at 30°C for 30 days at which time all the viable offspring had emerged. The sex of each brood cell inhabitant was determined by simple observation of the emerged adult and then the bees were further dried for one hour at 90°C. Adult emergence dry weight and head capsule width were measured in order to determine the mother's resource allocation response to increased flight distance. The dry weight of each bee was obtained using a Sartorius™ scale accurate to the nearest 0.0001g. Five nests were randomly selected using the role of a die from each replicate, and the head capsule width of each bee in the nest was measured using an ocular micrometer (95 females; 148 males). Bees that did not emerge were dissected to determine cause of death. Where possible, bees that did not hatch were sexed according to their position in the nest sequence as described by Frohlich and Tepedino (1986). This is possible because female bees are produced in the innermost cells and there is generally a single switch to the production of males in the outer cells. When an unhatched brood cell was found in a position between the switch from female to male, that cell was discarded from the sex ratio analysis. The sex ratio for each replicate was calculated as the mean of the proportion of daughters per nest in the replicate.

3.3.4 Statistical analyses

The unit of replication was the shelter. A t-test was used to compare the mean nest sex ratio (number of females in a nest/total number of offspring in the nest), number of nests-per-shelter, number of cells-per-nest, number of sons and daughters per nest, and son and daughter emergence dry weight and head capsule width between the near and far treatments. The proportion of cells that died during development, the proportion of nests that were capped, and the proportion of capped nests with space behind the cap were arcsine square root transformed and then analysed using a t-test.

3.4 RESULTS

One of the far (150m) treatments had a patch of alfalfa that emerged 25m from the shelter. Therefore, this treatment was excluded from analysis.

3.4.1 Nest production

In the near treatment, compared to the far, there was a significant increase in the proportional production of daughters per nest (female sex ratio), number of nests-per-shelter, mean number of cells-per-nest, average production of daughters-per-nest and a decrease in the proportion of brood cells in which the progeny died during development (Table 3-1). The primary (zygote) sex ratio was 0.49 ± 0.03 (mean +/- SE) in the near treatments and 0.31 ± 0.03 in the far treatments (t=4.12, *d.f.*=6, P=0.003). The difference in sex ratio was nearly identical using the secondary (adult) emergence sex ratio: 0.50 ± 0.03 for the near treatment, and 0.31 ± 0.03 for the far treatment (t=3.89, *d.f.*=6, P=0.004). The sex ratio also varied significantly depending on the week during the season when the nest was capped (i.e., completed) (Fig. 3-3A). There was a significant increase in the production of nests (t=2.88, *d.f.*=6, P=0.028) and brood cells (t=2.83, *d.f.*=6, P=0.030) in the near compared to the far treatments. The mean number of brood cells per nest was 5.5 ± 0.1 in the near and 3.9 ± 0.2 in the far treatments (t=3.10, *d.f.*=6, P=0.011), with a maximum of fifteen cells fitting in a single nest. The mean production of sons per nest was nearly identical in both treatments: 3.0 ± 0.1 and 3.0 ± 0.2 (t=0.004, *d.f.*=6, P=0.95, β=0.05), whereas 2.4 ± 0.1 daughters were produced per nest in the near treatments and 1.0 ± 0.1 daughters in the far treatment (t=7.44, *d.f.*=6, P<0.01). The proportion of cells that did not develop into viable adults was 0.23 ± 0.01 in the near treatment and 0.43 ± 0.05 in the far treatment (t=-4.95, *d.f.*=6, P=0.003). Females were less likely to establish nests when resources were further away; of the initial 50 females, an average of 47 started nesting within the first week in the near and 35 in the far treatment.

3.4.2 Distance effects on nest structure

Not all nests were capped with leaf pieces. In the near treatment, 52.6 ± 7.3% were capped, while 28.0 ± 1.6% were capped in the far treatment (t=2.49, *d.f.*=6, P=0.047) (Table 3-1). Although not significantly different between treatments, in both treatments the majority of capped nests had space left behind the cap (e.g., see Fig. 3-2(II)), near: 59 ± 5%, far: 75 ± 7% (t=-1.85, *d.f.*=6, P=0.11, β=0.34). The average amount of space left behind a cap was 22 ± 4mm in the near treatment and 33 ± 5mm in the far (t=-1.65, *d.f.*=6, P=0.15, β=0.29). Overall, the only significant difference between the treatments was an increased proportion of nests capped in the near treatment.

3.4.3 Distance effects on investment per offspring

The dry weight and head capsule width of sons and daughters increased in the near treatment compared to the far treatment. The average weight of daughters was 0.0127 ● 0.0001g in the near treatment and 0.0115 ± 0.0004g in the far treatment (t=3.53, *d.f.*=6, P=0.006). For males, the average weights were 0.0089 ± 0.0001g and 0.0081 ± 0.0004g for the near and far treatments respectively (t=2.698, *d.f.*=6, P=0.009). Female head capsule width was 3.07 ± 0.027mm in the near treatment and 2.89 ± 0.019mm in the far treatment (t=4.649, *d.f.*=6, P=0.002). Male head capsule width was 2.88 ± 0.026mm in the near treatment and 2.78 ± 0.033mm in the far treatment (t=2.43, *d.f.*=6, P=0.025).

Male and female weights were significantly different for both the near (t=316.3, *d.f.*=6, P<0.001) and far treatments (t=11.14, *d.f.*=6, P<0.001). This difference was also significant for head capsule widths in the near treatment (t=4.232, *d.f.*=7, P=0.004) and the far treatment (t=3.34, *d.f.*=3, P=0.044).

When the weights of adult progeny were compared based on the location within the nest, I found that female weight varied with location while male weight remained relatively constant (Fig. 3-4).

3.4.4 Distance effects on alfalfa pollination

The amount of alfalfa seed produced per square metre (i.e., number of flowers utilized) was greater in the near treatments 39 ± 5 g/m^2 than the far 22 ± 6g/m^2, although the difference was not significant ($t=2.14$, *d.f.*=6, $P=0.076$, $\beta=0.44$).

3.5 DISCUSSION

Follow up studies to Trivers and Willard's (1973) work on optimal sex allocation have revealed that ecological conditions can strongly influence sex allocation decisions (Kruuk et al., 1999; Koskela et al., 2004). I manipulated the flight distance required to obtain resources, altering the ecological conditions mothers faced. The result was a change in both resource allocation and sex ratio of offspring. Below, I consider the impacts and implications of flight distance on sex ratio and maternal investment.

Previous work on solitary bees has shown that the proportion of sons - the smaller sex - increased when food availability declined (Kim, 1999). I demonstrated, under field conditions, that increased flight distance has a similar effect. There are a number of possible explanations for the change in sex ratio with increased flight distance: decreased mating, dispersal of mothers in good condition, and my hypothesized change in the optimal sex ratio. 1) Adult males likely required more time to obtain food to maintain somatic function with increased flight distance; this may have decreased mating opportunity and thus increased the number of unfertilized eggs. However, numerous males were always present near the nest. As well, females only require a single mating to fertilize all eggs (Gerber & Klostermeyer, 1970; Krunic, 1971). Females were also produced in many of the nests (at a lower proportion); thus, some eggs were fertilized and females must have mated. 2) High-condition females may have abandoned the far treatment, as they would be most able to survive a search for a new nesting site. If such females tend to produce more daughters, then their departure would affect the population sex ratio. However, because the original 50 females were of similar size, a large difference in condition among them is not expected. It would also hold that the healthiest females would be the ones most able to tolerate the increased flight distance. 3) As predicted, females may use facultative sex ratio manipulation, producing more sons as a response to the increased costs of collecting resources.

Mothers were predicted to alter both the sex ratio and the size of offspring as resources became more difficult to obtain (i.e., when the curves in Figure 3-1 are shifted to the right). I found that as flight distance increased a greater proportion of sons were produced and fewer resources where allocated to each offspring. If we look at Figure 3-1, the optimal male size is controlled by the point on the curve where a line extending from the origin is tangent to the curve (indicating the highest rate of return per unit investment) (Smith and Fretwell, 1974) or the point where the daughter return curve rises above the son curve, whichever occurs first. In the case of the latter, for any investment greater than this point, more fitness will be gained by producing a daughter. This may have limited the increase in male investment when distance to resources decreased. The optimal daughter size will be the tangent of the female curve with the greatest slope that passes through the origin. The optimal daughter size is not limited by the male curve, which may account for the greater increase in weight in daughters. The one factor for which we do not have information is how spending more time and energy, perhaps while at the same time consuming less food, affects the maturation of oocytes.

Fisher's (1930) theory predicts that if the only cost of reproduction is oocytes, and oocytes are of equal size and cost, there should be a 1:1 investment in the sexes. It is possible that by providing large amounts of easily accessible resources, the cost of obtaining resources became less important compared to oocyte maturation, pushing the sex ratio closer to 1:1. This may explain why reported *M. rotundata* sex ratios include examples of extremely male-biased populations, but rarely female biased populations (e.g. Maki and Moffett, 1986; Jay and Mohr, 1987; Tepedino et al., 1994; McCorquodale and Owen, 1997). In the same year as this work was conducted, the average sex ratio, based on the total production of daughters divided by total production of offspring in a field (the standard for commercial sex ratio tests), in commercial fields in Southern Alberta was 0.37 (range 0.28 - 0.45) (Canadian Leafcutter Bee Cocoon Testing Centre, unpublished data). Presumably, oocyte maturation will become increasingly important as resources become more plentiful, and cell provisioning rates can exceed oocyte maturation rates (Rosenheim et al., 1996). More information is required as to what factors influence oocyte maturation in order to understand the quantitative effect on sex ratio.

The approaching end of the season may also play a role in the earlier switch to the production of sons in the far treatment compared to the near treatment. Larger leafcutter bee females are able to provision a brood cell at a faster rate than smaller females (Kim, 1997). Recall, however, that mothers do not receive any fitness return until after a brood cell is completed. Therefore, selection pressures may favour those females who produce males near the end of the season, because they are "capturing" the fitness benefits more frequently. This is important because older foragers often have a greater risk of death (Cartar, 1992) and provision cells at a slower rate (Tepedino and Torchio, 1982). Producing a male increases the probability that the brood cell will be completed. A large percentage of the nests contained partially complete cells suggesting that mothers are often unable to complete cells. As well, when resources were more distant, the proportion of capped nests was significantly lower. There were also cases where the mother apparently started building a brood cell and then switched to capping the nest. This may be the result of an attack by a parasitoid or predator, or simply a feature of running out of time. In the case of the latter, the asset protection principle (Clark, 1994) predicts that females can gain the most fitness by protecting the offspring already produced as opposed to producing more.

I assumed an increased cost in the production of brood cells when the bees were required to fly a greater distance. One way this might be manifested is in the production of brood cells near the minimum viable size. When resources were further away, there was an increase of more than 50% in the proportion of cells that did not develop into viable adults. Adult weight and head capsule size were lower in the far treatment, meaning the brood cells received fewer resources, possibly resulting in the increased number of offspring that died during development. Brood cells that receive more resources not only produce larger offspring, but may also be better able to maintain somatic function in adverse conditions during development and over-wintering (Tepedino and Torchio, 1982; Tepedino and Parker, 1986).

Parasitism is another factor causing mortality in brood. To defend brood mothers may leave empty spaces or empty cells in front of brood cells as a defence against parasitoids that can oviposit through a completed nest cap (Münster-Swendsen and Calabuig, 2000). When a parasitoid deposits an egg into this empty area, the parasitoid hatches but dies without access to a host. In both treatments, a large percentage of the space in each nest behind the cap was left empty, 18% and 27% for the near and far treatments respectively. This difference is not significant and we might assume that leaving this empty space is a luxury of having plenty of nesting sites available. However, in a similar experiment to the one described in this chapter and run simultaneously, but with increased numbers of bees such that there were not enough nesting sites for all the bees, there was still an average of 18% empty space behind caps. This suggests that even when nesting sites are limited it remains important to leave space behind the cap. Had no space been left, the last cell would likely be exposed to possible parasitoid attack, endangering other brood cells in the nest when these parasitoids emerged. This is because parasitoids hatch prior to the bees and can attack other brood cells in the nest. The presents of parasitoids can also result in offspring living in a more dangerous environment if they do not leave the area as adults. As the size of the cap increased, the amount of empty space left behind the cap decreased. This may suggest two alternative methods of accomplishing the goal of protecting the nest.

The application of the theory discussed above need not be limited to solitary bees. Although maternal allocation within a clutch has been an important topic in evolutionary ecology, the prevailing view has been that heterogametic sex determination severely constrains biased sex ratio production (Dyrcz et al., 2004). However, recently, numerous studies have demonstrated flexible sex ratio patterns in several higher vertebrates such as birds, in response to variable environmental conditions. (Komdeur et al, 1997, Oddie 1998, Sheldon et al, 1999; Hardy 2002; West and Sheldon 2002). Findings from a number of different bird species suggests that the theories utilized here may hold true for other groups as well. In sexually dimorphic bird species, the offspring of the larger sex generally have faster growth and increased metabolic rates, and thus a higher demand for resources, so they are usually more expensive to rear (Bortolotti, 1986; Anderson et al., 1993). How much more expensive will likely be determined by the cost of obtaining these resources. Birds have been shown to alter resource allocation based on sex (Boulet et al., 2001), and male-biased (the smaller sex) fledging sex ratios have been reported for birds nesting in areas with low food availability or with poor maternal condition (Korpimäki et al., 2000). In addition, increased flight costs have been shown to result in parents returning fewer resources per foraging trip to offspring (Weimerskirch et al., 2000). Further work needs to be carried out to see if flight distance and the costs of provisioning offspring impact resource investment and the sex of offspring in non-hymenopteran species.

Here I have demonstrated that flight distance has a significant impact on the sex ratio of offspring as well as the investment per offspring. We now need to investigate the shapes of the optimal sex ratio and allocation curves over a greater variety of distances. Does flight impact sex ratio and resource allocation because of an inability to carry large loads, an increase in the time nests are unprotected, greater energy expenditure or some other factor? These questions need to be addressed to allow a more complete understanding of how the issue of resource acquisition affects sex ratio and resource allocation in offspring.

3.6 REFERENCES

Alcock, J. 1979. The relation between female body size and provisioning behavior in the bee *Centris pallida* Fox (Hymenoptera: Anthophoridae). Journal of the Kansas Entomological Society 52:623-632.

Anderson, D.J., J. Reeve, J.E. Martinez Gomez, W.W. Weathers, S. Hutson, H.V. Cunningham and D.M. Bird. 1993. Sexual size dimorphism and food requirements of nestling birds. Canadian Journal of Zoology 71:2541-2545.

Barthell, J.F. and R.W. Thorp. 1995. Nest usurpation among females of an introduced leaf-cutter bee, *Megachile apicalis*. Southwestern Entomologist 20:117-124.

Bortolotti, G.R. 1986. Influence of sibling competition on nestling sex ratios of sexually dimorphic birds. American Naturalist 127:495-507.

Bosch, J. and N. Vicens. 2002. Body size as an estimator of production costs in a solitary bee. Ecological Entomology 27:129-137.

Boulet, M., P. Olsen, A. Cockburn and K. Newgrain. 2001. Parental investment in male and female offspring by the Peregrine Falcon, *Falco peregrinus*. Emu 101:95-103.

Campbell, D.R. 2000. Experimental tests of sex-allocation theory in plants. Trends in Ecology and Evolution 15:227-232.

Cartar, R.V. 1992. Morphological senescence and longevity: an experiment relating wing wear and life span in foraging wild bumble bees. The Journal of Animal Ecology 61:225-231.

Charnov, E.L. 1982. The theory of sex allocation. Princeton University Press, Princeton.

Charnov, E.L. and J. Bull. 1977. When is sex environmentally determined. Nature 266:828-830.

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

Clark, A.B. 1978. Sex ratio and local resource competition in a prosimian Primate. Science 201:163-165.

Clark, C.W. 1994. Antipredator behaviour and the asset-protection principle. Behavioral Ecology 5:159-170.

Clutton-Brock, T.H., S.D. Albon and F.E. Guinness. 1984. Maternal dominance, breeding success and birth sex ratios in red deer. Nature 308:358-360.

Cook, J.M. 1993. Sex determination in the Hymenoptera, a review of models and evidence. Heredity 71:421-435.

Cowan, D.P. 1981. Parental investment in two solitary wasps *Ancistrocerus adiabatus* and *Euodynerus foraminatus* (Eumenidae: Hymenoptera). Behavioral Ecology and Sociobiology 9:95-102.

Dyrcz, A., H. Sauer-Gürth, E. Tkadlec and M. Wink. 2004. Offspring sex ratio variation in relation to brood size and mortality in a promiscuous species: the aquatic warbler *Acrocephalus paludicola.* Ibis 146:269-280.

Fisher, R.A. 1930. The genetical theory of natural selection. Clarendon Press, Oxford.

Frank, S.A. 1990. Sex allocation theory for birds and mammals. Annual Review of Ecology and Systematics 21:13-55.

Frank, S.A. 1995. Sex allocation in solitary bees and wasps. American Naturalist 146:316-323.

Freeman, B.E. 1981a. Parental investment and its ecological consequences on the solitary wasp *Sceliphron assimile* (Dahlbom) (Sphecidae). Behavioral Ecology and Sociobiology 9:261-268.

Freeman, B.E. 1981b. Parental investment, maternal size and population dynamics of a solitary wasp. American Naturalist 117:357-362.

Frohlich, D.R. and V.J. Tepedino. 1986. Sex ratio, parental investment, and interparent variability in nesting success in a solitary bee. Evolution 40:142-151.

Gerber, H.S. and E.C. Klostermeyer. 1970. Factors affecting the sex ratio and nesting behaviour of the alfalfa leafcutter bee. Technical Bulletin No. 73: Washington Agricultural Experiment Station; Washington, DC.

Hamilton, W.D. 1964. The genetical evolution of social behaviour. Journal of Theoretical Biology 7:1-52.

Hamilton, W.D. 1967. Extraordinary sex ratios. Science 156:477-488.

Hamilton, W.D. 1979. Wingless and flightless males in fig wasps and other insects. *In* Sexual selection and reproductive competition in insects. Ed. M.S. Blum and N.A. Blum. Academic Press, New York.

Hardy, I.C.W. 2002. Sex ratios: concepts and research methods. Cambridge University Press, Cambridge.

Jay, S.C. and N. Mohr. 1987. The effect of nest replacement on the production of females of the alfalfa leaf-cutter bee *Megachile rotundata*. Journal of Apicultural Research 26:69-72.

Johnson, M.D. 1988. The relationship of provision weight to adult weight and sex ratio in the solitary bee *Ceratina calcarata*. Ecological Entomology 13:165-170.

Kim, J-Y. 1997. Female size and fitness in the leaf-cutter bee *Megachile apicalis*. Ecological Entomology 22:275-282.

Kim, J-Y. 1999. Influence of resource level on maternal investment in a leaf-cutter bee (Hymenoptera: Megachilidae). Behavioral Ecology 10:552-556.

Klostermeyer, E.C., S.J. Mech, Jr. and W.B. Rasmussen. 1973. Sex and weight of *Megachile rotundata* (Hymenoptera: Megachilidae) progeny associated with provision weights. Journal of the Kansas Entomological Society 46:536-548.

Komdeur, J., S. Daan, J. Tinbergen. and C. Mateman. 1997. Extreme adaptive modification in sex ratio of the Seychelles Warbler's eggs. Nature 385:522-525.

Korpimäki, E., C.A. May, D.T. Parkin, J.H. Wetton and J. Wiehn. 2000. Environmental and parental condition-related variation in sex ratio of kestrel broods. Journal of Avian Biology 31:128-134.

Koskela, E., O. Huitu, M. Koivula, E. Korpimaki and T. Mappes. 2004. Sex-biased maternal investment in voles: importance of environmental conditions. Proceedings of the Royal Society of London, Series B 271:1385-1391.

Krunic, M.D. 1971. Mating of the alfalfa leafcutter bee, *Megachile rotundata* (F.), in the laboratory. Canadian Journal of Zoology 49:778-779.

Kruuk, L.E.B., T.H. Clutton-Brock, S.D. Albon, J.M. Pemberton and F.E. Guinness. 1999. Population density affects sex ratio variation in red deer. Nature 399:459-461.

Larsson, F.K. 1990. Female body size relationships with fecundity and egg size in two solitary species of fossorial Hymenoptera (Colletidae and Sphecidae). Entomologia Generalis 15:167-171.

Maki, D.L. and J.O. Moffett. 1986. Nesting cell position, sex ratios, and emergence times of the alfalfa leafcutting bee, *Megachile rotundata* (F) in northwest Oklahoma. Southwest Entomologist 11:107-112.

Mayer, D.F. 1994. Effects of male to female ratio and number of females per nesting tunnel on sex ratio and number of progeny of the alfalfa leafcutter bee *Megachile rotundata* (Hymenoptera: Megachilidae). Journal of the Entomological Society of British Columbia 91:43-46.

McCorquodale, D.B. and R.E. Owen. 1997. Allozyme variation, relatedness among progeny in a nest, and sex ratio in the leafcutter bee, *Megachile rotundata* (Fabricius)(Hymenptera: Megachilidae). The Canadian Entomologist 129:211-219.

Minckley, R.L., W.T. Wcislo and D. Yanega. 1994. Behavior and phenology of a specialist bee (*Dieunomia*) and sunflower (*Helianthus*) pollen availability. Ecology 75:1406-1419.

Münster-Swendsen, M. and I. Calabuig. 2000. Interaction between the solitary bee *Chelostoma florisomne* and its nest parasite *Sapyga clavicornis* - empty cells reduce the impact of parasites. Ecological Entomology 25:63-70.

Oddie, K. 1998. Sex discrimination before birth. Trends in Ecological Evolution 13:130-131.

Orians, G.H. and N.E. Pearson. 1979. On the theory of central place foraging. *In* Analysis of ecological systems. Ed. D.J. Horn, R.D. Mitchell and G.R. Stairs. Ohio State University Press, Columbia, Ohio.

Pankiw, P. and B. Siemens. 1974. Management of *Megachile rotundata* in northwestern Canada for population increase. Canadian Entomologist 106:1003-1008.

Peterson, S.S., C.R. Baird, and R.M. Bitner. 1992. Current status of the alfalfa leafcutting bee, *Megachile rotundata*, as a pollinator of alfalfa seed. Bee Science 2:135-142.

Phillips, J.K. and E.C. Klostermeyer. 1978. Nesting behavior of *Osmia lignaria propinqua* (Hymenoptera: Megachilidae). Journal of the Kansas Entomological Society 51:91-108.

Richards, K.W. 1994. Ovarian development in the alfalfa leafcutter bee, *Megachile rotundata*. Journal of Apicultural Research. 33:199-203.

Rosenberg, D.K. and K.S. McKelvey. 1999. Estimation of habitat selection for central-place foraging animals. Journal of Wildlife Management 63:1028-1038.

Rosenheim, J.A., P. Nonacs and M. Mangel. 1996. Sex ratios and multifaceted parental investment. American Naturalist 148:501-535.

Schoener, T.W. 1979. Generality of the size-distance relation in models of optimal foraging. American Naturalist 114:902-914.

Schwarz, M.P. 1988. Local resource enhancement and sex ratios in a primitively social bee. Nature 331:346-348.

Sheldon, B.C., S. Andersson, S.C. Griffith, J. Örnborg, and J. Sendecka. 1999. Ultraviolet colour variation influences blue tit sex ratios. Nature 402:874-877.

Smith, C.C. and S.D. Fretwell. 1974. The optimal balance between size and number of offspring. American Naturalist 108:499-506.

Stearns, S.C. 1992. The evolution of life history strategies. Oxford University Press, Oxford.

Stephens, D.W. and J.R. Krebs. 1986. Foraging theory. Princeton University Press, Princeton.

Sugiura, N. and Y. Maeta. 1989. Parental investment and offspring sex ratio in a solitary Mason bee, *Osmia cornifrons* (Radoszkowski) (Hymenoptera, Megachilidae). Japan Journal of Entomology 57:861-875.

Tasei, J.N. and A. Delaude. 1984. Efficacité pollinisatrice de *Megachile rotundata* F. (Hymenoptera: Megachilidae) utilisée sur luzerne *Medicago sativa* L. Agronomie 4:653-662.

Tepedino, V.J., D.R. Frohlich and C.R. Baird. 1994. Effect of intertunnel distance and nest-surface aspect on progeny production rate and sex ratio in the alfalfa leafcutting bee (Hymenoptera: Megachilidae). Journal of Economic Entomology 87:27-30.

Tepedino, V.J. and F.D. Parker. 1986. Effect of rearing temperature on mortality second-generation emergence and size of adult in *Megachile rotundata* (Hymenoptera: Megachilidae). Journal of Economic Entomology 79:974-977.

Tepedino, V.J. and P.F. Torchio. 1982. Phenotypic variability in nesting success among *Osmia lignaria propinqua* females in a glasshouse environment (Hymenoptera: Megachilidae). Ecological Entomology 7:453-462.

Tepedino, V.J., R. Thompson and P.F. Torchio. 1984. Heritability for size in the megachilid bee *Osimia lignaria propinqua* Cresson. Apidologie 15:83-88.

Torchio, P.F. and V.J. Tepedino 1980. Sex ratio, body size and seasonality on a solitary bee, *Osmia lignaria propinqua* Cresson (Hymenoptera: Megachilidae). Evolution 34:993-1003.

Trivers, R.T. and D.E. Willard. 1973. Natural selection of parental ability to vary the sex ratio of offspring. Science 179:90-92.

Visscher, P.K. and B.N. Danforth. 1993. Biology of *Calliopsis pugionis* (Hymentoptera: Andrenidae): Nesting, foraging, and investment sex ratio. Annals of the Entomological Society of America 86:822-832.

Weimerskirch, H., P.A. Prince and L. Zimmermann. 2000. Chick provisioning by the yellow-nosed albatross *Diomedea chlororhynchos*: Response of foraging effort to experimentally increased costs and demands. Ibis 142:103-110.

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

Westrich, P. 1996. Habitat requirements of central European bees and the problems of partial habitats. Linnean Society Symposium Series 18:1-16.

Williams, G.C. 1966a. Adaptation and natural selection. Princeton University Press, Princeton.

Williams, G.C. 1966b. Natural selection, the costs of reproduction and a refinement of Lack's principle. American Naturalist 100:687-690.

3.7 FIGURES AND TABLES

Figure 3-1. Theoretical parental fitness returns with increasing resource investment in a single son and daughter (cumulative fitness of a single offspring). The solid line represents the sex where large investment provides the greatest payoff. In *Megachile rotundata* the solid curve would be for daughters and the dashed curve for sons. The optimal investment is determined using Smith and Fretwell (1974) (steepest slope). Using this method the optimal investment for sons is A, and for daughters B. However, depending on the position of the son and daughter curves in relation to one another, it is possible to have the optimal investment for a son decrease to C (As is the case for the curves drawn here). This will be the case when the daughter curve rises above the son curve, wherein any investment greater than that point makes it more beneficial to produce a daughter. The result is that C becomes the optimal investment in a son.

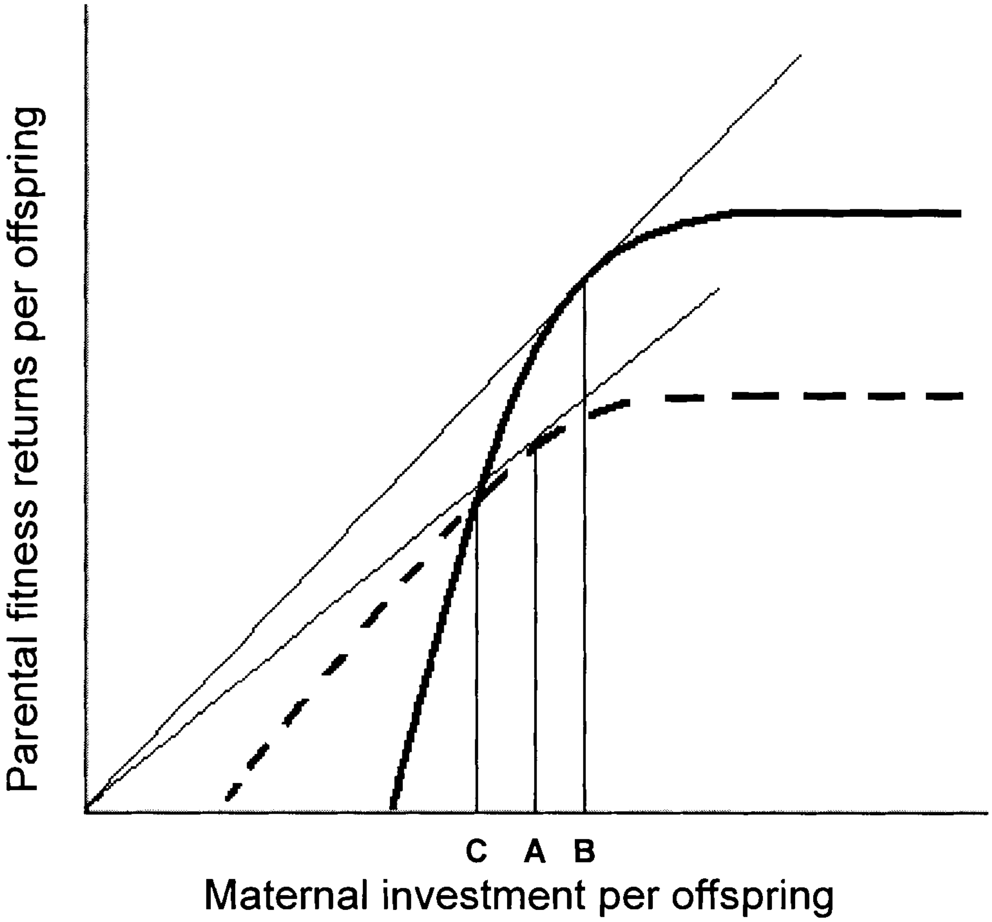
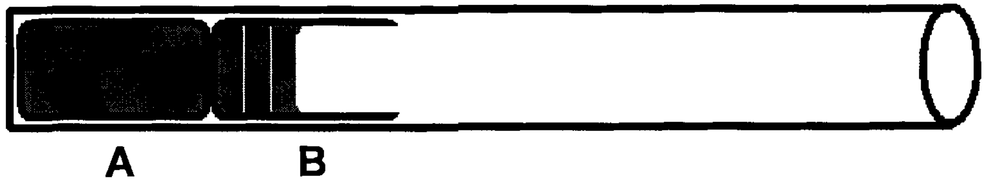

Parental fitness returns per offspring
C
A
B
Maternal investment per offspring

Figure 3-2. Representation of a linear nest created by the solitary bee *Megachile rotundata* (cross-section view).

A: Completed brood cell - A leaf-wrapped cell provisioned with pollen and nectar, with a tiny egg.
B: 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.
C: Space left behind cap - In some nests the mother leaves an empty space between the nest cell and the beginning of the cap.
D: Nest cap - 10-40 leaf pieces are plastered together to "cap" the outside of the nest and prevent access to the nest or further use.

I. Incomplete Nest



II. Completed Nest (with empty space behind cap)

III. Completed Nest (no space behind cap)

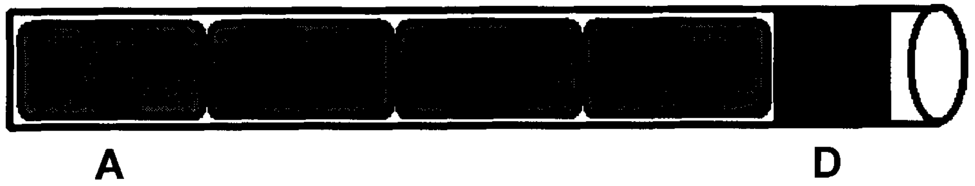
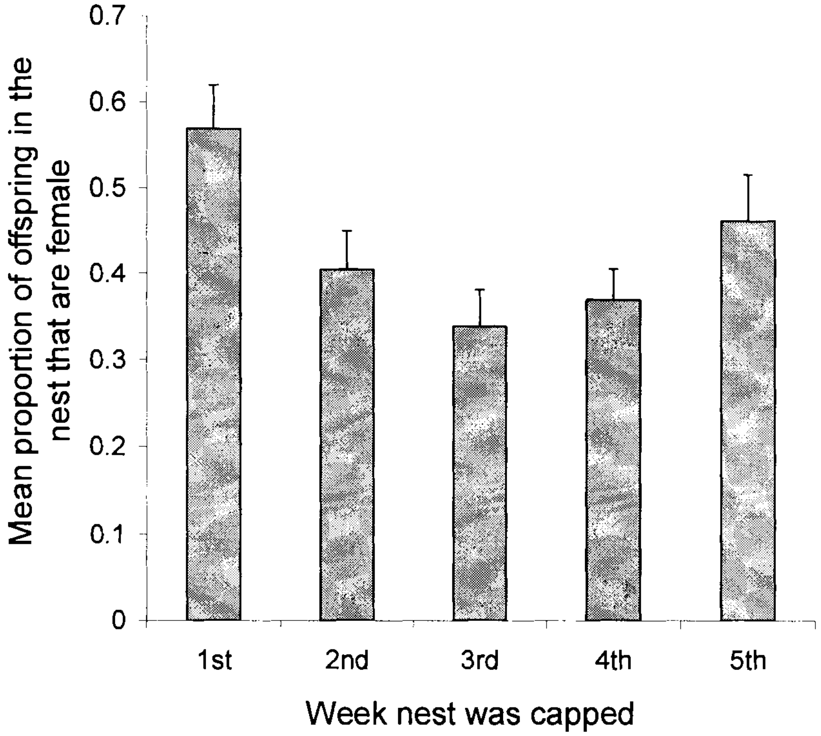


Figure 3-3. The two graphs demonstrate the mean proportion of females per nest (A) and the number of nests capped (B) during each week of the experiment involving the leafcutter bee *Megachile rotundata*. The 1st week column represents the first week after bee release. All data are from the Near treatments. Female sex ratio (mean proportion of progeny in the nest that are female) is significantly different between weeks (F=3.93, *d.f.*=4, P=0.016), while the number of nests capped was not significantly different (F=1.98, *d.f.*=4, P=0.118).

A.


0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Mean proportion of offspring in the nest that are female
1st
2nd
3rd
4th
5th
Week nest was capped

B.

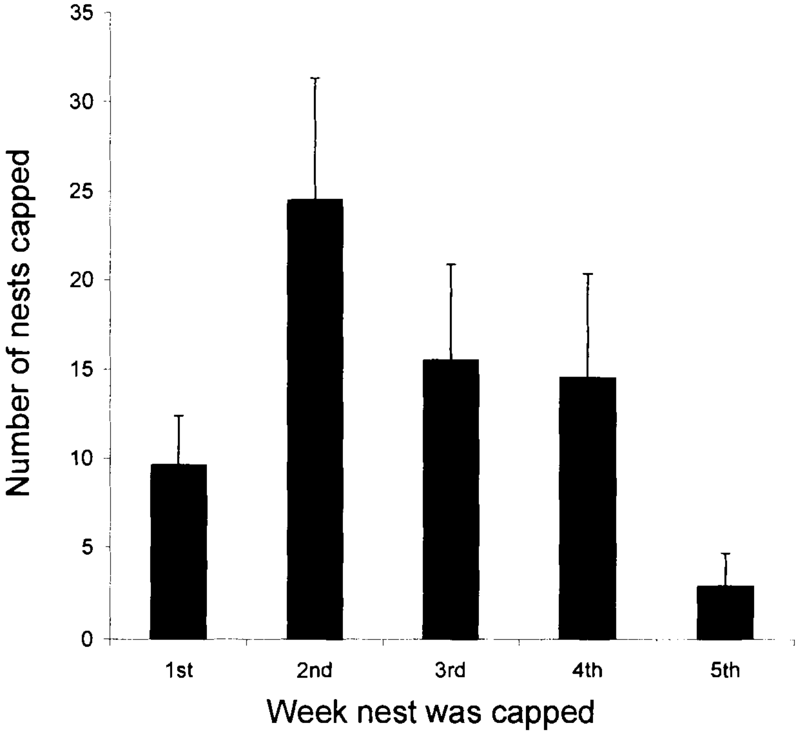
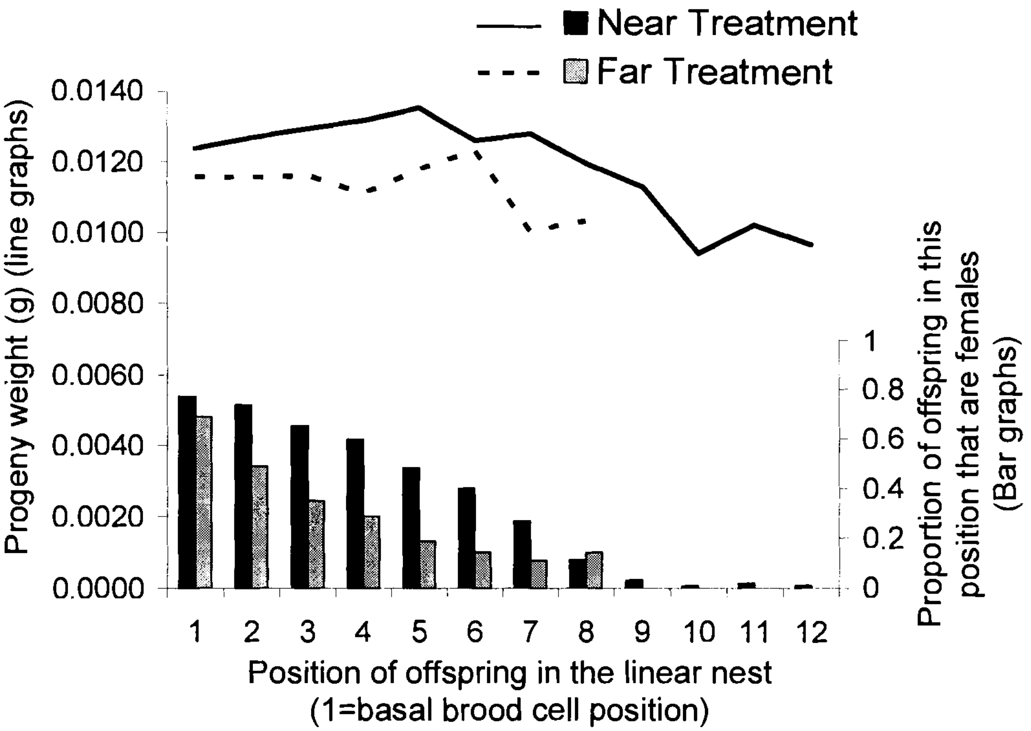

35
30
25
20
15
10
5
0
Number of nests capped
1st
2nd
3rd
4th
5th
Week nest was capped

Figure 3-4. The dry weight at emergence for adult daughters (A) and sons (B) of *Megachile rotundata* based on position within the linear nest. The figure also shows the proportion of each sex found at each nest position. The two distance treatments were: resource located adjacent to the nesting sites (Near treatment) and 150 metres away (Far treatment). Position 1 is the most basal position in the nest. The line graphs represent the average weight of bees found in that position. The bar graphs represent the proportion of the offspring from each position within the nest that were female (A) and males (B).

A. Daughters



B. Sons

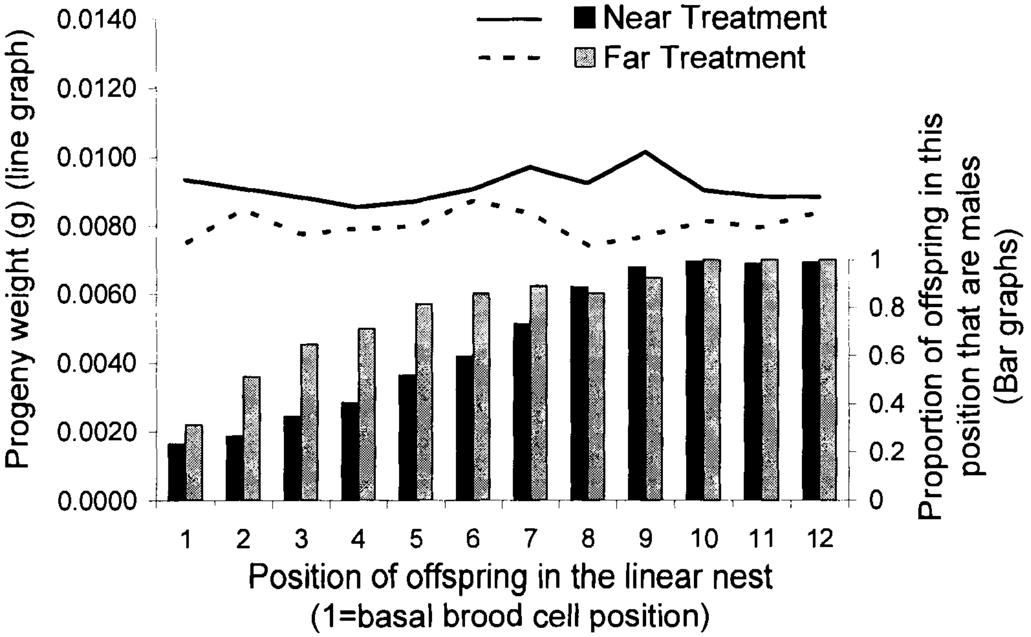


Table 3-1. Results from an experiment where leafcutter bees, *Megachile rotundata*, were either given foraging resources adjacent to nesting sites or 150 metres away (near n=5 and far n=3). Sex ratio is considered the average proportion of daughters per nest. Results from each site were based on the mean or proportion of all the bees in that replicate. P values were calculated using a t-test (the proportion of brood cells that did not hatch and the proportion of nests capped were arcsine square root transformed prior to analysis).

	Near Flight Distance Treatments		Far Flight Distance Treatments		P
	Mean	SE	Mean	SE	
Mean primary female sex ratio per nest	0.49	0.03	0.31	0.03	0.003
Mean number of brood cells per nest	5.5	0.14	3.9	0.19	0.011
Production of sons per nest	3.0	0.12	3.0	0.15	0.949
Production of daughter per nest	2.4	0.08	1.0	0.11	<0.0001
Proportion of brood cells that did not hatch	0.23	0.01	0.43	0.05	0.025
Proportion of nests capped (sealed)	0.53	0.07	0.28	0.02	0.025

Chapter 4
A Dynamic State Variable Model of Resource Allocation and Sex Ratio Decisions in a Solitary Bee

4.1 ABSTRACT

In situations where resource requirements differ for male and female offspring, a parent may be expected to alter sex allocation decisions based on current ecological conditions. A dynamic state variable model was developed to investigate factors that influence sex allocation in solitary bees. The factors examined were: time to collect a load of pollen, time to lay an egg, time to cap (seal) a nest, the risk of death to the mother, the increased value of a nest when capped, and total amount of time available to produce offspring. Field work was also carried out on the alfalfa leafcutter bee, *Megachile rotundata*, in order to parameterize the model. I found that the optimal size of both sexes was stable across a wide variety of assumptions about parameter values, while sex ratio was influenced by nearly all of these factors. In contrast to many dynamic state variable models, in my model current decisions strongly depend upon both future opportunities and past events.

4.2 INTRODUCTION

Fisher's (1930) theory of parental investment and sex allocation predicts equal investment in the offspring of each sex. However, a number of studies have shown that ecological conditions can result in unequal allocation between sexes (Komdeur et al., 1997; Kim, 1999; West and Sheldon 2002). Skewed investments are expected in situations where the fitness return on maternal investment is determined by functions that differ in shape between sons and daughters (Trivers and Willard, 1973; Charnov, 1982; Frank, 1990; Campbell, 2000). Investment is considered to be any activity involved in the production of offspring that decreases the parents' future ability to invest in offspring (Williams, 1966a,b). Life-history theory predicts that reproductive effort should be concentrated on the sex that generates comparatively larger reproductive payoffs (Charnov, 1982; Bortolotti, 1986; Frank, 1990; Stearns, 1992).

Sexually size-dimorphic species provide excellent systems to investigate sex ratio and resource allocation in offspring. As the larger sex likely requires greater parental investment, Fisher's (1930) theory predicts that greater numbers of the smaller sex will be produced in order to invest equally in both sexes. In sexually size-dimorphic species, the assumption is that a viable offspring (i.e., an offspring that will produce offspring of its own, thereby increasing the parent's fitness) of the smaller sex can be produced with fewer resources, but the larger sex provides greater fitness benefits to the mother from increased resource investment (Frank, 1995; Rosenheim et al., 1996). Therefore the maternal fitness return curves are likely to be different for each sex (e.g., Fig. 4-1). As well, changes in the costs of obtaining resources may impact the maternal fitness benefits of sons and daughters differently.

Ecological conditions (e.g., resource availability) have been suggested as having the potential to alter optimal sex ratio and resource investment in progeny (Rosenheim et al., 1996). However, the paucity of empirical studies on these factors has left many parameter values undetermined. In the absence of such information, it is useful to model the system to determine what parameters may be important in determining optimal sex ratio and resource allocation. Once these parameters have been determined we can also examine the directional impact of each factor and how they interact.

Dynamic state variable modelling has been shown to be an excellent tool for modelling optimal decisions in situations where a forager experiences different states (Clark and Mangel, 2000). The structure of these models provides flexibility to test numerous assumptions and scenarios. Sex ratio and resource allocation decisions are often state dependent, and thus, may be usefully examined using dynamic state variable models.

The alfalfa leafcutter bee, *Megachile rotundata*, has several features that make it an excellent subject for addressing questions of sex allocation and maternal investment. Females are, on average, 20-30% larger than males (Klostermeyer et al., 1973) and females control the sex of offspring by fertilizing the ovum (daughters) or not fertilizing the ovum (sons) (Cook, 1993). Singly mated females build linear twig nests in preformed cavities (McCorquodale and Owen, 1997) and provision the brood cells; male investment is limited to sperm. A single egg is laid per brood cell and the brood cell is completed before construction begins on the next, eliminating competition among offspring for food and unitizing investment along an easily followed timeline (Freeman, 1981). The leaf-wrapped brood cell is filled with pollen and nectar (referred to below collectively as pollen) collected during a number of foraging trips. After a given number of trips (i.e., a given amount of pollen amassed in the brood cell), the mother lays an egg onto this pollen "loaf", seals the brood cell and begins construction of the next brood cell (see Fig. 4-2 for a description of nests). The adult size of offspring is strongly correlated to the amount of pollen that is provisioned to the brood cell (Klostermeyer et al., 1973; Bosch and Vicens, 2002); all of the food consumed by the developing progeny prior to adulthood is provided by the mother.

The dynamic state variable model developed here addresses issues that are common in many other groups of organisms, and also considers an angle not commonly addressed in previous dynamic state variable models. Here, current decisions strongly depend upon not only future opportunities but also past events. During the construction of a nest, a female must decide when the nest should be capped. Capping involves blocking the single entrance to the nest with 10-40 leaf pieces to prevent predators and parasitoids from attacking the brood cells within the nest. The act of capping presumably increases the value of the nest already assembled, but precludes laying further offspring in the nest. This introduces an additional trade-off that may change behavioural decisions compared to those made in the building of a more traditionally studied nest (e.g., birds).

I have previously shown empirically that ecological factors can alter sex ratio and resource allocation decisions (see Chapters 2 and 3). Here, a dynamic state variable model allows me to test the impact on sex allocation of a number of specific ecological factors. Furthermore, using a sensitivity analysis, I examine how a range of values for these ecological factors (time required to collect pollen, time required to lay an egg, time required to cap a nest, risk of female mortality, value of capping the nest, and the length of the season) impact behavioural decisions.

Based on the general shape of the hypothesised maternal fitness curves for each sex (Fig. 4-1), I predicted that changes in factors which result in an increase in the cost of provisioning a single progeny will decrease the proportion of daughters produced.

4.3 THE MODEL

In order to determine the optimal schedule of allocation towards size, sex and number of offspring during an organism's lifetime, the implications of an investment decision on both current and future reproduction must be determined. While determining the impact on current reproduction is relatively easy, determining the impact on future reproduction can be more complicated. One method used to deal with such problems is dynamic state variable modelling. Dynamic state variable modelling is an optimization technique used to determine the optimal sequence of decisions (e.g., allocations to reproduction, foraging), based on the state of the organism (e.g., fat reserves) along a time axis.

I developed a dynamic state variable model to look at resource allocation and sex ratio decisions in *M. rotundata* (model is detailed in Appendix A; the computer code is in Appendix B). The model was developed with the assumption that females have evolved to maximize lifetime reproductive success, which is a function of the size, number, and sex of offspring produced. Time is divided into discrete units. In each time unit the mother is faced with a decision to: (1) collect more pollen, (2) lay a son and complete the brood cell, (3) lay a daughter and complete the cell, or (4) cap (seal) the nest completely. Some of these activities take longer than one unit of time; thus, completion of such behaviours moves the bee a number of time units into the future. Mothers also face the chance of death within each time unit.

Three states were considered: (1) pollen (resource) state, (2) nest state, and (3) time state. In this system, there are numerous fitness payoffs that are dependent upon current state. Based on the shape of the maternal fitness function for each sex, the current state of pollen in the brood cell being constructed determines the size of the offspring that will be produced. The nest state is specified by the number, size and sex of inhabitants in completed brood cells (offspring currently in the nest) and determines the value of capping the nest (i.e., the greater the value of the nest the greater the value of capping the nest). Time state goes from one (the beginning of time) to a predetermined maximum time (T) and the current state determines how much time an individual has remaining to carry out activities.

There are two parts to the model. The first part determines the best decision for every combination of the three state variables: pollen state, nest state and time state. Optimal decisions are placed in a 3 dimensional decision matrix (pollen, nest, time) as follows. Matrix cells are filled through backwards induction, starting at the end of time (where terminal fitness is 0) and working backwards to the beginning of time, determining the optimal behaviour for every combination of the three state variables. Thus, the procedure produces a matrix of the optimal decision (pollen, son, daughter or cap) for each of the possible combinations of the state variables (pollen state, nest state, time). Note that the model is deterministic; i.e., it solves for the single best policy for maximizing fitness in a stochastic world where death occurs with some probability.

Once the decision matrix is complete, it is incorporated into a related model termed a forward iteration (simulation). Here, a stochastic simulation is run for individual bees, starting at the beginning of time and moving forward (based on the optimal decisions determined in the first part of the model) until time expires or an individual dies due to some weighted random draw. As a result, there is variation among individuals; in some runs the bee dies before producing any brood cells, while those in other runs produce many brood cells.

4.3.1 Parameter estimation

A critical step in modelling a system involves determining what parameter values should be used to characterize the system. The paucity of studies on sex ratios and resource allocation in this system means that many of the parameter values to be used in the dynamic state variable model had to be empirically determined or estimated from preliminary simulations. Below is a description of how parameter values were obtained or estimated.

4.3.1.1 Field experiment

To solve for optimal maternal behaviours, field values for several parameters are required: time to collect resources, average number of trips to provision a brood cell, time to complete a brood cell, and time to cap a nest.

Flight times for various nest-building activities, as a function of distance to resources, are not well known in the megachilids. Thus, I carried out a field experiment to determine the amount of time 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 (54°23' North, 111°41' West). In order to test the effect of flight distance on the time required to carry out various nest-construction activities in female *M. rotundata*, I used the experimental setup describe in Chapter 3.

The bees were released into the shelters on the morning of July 6th. Bees were given 20 days before videotaping started to allow them to become established in the environment. Bee activity at each shelter was 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). These tapes allowed me to determine the exact time bees exited and entered the nest and what they were transporting (leaves, pollen or nothing). This information yielded the amount of time the mothers spent in and out of the nest when carrying out these various collection activities, the number of trips required for each of these activities, and the time spent between completing one brood cell and starting the next. The cameras were set up just outside each shelter below the observed flight path of the bees to avoid disturbing their flight patterns.

A t-test was performed to test for differences between the near and far treatments regarding: the number of pollen loads per brood cell, the amount of time bees took to collect a load of pollen, and the amount of time required to lay an egg and seal the brood cell.

4.3.1.2 Parameter estimation by simulation

Experimental data were not available for a number of parameters; therefore, I estimated values for these parameters. To carry out a sensitivity analysis, I tested a wide range of values for a single parameter by keeping all other parameters unchanged and incrementing the parameter I was evaluating to determine how stable the results were. The derived values used for these parameters are described below.

4.3.1.2.1 Maximum amount of time available (T)

Maximum production of offspring appears to be in the 30 - 40 progeny range; however, realized production is usually considerably less than this (Gerber and Klostermeyer, 1972; Peterson et al., 1992). I set the standard maximum available time (T) high enough to achieve this level of production (30-40 progeny) when there was no chance of mortality (T=250). Therefore, under realistic conditions, with a chance of death, realized production would be less than this maximum range. In the sensitivity analysis, I ran the model with values of T from 25 to 1000 in increments of 25 units.

4.3.1.2.2 Risk of mortality (μ)

Chance of death (μ) is obviously dependent on the specific environment; I therefore tested a wide range of μ values, from 0 - 0.1 in increments of 0.005 (with a value of 0.1 there is a 10% chance of mortality for every time unit and under these conditions the average mother will produce less than one offspring). In order to look at the impact of changing two factors, I repeated the above range of μ values for a range of k values (1.0 - 5.0 times increase in nest value from capping; see below). This procedure allowed me to determine how results from changing μ values are impacted by changes in the k value.

4.3.1.2.3 Value of capping the nest (k)

The value of capping a nest is a result of the increased protection of the brood cells, especially from predators and parasitoids, when the nest is capped. The value of capping is defined as the change in maternal fitness from offspring in an uncapped versus capped nest. There are no reliable data available on the value (fitness benefit) of capping a nest for solitary hymenopterans. Therefore, I ran a wide range of values from zero increase in nest value (k) to a fivefold increase in the value of the nest, incremented by 0.1. The biologically meaningful values of capping the nest (k) in the vast majority of situations are likely those below two. Otherwise, in nature, we would likely find capping occurring after every brood cell, barring factors such as nesting site limitations. This is because more value would be gained from capping a nest with a single brood cell than adding another offspring to the nest, as the events appear to take similar amounts of time.

4.3.1.2.4 Time required to find new nest site (NS)

Once a nest is capped the mother must find a new location to start another nest. The time required to carry out this activity was randomly drawn from a bell curve centred on the estimate mean time value for finding a new nest. I ran the model with values from 1 to 10 time units.

4.3.1.2.5 Shape of the maternal fitness returns curves

The general shape of the maternal fitness curves are shown in Figure 4-1. The equations used to produce the curves in the model are:

$$F(m) = (-4 + (a_m (1 - e^{-(p\gamma_m)}))) / 4; \qquad (1a)$$

$$F(f) = (-6 + (a_f (1 - e^{-(p\gamma_f)}))) / 4; \qquad (2a)$$

where a_m and a_f control the asymptote of the curves of males (m) and females (f), respectively, and γ controls the slope based on the number of pollen loads (p) and the sex. I altered both the a and γ values to increase and decrease the slope and asymptote of the curves to examine the change in sex allocation. I also tested fitness functions that were linear with the male curve having an earlier x intercept and the female curve having a greater slope and higher asymptote.

The simulation portion of the model was run 250 times with each set of parameter values in order to determine the average values for the female sex ratio (proportion of offspring produced by a mother in her lifetime that were female), nest size (number of offspring produced), brood cells produced per nest, and progeny size. Time values for carrying out various behaviours were determined using the results from the experimental work.

4.4 RESULTS

4.4.1 Field study

The purpose of the field experiment was to obtain approximate figures for the amount of time each activity takes and to test whether flight distance affects the time required to carry out an activity. This is important, because to simulate experiments with changing ecological factors (e.g., flight distance), one needs to know whether changing one factor requires altering other factors simultaneously.

The mean number of pollen loads per brood cell was not affected by flight distance, means (for the combination of sons and daughters) were 12.7 ± 0.9 (near treatment) and 12.4 ± 1.0 (far treatment) ($t=0.16$, *d.f.*=19, $P=0.87$, $\beta=0.05$). Flight distance had a significant effect on the amount of time bees spent to collect a load of pollen: 37 ± 4 minutes in the near treatment and 58 ± 4 minutes in the far treatment ($t=-3.99$, *d.f.*=73, $P<0.001$). The mean amount of time required to lay an egg and seal the brood cell (calculated as the time between the completion of provisioning one brood cell and initiation of the next) was 200 ± 23 minutes in the near treatment and 221 ± 19 in the far treatment (t=1.35, *d.f.*=25, P=0.19, β=0.25). I did not know the sex being laid; however, there is likely little difference in time between sexes because oviposition takes, on average, less than one minute (Klostermeyer and Gerber, 1969). I was only able to observe a few bees constructing a cap and there was a wide range of times, from 3 to 12 hours.

The construction of a brood cell involves building part of the leaf lining, then provisioning the cell, laying the egg and completing the leaf covering. As all these activities need to be carried out to complete the brood cell, I simplified the process in the model into provision collection and leaf lining construction/egg laying. Based on the above data, I used one hour as the basic time unit for the model. Using the results from the short distance treatment (normal conditions), I simplified pollen collection: a single pollen trip in the model reflects two pollen trips in nature (approximately double the 37 min collection time). Therefore, in the model one pollen trip takes one time unit. As time to collect a load of pollen was the only factor impacted by flight distance, I varied this time in the model without altering the time required to carry out other activities when simulating an increased distance to resources.

I therefore parameterized the basic setup of the model as follows: one time unit is required to collect a load of pollen, three to lay an egg (son or daughter) plus seal a brood cell, and four to cap the nest.

4.4.2 Dynamic state variable model simulations

I used a set of median values as a baseline and then varied chosen parameters to observe the impact. The baseline values are: T=250 (Total time available), P=1 (time to collect a load of pollen), E=3 (time to lay a male or female egg and seal the brood cell), C=4 (time to cap the nest), μ=0.02 (chance of death per unit of time), NS=3 (average time required to locate a new nest site after capping a nest), and k=1.2 (factor by which the value of the nest is increased when capped). In the sensitivity analysis, the range of times required to carry out the various activities incrementally increased by one time unit at a time (the only fractional value was 0.5 for pollen, which is explained below): pollen collection (0.5-10), laying a male or female egg (1-10), and capping the nest (1-50). The incremental increase of non-time factors is discussed below. A sensitivity analysis was carried out for changes in the time required for each activity (Table 4-1). The impact of variation in each of these individual factors is discussed below.

4.4.2.1 Effects of time to provision brood cell (P)

Increasing the amount of time required to collect a load of resources to provision the brood cell (e.g., because of increased flight time) had no impact on offspring size (i.e., number of pollen loads) of sons (4 pollen loads) or daughters (6 pollen loads). There was, however, an increase in the proportion of sons produced. A pollen collection time of one resulted in a female sex ratio just over 0.40, while increasing the time to collect pollen above one resulted in only sons being produced. When I simulated a run with time to collect pollen set at 0.5 units (by doubling all the other values), only females were produced (female sex ratio=1). Increase in pollen collection times also decreased the average size of nests in a logarithmic manner.

4.4.2.2 Effects of time to lay an egg and seal the brood cell (E)

An increase in the time required to lay an egg and seal the brood cell caused a slight increase in the optimal female size. There was an increase in size of daughters with a laying time of six (size=7) and with a laying time of eight (size=8). However, above this value optimal female size stabilized at eight. When the amount of time was set at 2 time units and below, males were produced exclusively; with a value of 4 and above, females were produced exclusively. Increased time to produce offspring also decreased the average number of offspring per nest.

4.4.2.3 Effects of time to cap nest (C)

There was no change in the size of sons or daughters with changes in the time required to cap the nest (sons=4; daughter=6). Sex ratio remained constant at around 0.4 until the capping time was increased to 10 time units; then there was a sharp drop to a sex ratio of 0.1 (Fig. 4-3). The sex ratio remained at this level as capping time increased to 30 time units. There was a continuous increase in the number of offspring per nest, with fewer nests produced, as the time to cap increased.

4.4.2.4 Effects of amount of time available (T)

Increasing time (T) had no impact on offspring size. There was also no effect on the optimal sex ratio (other than at very low T values when the last few time units are very influential, as the model tries to optimize each time unit available), the number of brood cells produced per nest, or the number of nests produced (Fig. 4-4a). In order to test that this was not simply the effect of the chance of death, another run of the model was done with the chance of death reduced to 0.001 (Fig. 4-4b). This ensured a large percentage of the bees were able to survive until near the end of time, even with high T values. The results were the same, the only difference being that because the bees lived longer they were able to produce more offspring per nest with increased time. Because more bees were produced per nest, when time was greater than 500 units the nest was so large that the bees began producing two nests instead of one. All other factors were unaffected by T.

4.4.2.5 Effects of risk of death (μ)

Varying the chance of death per time unit had no impact on the size of offspring (sons=4; daughter=6). However, increasing such risk did have an impact on the sex ratio of offspring (Fig. 4-5). There were three different phases in the sex ratio response. Daughters were produced almost exclusively at low risk levels (<0.015). Female sex ratio decreased to around 0.5 with medium risk (0.016-0.02); beyond this risk level only males were produced. Increased risk per unit time also resulted in a decreased average nest size. The impact of an increased k value on the effect of risk was to increase the risk value required before the proportion of daughters produced became zero (Fig. 4-5). When k was set at 1.5, mothers did not produce all sons until chance of death reached 0.035. This was three times the chance of death that resulted in only sons being produced when there was no added value of capping the nest.

4.4.2.6 Effects of value of capping nest (k)

Over the range of k values there was no change in the size of progeny and the values stayed constant for both sexes: sons (4) or daughters (6). There was a shift in the optimal sex ratio with changing k values (Fig. 4-6). At very low k values (<1.2) the optimal female sex ratio was 0, increasing to the 0.4 to 0.5 range, until k=2, when the sex ratio drops back to around 0.4. The size of the nest decreased logarithmically as k increased. There was also a decrease in the total number of offspring produced because of the increased time allocated to capping nests. However, there was an increase in the number of nests produced with increased k values.

4.4.2.7 Effects of time required to locate new nesting site (NS)

The time required to locate a new nest did not alter the size of progeny and the values stayed constant for both sexes: sons (4) or daughters (6). There was a shift in the optimal sex ratio. With NS values less than 6, the female sex ratio was around 0.4; this value dropped to around 0.1 with values greater than 6 (Fig. 4-7). The average number of offspring per nest increased as the time required to locate a new nest increased, while the average number of completed nests decreased.

4.4.2.8 Shape of curves

Increasing the slope of the maternal fitness return curve for males increased the proportion of sons produced. Major increases to the slope decreased the optimal size of males; however, there was little impact on the size of nests. Increasing the slope of the female curve similarly increased the proportion of daughters and increased the optimal female size. Changes in the shape of the curves of one sex had no impact on the optimal size of the other sex; there was simply a change in sex ratio.

I also used linear offspring payoff functions that were constrained at a maximum. The general response did not change from the results using curved functions.

4.5 DISCUSSION

The dynamic state variable model revealed a number of interesting factors that influence sex allocation decisions. Optimal size of both sons and daughters is controlled mainly by the shape of the maternal fitness returns function. The optimal size is stable across a wide variety of assumptions about other life history characteristics and ecological parameters. Sex ratio decisions, however, appear to be impacted by a number of different factors.

The stability of optimal size across numerous assumptions suggests that changes observed in offspring size in the field with changing ecological factors (Chapter 2 - resource levels; Chapter 3 - distance to resources) are, in fact, a result of ecological conditions altering the maternal payoff curves. Such an effect is likely, as ecological conditions change the amount of resources that can be obtained with a given amount of investment change as well, altering the maternal fitness payoff of that level of investment. It is therefore important to realize that because maternal fitness curves are derived through empirical research, the ecological conditions under which that work is carried out may have a dramatic impact on the shape of the curves produced. Caution needs to be exercised when trying to apply these types of curves across areas with drastically different ecological conditions.

The sex ratio of offspring produced was heavily influenced by the values of a number of factors. In general, the greater the investment involved in building a brood cell, the greater the proportional production of sons. The one case where this was not true was when the time to lay an egg and seal the brood cell increased. This may be because as the production time increases equally for both sexes, the difference in the proportional amount of time to produce sons and daughters (time to collect pollen + time to lay egg and seal cell) decreases, and the higher payoff of producing a daughter becomes more important. However, if the time to collect pollen increases (simulating, e.g., increased flight distance), less pollen has to be collected for males so the increase in time will not be as great for males and proportionally more sons are produced. This partially corroborates the idea that increasing the investment required to collect a load of resources will decrease the optimal amount of resources to invest. What I found is that the optimal size does not change, but there is a proportional increase in the production of the smaller sex (males), thereby decreasing the amount of resources invested per average progeny. In my field work (Chapters 2 and 3) I had predicted that there would be a decrease in both the optimal size and proportion of daughters produced with increased amounts of time to collect resources; however, it appears this is only true when the shape of the fitness function changes. In other words, factors such as pollen collection time may have an impact on offspring size, but only if the changes to that factor alter the shape of the maternal fitness returns curves. I also found under a variety of conditions that investment in each sex (i.e., time) was relatively equal.

In this model mothers were not constrained to invest equally in each sex. With extreme values of most of the parameters, mothers either produced all sons or all daughters. However, for median values that are within the range we might expect to find in nature, female sex ratio was around 0.4, with 6 units of pollen invested per female and 4 units of pollen per male. The investment was not always equal; however, it is interesting to note that even without an increase in the value of sons when a population produces a greater proportion of daughters (i.e., frequency dependence), relatively equal investment in each sex is optimal. In nature, this range where the sex ratio is not extremely skewed is likely greater because of the increasing value of the under-produced sex, resulting in increased selection pressure against extreme skew of sex ratio. This lack of frequency dependence in the model is a shortcoming that should be addressed in the future. One factor that increased the range of conditions that produced relatively equal investment in each sex was the value of capping the nest (k).

One of the unique features of this model is based on the assumption that a bee is able to increase her fitness by producing offspring and later gain more fitness from those same offspring by the decision to cap the nest. In most dynamic programming models, the benefits from decisions only occur once. In other words, once fitness is accrued, for example, from one offspring, no further fitness from that offspring can be accrued in the future. However, in this model bees have the option of employing a form of Clark's (1994) asset protection principle. In other words, they can spend time not producing more offspring but gain fitness by protecting the offspring they already have, increasing the fitness value of what they have already produced. The solitary bee system allows us to quantify this cost of protection, because mothers spend time building a physical structure to better protect the nest. This same type of trade-off is found in other systems, e.g., parents must choose between care for current offspring versus weaning them and producing more offspring.

The impact of increased nest value from capping (k) on the sex ratio was limited to low values of k. In these cases (<20% increase in nest value) the optimal female sex ratio was 0. With higher k values, the optimal female sex ratio was relatively stable around 0.4 (Fig. 4-6), while optimal progeny size was stable across all k values. This suggests that part of the gain from producing high value daughters is that a larger fitness benefit per offspring is obtained when the nest is capped. However, if the benefit of capping is extremely low, daughters are less advantageous to produce. This appears to be a threshold function where, so long as the benefit is greater than some minimal value, a sufficient benefit is received from capping to make daughters beneficial to produce. However, there is no further increase in benefit to producing daughters once this threshold has been reached, and as a result the sex ratio stabilizes. Changes in the value of capping the nest also impacted how frequently the capping behaviour occurred. When the value of k increased, mothers capped nests more frequently, thereby banking the bonus fitness more often. This also means that fewer offspring are produced overall (as there is less time available to invest in them), and fewer offspring are placed in each nest (Fig. 4-6). These factors were also affected by the risk of mortality.

Risk of mortality has a strong impact on the trade-off between making one large nest or several small nests. If there is no chance of death, maximum fitness will always be gained by producing one large nest and then capping it. This is because the act of capping takes the same amount of time regardless of the size of the nest; however, the payoff increases with increased nest size where no inter-nest travel cost is suffered. Therefore, one might expect the greatest benefit per unit of time spent capping, would be capping a very large nest. However, in nature there is a trade-off, the greater the amount of time the bee devotes to building a larger nest, the less likely the bee will survive to cap the nest and gain that extra fitness. Therefore the trade-off is between increasing the fitness benefits from time spent capping and the chance of dying before gaining any fitness benefit from capping. As a result, the greater the fitness bonus for capping the nest (k), the more frequently bees capped nests. This also holds true for increased risk of mortality. This trade-off may be further complicated in situations where nesting sites are limited.

According to Clark's (1994) asset protection principle, the more valuable the nest, the more risk averse the mother should become. In my model the most risk averse behaviour is to cap the nest. Protecting all brood cells is important because if any cell becomes parasitized, in many groups, e.g. *Pteromalus* spp., the parasitoid offspring will emerge as adults before the other bees in the nest, allowing further brood cells to be parasitized. If a mother is killed just after capping a nest, then she has obtained the highest possible fitness to that point. However, if she dies while adding another brood cell to a large nest it would have been more advantageous to have spent that time capping the nest. Of course, in a stochastic world bees do not know exactly when they are going to die. Therefore, they should alter their actions based on the perceived degree of risk and the importance of the activity.

This type of result, where an immediate reward of smaller magnitude is preferred to a delayed reward of greater magnitude, is referred to as discounting (Logan, 1965; Mazur, 1987) and is typical of a number of life history problems. In the case of reproductive rewards, there is a risk an animal will die before realizing the reward (Iwasa et al., 1984; Houston and McNamara, 1986; Candolin, 1998). Therefore, natural selection puts a premium on immediate reproduction. The trade-off is that females that reproduce earlier in life can experience a survival cost that increases steeply with the size of their reproductive effort (Lambin and Yoccoz, 2001). However, female voles of the species *Microtus townsendii*, that reproduce early in life have been shown to obtain greater lifetime fitness than females that wait until full maturity to reproduce (Lambin and Yoccoz, 2001). In my model, I found that the greater the benefit of capping or chance of mortality, the more often female bees should cap nests to ensure they receive at least some of the added fitness benefit. This tendency should increase if bees senesce via wing wear or some other physiological breakdown; in this model the probability of death was independent of age.

One of the unique features of the system modelled here is the value of capping the nest. This appears to be a very interesting and potentially important facet of organisms such as solitary bees. However, we understand very little of the quantitative benefits of capping a nest; I simply assume some fitness benefit is obtained by this act. This issue may become even more important in situations where foraging for food and nest materials occur in disparate areas, such as on different plants. In this case, risk between the two activities may be completely independent, altering behaviours in a way not predicted by the assumptions of the model.

Attacking the sex allocation decision problem using a dynamic state variable model allowed me to ascertain how ecological factors impact progeny size, sex and number. Field studies documented in Chapter 2 and 3 showed how ecological circumstances affect these two decisions. Here, modelling allowed me to look at specific factors over a range of values. Numerous factors impacted the optimal sex ratio, suggesting an evolutionary pressure for facultative sex allocation. Further work needs to be carried out to establish the exact shape of the maternal fitness return curves for both sexes.

4.6 REFERENCES

Bortolotti, G.R. 1986. Influence of sibling competition on nestling sex ratios of sexually dimorphic birds. American Naturalist 127:495-507.

Bosch, J. and N. Vicens. 2002. Body size as an estimator of production costs in a solitary bee. Ecological Entomology 27:129-137.

Campbell, D.R. 2000. Experimental tests of sex-allocation theory in plants. Trends in Ecology and Evolution 15:227-232.

Candolin, U. 1998. Reproduction under predation risk and the trade-off between current and future reproduction in the threespine stickleback. Proceedings of the Royal Society of London B 265:1171-1175.

Charnov, E.L. 1982. The theory of sex allocation. Princeton University Press, Princeton.

Clark, C.W. 1994. Antipredator behaviour and the asset-protection principle. Behavioral Ecology 5:159-170.

Clark, C.W. and M. Mangel. 2000. Dynamic state variable models in ecology: Methods and applications. Oxford University Press, New York.

Cook, J.M. 1993. Sex determination in the Hymenoptera, a review of models and evidence. Heredity 71:421-435.

Fisher, R.A. 1930. The genetical theory of natural selection. Clarendon Press, Oxford

Frank, S.A. 1990. Sex allocation theory for birds and mammals. Annual Review of Ecology and Systematics 21:13-55.

Frank, S.A. 1995. Sex allocation in solitary bees and wasps. American Naturalist 146:316-323.

Freeman, B.E. 1981. Parental investment, maternal size and population dynamics of a solitary wasp. American Naturalist 117:357-362.

Gerber, H.S. and E.C. Klostermeyer. 1972. Factors affecting the sex ratio and nesting behaviour of the alfalfa leafcutter bee. Washington Agricultural Experiment Station, Washington, DC; Technical Bulletin No. 73.

Houston, A.I. and J.M. McNamara. 1986. The influence of mortality on the behaviour that maximises reproductive success in a patchy environment. Oikos 47:267-274.

Iwasa, Y., Y. Suzuki and H. Matsuda. 1984. Theory of oviposition strategy of parasitoids. I. Effect of mortality and limited egg number. Theoretical Population Biology 26:205-227.

Kim, J-Y. 1999. Influence of resource level on maternal investment in a leaf-cutter bee (Hymenoptera: Megachilidae). Behavioral Ecology 10:552-556.

Klostermeyer, E.C. and H.S. Gerber. 1969. Nesting behavior of *Megachile rotundata* (Hymenoptera: Megachilidae) monitored with an event recorder. Annals of the Entomological Society of America 62:1321-1325.

Klostermeyer, E.C., S.J. Mech, Jr. and W.B. Rasmussen. 1973. Sex and weight of *Megachile rotundata* (Hymenoptera: Megachilidae) progeny associated with provision weights. Journal of the Kansas Entomological Society 46:536-548.

Komdeur, J., S. Daan, J. Tinbergen. and C. Mateman. 1997. Extreme adaptive modification in sex ratio of the Seychelles Warbler's eggs. Nature 385:522-525.

Lambin, X. and N.G. Yoccoz. 2001. Adaptive precocial reproduction in voles: reproductive costs and multivoltine life-history strategies in seasonal environments. Journal of Animal Ecology 70:191-200.

Logan, F.A. 1965. Decision making by rats: delay versus amount of reward. Journal of Comparative and Physiological Psychology 59:1-12.

Mazur, J.E. 1987. An adjusting procedure for studying delayed reinforcement. *In* Quantitative analysis of behaviour: V. The effect of delay of intervening events on reinforcement value. Ed. M.L. Commons, J.E. Mazur, J.A. Nevin and H. Rachlin. Lawrence Erlbaum, Hillsdale, New Jersey.

McCorquodale, D.B. and R.E. Owen. 1997. Allozyme variation, relatedness among progeny in a nest, and sex ratio in the leafcutter bee, *Megachile rotundata* (Fabricius) (Hymenoptera: Megachilidae). Canadian Entomologist 129:211-219.

Peterson, S.S., C.R. Baird, and R.M. Bitner. 1992. Current status of the alfalfa leafcutting bee, *Megachile rotundata*, as a pollinator of alfalfa seed. Bee Science 2:135-142.

Rosenheim, J.A., P. Nonacs and M. Mangel. 1996. Sex ratios and multifaceted parental investment. American Naturalist 148:501-535.

Stearns, S.C. 1992. The evolution of life history strategies. Oxford University Press, Oxford.

Trivers, R.T. and D.E. Willard. 1973. Natural selection of parental ability to vary the sex ratio of offspring. Science 179:90-92.

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

Williams, G.C. 1966a. Adaptation and natural selection. Princeton University Press, Princeton.

Williams, G.C. 1966b. Natural selection, the costs of reproduction and a refinement of Lack's principle. American Naturalist 100:687-690.

4.7 FIGURES AND TABLES

Figure 4-1. Theoretical parental fitness returns with increasing resource investment in a single son and daughter (fitness of a single offspring). The solid line represents the sex where large investment is most important. In *Megachile rotundata* the solid line would be for daughters and the dashed line for sons.

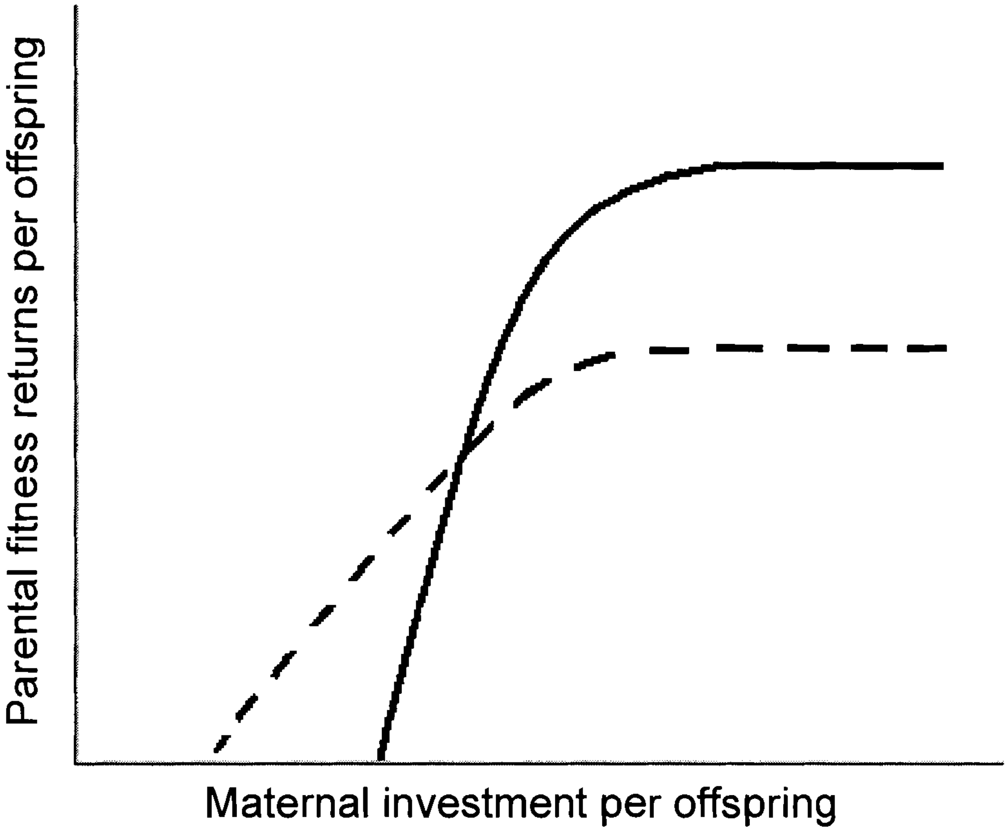

Parental fitness returns per offspring
Maternal investment per offspring

Figure 4-2. Representation of a linear nest created by the solitary bee *Megachile rotundata* (cross-section view).

A: Completed brood cell - A leaf-wrapped cell provisioned with pollen and nectar, and with a tiny egg.
B: 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.
C: Nest cap - Leaf pieces are plastered together to “cap” the outside of the nest and prevent access to the nest or further use.

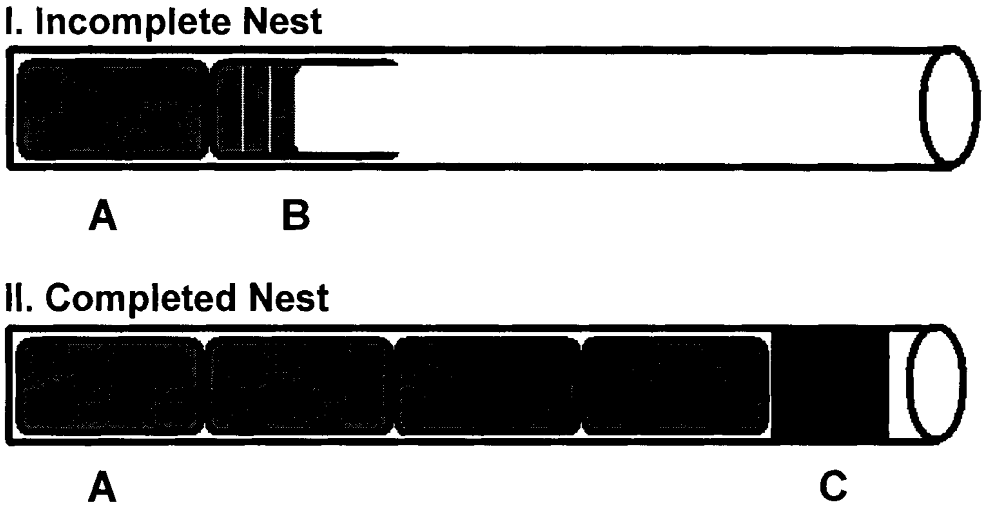

I. Incomplete Nest
A
B
II. Completed Nest
A
C

Figure 4-3. Changes in the optimal sex ratio of offspring and number of progeny produced per nest by theoretical leafcutter bees (n=250) with increasing amounts of time required to cap (seal) the nest (results are from multiple runs of the forward iteration (simulation)). Sex ratio is considered the mean proportion of daughters produced in a mother's lifetime. The model considers the following parameters: μ - chance of death per time unit, T - total time available for the bees to build nests, P - time to collect a load of pollen and nectar, E - time to lay an egg of either sex and seal the brood cell, C - time required to cap (seal) the nest, NS - time required to locate a new nest site after capping nest, k - factor by which the value of the nest increases when capped. I incrementally increased the value of C, while all other parameters were set at baseline levels (T=250, μ-0.02, P=1, E=3, NS=3, k=1.2).

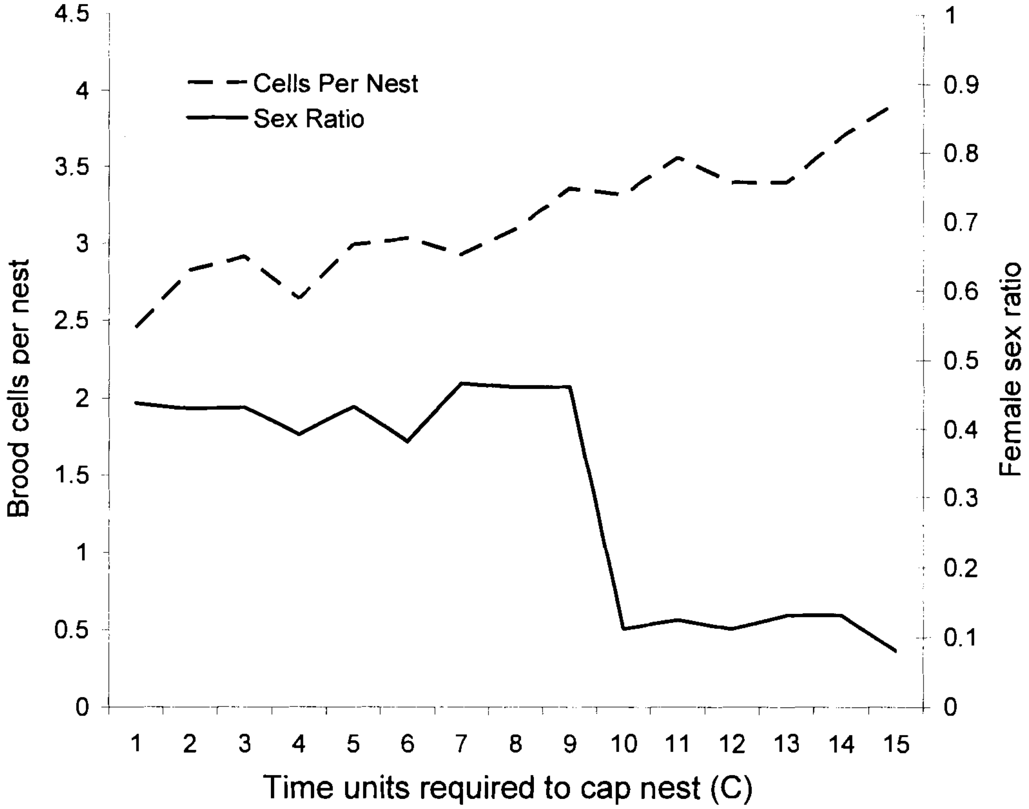
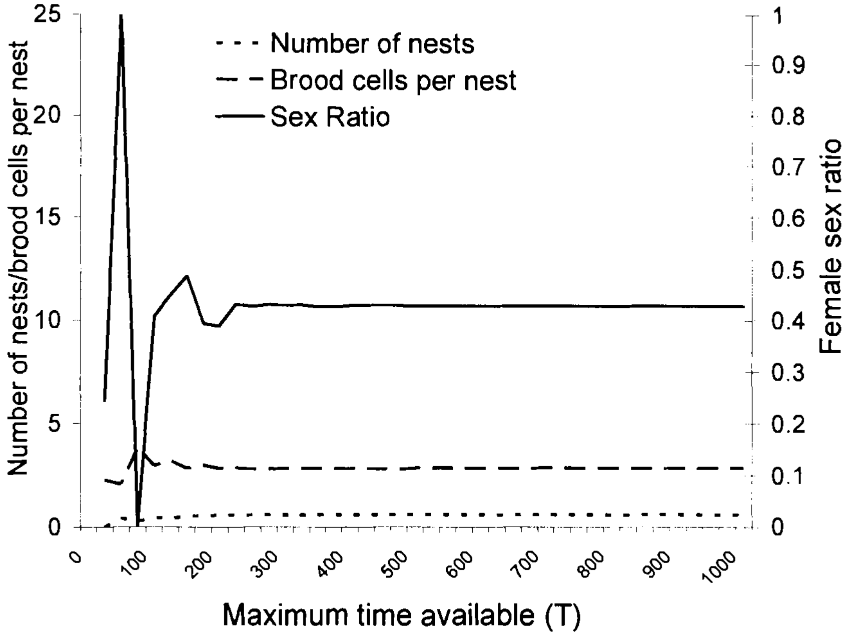

Cells Per Nest
Sex Ratio
Brood cells per nest
Female sex ratio
Time units required to cap nest (C)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Figure 4-4. Results produced in the forward iteration (simulation) by theoretical leafcutter bees (n=250) with increasing values of T (total amount of available time). A complete set of runs were done for two different chances of death. In Graph A, μ=0.02 and in Graph B, μ=0.001. Sex ratio is considered the mean proportion of daughters produced in a mother's lifetime. With very low T values there is a lot of variation because the end of time is the main controlling factor. With greater values of T, results stabilize as the decisions at the end of T become less important. The model considers the following parameters: μ - chance of death per time unit, T - total time available for the bees to build nests, P - time to collect a load of pollen and nectar, E - time to lay an egg of either sex and seal the brood cell, C - time required to cap (seal) the nest, NS - time required to locate a new nest site after capping nest, k - factor by which the value of the nest increases when capped. I incrementally increased the value of T, while all other parameters were set at baseline levels (P=1, E=3, C=4, NS=3, k=1.2).

A. μ=0.020



B. μ=0.001

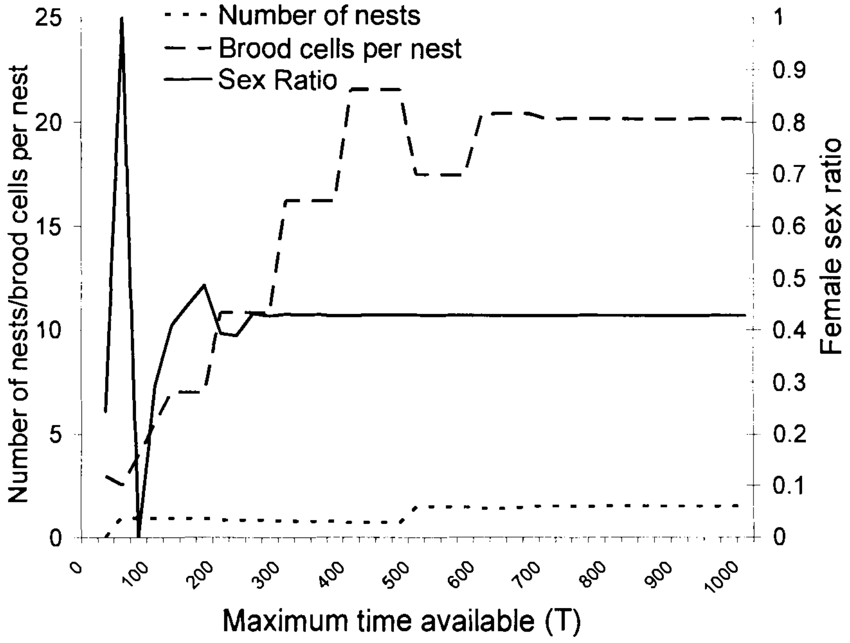
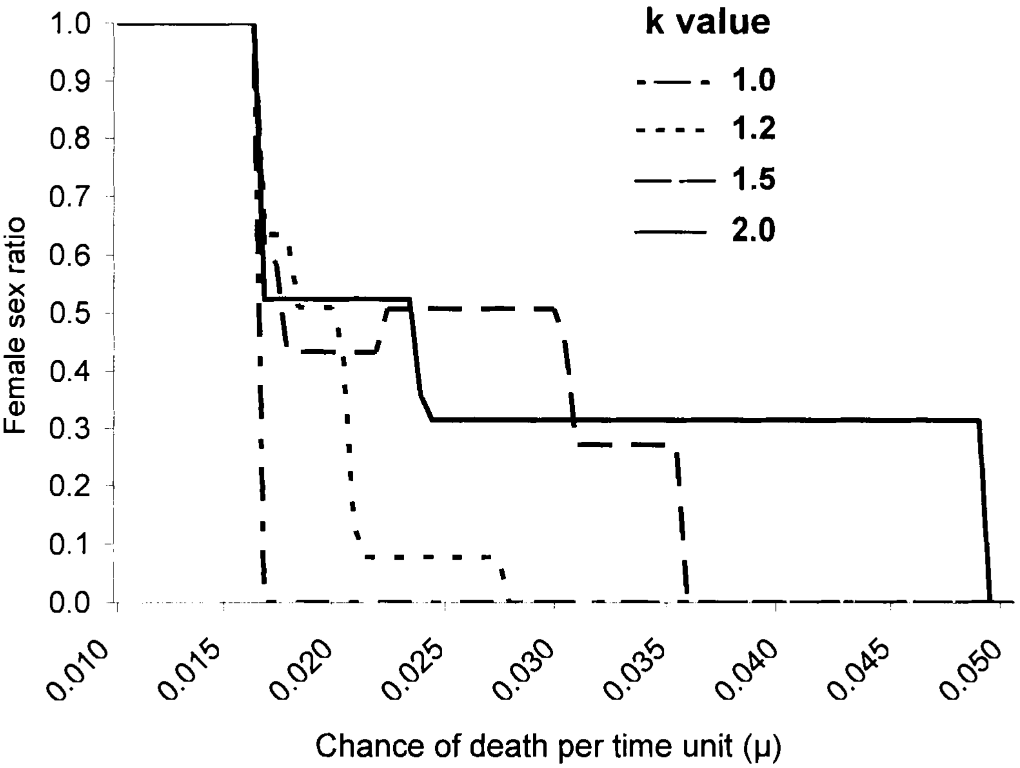
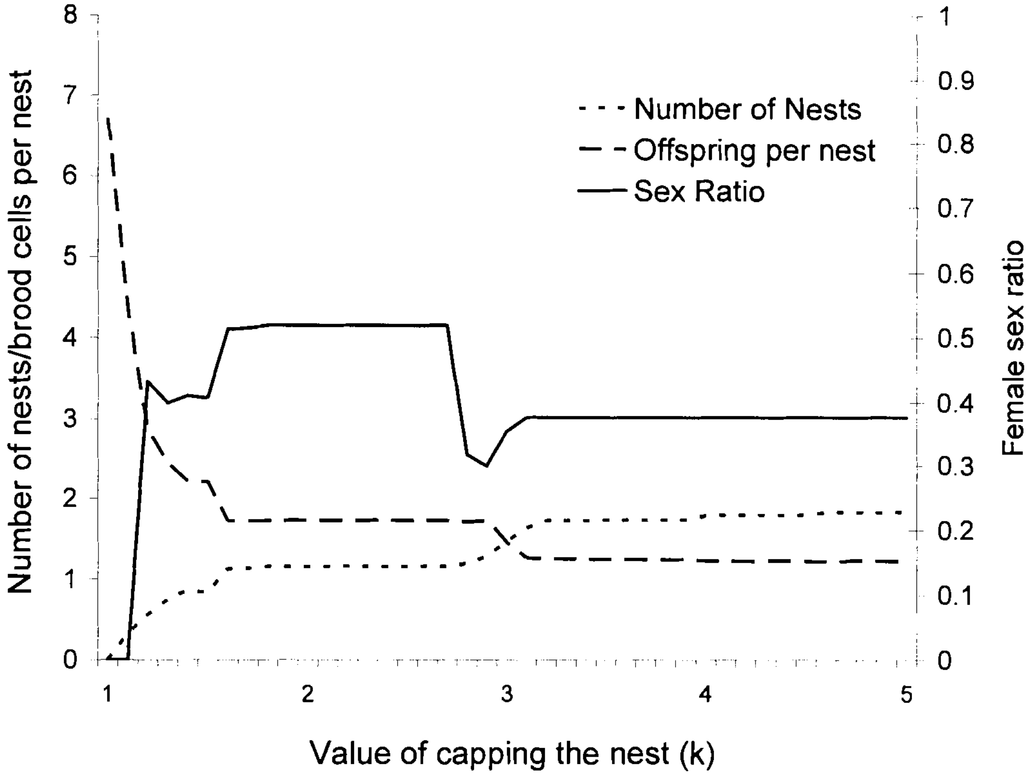


Figure 4-5. Sex ratio of offspring produced in the forward iteration (simulation) by theoretical leafcutter bees (n=250) with incremental increases in the chance of death (μ), using a dynamic state variable model. Each line represents a different value of k (increase in nest value when capped (sealed)). Multiple runs of the model were carried out, incrementally increasing the chance of death (μ) for each k value. Sex ratio is considered the average proportion of daughters produced in a mother's lifetime. The model considers the following parameters: μ - chance of death per time unit, T - total time available for the bees to build nests, P - time to collect a load of pollen and nectar, E - time to lay an egg of either sex and seal the brood cell, C - time required to cap (seal) the nest, NS - time required to locate new nest site after capping nest, k - factor by which the value of the nest increases when capped. I incrementally increased the value of μ, while all other parameters were set at baseline levels (T=250, P=1, E=3, C=4, NS=3).



k value
1.0
1.2
1.5
2.0
Female sex ratio
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.010
0.015
0.020
0.025
0.030
0.035
0.040
0.045
0.050
Chance of death per time unit (μ)

Figure 4-6. Results produced in the forward iteration (simulation) by theoretical leafcutter bees (n=250), with increasing bonus value from sealing a nest (k). A complete run was done for each value of k, and k was incrementally increased 0.1 at a time. Sex ratio is considered the mean proportion of daughters produced in a mother's lifetime. The model considers the following parameters: μ - chance of death per time unit, T - total time available for the bees to build nests, P - time to collect a load of pollen and nectar, E - time to lay an egg of either sex and seal the brood cell, C - time required to cap (seal) the nest, NS - time required to locate new nest site after capping nest, k - factor by which the value of the nest increases when capped. I incrementally increased the value of k, while all other parameters were set at baseline levels (T=250, μ-0.02, P=1, E=3, C=4, NS=3 k=1.2).



8
7
6
5
4
3
2
1
0
Number of nests/brood cells per nest
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Female sex ratio
- - - Number of Nests
— — Offspring per nest
—— Sex Ratio
1
2
3
4
5
Value of capping the nest (k)

Figure 4-7. Results produced in the forward iteration (simulation) by theoretical leafcutter bees (n=250), with increasing amount of time required to find a new nest after completing a nest. Sex ratio is considered the mean proportion of daughters produced in a mother's lifetime. The model considers the following parameters: μ - chance of death per time unit, T - total time available for the bees to build nests, P - time to collect a load of pollen and nectar, E - time to lay an egg of either sex and seal the brood cell, C - time required to cap (seal) the nest, NS - time required to locate new nest site after capping nest, k - factor by which the value of the nest increases when capped. I incrementally increased the amount of time required to find a new nest, while all other parameters were set at baseline levels (T=250, μ-0.02, P=1, E=3, C=4 k=1.2).

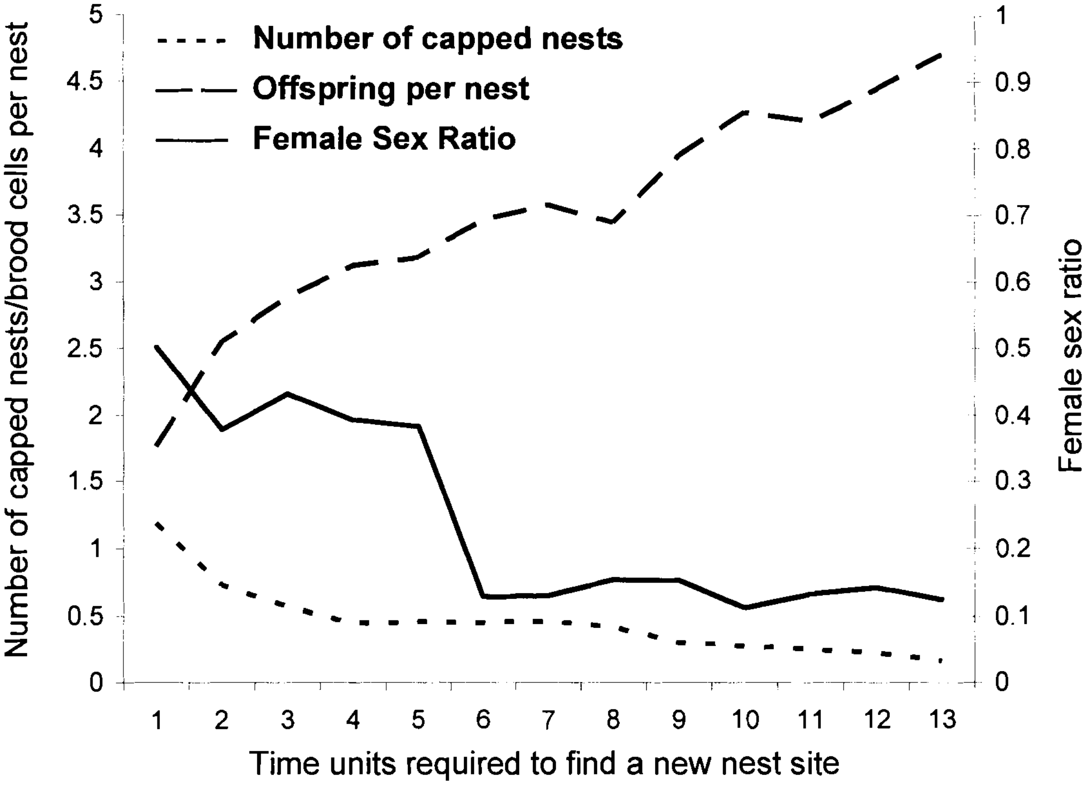

Number of capped nests
Offspring per nest
Female Sex Ratio
Number of capped nests/brood cells per nest
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Female sex ratio
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
5
6
7
8
9
10
11
12
13
Time units required to find a new nest site

Table 4-1. Sensitivity analysis for the dynamic state variable model, testing the impacts of risk and the time required to carry out various nest building activities. Sex ratio is considered the mean proportion of daughters produced in a mother's lifetime. The model considers the following parameters: μ - chance of death per time unit, T - total time available for the bees to build nests, P - time to collect a load of pollen and nectar, E - time to lay an egg of either sex and seal the brood cell, C - time required to cap (seal) the nest, k - factor by which the value of the nest increases when capped. The baseline values for each parameter are: T=250, μ-0.02, P=1, E=3, C=4, k=1.2). (Size values that are missing from the optimal size columns are because no offspring of that sex were produced.)

Description	Optimal Size		Female Sex Ratio	Nest Size (# of cells)
	Daughters	Sons		
Baseline run	6	4	0.43	2.8
μ=0.001	6		1	13.6
μ=0.010	6	4	0.99	4.9
μ=0.03	6	4	0.00	3
T=125	6	4	0.41	3.0
T=500	6	4	0.43	2.8
T=1000	6	4	0.43	2.8
P=0.5	6	-	1	-
P=2	-	4	0	2.0
P=4	-	4	0	1.4
E=2	-	4	0	5.6
E=4	6	-	1	2.4
E=6	7	-	1	1.5
E=8	8	-	1	1.4
C=2	6	4	0.41	2.7
C=6	6	4	0.38	3.0
C=12	6	4	0.11	3.4
k=1.0	6	4	0	6.6
k=1.5	6	4	0.42	2.2
k=2.0	6	4	0.52	2.7
k=3.0	6	4	0.36	1.5

Chapter 5
Thesis Summary and Suggested Future Work

5.1 INTRODUCTION

Sexual organisms are faced with the question of how to allocate investment between offspring of the two sexes. The goal of this thesis was to elucidate how ecological factors impact maternal allocation decisions related to the size, sex and number of offspring produced. This was accomplished through both empirical and theoretical work on the alfalfa leafcutter bee, *Megachile rotundata.* This chapter summarizes the thesis and suggests approaches for future work on sex allocation.

5.2 THESIS SUMMARY

In this thesis I examined how ecological factors can impact resource allocation and sex allocation in offspring. *M. rotundata* was used to examine these questions both empirically and theoretically. Previous work has suggested that resource levels can alter sex ratio and possibly resource allocation in solitary bees (Tepedino and Torchio, 1982; Rosenheim et al., 1996; Kim, 1999). The suggestion from this previous work was that when females are the larger sex and require greater investment, a decrease in resource levels results in a greater proportion of males being produced. This type of alteration in the sex ratio has been suggested to be a result of different maternal fitness payoffs from the two sexes (Rosenheim et al., 1996).

In the first chapter I introduced some of the basic concepts of sex ratio theory. The subsequent chapters addressed sex allocation with experimental work. In Chapter 2 I examined the impact of three different resource levels on sex allocation in the field using bees confined to covered tents. The results of this work showed no significant difference in the nest sex ratios between the treatments. There was, however, a significant increase in resource allocation to the average daughter, but not son. Previous work has also suggested that the investment in daughters may change more under different resource levels than investment in sons (Kim, 1999). This type of result may be related to the shape of the maternal fitness curves for each sex and how these are altered by changes in resource availability. Despite what some authors have found, I did not see an alteration in nest sex ratio with changing resource levels. This is possibly because all three of the treatments represented low resource conditions. During the season, by late morning very few flowers remained available for pollen and nectar exploitation. It is possible that changes in the sex ratio would require larger differences between treatments or possibly simply higher levels of resources across the treatments to cause an observable effect. This notion is partly supported by the fact that all treatments had sex ratios that were heavily skewed toward the production of sons.

In the third chapter I looked at flight distance to resources as a factor that may alter sex allocation decisions. I predicted that increased flight distance would result in a decrease in the proportion of daughters produced, the investment in individual offspring, and in the total number of offspring produced. Although the predictions are similar to those in Chapter 2, flight distance likely affects bees differently than changes in resource levels. When resource levels are low foraging females may simply determine that no resources are available and spend more time in the nest. Increased flight distance requires all bees to invest extra effort to obtain resources. By increasing the flight distance to pollen, nectar and nest construction materials (leaves) I increased the amount of investment required to provision offspring. This difference between resources levels and flight distance may also explain differences in results between the two experiments. Here I found a significant decrease in the female sex ratio, brood cells per nest and investment per daughter and son. Increasing the investment required to produce a single offspring decreased the resources that were allocated to each individual offspring. A greater proportion of sons were produced, requiring less investment on average compared to daughters. Armed with information about the various activities involved in producing brood cells, I moved to developing a computer model to analyse a wider range of factors that may impact sex allocation.

In Chapter 4 I used a theoretical approach to look at how a number of different factors affect sex allocation and the interactions between them. The dynamic state variable model enabled me to examine both the current and future implications of allocation decisions. I examined the effects of time required to carry out various nest building activities, the risk of mortality, length of the season, the value of capping the nest and the shape of the maternal fitness return curves. The model predicted an optimal strategy based on specific values for these characteristics. I ran the model numerous times varying a single characteristic per run to examine the implications on sex allocation. The amount of resources allocated to both sons and daughters was stable across a wide variety of assumptions and appeared to be controlled mainly by the shape of the maternal fitness return curves. By contrast, the sex ratio of offspring was influenced by most of these factors; in general, the greater the investment required to obtain a load of resources, the greater the proportion of sons produced.

The dynamic state variable model gave me the ability to run thousands of simulations, looking at numerous factors, and to determine possible approaches for future work. In Chapter 2 and 3 I discovered changes in resource investment with both changes in the amount of resources available and the flight distance required to obtain resources. The dynamic state variable model suggests that this is the result of changes in the shape of the maternal fitness payoffs for each sex as resource accessibility changes. If this is true, decreasing resource accessibility decreases the slope of the maternal payoff curves and decreases the optimal amount of resources to invest per offspring. The number of offspring produced decreased as resource availability decreased, in both theoretical and empirical work. This is likely because, although the amount of resources invested per offspring decreased, more time and effort was required to obtain those resources. As a result, mothers were unable to provision as many offspring as under higher resource conditions. The amount the mother invested per offspring may actually increase when resource accessibility is limited, due to increased effort. The actual amount of resources invested decreases as a result of the extra time and effort required to obtain those resources.

5.3 SUGGESTIONS FOR FUTURE WORK

One of the major challenges for the future is to understand the exact shape of the maternal fitness return curves for sons and daughters and how these curves change under different ecological conditions. I have assumed maternal fitness return curves for sons and daughters that are similar to those predicted by Rosenheim et al. (1996). However, there are few empirical data to determine the exact nature of these curves. Some of the challenges related to constructing these curves are determining the most appropriate proxy for maternal fitness and determining how parental investment is to be measured. Understanding these curves would give us the ability to test much more specific hypotheses related to sex allocation. This would allow for an understanding of the degree to which the shape of these curves influences sex allocation decisions.

A better understanding of the maternal fitness curves will also give us the ability to address other questions such as (1) which allocation decisions are most sensitive to changes in ecological conditions, and (2) which ecological conditions are most influential in maternal allocation decisions? My work on resource levels in Chapter 2 suggests the possibility that allocation to daughters and the total number of offspring produced are more sensitive to resource levels than are other sex allocation decisions. Sex ratio and investment in sons may be less sensitive to resource levels or simply be insensitive to this ecological factor. However, past work suggests that sex ratio is affected by resource levels (Torchio and Tepedino, 1980; Kim, 1999). Therefore, experiments need to be carried out that look at a wider range of treatment levels, in order to evaluate this issue of sensitivity. This information will allow us to understand behavioural reproductive decisions, as they relate to an organism's ecological condition, in an evolutionary context.

We currently do not understand the exact features of resource levels and flight distance that result in changes in sex allocation decisions. I therefore do not yet know whether the sensitivity of different allocation decisions is the same for resource levels, flight distance or other ecological factors. Is allocation to daughters more sensitive to ecological factors than allocation to sons? Are the changes in sex allocation I found simply a response to the organism's general assessment of resource availability or are the sex allocation changes reported in Chapters 2 and 3 actually a response to independent factors? When extra flight distance is required to obtain resources, there may be other factors not considered here such as increased wing damage and higher risk of predation. These factors may make it more important to complete brood cells as quickly as possible, which is best done by producing small males.

Finally, can my insights from leafcutter bees be generalized to other groups of organisms? To understand this we need a better understanding of the degree of maternal control over offspring sex in other groups of organisms. The results here suggest an evolutionary argument for facultative sex allocation, as mothers appear to be able to increase their fitness by altering sex allocation based on current conditions. However, we do not have a complete understanding of the capability of other groups to control the sex of their offspring. Evolutionarily, facultative sex allocation would appear to be beneficial in organisms where the maternal payoff for offspring varies depending on sex and these benefits change depending on conditions. In this situation the ability to produce the more beneficial offspring would improve the fitness of the parents. Numerous taxa have been shown to be more likely to produce a certain sex of offspring depending on conditions (Paul and Kuester, 1987; Maestripieri, 2001; Clutton-Brock et al., 1984). These findings suggest that other groups do have the capability of altering the sex of their offspring; however in many cases, we do not understand the mechanism for doing so.

Leafcutter bees mitigate resource deficiencies by altering sex allocation strategies. In the light of differing ecological conditions, these bees appear to have evolved the ability to alter the number and size of offspring based on the sex of the progeny. There are numerous other groups in which the questions asked here need to be addressed so that we can better understand sex allocation in a wider variety of taxa.

5.4 REFERENCES

Clutton-Brock, T.H., S.D. Albon, and F.E. Guinness. 1984. Maternal dominance, breeding success and birth sex ratios in red deer. Nature 308:358-360.

Kim, J-Y. 1999. Influence of resource level on maternal investment in a leaf-cutter bee (Hymenoptera: Megachilidae). Behavioral Ecology 10:552-556.

Maestripieri, D. 2001. Female-biased maternal investment in rhesus macaques. Folia Primatologica 72:44-47.

Paul, A. and J. Kuester. 1987. Dominance, kinship and reproductive value in female barbary macaques (*Macaca sylvanus*) at Affenberg Salem. Behavioral Ecology and Sociobiology 21:323-331.

Rosenheim, J.A., P. Nonacs and M. Mangel. 1996. Sex ratios and multifaceted parental investment. American Naturalist 148:501-535.

Tepedino, V.J. and P.F. Torchio. 1982. Phenotypic variability in nesting success among *Osmia lignaria propinqua* females in a glasshouse environment (Hymenoptera: Megachilidae). Ecological Entomology 7:453-462.

APPENDIX A
Description of Model

To qualitatively explore the decisions involved in the nest construction of the solitary bees, a dynamic state variable model was used (Clark and Mangel, 2000), using *Megachile rotundata* as a model species. This approach provides a means of calculating optimal decisions and seeing how these decisions change across different combinations of state variables. The model works under the assumption that the focal organism will choose the decisions that result in the highest total fitness. Fitness of the test subject at the end of the available time (T) for all combinations of state variables is 0 (terminal fitness function) because no time is available to accrue further fitness (i.e., the bee dies). To determine the optimal decision and fitness of that decision for a given combination of states, the model is solved through backwards induction from time $t = T$. The fitness at time $t = T-1$ is calculated as the fitness at time T, multiplied by the probability of surviving from T-1 to T. However, fitness at time T will depend on state variable values; therefore, we must take into account any changes in state variables between T-1 and T. As a number of time units are required to produce an offspring, potential fitness will remain zero until we go back in time far enough to allow for sufficient time to produce an offspring.

A simple example is a situation where a mother has a fitness value of 5 at T-1 if she caps (seals) a nest at time T-2, then the fitness value at T-2 is 5, discounted by the chance of surviving to T-1. The process is repeated for T-2, T-3, T-4 and so on (backwards induction) for the entire range of time values. This provides a matrix of fitness values for each unique combination of time and values of the state variables. Thus, different behavioural decisions may lead to different state variable values, subsequently leading to different fitness values. The state variables in the model are pollen state (p), nest state (v) and time (t). In each time unit the bee experiences a fixed probability of death. For each time unit, the choice of decisions are whether to (1) add more pollen to the current brood cell, (2) lay a male egg and complete the brood cell, (3) lay a female egg and complete the brood cell, or (4) cap the nest and search for a new nesting site. Some of these behaviours take more than one unit of time, thus certain decisions will take the bee greater than one time unit into the future. The decisions are mutually exclusive, and once a decision has been made, the activity must be completed.

The matrix construction process described earlier stores values of decisions for integer values of nest states. There are two matrices: one stores optimal decisions and the other stores fitness values for those decisions. During the process of determining the optimal behaviour, decisions can be made that cause the bee's fitness (nest value) to be increased by a fractional value. Here, linear interpolation is used to calculate the fitness (Mangel and Clark, 1988). The range of state variables are set large enough to allow sufficient resolution to see changes occur when conditions are altered (pollen values 1-15; nest values 1-50; time 1-250). At T (end of time) all bees are assumed to die (end of the season). The dynamic programming equation is thus:

$$\begin{aligned} F(p,v,t)=\max\,[&(1-\mu_p)\, F(p+1,\, v,\, t+t_p); \\ &f_m + (1-\mu_p)^{tm}\, F(0, v+o_m(p),\, t+t_m); \\ &f_f + (1-\mu_p)^{tf}\, F(0, v+o_f(p),\, t+t_f); \\ &(1-\mu_p)^{ts}\, k_v + (1-\mu_n)^{tn}\, F(0,\, 0,\, t+t_n)] \end{aligned} \qquad (1a)$$

The times required to carry out the various behaviours are t_p (time to collect a pollen load), t_m (time to lay a male egg and seal the brood cell), t_f (time to lay a female egg and seal the brood cell), and t_c (time required to cap the nest). F(p,v,t) represents the expected lifetime fitness of the mother at time t, with a certain pollen value in the current brood cell and with the current value of her nest. There are fitness increments for the final three options, therefore f_m and f_f are the fitness increments of producing a male and a female, while k_v is the fitness increment of capping the nest at the current value of the nest. $1-\mu$ is the chance of survival in each time unit and the subscript on μ indicates chance of death for that particular behaviour (the basic model uses the same μ value for all activities). This value is then raised to the power of the time the activity will take. After capping the nest, a number of time units are required to find another nest site (t_n). The basic parameter values are estimated when data were not available; values were chosen which would produce biologically meaningful results (e.g., reasonable number of offspring produced in a lifetime), making sensitivity analysis more informative.

At any state, the bee has the option of choosing to perform any of several defined behaviours (forage for pollen, lay an egg or cap (seal) the nest). By examining optimal decisions as a function of time, one can determine the general sequence of optimal decisions that each bee will make. The optimal sequence includes bees starting a brood cell by adding provisions (pollen). After some number of pollen loads have been collected (pollen state) a decision is made to lay a son or a daughter. After this is completed the bee can either provision a new cell with pollen or cap the nest. When a nest is sealed the bee then spends time searching for a new nesting site and beginning the process again.

Fitness Function from offspring production

Empirical curves for fitness as a function of the size and sex of offspring are usually not available. However a number of authors have proposed fitness curves for species such as *M. rotundata* which have larger females than males (Frank, 1995; Rosenheim et al., 1996). The general shape of these maternal fitness returns for producing sons and daughters of increasing size are summarized in Figure 4-1. I assume there is some minimum level of food resources needed before a viable offspring can be produced and that this minimum is lower for the smaller sex, males. As a non-territorial species, the fitness of daughters is expected to increase at a faster rate than that for males, as increased size results in increased egg capacity and the ability to provision offspring at a higher rate.

$$F(m) = (-4 + (a_m (1 - e^{-(p\gamma_m)}))) / 4; \qquad (2a)$$

$$F(f) = (-6 + (a_f (1 - e^{-(p\gamma_f)}))) / 4; \qquad (3a)$$

where 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.

I also tested fitness functions that were linear with the male curve having an earlier x intercept and the female curve having a greater slope and higher asymptote.

Risk of death

There are numerous mortality factors that can impact mothers while they are producing a nest. There were generalized into a background mortality factor that assumes there is a given chance of death for every time unit, allowing the model to be more generally applicable. Therefore the chance of death for a given activity is a function of how long it takes to carry out that activity. This assumption seems reasonable because all decisions require the bee to carry out foraging where they are exposed to predators and parasitoids.

Simulation Model

The dynamic program produces a matrix of decisions for every possible combination of pollen, nest and time state. To gain an appreciation of offspring production at the population level, a stochastic forward iteration program was developed to follow a group of bees (n=250) from t=1 until the end of time (T) or whenever the individual bee dies. The one stochastic element in the model is the chance of death, so the mother will always follow the same path but multiple runs are required because the mother will die at different points along the optimal path. When a nest is capped, the time taken to locate a new nest is randomly drawn from a bell curve of values. In this case the time to find a new nest varies between bees and therefore each bee will be sent to a new location within the matrix depending on the time required to find a new nest.

Limitations

This model is basically heuristic. However, where possible, real time values were used in an attempt to more closely represent reality. This is an individual-based tactical model that does not take into account the decisions made by other females in the population (i.e., no frequency dependence). I assume that the sex ratio of the breeding population as a whole is stable and that the population is large and therefore the relative values of sons and daughters are not being altered by one sex becoming more rare.

The range of pollen and nest values did put some limits on the parameter values that could be used. However, the range was made large enough that the maximum values would not be reached using what appear to be biologically reasonable values for the parameters (i.e., a nest with a value of v=50, requires 75 large offspring). Bees were also not permitted to choose decisions for which there was not enough time remaining to complete, i.e., focal individuals were not allowed to exceed the maximum time (T), decisions near the end of time were very much influenced by maximizing the remaining time. This did not, however, influence results as the baseline values for life duration (T=250) and the chance of death per time unit (μ=0.02) meant that during the simulation very few bees lived long enough to be constrained by the end of time.

References

Clark, C.W. and M. Mangel. 2000. Dynamic state variable models in ecology: Methods and applications. Oxford University Press, New York.

Frank, S.A. 1995. Sex allocation in solitary bees and wasps. American Naturalist 146:316-323.

Mangel, M. and W.C. Clark. 1988. Dynamic modeling in behavioral ecology. Princeton University Press, Princeton.

Rosenheim, J.A., P. Nonacs and M. Mangel. 1996. Sex ratios and multifaceted parental investment. American Naturalist 148:501-535.

APPENDIX B
Computer Code for Model

See CD in inside back cover.