uOttawa

L'Université canadienne
Canada's university

FACULTÉ DES ÉTUDES SUPÉRIEURES ET POSTOCTORALES

uOttawa

L'Université canadienne
Canada's university

FACULTY OF GRADUATE AND POSDOCTORAL STUDIES

Dalit Weinberg

AUTEUR DE LA THÈSE / AUTHOR OF THESIS

Ph.D. (Psychology)

GRADE / DEGREE

School of Psychology

FACULTÉ, ÉCOLE, DÉPARTEMENT / FACULTY, SCHOOL, DEPARTMENT

Simultaneous Deprivation of Pollen and Nectar: Pollen Collection Behaviour and Development in Bumblebee Colonies (*Bombus impatiens cresson)*

TITRE DE LA THÈSE / TITLE OF THESIS

Catherine Plowright

DIRECTEUR (DIRECTRICE) DE LA THÈSE / THESIS SUPERVISOR

CO-DIRECTEUR (CO-DIRECTRICE) DE LA THÈSE / THESIS CO-SUPERVISOR

EXAMINATEURS (EXAMINATRICES) DE LA THÈSE / THESIS EXAMINERS

C. Bielajew

J. Picman

R. Dukas (teleconference)

G. Fouriezos

Gary W. Slater

Le Doyen de la Faculté des études supérieures et postdoctorales / Dean of the Faculty of Graduate and Postdoctoral Studies

Simultaneous Deprivation of Pollen and Nectar: Pollen Collection Behaviour and Development in Bumblebee Colonies (*Bombus impatiens* Cresson)

Dalit Weinberg

Thesis submitted to the

Faculty of Graduate and Postdoctoral Studies

In partial fulfillment of the requirements

For the PhD in Clinical Psychology

School of Psychology

Faculty of Social Sciences

University of Ottawa

Your file Votre référence
ISBN: 978-0-494-34152-0
Our file Notre référence
ISBN: 978-0-494-34152-0

To my parents, Dora and Zvi, with love and admiration

TABLE OF CONTENTS

Acknowledgments

I was very fortunate to be surrounded and supported by people who helped me feel at home away from home. Although far away, my parents were here with me all along. Their daily spiritual support, constant interest in my work, and never-ending trust in my abilities to finish what was started fuelled my hopes and determination. They have been my source of inspiration, comfort, and pillars of support throughout my life and never showed a sign of fatigue... Spasiba Mama, Spasiba Papa.

My supervisor, Dr. Catherine Plowright, was the first person to greet me into the program. I remember the day we met. She was soft-spoken, gently interacting with students (and stinging bees!), and everything about her said "motherly". And she was. Supportive, caring, compassionate, encouraging, constructive, celebratory; Catherine, my "second mother", had all the wonderful qualities good supervisors (and good parents) posses. She taught me to love working with bees and I loved working with her. Thank you Catherine.

Thank you to my "second sister", Natasha, for cheering me and being there for me when I needed you the most. Thank you to Danni, for encouraging and taking interest in my work throughout most of the years of this project—you will always have a special place in my heart. A warm thank you to my former and present lab mates Ginny, France, Dana, Martine, Emily, and Mathieu, who made me forget that I was actually working...

My committee members Dr. Kate Bielajew, Dr. Jeroslav Picman, and Dr. George Fouriezos, were a great help in shaping and improving my research. Thank you all.

Abstract

Barely touched on and resulting in unsettled findings in previous research, the effect of simultaneous deprivation of pollen and nectar on pollen collection in bumblebee colonies is investigated in the present thesis. This investigation is further expanded to include the roles of colony growth and foraging experience in mediating the effect of deprivation on foraging behaviour. In all experiments colonies were presented with one of two treatment conditions: Pollen-deprivation (-P) or pollen-and nectar-deprivation (-P-N). In Study One, the effect of type of deprivation on colony growth was examined. Results showed that colony growth in -P-N did not differ from colony growth in -P. Study Two includes two experiments. Experiment 1 examined the role of prior foraging tasks combined with resource deprivation in pollen collection. Colonies were first assigned to either -P or -P-N treatment conditions and were subsequently tested while deprived of both resources (-P-N). Colonies that had experience in managing both foraging tasks collected more pollen and allocated greater foraging effort than did colonies that only had experience in collecting pollen. Experiment 2 examined the role of colony size combined with resource deprivation in pollen collection. Larger colonies collected more pollen although smaller colonies collected more pollen relative to their colony size. Taken together, these experiments provide a comprehensive understanding of the interrelatedness between colonies' nutritional requirements, foraging experience, colony development and foraging behaviour. Also, they clarify the effect of simultaneous deprivation on pollen collection. Contributions of this thesis are placed within the context of practical implications for greenhouse pollination, as well as research implications for bumblebees' foraging currencies and foraging decisions.

General Introduction

Bumblebee colonies require two resources to grow and survive: pollen and nectar (Free, 1993). Nectar provides most of the energy in the form of carbohydrates for activity and thermoregulation (Seeley & Heinrich, 1981) and pollen provides protein for egg production, promotes bees' growth and longevity, brood rearing, ovary development, body fat, and development of hypopharyngeral glands (Knox, Shimanuki & Herbert, 1971; Maurizio, 1950, 1951; Plowright & Pendrel, 1977; Ribeiro, Duchateau & Velthuis, 1996; Standifer, 1967; Sutcliffe & Plowright, 1988, 1990). Hence, nectar serves as an immediate energy resource and pollen serves as a long-term resource for growth and longevity (Heinrich, 1979; Ribeiro et al., 1996; Sutcliffe & Plowright, 1988, 1990). Given their pivotal role in colony survival, it is not surprising that pollen and nectar availability affect bumblebees' foraging behavior (Cartar & Dill 1990a; Cartar & Dill 1991; Free, 1955; Plowright, Thomson, Lefkovitch & Plowright, 1993), which is the focus of the present study.

In order to understand the interrelatedness of pollen and nectar availability, colony growth and foraging behavior in bumblebee colonies, it is important to understand their life cycle and division of labor. Bumblebees have two important periods in their annual reproductive cycle in which one generation of bumblebees is produced in each cycle. In the first period, the queen produces worker (female) eggs. During this period, the queen is responsible for collecting pollen and nectar, constructing food pots, warming, and feeding the first brood. When the first workers emerge, the social phase of the colony begins. The workers help the queen to rear other workers and collect food. In the second period of the colony's cycle (the reproductive period), production of sexuals (males and new queens) occurs. As in the first period, workers help to rear sexual reproductives. In the reproduction

phase, the colony has reached the end of its life cycle and rarely if ever returns to pure worker production. Younger queens leave the colony, then mate and go into hibernation during the winter. The young queens start new colonies in the following spring (Alford, 1975; Free & Butler, 1959; Macevicz & Oster, 1976; Michener, 1974; Plowright & Laverty, 1984; Sladen, 1912).

Workers, males, and queens have different roles in the colony. Research has suggested that workers may be classified according to their duties as foragers or non-foragers (Cartar, 1992; Free, 1955). Nevertheless, non-foraging household workers are capable of foraging, and they do so especially when the colony is in need of more resources. Foragers may be further distinguished according to their specific foraging activities as pollen collectors or nectar collectors. However, when in need, foragers can switch from pollen collection to nectar collection and vice versa (Cartar, 1992). Having said this, all workers are likely capable of foraging for all resources, yet at times some workers forage for one resource while others forage for another or do not forage at all. The queen (except for the period before the emergence of the first brood), new queens, and males do not have a role in resource collection for the colony, though they do forage for themselves (Free, 1955; Sladen, 1912).

Resource Availability and Colony Growth

Resource availability affects colony growth and development (Plowright & Jay, 1968). For example, larvae in colonies develop more slowly and become smaller workers as pollen availability decreases (Sutcliffe & Plowright, 1988, 1990). Similarly, queens in pollen-deprived colonies have a longer larval stage than queens in pollen-supplemented

colonies (Sutcliffe & Plowright, 1990), and pollen-supplemented larvae are more likely to be larger and become queens than pollen-deprived larvae (Katamaya, 1966, 1973, 1975; Plowright & Jay, 1977). When nectar-deprived, colonies have a lengthened developmental period resulting in smaller colony size at the time of reproduction (Cartar & Dill, 1991). Because reproduction depends on the number of workers in the colony (Pomeroy, 1979; Pomeroy & Plowright, 1982; Shykoff & Muller, 1995), the size of the worker population is associated with reproductive success and timing of the change from worker production to sexual production (Pomeroy, 1979; Pomeroy & Plowright, 1982). That is, new queens are produced sooner when more workers are present (Pomeroy & Plowright, 1982). In summary, pollen and nectar are vital resources to colony growth and development in that they affect larvae and workers' size, as well as its reproduction.

The relationship between resource availability and colony development is reciprocal: Resource availability determines colony's growth and development, and in turn, colony development determines resource intake. For example, larger larval biomass predicts higher pollen consumption (Pendrel & Plowright, 1981). Also, queens are heavier and so require more food intake and care (Fisher & Pomeroy, 1989), and therefore, queen larvae are fed more often during the last phase of the larval stage than are worker larvae (Ribeiro, 1997). In short, increased resource intake leads to an increase in colony growth, which results in a greater need for resource intake and consequently an increase in foraging activity.

Another factor associated with colony growth and resource collection is workers' size. Large workers are more likely to become foragers and small workers are more likely to become household workers (Reviewed in Goulson, 2003; Free, 1955b). Furthermore, recent findings show that larger body size in bumblebee workers is associated with a higher

foraging performance (Goulson et al., 2002; Peat & Goulson, 2005; Spaethe & Weidenmuller, 2002). Spaethe and Weidenmuller (2002) examined foraging activity in two free-flying bumblebee colonies and found a significant correlation between body mass and nectar foraging rate. Compared to small workers, large workers had higher nectar return by volume, perhaps because of their capacity to collect larger nectar loads per trip and handle larger flowers with large nectar stores. Similar results in another study showed that nectar loads per time unit are positively correlated with worker size (Peat and Goulson, 2005). These findings suggest that colonies in which large workers are produced may have a higher foraging activity than colonies with small workers.

In short, resource collection and colony growth are interrelated. Colonies with ample resource availability of either pollen or nectar produce larger workers and colonies. They are also more likely to have an early reproduction stage than resource-deprived colonies. In turn, large workers and large colonies maximize resource influx. Nevertheless, it is unclear how colony growth is affected by as well as affects pollen and nectar collection when both resources are in need. Which resource is more in demand in different developmental stages? Is it nectar as an immediate energy resource or is it pollen as a growth resource? And how does simultaneous deprivation of pollen and nectar affects colony growth? These questions are examined in this thesis.

Foraging Behaviours for Pollen and Nectar Collection

Pollen and nectar collection require different foraging behaviours. Bumblebees' pollen manipulation varies depending on flower's morphological structure. For example, when collecting pollen from flowers with challenge-access anthers, such as blueberry,

kiwifruit, cranberry, and tomato, bumblebees are required to "shake" the flower in order to release their pollen. They gather its anthers holding it tightly against their abdomen and thorax producing small vibrations, or a "buzz" sound (Buchmann, 1985). The "buzz", or the "shake", elicits resonance within the flowers' anther causing pollen to be ejected. On flowers with easy-access anthers, or "platform flowers" such as grass flowers, bumblebees scrabble for pollen (Heinrich, 1976). When scrabbling, bees may walk up on a flower's inflorescence as they become dusted with pollen, or they may press their bodies onto the inflorescence while rapidly walking on it, which, as noted earlier, depends on flower's structure. Following buzzing and scrabbling the flower for pollen, bumblebees groom and scrape pollen from their body and hind legs packing it on their corbiculae (reviewed in King, 1993 and in Rasheed and Harder, 1997).

To collect nectar, bumblebees extend their proboscis (i.e., the bee's "tongue") to access this resource by probing the flower's corrola. Although pollen and nectar collection require two distinct behaviours, bumblebees' probing and buzzing often co-occur when visiting flowers offering both of these resources (Heinrich, 1976). Resource collection, especially pollen collection, is energy costly in that it is physically demanding for bees to collect (i.e., requires grooming behaviour and buzzing on some flowers) and it is mainly used as a protein resource. Therefore, compared with nectar collection, pollen collection entails a relatively large energetic investment yet provides a poor utility as an energy source for bumblebee colonies (Rasheed & Harder, 1997).

Foraging efficiency

Bumblebees' success in resource collection may be affected by their different characteristics, such as innate physiological characteristics as well as their capability to adapt

to a variety of environmental conditions. With regard to the first factor, physiological features such as tongue length may affect bee's access to nectar in flowers. For example, short-tongued bees may be limited in their success in obtaining nectar from long-corolla flowers compared with long-tongued bees (Heinrich, 1976). Conversely, long-tongued bees are comparatively inefficient when foraging on short-corolla flowers (Plowright & Plowright, 1999). Another important physiological characteristic affecting foraging success is body size. Body size may limit bees' successful entrance to structurally complex flowers, and therefore, small bees handle structurally complex flowers more successfully and efficiently than large bees (Stout, 2000).

Foraging theory

With regard to bees' second characteristic affecting resource collection, the capability to adapt to the environment, foraging theories assert that bumblebees' foraging behaviour involves adaptive features to optimize and improve foraging efficiency. In his theory of classical optimal foraging, Charnov (1976a, 1976b, also see a review of the literature by Bateson & Kacelnik, 1998) presented a model in which long-term energy gain rate is a ratio of energy obtained from food to the expected time spent foraging. The model assumes that animals that maximize this currency are fit and more likely to survive and reproduce. Therefore, the more net energy an animal is gaining, the higher its probability to survive and allocate more energy into reproduction and predator avoidance. Also, according to this model, animals should display a foraging behaviour that will maximize their long-term rate of energy gain because it will result in a minimized foraging time and effort.

Elaborating on and modifying Charnov's model, risk sensitivity theorists (e.g., Stephens, 1981, refer to reviews by Bateson & Kacelnik, 1998, and Plowright & Laverty,

1984) argue that the function between energy gained to fitness is nonlinear, and therefore animals engage in foraging behaviours that are either risk-prone (i.e., preference towards a more variable option) or risk-averse (i.e., a preference towards a less variable option). Studies examining this notion mostly focus on bumblebee's nectar collection and address questions, such as what rules or "decisions" bumblebees use to modify their foraging behaviour, and what factors or information affect their foraging decisions. Early findings by Hodges (1981, 1985) as well as contemporary research (e.g., Niv, Joel, Meilijson, & Ruppin, 2002) suggest that bees' decisions in foraging behaviour are consistent with their expected rate of cost and benefits. For example, Hodges (1981) found that bees were likely to stay on a plant and visit another flower if nectar was available in the first flower, but they were likely to leave and forage elsewhere if nectar was unavailable.

In another study examining bumblebees' ability to discriminate between flowers, bees' choice of flowers depended on pollen quality and pollen quantity within the flower species (Robertson et al., 1999). Similarly, Keasar, Rashkovitch, Cohen, and Shmida (2002) found that bumblebees gradually increased their visits to a higher rewarding food source when presented with two food sources differing in reward probabilities. The bees did not, however, exclusively choose the higher rewarding food resource as would be predicted by optimality models (Krebs, Kacelnik, & Taylor, 1978). Instead, their choice ratio was in tandem with resources' ratio of reward probability. Keasar et al. (2002) suggest that in terms of proximate causes of behaviour, the foraging choices of rewarding flowers depend on past experiences and may involve long-term memory processes. In general, explaining discrepancies between optimality models and observed behaviours is an area of fruitful

collaboration between behavioural ecologists and psychologists (e.g., Shettleworth, 1988, 1989).

The role of experience

Heinrich (1976) suggested that bumblebees' foraging behaviour is flexible and hence adaptive because their habitat's environmental conditions are generally unpredictable. He described bumblebee colonies as "floral generalists" and their workers as "specialists", that is, bumblebees at the colony level forage on a large variety of flowers efficiently, but at the individual level they have a limited behavioural repertoire and tend to concentrate on a narrow spectrum of flowers[1]. Heinrich argued that this social structure of bumblebee colonies, in which colonies are both floral generalists and specialists provides them with the plasticity to adapt their foraging behavior to food resources in the environment and maximize resource influx into the colony. Furthermore, he postulated that despite their limited foraging repertoires as specialists, bumblebee workers develop their foraging "know-how" following repeated interactions with the environment. In his examination of bumblebees' foraging acquisition skills, Heinrich (1976) found that inexperienced bumblebee workers were unsuccessful in their first foraging attempts on monkshood flowers. However, once made a correct entry to the flower and were rewarded with a successful resource collection, workers made successive visiting trips in which they efficiently manipulated these flowers (see also Laverty, 1980; Laverty & Plowright, 1988; and a review by Plowright & Laverty, 1984).

In accord with Heinrich's and Hodges' classic work on bumblebee foraging behavior, contemporary research findings confirm that bumblebees' foraging behaviour is

[1] The terms "generalist" and "specialist" here are used loosely; the only known specialist bumblebee is *Bombus consobrinus* that feeds primarily on monkshood, unlike other bumblebees that feed on a variety of flowers (Dukas, 1998).

modified and foraging efficiency is maximized following experience in resource collection (Cresswell & Robertson, 1994; Harder, 1990; Keasar et al., 2002; Peat & Goulson, 2005; Rasheed & Harder, 1997; Robertson et al., 1999). For example, bumblebees detect and respond to differences between flowers in their revisits according to nectar and pollen quality and availability (Cresswell & Robertson, 1994; Harder, 1990; Robertson et al., 1999).

Other studies illustrating the effect of experience on bumblebees' foraging behavior examined the adaptation of naïve bumblebees to resource collection (Cartar, 1992; Heinrich, 1976; Peat & Goulson, 2005). Peat and Goulson (2005) examined factors influencing nectar collection in three colonies of free-flying bumblebees. Nectar-foraging rate for each trip was calculated by pairing out-goings and in-comings for each bee returning with nectar, which was evaluated by weighing bees before and after the trip. During their first few trips, naïve bees had low foraging rates. Moreover, they lost more energy (i.e., weight) than they collected (i.e., nectar). However, as time passed, foraging rates increased and reached a plateau after approximately 30 trips. Congruent with findings in Peat and Goulson (2005), other research findings show that inexperienced bumblebee workers are less efficient than forage-experienced workers (Heinrich, 1976, Cartar, 1992). For example, Cartar (1992) examined foraging effort and task switching in energy-manipulated bumblebee colonies. Following nectar depletion, some workers that typically collected pollen switched to nectar collection and household workers that did not forage prior to manipulation, switched to foraging. Switching workers were less efficient in resource collection than continuing foragers in that they made longer and fewer trips and collected smaller pollen and nectar loads. Interestingly, switching-task workers were the least productive workers pre-and post-

manipulation. Based on these and other research findings (Free, 1955), Cartar (1992) suggested that bumblebees' foraging may involve a learning component in the efficiency of task performance, and therefore, foragers that have reached competency in foraging for one resource may be less likely to engage in a novel foraging task.

To summarize the above, experience has an important role in shaping bumblebee's foraging behavior. Bumblebees choose flowers based on previous visits and increase their visits to higher-rewarding resources. In addition, findings suggest that foraging behaviour is a malleable skill in that foraging efficiency increases with number of trips. In their adjustment to resource manipulation, workers' differences in foraging abilities depend on their pre-manipulation skills. The present study examines the role of experience in pollen collection by foraging bumblebees, which, compared to nectar collection, has received little attention by foraging theorists (Plowright & Plowright, 1999). The next section discusses in further detail the effect of resource manipulation on foraging.

Resource Deprivation and Foraging Behaviour

Deprivation of either pollen or nectar

Bumblebees' foraging behaviour is sensitive to changes in colony resource reserves (Cartar, 1991; Cartar & Dill, 1990a; Cartar & Dill 1991; Free, 1955; Plowright et al., 1993). Studies examining the relationship between resources and foraging behaviour typically manipulate resource availability in colonies using one of three treatments. In the pollen-deprivation treatment the reserves of pollen are removed from the colony by the experimenter, and the colony forages for the resource in need (pollen) outside of the colony. In a second treatment, nectar-deprivation, nectar reserves are removed from the colony, and

the colony forages for the resource in need (nectar) outside of the colony (for example of both treatment conditions see Plowright et al., 1993). The third treatment, pollen-and nectar-deprivation, is a simultaneous deprivation of both resources within the colony. The colony forages for both resources in need outside of the colony (for example see Cartar, 1992; Plowright & Silverman, 2000). Usually, these treatment conditions are compared to conditions in which the colony is supplemented (i.e., non-deprived) with pollen, nectar, or both.

Bumblebees modify their foraging behaviour following resource manipulation. When pollen-deprived, bumblebee colonies collect more pollen in comparison to nectar-deprived and pollen-supplemented colonies (Landry, Plowright & Plowright, 2000; Plowright et al., 1993; Plowright, Cohen-Salmon, Landry & Simonds, 1999). For example, Plowright et al., (1993) examined the effects of pollen-deprivation and nectar-deprivation in two bumblebee colonies. Pollen or nectar stores were removed on alternate days and colonies were to forage freely for the needed resource at a field site. Pollen collection was influenced by pollen reserves within colonies with workers' pollen load size significantly increased in their incoming trips when the colony was pollen-deprived compared to when it was nectar deprived.

In a follow-up study, Plowright et al., (1999) examined the question of how the response to pollen deprivation was achieved. They compared the effects of pollen deprivation vs. nectar deprivation on workers' flower handling and choice in two experiments. In the first experiment, colonies were resource-manipulated on alternate days (e.g., days 1 and 2 pollen deprived, days 3 and 4 nectar deprived) and presented with fresh and day-old thistle flowers as their foraging source. Type of deprivation affected colonies'

foraging behaviour in two ways. When the colony was pollen-deprived, workers opted for day-old flowers over fresh flowers for pollen scrabbling, perhaps because dry pollen was easier to gather. Also, the relative frequency of scrabbling for pollen rather than probing for nectar was greater when the colony was deprived of pollen.

In their second experiment, Plowright et al., (1999) replicated colonies' resource manipulation (i.e., pollen deprivation vs. nectar deprivation), but replaced the foraging resource (i.e., thistle flowers) with artificial flowers containing either pollen or pollen-and nectar stores. As in the first experiment, colonies' flower handling and choice depended on type of deprivation. Scrabbling behavior was significantly elevated and probing was much less frequent in the pollen deprivation condition than in the nectar deprivation condition. The bees also tended to avoid flowers that contained no pollen when the colony was pollen-deprived. In short, research findings show that when pollen-deprived, colonies collect larger pollen loads: This occurs because workers choose pollen-rich flowers and they engage in floral handling behaviours (buzzing and scrabbling) associated with pollen collection.

Deprivation of both pollen and nectar

Although findings in previous studies show that pollen deprivation leads to increased pollen collection and nectar deprivation leads to increased nectar collection, they do not predict how deprivation of one resource affects collection of the other. In other words, previous studies, by studying the two resources in isolation, are silent on the interrelationship between pollen and nectar collection. Having said this, which resource is sought out by foragers when the colony is in need of both? Would pollen collection be further enhanced or depressed if colonies were both pollen and nectar depleted? In nature, colonies are likely to face shortages of both resources and so these questions are of

ecological importance. Also, the present study focuses on pollen collection because bumblebees are widely used as natural pollinators in buzz-pollinated greenhouse crops, and hence, it examines selected factors, such as resource deprivation and colony size, which may contribute to the enhancement of pollen collection. The discussion on the implications of bumblebee pollination greenhouses is expanded further in the paper. The next section reviews studies examining the effect of simultaneous pollen-and nectar-deprivation on pollen collection.

Cartar (1992) examined the effects of energy manipulation on bumblebees' pollen and nectar foraging behaviour in free-foraging colonies. Natural-like conditions provided the baseline comparison condition in which colonies were not supplemented with either pollen or nectar and required to forage for both resources in the field. Manipulation consisted changes in energy stores, specifically, colony's nectar stores were either removed or enhanced on alternate days for a period of two hours. Following nectar depletion, foragers had more frequent foraging trips and shorter visits inside the colony and frequency of pollen collection trips and pollen-load size decreased. In contrast, when nectar enhanced, frequency of pollen collecting trips and size of pollen loads was increased. Cartar's findings suggest that when nectar is in greater demand, colonies compromise pollen collection.

Other research using laboratory settings has found significant, yet conflicting results in experiments on pollen collection in pollen-and nectar-deprived colonies. In accord with Cartar's findings, Plowright and Silverman (2000) have found that nectar collection in pollen- and nectar-deprived *Bombus impatiens* colonies was maintained at a high level, but pollen collection was compromised. In this study there was a foraging trade off in that nectar was collected at the expense of pollen. When deprived of both pollen and nectar,

workers collected less pollen in comparison to when the colony was deprived only of pollen. In contrast, Landry et al., (2000) found that when deprived of both resources, a few workers collected more pollen, not less, than when the colony was deprived only of pollen. These mixed findings of the effect of pollen-and nectar-deprivation on pollen collection require further investigation.

Several methodological considerations may be raised in understanding the differences in Landry et al.(2000) and Plowright and Silverman (2000). First, pollen deprivation was followed by nectar deprivation in the same colony in these studies. Therefore, it is possible that the first type of deprivation carried over to the next type of deprivation and affected foraging behavior. Second, Landry et al. (2000) and Plowright and Silverman (2000), selected and examined active foragers. Extrapolating from research on task switching in bumblebees (Cartar, 1992), it is possible that the workers' selection prevented colonies from allocating additional naïve workers, such as household workers to foraging. Therefore, the selection of active foragers might have restricted colony's resource collection. Third, Landry et al. (2000) and Plowright and Silverman (2000) used small sample sizes in their studies (i.e., two and one colonies, respectively), which may have contributed to the conflicting findings of pollen collection in resource-manipulated colonies.

In a similar line of investigation, a pilot study by Weinberg and Plowright (2002, see Appendix A) examined pollen collection in pollen-deprived colonies in two conditions: nectar was available in a feeder attached to the colony versus nectar was available in the flight cage. The former condition mimics foraging conditions in greenhouses in which nectar supplies are attached within the hive. The study compared six colonies of *Bombus impatiens*. Colonies were paired and randomly assigned to nectar in colony versus nectar in

cage. That is, nectar was readily available in the colony or the bees had to forage to obtain it. For 20 days, paired colonies placed side by side in a divided flight cage were let to forage for 40 minutes. Bees that worked to collect nectar collected also less pollen than bees that had nectar provided to them. This effect may have been due either to the placement of the feeders, or to a difference in how much nectar the bees had stored. What was striking was that pollen collection was affected by presentation of nectar, thus suggesting that the two resources are not independent. There may be additional methodological considerations contributing to this difference, such as foraging time. Specifically, 40 minutes of foraging may have been insufficient for collection of both pollen and nectar, and therefore, colonies that had to forage for both resources in the flight cage may have collected nectar at the expense of pollen due to pressing energy demands. Also, workers may have had insufficient time to gain efficient foraging skills when required to collect both resources outside the colony. The present thesis investigates these suggestions by improving on the method used pilot study (specifically Study Two).

In summary, research is inconsistent in findings on the effect of simultaneous pollen- and nectar-deprivation on pollen collection. Results in two studies show that when colonies were deprived of both resources workers collected less pollen, however, findings in another study show the opposite. Also, previous studies examining simultaneous deprivation in a lab setting present with several methodological considerations presenting the need to expand on existing research in the field.

The present thesis compares the effect of simultaneous deprivation of pollen and nectar with treatment conditions similar to those presented in greenhouses. Bumblebees in experiments presented here are required either to forage for both resources outside the

colony or to forage for pollen outside the colony while being supplemented with nectar inside the colony. The next section reviews the important role of bumblebees in greenhouse crop pollination.

Greenhouse Crop Pollination by Bumblebees

Natural pollinators, especially bumblebees, have gained an important role in commercial crop pollination since 1987 in Europe, where they were first introduced to the industry, and since 1990 in North America. For example, commercial crops that are pollinated by bumblebees include tomatoes, peppers, eggplants, melons, watermelons, cucumbers, zucchinis, strawberries, raspberries, blackberries, currants, cranberries, blueberries, apples, pears, cherries, kiwifruit, peaches, apricots, and plums (reviewed in Velthius & van Doorn, 2006).

With regard to cost-efficiency, mechanical pollination of crops exceeded €10,000 per hectare per year in 1991 in comparison to bumblebee pollination costs estimated at €9000 (Velthuis & van Doorn, 2006). Today, colonies can pollinate from 1000 square meters to 3000 square meters of crop for up to 12 weeks. Cost of a commercially reared colony ranges between $65-$200 depending on size and longevity, and typically contains between 30- 100 workers (Biobest Biological Systems Ltd). Number of colonies required to pollinate crops depends on several factors, such as the size of the greenhouse, season, variety of crops in greenhouse, number of plants for square meter, and outside-greenhouse plant competition. Although colonies can be self-reared, growers typically prefer commercial colonies, as they do not require special apparatus and handling procedures during the rearing period. Another

appealing factor in commercial colonies is its prompt availability; colonies can be ordered on a need basis. Typical specie used for pollination in North America is *B. impatiens* Cresson.

Bumblebee pollination is superior to mechanical pollination methods (i.e., manual use of vibrators, hormone application) and is shown to improve yield and quality of a variety of fruits and vegetables, thus increasing produce market value and decreasing labor costs (Abak et al., 1997; Asada & Ono, 1996; Dogterom, Matteoni, & Plowright, 1998; Ecran & Onus, 2003; Kevan, Gadawski, Kevan, & Gadawski, 1983; Kevan, Starver, Offer, & Laverty, 1991; Macfarlane, 1992; Paydas, Eti, Kaftanogul, Yasa, & Derin, 2000; Plowright & Laverty, 1987; Sampson & Spiers, 2002). For example, pollination by the bumblebee *Bombus impatiens* Cresson enhances berry qualities. Blueberry flowers visited by bumblebees reached earlier fruit maturation (i.e., 11 to 13 days earlier) in comparison to flowers receiving no bumblebee pollination (Sampson & Spiers, 2002). Other fruit quality enhancement attributed to bumblebee pollination includes increased number of mature seeds per berry, as well as yield of heavier and larger fruit (Paydas et al., 2000; Sampson & Spiers, 2002). The advantages of bumblebee-pollinated crops were also observed in self-pollinating crops, such as peppers (Abak et al., 1997; Ercan & Onus, 2003). Specifically, total fruit yield, fruit size and weight, seed number, and flesh thickness were all positively affected by bumblebee pollination. Similar fruit-enhancement results were obtained in bumblebee-pollinated tomato crops (Dogterom et al., 1998).

Bumblebees are also superior pollinators to other natural pollinators, such as hummingbirds. Unlike birds, bumblebees are frequent visitors on flowers and can pollinate a variety of plants including buzz-pollinated flowers, such as berries and tomatoes (see Castellanos, Wilson, & Thomson, 2003). Bumblebees are also superior pollinators to other

social bees, such as honeybees, because of their physiological characteristics. For example, bumblebees' tongues are longer than those of honeybees, and hence are more suitable for pollinating deep corolla flowers, such as red clover (Velthuis & van Doorn, 2006). Also, bumblebees are superior pollinators to honeybees in that their pollinated fruit are heavier on average than honeybee-pollinated fruit. And, in contrast to honeybees, bumblebees are resilient to cold climate conditions (Paydas et al., 2000; also see review by Richards & Kevan, 2002). In accord with Paydas et al., (2000), other findings show that in greenhouses, bumblebees are efficient foragers in temperatures ranging from 6 to 30 degrees Celsius, making bumblebees suitable pollinators for northern countries, such as Canada, as well as Mediterranean countries, such as Turkey and Israel (Bal, 1997; Sampson & Spiers, 2002). Hence, bumblebees' frequent visitation rate, ability to collect pollen from a large number of plants including buzz-pollinated flowers, and their suitability to a variety of climate conditions contributes to their appeal as natural commercial crop pollinators.

In light of all the above advantages as well as their economic advantages (i.e., cheap and low-maintenance labour, produce of larger fruit, facilitator of early fruit maturation), it is not surprising, that bumblebees are widely used in greenhouses to pollinate tomatoes, which are buzz-pollinated. Bumblebee-pollinated tomatoes represent about 95% of sales and their crop value is estimated to be €12,000 million per year (Velthuis & van Doorn, 2006). Because tomato crops provide only pollen, bumblebees are provided with an inside supply of nectar within their colonies. In other words, in greenhouses, bumblebee colonies are supplemented with nectar and are to forage for the resource in need (i.e., pollen) on crops. This condition resembles the experimental condition described earlier, pollen deprivation, in which colonies are pollen-deprived and nectar-supplemented. As mentioned

earlier, deprivation of both resources and their presentation outside the colony may possibly enhance bumblebees' pollen collection. Therefore, pollen collection by bumblebees may possibly be improved by further resource manipulation, namely simultaneous deprivation of pollen and nectar, resulting in bumblebees foraging for pollen on flowers and nectar on feeding stations.

In a personal communication with Mr. Gordon Yakel on June 13, 2005, principal greenhouse technician at Gipaanda Greenhouses LTD, British Columbia, he stated that growers have not considered providing nectar outside colonies, perhaps to avoid care and maintenance. He noted that the only method used to improve bumblebee pollination in the greenhouse is temperature manipulation so that optimal foraging conditions are mimicked (i.e., fall-like temperatures apprx. between 18 and 22°C). Another factor Mr. Yakel reported to be associated with greater foraging is plant species. For example, he reported noticing that bumblebees tend to have a higher foraging rate on cherry tomatoes than on beefsteak tomato crops, perhaps due to differences in pollen quality. In sum, bumblebees are widely used as natural pollinators in greenhouses and applied research has examined factors affecting their foraging behaviour in greenhouses, such as bee species, temperature, and lightening conditions (e.g., Morandin, Laverty, Gegear, & Kevan, 2002). However, the conditions addressed in the present study, namely simultaneous deprivation and colony size and their effect on pollination have not received attention in the research literature, hence the impetus for the present thesis.

Summary and Focus of Present Study

The foraging behaviour of bumblebees is affected by a number of factors, such as colony size and growth, resource availability and colony's nutritional demands, and exposure to foraging tasks. Some work has been done to investigate these factors, but has not established a comprehensive examination of the relationship between them. In addition, there is as yet no clear understanding of the factors that elevate pollen collection in bumblebee colonies, namely the effect of simultaneous deprivation on resource collection. Furthermore, investigation of contributing factors to improvement in pollen collection is of economic importance because pollination by bumblebee colonies is practiced in greenhouses. Having said all that, the main objective of the present study is to examine the relationship between pollen and nectar deprivations (pollen-and nectar-deprivation vs. pollen-deprivation), colony growth and foraging experience, and their effect on pollen collection in *Bombus impatiens* colonies. This study expands on previous research on pollen-deprivation (Plowright et al., 1993; Plowright et al., 1999) and pollen-and nectar-deprivation in bumblebee colonies (Landry et al., 2000; Plowright & Silverman, 2000), and incorporates the role of experience in foraging behavior and developmental factors in affecting foraging behaviour and resource intake (Cartar, 1992; Peat & Goulson, 2005; Ribeiro, 1994; 1996; Pomeroy & Plowright, 1982; Shykoff & Muller, 1995).

Three experiments were designed to examine the effects of resource manipulation, colony size and foraging experience on pollen collection. In all studies, resource manipulation served as a common thread. In one condition, colonies were pollen-deprived (i.e. supplemented with nectar but not pollen). In the other condition, colonies were deprived of both pollen and nectar.

Study One examines the effects of simultaneous deprivation of pollen-and nectar on free-foraging bumblebee colonies' growth. Findings pointing to the effect of pollen on colony development on the one hand, but the absence of previous research on simultaneous deprivation on the other hand, lead to the following hypothesis: Type of deprivation will affect colony growth. Specifically, pollen-deprived colonies will differ from pollen-and nectar deprived colonies in their pollen collection and consequently result in a different colony size. Hence, if colonies in one condition collect more pollen then they will produce larger colonies (i.e., more workers and queens) than colonies in the other condition. Two possibilities then arise. Assuming that managing one task (i.e., pollen collection in pollen-deprived colonies) is easier than collecting both pollen and nectar, then pollen-deprived colonies will collect more pollen and consequently produce a greater number of workers and queens in comparison to pollen-and nectar-deprived colonies. Alternatively, colonies might, for instance, allocate a greater foraging force according to their nutritional demands. If so, pollen-and nectar-deprived colonies will allocate a greater foraging force and collect more pollen in comparison to pollen-deprived colonies.

In Study Two, a set of two experiments is presented. The first experiment examines the effect of resource manipulation and foraging experience on pollen collection and suggests that type of deprivation and prior exposure to type of deprivation will affect pollen collection. Given the presence of mixed findings in previous studies on pollen collection in resource manipulated colonies and presence of only one study examining the effect of prior experience on pollen collection the hypothesis is as following: Type of deprivation and prior experience will affect pollen collection. Thus, if pollen collection depends on experience, then colonies exposed to different resource manipulation will differ in their pollen collection.

As in Study One, two possibilities arise. One possibility follows the assumption that managing one task is easier than the "balancing act" of collecting two resources. Therefore, colonies that have been deprived of pollen, but not nectar, would be proficient pollen collectors and therefore will collect more pollen when exposed to deprivation of both resources compared with colonies that have been only exposed to the deprivation of pollen-and nectar. The second possibility follows the assumption that the introduction to an additional foraging task (i.e., additional depletion of resources) will compromise pollen collection in colonies exposed to deprivation of only one resource. Thus, pollen-deprived colonies would experience greater difficulty in managing pollen collection when introduced with new nutritional demands (i.e., depletion of both pollen and nectar) and will collect less pollen in comparison to pollen-and nectar-deprived colonies. To test the above assumptions, a standard test procedure allowing the reading of change in behaviour following experience is introduced.

The second experiment in Study Two examines the effect of colony growth on pollen collection. In the absence of previous research examining the effect of colony size and simultaneous deprivation on pollen collection, the hypothesis states that colony size and type of deprivation will affect pollen collection. Colonies will differ in pollen collection depending on colony size and type of deprivation. Several predictions are outlined. If colonies are in their first stages of their development then they may require more protein and therefore will collect more pollen than colonies in advanced stages of development. In contrast, large colonies, by the virtue of having more foraging force, will collect more pollen than small colonies. With regard to the effect of deprivation, predictions are as previously stated in Study One and Experiment 1. If managing one task (i.e., pollen collection in

pollen-deprived colonies) is easier than collecting both pollen and nectar, then pollen-deprived colonies will collect more pollen than pollen-and nectar deprived colonies. Alternatively, assuming that colonies allocate a greater foraging force according to their nutritional demands, pollen-and nectar-deprived colonies will collect more pollen in comparison to pollen-deprived colonies.

A schematic illustration of the experiments and the proposed relationship between the variables is presented in Figure 1.

A Word on Methodological Considerations

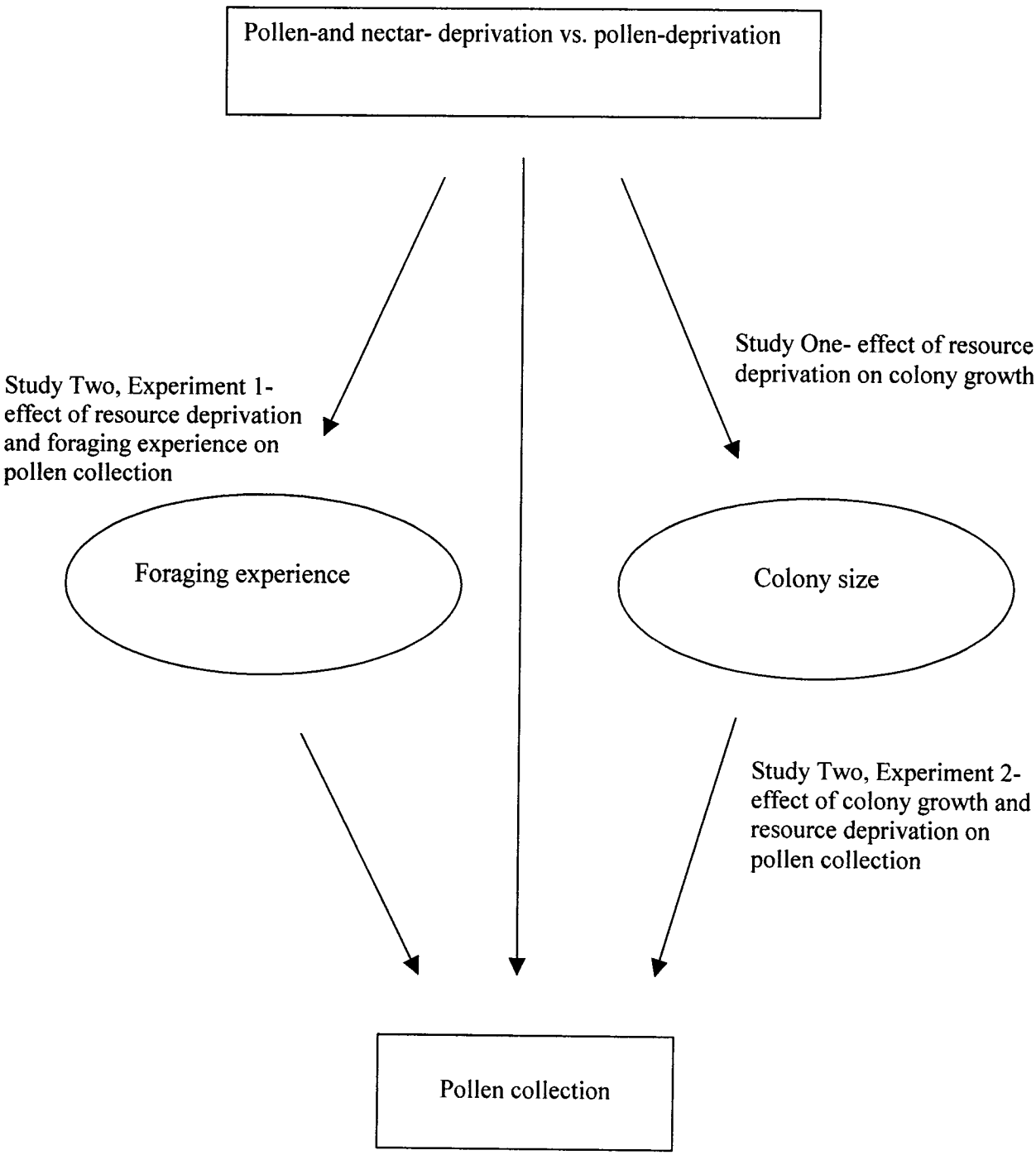
As noted earlier, resource manipulation in colonies is consistent in all experiments presented here. Colonies are either pollen-deprived or pollen-and nectar-deprived. Deprivation in the context of this thesis means that the colony has to forage for the resource in need outside of the colony (i.e., field in Study One, flight cage in Study Two). Pollen-deprived colonies are supplied with an *ad libitum* supply of sugar solution (heretofore referred to as "nectar") inside the colony, but are not supplied with an *ad libitum* supply of pollen inside the colony, and are required to forage for this resource outside the colony. Similarly, pollen-and nectar-deprived colonies are not supplied with an *ad libitum* supply of either pollen or nectar in the colony, and are required to forage for these resources outside the colony. In both conditions pollen and nectar are available in the flight cage and are assumed to be available in the field.

However, deprivation in the context of this thesis also means that colonies are allowed to keep and store collected resources; pollen lumps were not removed from workers' legs. Furthermore, because colonies in Study Two were restricted in foraging time, they were

supplemented on a need basis with small amounts of the resource in need to prevent larval expulsion and premature colony's death. This procedure is typical for bee studies in laboratory settings (e.g., Laundry et al., 2000; Plowright & Silverman, 2000). Having said all this, "deprivation" in this thesis does not refer to the traditional procedure in which resources are completely being withheld from the animals.

As for measuring resource intake (i.e., pollen collection), the procedure in this thesis involved counting number of entries with pollen on workers' legs and estimating pollen load size. This method may not be as accurate as weighing the loads, but it follows the rationale that direct contact with workers (e.g., scraping pollen of workers' legs and weighing it) may affect their foraging behaviour. This rationale was confirmed in a personal communication with Dr. James Thomson, University of Toronto (May 12, 2006), who noted that workers in one of his experiments were "hesitant" to enter the colony with pollen loads after having the experience of it being scraped of their legs.

The comparison group in the experiments is colonies that are pollen-deprived and nectar-supplemented. The choice of this comparison group is based on consistent research findings showing that pollen-deprived colonies collect more pollen than pollen-supplemented colonies or nectar-deprived colonies, as elaborated earlier in the Introduction. Furthermore, personal communication with other experts in the field, such as Dr. Chris Plowright and Dr. Catherine Plowright, provided the impetus for limiting comparison groups to resource manipulated colonies and excluding a group in which both resources are supplemented *ad libitum* inside the colony. The rationale follows experts' observations in which colonies that are being supplemented at all times with both pollen and nectar do not forage. Nevertheless, Experiment 2 in Study Two examines this notion.



Introduction- Figure 1. Proposed relationships between primary variables and experiments

STUDY ONE

Growth and Reproduction in Resource Manipulated Field-Foraging Bumblebee Colonies

Dalit Weinberg and C. M. S. Plowright

Unpublished Paper

Abstract

The study examines the effect of simultaneous deprivation on colony growth in five pairs of pollen-deprived colonies (-P) and pollen-and nectar-deprived colonies (-P-N). Colonies were left to free forage in a field setting for 24 hours for a period of five weeks. To estimate colony growth, workers were counted each week. Cocoons were also counted to better estimate colony size at the end of the experiment. Results showed that colony growth in -P-N did not differ from colony growth in -P. Results are discussed in terms of colony's adaptation to resource availability.

Growth and Reproduction in Resource Manipulated Field-Foraging Bumblebee Colonies

Introduction

In order to grow and survive, bumblebee colonies require pollen as a source of protein and nectar as a source of carbohydrate (Heinrich, 1979, Sutcliffe & Plowright, 1988, 1990). Pollen and nectar depletion affects colony growth, specifically, pollen-deprived colonies produce smaller workers and their developmental period is lengthened (Cartar & Dill, 1991; Plowright & Jay, 1977). The notion that colonies' growth is affected by resource availability is further supported in research on honeybees showing that colony's growth was increased when supplemented with high levels of pollen (Fewell & Winston, 1992).

The size of a colony is often a reliable indicator of its foraging workforce, which is usually a third of the total number of workers in the colony (Alford, 1975). Therefore, naturally, larger colonies have a larger foraging force than smaller colonies. The availability of pollen and nectar indirectly affects colony's work force in that it affects the growth of a colony and size, and consequently its foraging workforce.

The size of a colony, however, does not necessarily imply a higher volume of pollen collection. Pollen collection is strongly affected by the availability of pollen and nectar in the colony as demonstrated in resource-manipulation research studies (Cartar, 1992; Landry, Plowright, &Plowright., 2000; Plowright, Thompson, Lefkovitch, & Plowright, 1993; Plowright, Cohen-Salmon, Landry, & Simonds, 1999; Plowright & Silverman, 2000). For example, pollen-deprived colonies collect more pollen in comparison to pollen-supplemented colonies (Plowright et al., 1993; 1999) and colonies that are further depleted of nectar collect less pollen in comparison to nectar-supplemented colonies (Cartar, 1992). Research on simultaneous deprivation of pollen and nectar has reached inconclusive results.

In one study, pollen-and nectar-deprived colonies collected more pollen than pollen-deprived colonies (Landry et al., 2000), but in another study results showed the opposite effect (Plowright & Silverman, 2000).

Extrapolating from the above, findings suggest that the energy state of the colony determines colony's growth and its workers allocation to resource collection. But, which resource is more in demand when the colony is deprived of both pollen and nectar? Which resource, pollen or nectar, would colonies collect under simultaneous deprivation of resources, and what would be the subsequent effect on their growth?

The objective of this study is to examine the effects of simultaneous deprivation of pollen and nectar on colonies' growth. Absence of previous research on simultaneous pollen and nectar deprivation and colony growth provides the impetus for the study. Findings pointing to the effect of pollen on colony development on the one hand, and the absence of previous research on simultaneous pollen and nectar deprivation on the other hand, lead to the following hypothesis: Type of deprivation will affect colony development and growth. Specifically, pollen-deprived colonies will differ from pollen-and nectar-deprived colonies in their pollen collection and therefore will result in a different colony size. If colonies in one condition collect more pollen then they will produce larger colonies (i.e., more workers and queens) than colonies in the other condition. Two possibilities then arise. Assuming that managing one task (i.e., pollen collection in pollen-deprived colonies) is easier than collecting both pollen and nectar, then pollen-deprived colonies will collect more pollen and consequently produce a greater number of workers and queens in comparison to pollen-and nectar-deprived colonies. Alternatively, assuming that colonies allocate a greater foraging force according to their nutritional demands, pollen-and nectar-deprived colonies will

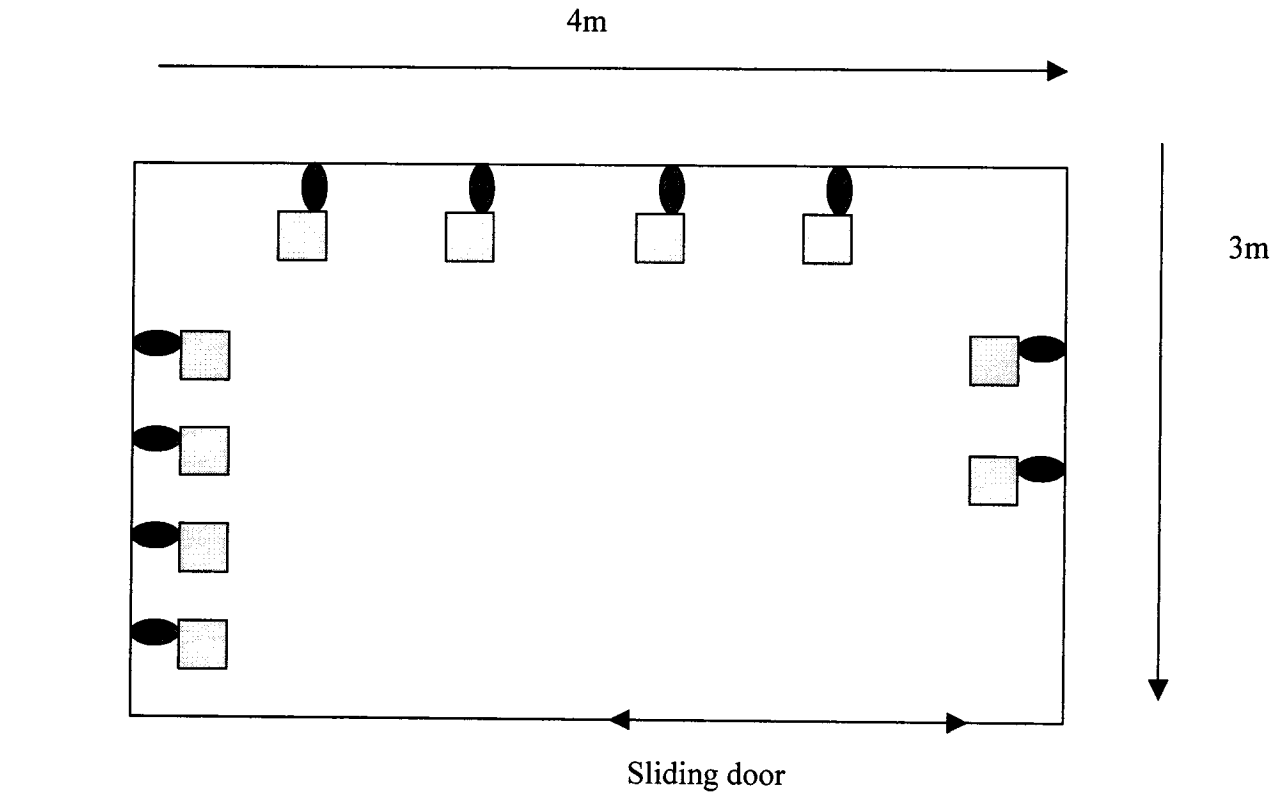
allocate a greater foraging force, collect more pollen and consequently produce a greater number of workers and queens in comparison to pollen-deprived colonies.

Method

Subjects Initially, twelve colonies of *Bombus impatiens* were examined in the experiment, but during the procedure one colony had lost its queen and therefore the pair including this colony was eliminated from the study and the analysis. The colonies were from wild-caught queens reared in the lab following the methods of Plowright and Jay (1966), and kept in a wooden domicile (30 cm x 15 cm x 15 cm) in the lab. The colonies were fed with fresh frozen pollen and "Bee Happy" solution/sugar water solution (2:1 vol:vol). The numbers of workers in colonies prior to the experimental manipulation in the remaining ten colonies were 11, 12, 12, 15, 20, 27, 29, 29, 36, and 75. Colonies that were excluded from the analysis included 16 and18 workers.

Materials Colonies were transported to a field site in Cantley, Quebec, and were housed within a shed (4x3x2.5m) with connecting tubes leading to the out side. See Figure 1 for illustration of the setting.

Procedure The study was conducted in July- August 2001 in Cantley, Quebec. Colonies were assigned into pairs according to similarity in workers' number in colonies: 11 and 12 workers; 12 and 15; 20 and 27; 29 and 29; 36 and 75. Although colonies in the last pair (36 and 75 workers) were markedly different in size, this issue was addressed in the analysis by



Study 1- Figure 1. Illustration of housing and testing environment (bird's eye view)

calculating growth in proportion to colony size. The colonies were placed equidistant from one another. Each colony in each pair was randomly assigned by a coin flip into one of two conditions: (1) pollen-and nectar-deprivation (-P-N) or (2) pollen-deprivation (-P). In -P-N, pollen lumps and nectar tubes were removed. In -P colonies, only pollen lumps were removed and sugar solution was supplied *ad libitum* inside the colony.

Colonies in both conditions were left to free forage 24 hours each day in the field. Data were collected weekly for a period of five weeks and consisted of weekly census count of workers in each colony, a record of date of emergence of new queens and males in each colony, and their number. Each week, new workers were colored with a non-toxic paint or numbered with labels glued onto their thorax in order to track the colony size.

Following one month of treatment, colonies were frozen in order to allow a count of cocoons. The final data collection of cocoons allowed for a better estimation of colonies' size and number of new queens produced (workers' and males' cocoons differ morphologically from queens' cocoons). This final estimation of colonies' size was essential, because new queens and males may have been absent from the colony permanently when bees were counted.

Data analysis Colony growth (number of new workers) was calculated each week using the following formula:

$$\%F = [(current - past) \div past] \times 100$$

The percentage of new workers each week was first treated as a binominal proportion. Because the residual was much greater than the degrees of freedom, the variability was greater than the theoretical variability of a binomial distribution. Similarly,

when the data were treated as counts reflecting a Poisson process, the variability was greater than predicted. Accordingly, the data were treated to a square root transformation. Normal error distribution and log link were used (Baker & Nelder, 1978). See Appendix B for a sample of statistical output.

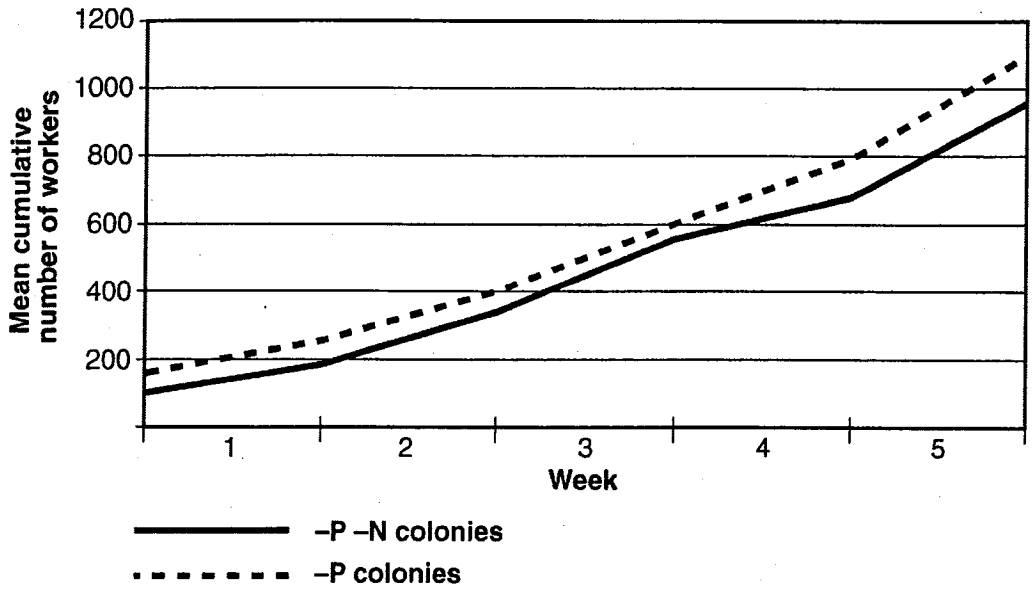
Results

The counts of queen cocoons vs. males or workers are given in Table 1. Despite an almost two fold difference in the average number of queen cocoons and the number of workers/male cocoons (more individuals in -P colonies), the differences were non-significant for the worker/male cocoons ($F_{1,4}$= 3.64, P= .13, n.s.) as well as for the queen cocoons ($F_{1,4}$= .22, P = .67, n.s.). Figure 2 shows a similar pattern in colony growth in both treatment conditions, resulting in an insignificant interaction between time and treatment condition ($F_{1,16}$= 1.98, P = .18, n.s.).

We also evaluated these findings by (1) using the total number of workers at the start of the experiment as a co-variate (2) ignoring the pairing of the colonies and treating the experiment as a between-groups design rather than a paired-colonies design (3) looking for a main effect at just the beginning of the experiment and discarding the data for weeks five and six (4) excluding one pair of colonies where the starting sizes were less well matched than in the other.

	Workers/males		Queens	
Pair #	–P	–P –N	–P	–P –N
1	153	81	16	26
2	666	336	11	123
3	836	486	366	55
4	1039	200	17	16
5	1082	1142	61	133

Study 1- Table 1. Number of worker and male cocoons and number of queen cocoons for each pair of colonies, one of which was pollen deprived (-P) and the other of which was deprived of both pollen and nectar (-P-N).



Study 1- Figure 2. Cumulative number of workers in -P colonies and -P-N colonies.

Discussion

Briefly, no significant differences between treatment conditions were obtained in this experiment, even though the manipulation could not have been stronger (colonies either had to collect all their nectar in the field for a period of 5 weeks or had it supplied to them directly *ad-libitum*). One possible interpretation of the failure to find an effect is that colonies are resilient to resource deprivation. Furthermore, colonies were let to free forage 24 hours a day during the experimental period. Hence, as in natural habitats, deprived colonies had no time or labor restrictions to obtain the resource in need.

The suggestion that colonies will change their foraging strategy and adapt to a novel storage condition is supported by findings in Fewell and Winston (1992). In their experiment on honeybee colonies, Fewell and Winston (1992) found that pollen-deprived colonies collected more pollen than pollen-supplemented colonies, but pollen-supplemented colonies collected pollen with higher nutritional value than pollen-deprived colonies. Colonies returned to their pre-experimental levels of pollen storage within 16 days. Two conclusions can be drawn from their findings. First, colonies change their foraging behavior and adjust to new storage conditions. Second, as noted by Fewell and Winston (1992), colonies regulate pollen storage levels around a homeostatic set point. Therefore, with regard to the present experiment, no differences in reproduction were evident possibly because colonies were able to regulate their resource homeostatic set point over time, and thus, growth was not affected by the experimental treatment.

It was not feasible to monitor foraging activity in 10 colonies, but perhaps pollen-and nectar-deprived colonies worked harder than pollen-deprived colonies and provided

sufficient pollen and nectar for colony development to be unaffected. The interpretation that colonies adapt to shortage in resource is the subject of Study Two.

Statement of authors' contributions

A shorter version of the following manuscript, Pollen Collection by Bumeblbees (Bombus impatiens): The Effects of Resource Manipulation, Foraging Experience and Colony Size, was published in the *Journal of Apicultural Research* (2006). I collected the data, with the assistance of two summer students, analyzed the data, and wrote the manuscript. My supervisor, Dr. Catherine Plowright, assisted in the design of Experiment 1 and provided editorial comments.

STUDY TWO

Pollen Collection by Bumblebees (*Bombus impatiens*): The Effects of Resource Manipulation, Foraging Experience and Colony Size

Dalit Weinberg and C. M. S. Plowright

School of Psychology, University of Ottawa

Abstract

To examine factors influencing pollen collection by bumblebees (*Bombus impatiens*), we compared pollen collection in free-foraging resource-manipulated colonies (i.e., pollen-and nectar-deprived vs. pollen-deprived only). Additional manipulations included prior foraging experience in Experiment 1, and colony size in Experiment 2. In Experiment 1, colonies were either deprived of both resources or deprived of only pollen and were subsequently tested while deprived of both resources. Colonies that had experience in managing both foraging tasks subsequently collected more pollen and allocated greater foraging effort than did colonies that only had experience in collecting pollen. In Experiment 2, larger colonies collected more pollen although smaller colonies collected more pollen relative to their colony size. Results show that colonies collect more pollen when deprived of both resources, exhibit foraging efficiency depending on previous tasks, and allocate foraging effort according to their nutritional and energetic demands. Results in our study demonstrate that pollen collection is dependent on nectar availability and have practical implications for growers of bumblebee-pollinated crops: Pollination may possibly be improved by causing colonies to forage for nectar as well as for pollen.

Keywords: Bumblebees, *Bombus impatiens*, pollen collection, pollen and nectar deprivation, colony requirements, colony size, foraging experience.

Pollen Collection by Bumblebees (*Bombus impatiens*): The Effects of Resource Manipulation, Foraging Experience and Colony size

Introduction

In recent years, bumblebee colonies have gained an important role in greenhouses. Bumblebee pollination, especially by *Bombus impatiens*, has shown to improve greenhouse crops (Dogterom, Matteoni, & Plowright, 1998; Macfarlane, 1992; Plowright & Laverty, 1987; Kevan, Straver, Offer, & Laverty, 1991). For example, tomato flowers that were pollinated by bumblebees produced larger fruits than flowers that were pollinated manually (Dogterom et al., 1998). Bumblebee pollination also increases the yield and quality of self-pollinating crops, such as peppers (Abak et al., 1997). Given these considerable economic and agricultural implications for greenhouses, it is important to understand factors affecting colonies' pollen collection.

In greenhouses, bumblebees collect pollen from flowers, but are provided with a supply of sugar solution (here simply referred to as "nectar") within the colony. We assume that the purpose for supplementing the colonies is implicit and twofold. First, growers purchase colonies with an *ad libitum* nectar supply attached to them. A "built-in" nectar supply may be more convenient to growers, because in this way, colonies do not require constant handling and feeding. Second, implicit in the practice is also the assumption that if colonies were to collect both pollen and nectar outside the colony, then there would be a trade-off between pollen and nectar collection (Stephens & Krebs, 1986). In other words, when deprived of both resources within the colony, bumblebees may collect nectar at the expense of pollen when foraging in the greenhouse. However, research findings are not consistent in supporting this assumption (see below).

Plowright and Silverman (2000) compared foraging behaviours under conditions of single resource vs. two-resource deprivation. When a colony was deprived of both nectar and pollen, bees spent just as much time foraging for nectar as when they were deprived only of nectar. The results were different, however, for pollen foraging. When the colony was deprived of both nectar and pollen, pollen foraging was compromised compared to when the colony was only in need of pollen. Nectar was apparently collected at the expense of pollen. In contrast, Landry, Plowright, and Plowright (2000) found no foraging trade-off in a sample of two bumblebee colonies foraging on blueberry flowers. Specifically, when colonies were deprived of both resources, a few workers collected more pollen, not less, than when colonies were deprived only of pollen. In this case, pollen may have been used as a substitute for nectar.

A variety of uncontrolled variables may account for the different findings in the two studies. As noted by Plowright and Silverman (2000), the value of pollen to the colony depends on amount and age of brood, in that younger colonies require more pollen in order to grow and develop than older colonies (Free, 1967; Pendrel & Plowright, 1981). Foraging experience by individual workers may also be important. Indeed, in one of the studies, the bees that collected more pollen when the colony was deprived of both resources tended to be the most experienced (See Table 2 in Landry et al., 2000). Accordingly, the present studies expand on Plowright and Silverman (2000) and Landry et al., (2000).

Our studies compare pollen collection under conditions of deprivation of pollen (with nectar being supplemented to the colony) vs. deprivation of both pollen and nectar. The previously uncontrolled variables of foraging experience and colony size are systematically manipulated in Experiment 1 and 2, respectively.

Experiment 1: The effects of resource manipulation and foraging experience

Bumblebees' foraging behaviour may depend on their past foraging experiences (Cartar, 1992; Jones, Reithel, & Irwin, 1998; Landry et al., 2000) and resource availability within the colony (see Plowright & Plowright, 1999 for a review of the literature). For example, following nectar depletion, number of foraging workers and nectar collection increased, but inexperienced foraging workers were less efficient in resource collection than experienced foraging workers (Cartar, 1992). Thus, bumblebees adjust their foraging effort and resource collection according to colonies' nutritional demand, yet foraging efficiency may depend on workers' experience in managing previous tasks.

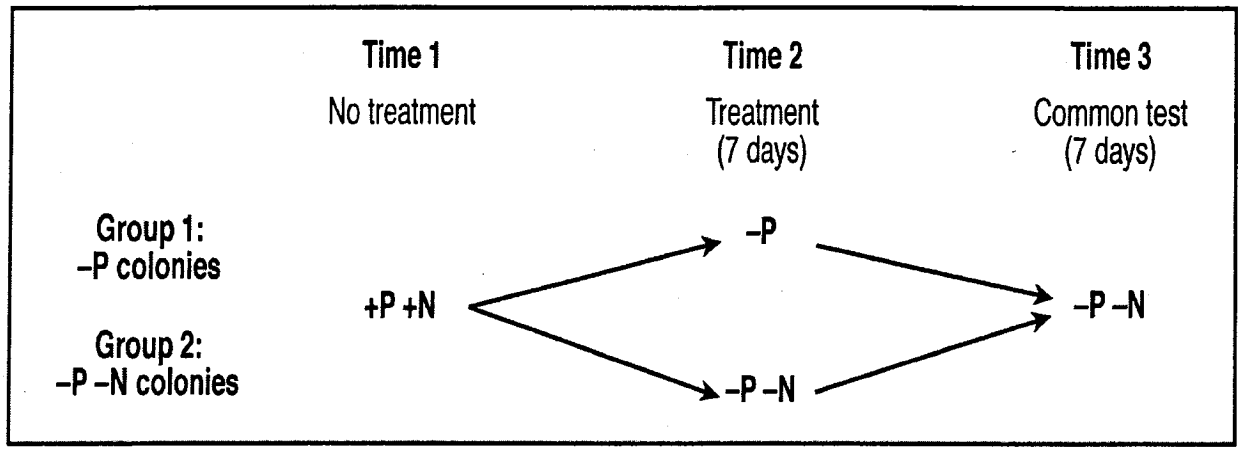
The above proposed foraging principles stating that (1) colonies allocate foraging effort according to their nutritional demands, and (2) colonies' foraging efficiency depends on experience in previous tasks, are the aim of investigation in the present study. First, we follow previous research and examine pollen collection in pollen-and nectar-deprived colonies vs. only pollen-deprived colonies. Then, because previous research does not untangle effects of prior experience from effects of current environmental conditions, a standard test procedure is introduced in this study (Shettleworth, 1998). A standard test is a procedure allowing the reading of change in behaviour following experience by comparing two groups at two times. Specifically, experience is manipulated between groups at Time A. The two groups are then compared on an identical task at Time B. In our experiment, the effect of foraging experience on pollen collection in colonies subsequently deprived of both resources is examined following the principles of a standard test. At Time 1 colonies receive no treatment. At Time 2 one group is deprived of both pollen and nectar and the other group

is deprived only of pollen. At Time 3 both groups are deprived of both pollen and nectar. The use of a standard test at Time 3, or a common test, is of great methodological importance because it enables an examination of the effect of prior foraging experience on pollen collection efficiency when both pollen and nectar are in current demand (see Figure 1 for an illustration of the experimental conditions and procedures).

We envisaged two possible scenarios regarding pollen collection behaviour at Time 3. One possibility was that the bees in Group 1 colonies would be proficient pollen collectors, having "focus their energies" on pollen collection at Time 2. Conversely, workers in Group 2 colonies, by virtue of having had to manage a "balancing act" between nectar and pollen collection at Time 2, would be comparatively inefficient at pollen foraging at Time 3. The other possibility was that the opposite might be observed. The Group 1 colonies were introduced to an additional foraging task (nectar collection) at Time 3, whereas the Group 2 colonies had been foraging for both nectar and pollen all along Times 2-3. Perhaps the bees in the Group 1 colonies would experience greater difficulty, not less, in managing pollen collection than the bees in the Group 2 colonies once the additional demand of nectar collection was placed upon them.

Material and methods

Subjects Four *Bombus impatiens* colonies were reared in the laboratory from wild caught queens during spring-summer 2002. The colonies were reared following the methods of Plowright and Jay (1966). They were transferred into a wooden domicile (30cm x 15cm x 15cm) and fed with honey-water solution (2:1 by volume) and fresh frozen pollen. Both of



+P +N Pollen and nectar supplemented
–P –N Pollen and nectar deprived
–P Pollen deprived and nectar supplemented

Study 2- Experiment 1- Figure 1. Illustration of experimental sequence in Experiment 1.

the resources were available *ad libitum* prior to manipulation. Number of workers in colonies prior to experimental manipulation was 18, 23, 26, and 29.

Testing apparatus A 165 x 165 x 166 cm flight cage with wooden frame and screen was divided by a 66 x 66.5 cm wooden frame and screen partition into two 165 x 82.5 x 166 cm flight cages. The two flight cages had a connecting door. Each division in the flight cage contained two stands, one for each resource. One white wooden stand (84 cm high) was used as a source of pollen. Frozen fresh pollen was ground and put on cheesecloth on the top of a basin vat full of water. The pollen was ground and changed daily. The second white wooden stand (140 cm high) supported a feeder containing honey solution. In addition to the fluorescent lighting in the lab, a fluorescent lighting fixture was assembled on the top of the flight cage.

Procedure Colonies were paired according to their size (18 and 23; 26 and 29) and then randomly assigned by coin tossing to one of two conditions: (1) -P (pollen-deprived), or (2) -P-N (pollen-and nectar-deprived). Colonies in the -P condition were supplemented directly with sugar solution inside the colony. -P-N colonies were not supplemented with either resource inside the colony. Both fresh ground pollen and nectar were available in the flight cage for the two conditions during the experiment.

The experiment proceeded for 17 consecutive days. The experiment consisted of three stages (see Figure 1 for a schematic illustration of the procedure). In the first stage (Time 1) each of two pairs of colonies were connected to the flight cage, one pair at a time, and left to free forage 3 hours a day. During this stage, which lasted for three days, colonies were supplemented with pollen and nectar. For one hour out of the 3-hour foraging time blocks, two observers recorded bees leaving and entering the colonies, time in which bees

left/entered the colony, bees' returns with pollen and size of pollen load. Assessment of pollen size was adapted from Plowright et al., (1993), and Cartar (1992). Pollen load size was classified according to the following categories: small, medium, large, and extra-large. Observers agreed that the criteria for pollen load size was as following: 1mm in diameter for a small load, 2mm for medium, 3mm for large, and 4mm or greater for extra-large. To assure adequate inter-rater reliability, we counterbalanced observations. Specifically, observer A would rate –P colonies and observer B would rate –P-N colonies on day 1, and on day 2 observer A would rate –P-N colonies and observer B would rate –P colonies. Hour of recording foraging activity was also counterbalanced. Each day, hour of recording was changed so on Day 1 we recorded during the first hour of the experiment, on Day 2 we recorded during the second hour of the experiment, and so on. Workers were counted and individually labelled every day.

After three days the manipulation began (Time 2). Randomly assigned, two colonies were -P for seven days and two colonies were -P-N. During each of the two 3-hour daily foraging periods, colonies in the two treatments were paired. The observers recorded foraging behaviour as recorded in the first stage of the experiment. After seven days, the common test began (Time 3). -P colonies were deprived not only of pollen but also nectar for seven days, and -P-N colonies remained deprived of both resources. Because the foraging periods were restricted to three hours per day, colonies were given daily small supplements of pollen (3 mm ball diameter) and nectar on a need basis (2 cc) to ensure that they would not expel larvae in response to drastic pollen and nectar shortage.

Data analysis The frequencies of pollen collection trips were treated as a Poisson process. The variance was greater than predicted by a Poisson model, and so the data were square-

root transformed, which stabilized the variance, and a normal error distribution with log link were used (Baker & Nelder, 1978). The data were analyzed using GLIM version 4.0 (Generalised Linear Interactive Modelling; Francis, Green, & Payne, 1993). We first tested for a difference between groups in pollen trip frequency at each time. Secondly, to examine whether the resource manipulation at Time 2 prompted the bees to collect larger or smaller pollen loads, the pollen collection trip counts were classified according to condition and load size. Even though pollen load size is a response variable, for purpose of statistical analysis it was treated as an independent variable (McCullagh & Nelder, 1989). We tested for whether the classification of trip counts according to pollen load size depended on condition, i.e. we tested for an interaction between load size and condition.

Results

Although the colonies were roughly matched at the beginning of the experiment, -P-N colonies were slightly smaller (mean = 22 workers) than -P colonies (mean= 26 workers). The growth rates were no different for the two conditions (Day x Condition interaction $F_{1, 30} = 2.21$, $P = .15$, n.s.). Accordingly, the absolute frequencies of pollen foraging trips were corrected for the number of workers in the colonies thus resulting in percentage of pollen foraging workers. Specifically, number of trips with pollen were divided by number of workers in colony and then multiplied by 100. Figure 2 shows that the frequency of foraging trips per worker is higher in -P-N colonies.

Time 1: No treatment

At Time 1, when colonies were supplemented with pollen and nectar (i.e., no treatment) there was no record of foraging behaviour except for one worker.

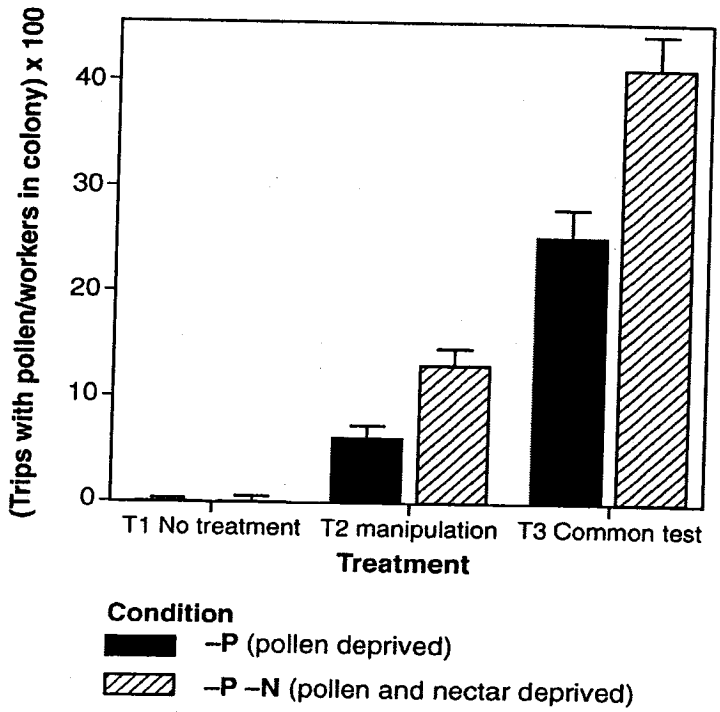
Time 2: Treatment (-P colonies vs. -P-N colonies)

At Time 2, when colonies were treated with different tasks (i.e., pollen deprivation vs. pollen and nectar deprivation), the effect of condition on pollen collection was significant, ($F_{1, 102} = 7.05$, $P < 0.025$) with -P-N colonies collecting more pollen loads than -P colonies (see Figure 2).

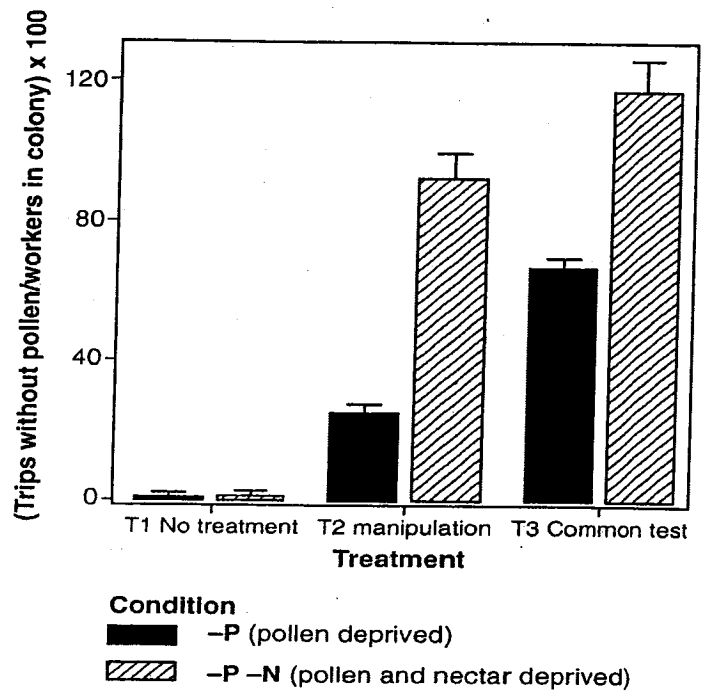
The effect of colony within condition was not significant ($F_{2, 102} = 0.97$, $P = .39$, n.s.) Furthermore, it appears that -P-N colonies had a general higher foraging activity than -P colonies (i.e., incoming trips without pollen, see Figure 3). The tendency by -P-N colonies to collect larger pollen loads was not significant ($F_{3, 102} = 0.83$, $P = .49$, n.s.) (see Figure 4).

Time 3: Common test (all colonies are -P-N deprived)

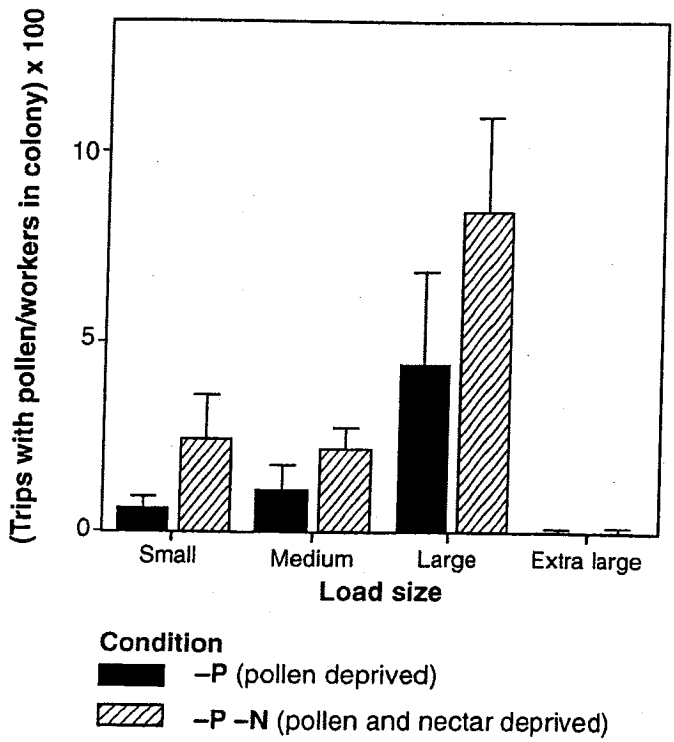
At Time 3, when colonies were introduced with a common test (i.e., pollen-and nectar-deprivation) the difference between groups was significant for pollen collection, ($F_{1, 102} = 25.12$, $P < 0.001$). Colonies that had been -P-N at Time 2 collected more pollen at Time 3 (see Figure 2) and no differences among colonies within condition were found ($F_{2, 102} = 0.68$, $P = .51$, n.s.). Finally, they collected larger loads at Time 3 than did -P colonies, ($F_{3, 102} = 3.34$, $P < 0.025$) (see Figure 5). Specifically, more trips with extra-large pollen loads were evident in -P-N colonies than in -P colonies. Also, -P-N colonies showed higher general foraging activity than -P colonies when trips with pollen were excluded (see Figure 3). However, -P colonies seemed to have a higher proportional increase in pollen collection between Time 2 and Time 3 in comparison to –P-N colonies.



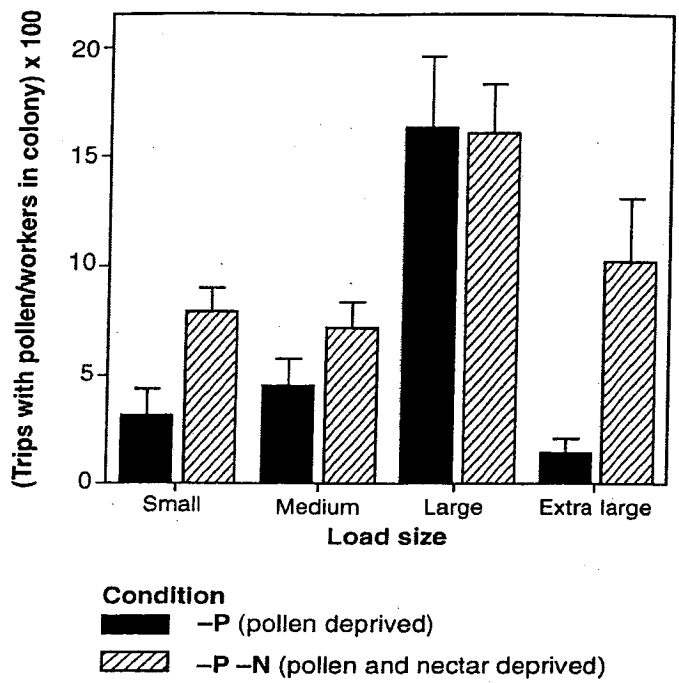
Study 2- Experiment 1- Figure 2. Mean frequency of trips with pollen with standard error bars in -P colonies and -P-N colonies (corrected for number of workers in the colony).



Study 2- Experiment 1- Figure 3. Mean frequency of foraging trips without pollen, with standard error bars, in -P colonies and -P-N colonies (corrected for number of workers in the colony).



Study 2- Experiment 1- Figure 4. Mean frequency of pollen loads of different sizes, with standard error bars, for -P colonies and -P-N colonies at Time 2.



Study 1- Experiment 1- Figure 5. Mean frequencies of pollen loads of different sizes, with standard error bars, for -P colonies and -P-N colonies at Time 3.

Discussion

In summary, -P-N colonies at Time 2 collected more pollen and appeared to allocate more foraging trips than -P colonies. Also, the overall foraging activity in -P-N colonies was higher in comparison to -P colonies. Moreover, results in Time 3 show that experience affects pollen collection. Colonies with previous experience in pollen and nectar collection when deprived of both resources at Time 2 collected more pollen and bigger loads in the common test (-P-N) than those that were experienced in pollen collection under deprivation of only pollen at Time 2. These results show that type of deprivation and experience affect pollen collection and colonies do not necessarily trade-off pollen collection for nectar collection when deprived of both resources.

Experiment 2: The effects of resource manipulation and colony size

Bumblebees modify their pollen collection according to requirements inside the colony. In particular, different developmental stages and changes in food resources in the colony affect bumblebees' pollen collection (Pendrel & Plowright, 1981; Plowright & Plowright, 1999). For example, the presence of brood in colonies is associated with greater pollen and nectar intake (Cartar & Dill, 1991; Pendrel & Plowright, 1981). However, it is not clear how bumblebee colonies in different developmental stages modify their pollen foraging behaviour when deprived of both resources. Accordingly, Experiment 2 examines the effects of colony development and simultaneous deprivation of pollen and nectar.

In the absence of previous research examining the effect of colony size and simultaneous deprivation on pollen collection, the hypothesis is as following: colony size and type of deprivation will affect pollen collection so that colonies will differ in pollen

collection depending on colony size and type of deprivation. Several predictions are outlined. If colonies are in their first stages of their development then they may require more pollen and therefore will collect more pollen than colonies in advanced stages of development. In contrast, large colonies, by the virtue of having more foraging force, will collect more pollen than small colonies. With regard to the effect of deprivation, predictions are as previously stated in Study One and Experiment 1. If managing one task (i.e., pollen collection in pollen-deprived colonies) is easier than collecting both pollen and nectar, then pollen-deprived colonies will collect more pollen than pollen-and nectar-deprived colonies. Alternatively, assuming that colonies allocate a greater foraging force according to their nutritional demands, pollen-and nectar-deprived colonies will collect more pollen in comparison to pollen-deprived colonies.

Materials and Methods

Subjects Twelve new *Bombus impatiens* colonies were reared as in Experiment 1. Number of workers in each colony prior to experimental manipulation was 6, 7, 7, 8, 9, 13, 31, 36, 37, 55, 58, and 59. Colonies with less than 15 workers, presence of worker larvae, and no presence of sexual reproductives were considered "small", and colonies with more than 30 workers with sexual reproductives were considered "large"; number of workers and the presence of reproduction are differential criteria for the developmental stage and hence the size of the colony.

Testing apparatus see Experiment 1.

Procedure Colonies were paired according to their developmental stage. "Small" colonies with less than 15 workers, presence of worker larvae, and no presence of sexual

reproductives were paired together (6 and 7, 7 and 8). "Large" colonies with more than 30 workers and with the presence of sexual reproductives were paired together (37 and 55, 58 and 59). In all, there were six pairs of colonies, with three "small" pairs and three "large" pairs. Eight colonies, four large and four small, were assigned to one of two possible conditions: (1) -P (pollen-deprived), or (2) -P-N (pollen-and nectar-deprived). Within each pair of colonies assignment to condition was made by a coin toss. The remaining four colonies were assigned to the no treatment group (+P+N), a condition in which colonies were supplemented with an *ad libitum* supply of pollen and nectar inside the colony. Number of workers in +P+N was 9 and 13 in "small" colonies, and 31 and 36 in "large" colonies.

The treatment began the afternoon before data collection. Pollen lumps were removed from both treatment conditions. Nectar tubes were removed in the -P-N condition. In the -P condition, nectar was supplied *ad libitum.* Bees were counted and recorded daily in order to monitor colony's growth. For 14 consecutive days, each pair was let to forage simultaneously for three hours. Each pair was tested every day. Because the foraging periods were short, colonies were given small supplements to avoid possible starvation and larval expulsion. Other details on method of data collection were the same as Experiment 1.

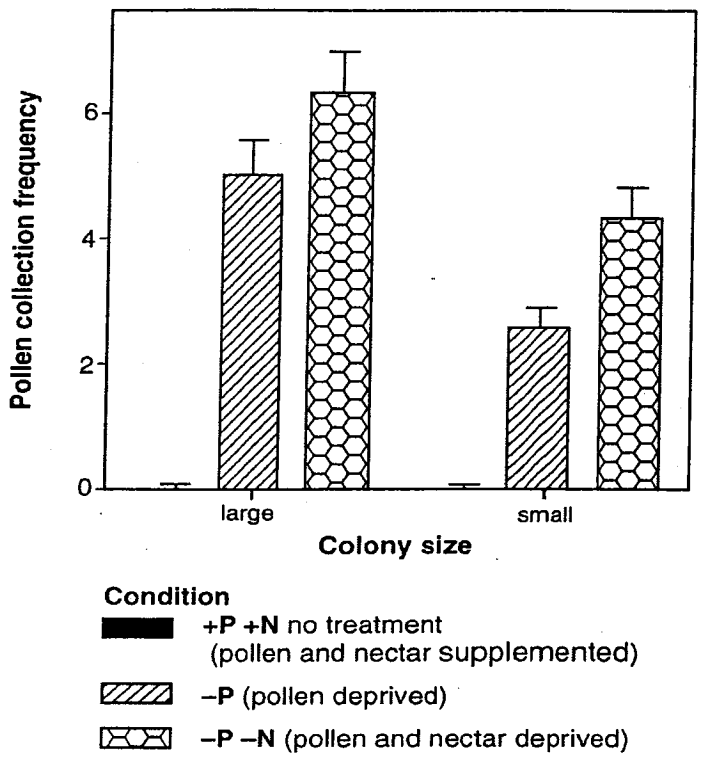
Data analysis The frequencies of pollen collection trips were treated as a Poisson process. The data were analyzed using GLIM version 4.0 (Generalised Linear Interactive Modelling; Francis, Green, & Payne, 1993). Even though pollen load size is a response variable, for purpose of statistical analysis it was treated as an independent variable (McCullagh & Nelder, 1989). We tested for whether the classification of trip counts according to pollen

load size depended on condition, i.e. we tested for an interaction between load size and condition.

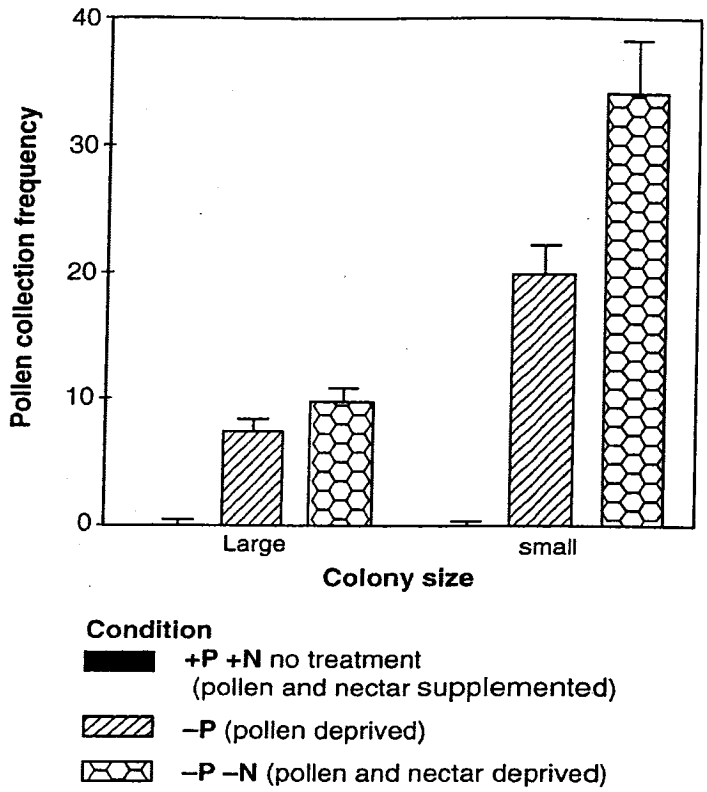
Results

A manipulation check revealed that mean number of workers was indeed significantly different between colony size conditions ($F_{1, 256} = 146.63$, $P < 0.001$). Workers' number in small -P colonies ranged from 6 at the beginning of the experiment to 20 workers at the end of the experiment, and from 6 to 22 in small -P-N colonies. The number of workers number in large -P colonies ranged from 54 at the beginning of the experiment to 85 workers at the end of the experiment, and from 37 to 80 in large -P-N colonies.

The effect of experimental condition was significant ($F_{1, 440} = 4.10$, $P < 0.05$). In keeping with the trend in Experiment 2, -P-N colonies collected more pollen in comparison to -P colonies. The effect of colony size on pollen collection was significant ($F_{1, 440} = 8.46$, $P < 0.005$). Not surprisingly, larger colonies collected more pollen loads than smaller colonies (see Figure 6). However, when corrected for colony size, smaller colonies collected more pollen loads than larger colonies relative to their colony size (Figure 7). The interaction of condition by colony size, however, was not significant ($F_{1,440} = .26$, $P = .62$, n.s.). Even when condition and colony size had been taken into account, there were significant colony differences (colony within size group and condition ($F_{4, 440} = 6.36$, $P < 0.001$).



Study 2- Experiment 2- Figure 6. Mean frequencies of pollen collection trips, with standard error bars, in -P colonies, -P-N colonies and control colonies.



Study 2- Experiment 2- Figure 7. Mean frequencies of pollen collection trips, with standard error bars, in -P colonies, -P-N colonies and control colonies corrected for number of workers.

Discussion

In summary, -P-N colonies collected more pollen than -P colonies. Large colonies collected more pollen than small colonies. Small colonies, however, collected more pollen relative to their colony size. Furthermore, when colony size is experimentally controlled and manipulated the same effect of -P-N deprivation emerges as in Experiment 1.

General Discussion

Under which conditions do bumblebees improve their pollen collection? Our results clarify the effects of simultaneous deprivation, prior experience, and colony size on pollen collection in bumblebee colonies. Deprivation of both resources as compared to just pollen deprivation improved foraging on three measures: frequency of foraging trips, frequency of trips with pollen and pollen size. Furthermore, pollen collection under dual resource deprivation was magnified when paired with other variables, namely prior experience or colony size.

Type of deprivation and prior experience in collecting resources in need seem to determine colonies' foraging allocation and efficiency when introduced with new nutritional demands. In Experiment 1, colonies that were deprived of both resources exhibited higher foraging efficiency (i.e., more frequent trips with pollen and larger pollen loads) in comparison to pollen-deprived colonies that were introduced with a novel task (i.e., nectar depletion). The notion that -P-N colonies will collect nectar at the expense of pollen ("trade-off") is rejected in our study. In fact, our findings show that pollen collection is intensified vis-à-vis colonies' energetic demand (i.e., nectar depletion) thus suggesting that nectar collection may be strongly linked to the efficiency of pollen collection. Apparently, pollen collection depends not only on pollen reserves but also on nectar reserves- a conclusion that

would have eluded us had pollen and nectar requirements and foraging behaviour been studied independently as they often are (Plowright & Laverty, 1984; Plowright & Plowright, 1999). Our findings here suggest that in the absence of pollen *and* nectar colonies enhance their collection of pollen. These findings are important for a couple of reasons. First, they elucidate previous findings on simultaneous deprivation (Landry et al., 2000; Plowright & Silverman, 2000), in that under identical conditions, pollen-and nectar-deprived colonies collect more pollen than pollen-deprived colonies. Second, they support previous suggestions (Cartar, 1992), in which bumblebees adapt their foraging behaviour depending on colonies' needs and exposure to previous tasks.

Why did Group 1 in Experiment 1 collect less pollen after further depletion of nectar than Group 2 at Time 3? Pollen collection and nectar collection are energy costly and entail acquisition of foraging skills (Heinrich 1976). Therefore, colonies deprived of only pollen at Time 2 may have had to elaborate on their foraging repertoire after further depletion of nectar by including non-experienced foragers to their foraging. In contrast, colonies deprived of both resources at Time 2 were not required to alter their foraging force and skills, which may have allowed them to strengthen their mastery in pollen collection resulting in greater collection of pollen in comparison to -P colonies. This suggestion, however, requires further observation at the individual level to examine each worker's performance at Times 2 and 3.

The role of nectar availability in enhancing pollen collection suggests that workers' nectar collection facilitates and improves their collection of pollen. How does the need for nectar contribute to pollen collection? Findings in Cartar (1992) show that workers who switched from nectar to pollen collection were as efficient in resource collection as workers

who did not switch tasks. In contrast, workers who switched from pollen to nectar collection were less efficient in resource collection after task switching. Extrapolating from Cartar's (1992) and our findings, workers specializing in nectar collection may display higher versatility in resource collection efficiency than workers specializing in pollen collection. Therefore, colonies in need for both pollen and nectar may have allocated "pollen workers" and "nectar workers" for resource collection, and therefore pollen collection was further enhanced with the "helping hands" of nectar workers. An inclusion of a comparison group in which colonies are depleted of only nectar at Time 2 and then depleted of both pollen and nectar at Time 3 to the method presented here may assist in confirming interpretations of our findings.

Another possible explanation is that the availability of both resources outside the colony may have been a reinforcing condition, in that it may have increased the likelihood of workers to collect more pollen (Shettleworth, 1998). The availability of both resources in need outside the colony may have had "a positive feedback" effect (for example see Plowright & Plowright, 1988): A successful foraging trip on one resource may have led to a successful foraging trip on another resource, thus resulting in greater foraging activity in general over time. The "positive feedback" principle is observed in nature. Specifically, the availability of nectar in flowers is strongly linked to bumblebees' foraging behaviour in that bumblebees increase their visits on flowers when nectar volumes are higher (Cresswell, 1999; Hodges, 1985). However, findings in this study showing the –P colonies had a higher proportional increase in pollen collection compared with –P-N colonies do not support the "positive feedback" notion. Also, nectar availability does not necessarily reflect on bumblebees' pollination activity on a flower. The latter is affected by pollen levels

presented in the flower, as well as it depends on the flower's morphological structure (Cresswell, 1999). Hence, nectar may be "hooking" bumblebees to visit flowers, but its availability does not guarantee pollination.

In generalizing the above findings and notions to the context of greenhouses, the availability of nectar outside the hive is likely to enhance bumblebees' pollination of crops, especially on nectarless plants presenting with pollen only, such as tomatoes. By removing the nectar supply from inside the colony and presenting it next to tomato crops, foraging activity, including pollination, may be significantly increased.

Colonies' size determines how much pollen foragers collect, and how many foragers are allocated for resource collection. An interesting finding in our study shows that colony's foraging force is reflective of its size and nutritional needs. In Experiment 2, small colonies allocated a greater foraging force relative to their colony's size in comparison to large colonies. It stands to reason that colonies in their first and second brood stages will increase their foraging force because these resources are vital to their healthy development (Cartar & Dill, 1991). However, in the case of greenhouses, large colonies are more suitable than small colonies for pollination, because larger colonies have a greater absolute number of foragers than small colonies.

The results in our experiments fill a void in research on the effect of simultaneous deprivation on pollen collection in bumblebee colonies. Thus far, only two studies examined the effects of pollen and nectar deprivation on pollen collection resulting in divergent findings (Landry et al., 2000; Plowright & Silverman, 2000). Expanding on their research, our study controlled for type of deprivation, prior experience, and colony size. Our findings suggest that bumblebees' pollen collection depends on several key variables

that should be controlled in future research. These variables include type of deprivation and it is sensitive to their familiarity with the foraging task and colony size.

Results in our study may have potential applications for practices in greenhouses. Our findings suggest that pollen collection is increased when bumblebees not only must collect pollen but also must leave the colony to forage for nectar. Accordingly, greenhouse growers might do well to remove the *ad libitum* supply of sugar solution to colonies at delivery, and to place nectar reserves away from the colony. As counter-intuitive as it may seem, crop pollination may possibly be improved by requiring bumblebees to do more, not less, for themselves.

Acknowledgements

We thank Dr. Chris Plowright for sharing his extensive knowledge on bumblebees and bumblebee research with us. We thank Drs. Kate Bielajew, Jaroslav Picman, and George Fouriezos for their helpful comments on the design of the experiment. A warm thank-you to Dr. Dana Church, Martine Perrault, Sola Sogbein, Martha Butler, Audray Potvin, and Lyssa Gagnon for their technical assistance. This research was supported by a research grant to C.M.S. Plowright from the Natural Sciences and Engineering Research Council of Canada.

General Discussion

Summary of Findings and Contributions

The meta-question binding all three experiments together was "Under which resource deprivation condition, pollen-deprivation, or pollen-and nectar-deprivation, is pollen collection enhanced?" Each experiment examined mediating factors, namely colony size and foraging experience, to allow a more comprehensive understanding of the interplay between resource availability and resource collection introducing sub-questions, such as "How does resource manipulation affect colony growth?" and in turn, "How does colony size affect resource collection?" and "How does foraging experience affect pollen collection?" In the first experiment, results showed that simultaneous deprivation of pollen and nectar did not differ from pollen-deprivation in its effect on colony growth in field free-foraging bumblebee colonies. Results from the second and third experiments showed that foraging activity for pollen is higher in colonies deprived of both pollen and nectar, and that larger colonies collect more pollen than small colonies, but small colonies collect more pollen in proportion to their colony size than large colonies. The results of the second experiment also showed that previous foraging experience enhanced pollen collection when colonies were not confronted with new energy demands.

As noted above, the experiments provide a comprehensive examination of the relationship between resource availability, colony growth, foraging experience, and pollen collection. While these factors were examined independently in previous studies, their reciprocal relationship as well as their facilitating role in pollen collection has been ignored in the literature. Furthermore, the experiments expand on previous research on simultaneous deprivation that resulted in unsettled findings (i.e., Landry et al., 2000; Plowright &

Silverman, 2000). By systematically manipulating variables that were previously allowed to vary freely, consistent findings emerged: Simultaneous deprivation of pollen-and nectar in colonies results in greater pollen collection compared to pollen collection in colonies that are pollen-deprived and supplied with nectar inside the hive. These counter-intuitive findings have an important practical implication for greenhouses using bumblebee colonies for crop pollination. As discussed in the second paper, growers might do well to remove the *ad libitum* supply of nectar to colonies at delivery, and to place nectar reserves away from the colony thus possibly enhancing pollen collection on nectarless crops. In addition, results from the first study in which treatment condition had no effect on colony growth further strengthen the impetus for the applicability of the findings to colonies in greenhouses in that growth (and therefore colonies' foraging force) in pollen-and nectar-deprived colonies, in comparison to pollen-deprived colonies, was not hindered. In other words, there is no reason to expect that gains to the grower in terms of pollen foraging would be offset by a decline in colony size.

With regard to research implications, the majority of studies on pollination have mainly investigated pollen collection independently from nectar (Plowright & Laverty, 1984; Plowright & Plowright, 1999). Hence, the findings in this thesis corroborated the principle stemming from the first preliminary study (Appendix A): Pollen collection depends not only on pollen reserves but also on nectar, and that foraging for one resource is not independent of the other.

Strengths, Limitations, and Future Directions

The strengths of the thesis are the simplicity of the testing environment, variety of experimental settings, as well as the consistency of the methodological design. The testing environment in the first experiment consisted of a shed to house colonies collecting their resources from the field. The use of a laboratory setting allowed for examination of the effect of resource manipulation on foraging behaviour in a controlled environment on the one hand, and on the other hand, the use of a field setting allowed for examination of the effect of resource manipulation on growth in natural-like conditions. The repeated comparison of pollen-deprived colonies to pollen-and nectar-deprived colonies in all three experiments allowed for the search of congruency between findings hence leading to robust conclusions with respect to the effect of the manipulation.

Although the method did not assist in detecting an effect of resource manipulation on colony size in Study One, it did successfully examine the effects of resource availability, foraging experience, and colony size on pollen collection in Study Two. To further explore the link between resource availability and colony growth as well as to further refine the study of factors shown here to affect pollen collection, the following methodological improvements are suggested: (1) examination of simultaneous deprivation and colony growth in a better controlled environment, such as in a laboratory flight cage to allow a closer monitoring of colonies' foraging behaviour; (2) examination of pollen collection at the individual level, for example, measure of time elapsed between workers' departure from and return to the hive over time to further explore the role of foraging experience on resource collection. Also, examination of pollen collection in "skilled" foragers vs. "naïve"

foragers to further disentangle the effects of new foraging tasks on workers' resource collection; (3) monitoring of change in foraging force allocation over time (i.e., how many workers are allocated to resource collection under which condition and for how long) to further reveal the relationship between experience, efficiency, and energy intake, and; (4) inclusion of a nectar-deprived and pollen supplemented (-P+N) comparison group to the experimental design presented in Study One, Experiment 1 (i.e., -P+N at Time 2 and -P-N at Time 3), to better understand how does the need for nectar contribute to pollen collection.

In accord with findings in the research literature (e.g., Cartar, 1992; Heinrich, 1976; Peat & Goulson, 2005), the results of the second experiment show that experience facilitates bees' foraging behaviour. Do these findings also point to learning processes involved in bees' pollen collection behaviour? In his review of the literature, Dukas (1998) noted that learning is a change in behaviour as a result of experience. Bees in Experiment 1 indeed showed foraging plasticity in response to resource manipulation, but the method used here is not sufficient to demonstrate whether learning was involved in their behavioural change. Specifically, the subsequent response in bees' foraging behaviour may have been adaptive, and did not necessarily involve change in memory. To examine presence of learning the method should reflect whether foragers "record" their experience as response to resource manipulation and on the basis of that memory modify their response to the manipulation (Dukas, 1998). Based on that notion, future research may include the use of an ABAB design (i.e., resource-supplemented; pollen-and nectar-deprivation; resource-supplemented; pollen-and nectar-deprivation) to expand on the study of workers' foraging response to change in resource availability. Such a design will also distinguish motivation from learning in bees' foraging behaviour. That is, learning processes in bees' foraging behaviour may be

detected if workers demonstrate an increased performance as a result of experience (i.e., increased pollen collection in B at time 2) and a lowered performance as a result of resource manipulation (i.e., lowered pollen collection in A at time 2).

Other future directions for the study of bumblebee foraging behaviour include colonies' decision making around resource collection vis-à-vis possibly multiple currencies (i.e., energy and protein intake), an area of research that has been overlooked in the literature. The introduction of nectar collection as a dependent variable in addition to pollen collection in a similar research context to the one presented here may help establish a better understanding of foraging currencies, fitness and energetic costs in resource manipulated colonies. A practical research avenue is the replication of the experiments presented here in a greenhouse setting, but perhaps a more simplified design in which pollen-and nectar-deprived colonies are compared with pollen-deprived colonies on pollen collection behaviour would suffice. Such replication may be helpful in clarifying whether treatment methods used here and shown to enhance pollen collection are portable and useful to greenhouse settings.

Although this thesis did not aim to examine theoretical questions, it is important to refer to foraging theories in understanding the results. However, interpretation of the results in the present study by looking to foraging theories is encountered with some difficulty. The basic assumption of some foraging models, in particular models of risk-sensitivity foraging, is that if forager's total intake of resources reaches a positive budget before foraging time expires then the forager must rest for the remainder of the day (reviewed in Ydenberg, 1998). This assumption is not confirmed here. Although colonies needs for pollen were

presumably similar, pollen-and nectar-deprived colonies collected more pollen than pollen-deprived colonies under identical time restraints.

Why, then, did pollen-and nectar-deprived colonies show a higher foraging rate for pollen than pollen-deprived colonies? There are several possible explanations for this finding, including learning, motivation, and subject self-selection processes. First, as discussed in the second and third experiments, the availability of both resources outside the colony may have been a reinforcing condition, in that it may have increased the likelihood of workers to collect more pollen as well as nectar (Shettleworth, 1998). Second, the availability of both resources in need outside the colony may have had "a positive feedback" effect (for example see Plowright & Plowright, 1988): A successful foraging trip on one resource may have led to a successful foraging trip on another resource, thus resulting in greater foraging activity in general over time. However, this explanation does not fit with the finding showing the –P colonies had a higher proportional increase in pollen collection in comparison to –P-N colonies. Third, the need for nectar in -P-N colonies may have enhanced pollen collection by nectar-foraging workers who demonstrated greater versatility in task switching and therefore efficiently collected pollen and nectar (as found in Cartar, 1992). Also, pollen-and nectar-deprived colonies' decisions on foraging force allocation may have contributed to maximization of the total net gain of resources while foraging. In turn, greater foraging performance may have resulted in minimum probability of energy shortfall thus perpetuating an enhanced foraging activity compared to pollen-deprived colonies.

As noted earlier, this thesis focused on foraging behaviour and the factors affecting it. It has not delved into functional questions that preoccupy foraging theorists, though the

interplay between psychological and biological approaches to behaviour is now a fertile ground (Chittka & Thomson, 2001). The main difficulty in the applicability of foraging theories to the present findings may be linked to its constructs, in that they do not reflect decision-making in foraging behaviour from a social-colony perspective, a point that has been raised by other researchers (Plowright & Plowright, 1999; Seeley, 1995). For example, in the risk sensitivity model (Stephens, 1981) foraging behaviour is explained in the individual level (i.e., foragers' choices and survival probability). Having said this, questions raised from findings in the present thesis, such as "For how long/how many/ which workers should be allocated for pollen collection" and "how much pollen should be collected when the colony is deprived of both resources" are not easily answered by existing foraging models. This difficulty in understanding findings in this thesis in a theoretical framework may present the need for elaboration on existing foraging models and inclusion of social-sensitive constructs. Ydenberg and his colleagues (1994, 1998) have already pointed out to factors ignored in foraging models, such as efficiency as well as the social aspects in foraging behaviour. Here, I suggest the inclusion of social decision mechanisms (i.e., foraging force allocation) as well as learning, experience, and task efficiency to foraging models. Such a social perspective has begun to be developed in research on honeybee behaviour (Moritzt & Southwick, 1992; Seeley 1995).

Summary

The main contribution of the thesis is its comprehensive examination of foraging behaviour and the findings that colonies under simultaneous deprivation of pollen-and nectar collect more pollen, not less, than pollen-deprived colonies. In addition, the findings

challenge "common knowledge" assumptions about the effect of resource manipulation on bumblebee's foraging behaviour and more indirectly the assumptions underlying foraging theories. Follow-up studies could further refine and expand on the understanding of bumblebee colony's economy and its foraging behaviour.

References

Abak, K., Dasgan, H. Y., Ikiz, O., Uygun, N., Sayalan, M., Kaftanoglu, O., & Yeninar, H. (1997). Pollen production and quality of pepper grown in unheated greenhouse during winter and the effects of bumblebees (*Bombus terrestris*) pollination on fruit yield and quality. *Acta Horticulturae 437*, 303-307.

Alford, D. V. (1975). *Bumblebees.* Davis-Poynter; London, UK.

Asada, S., & Ono, M. (1996). Crop pollination by Japanese bumblebees, Bombus spp.: tomato foraging behavior and pollination efficiency. *Applied Entomology and Zoology, 31(4)*, 581-586.

Baker, R. J., & Nelder, R. A. (1978). *The GLIM system, release 3: generalised linear interactive modelling.* Numerical Algorithms Group; Oxford, UK.

Baker, R. J., & Nelder, R. A. (1994). *The GLIM system, release 4: Generalized linear interactive modeling.* Numerical Algorithms Group, Oxford, UK.

Bal, J. M. (1997). Blueberry culture in greenhouses, tunnels, and under raincovers.

Bateson, M., & Kacelnik, A. (1998). Risk-sensitive foraging: decision making in variable environments. In R. Dukas (Ed.,), *Cognitive Ecology* (pp. 297-341). Chicago: The University of Chicago Press.

Buchmann, S. L. (1985). Bees use vibrations to aid pollen collection from non-poricidal flowers. *Journal of the Kansas Entomological Society 58(3)*, 517-525.

Cartar, R. V. (1991). A test of risk-sensitive foraging in wild bumblebees. *Ecology, 72*, 888-895.

Cartar, R. V. (1992). Adjustment of foraging effort and task switching in energy-manipulated wild bumblebee colonies. *Animal Behaviour, 44*, 75-87.

Cartar, R. V. & Dill, L. M. (1990a). Why are bumblebees risk sensitive foragers? *Behaviour Ecology and Sociobiology, 26*, 121-127.

Cartar, R. V., & Dill, L. M. (1991). Costs of energy shortfall for bumblebee colonies: Predation, social parasitism, and brood development. *Canadian Entomologist 123,* 283-293.

Castellanos, M. C., Wilson, P., & Thomson, J. D. (2003). Pollen transfer by hummingbirds and bumblebees, and the divergence of pollination modes. *Penstemon. Evolution, 57 (12),* 2742-2752.

Charnov, E. L. (1976a). Optimal foraging: Attack strategy of a mantid. *American Naturalist, 110*, 141-151.

Charnov, E. L. (1976b). Optimal foraging: The marginal value theorem. *Theoretical Population Biology, 9*, 129-136.

Chittka, L., & Thomson, J. D. (2001). *Cognitive ecology of pollination.* Cambridge University Press.

Cresswell, J. E. (1999). The influence of nectar and pollen availability on pollen transfer by individual flowers of oil-see rape (*Brassica napus*) when pollinated by bumblebees (*Bombus lapidaries*). *Journal of Ecology, 87*, 670-677.

Cresswell, J. E., & Roberston, A. W. (1994). Discrimination by pollen collecting bumblebees among differentially rewarding flowers on alpine wildflowers, *Campanula rotundifolia* (Campanulaceae). *Oikos, 69*, 304-308.

Dogterom, M. H., Matteoni, J. A., & Plowright, R. C. (1998). Pollination of greenhouse tomatoes by the North American *Bombus vosnesenskii* (Hymenoptera: Apidae). *Journal of Economic Entomology, 91*, 71-75.

Dukas, R. (1998). Evolutionary ecology of learning. In R. Dukas (Ed.,), *Cognitive Ecology* (pp. 129-174). Chicago: The University of Chicago Press.

Ercan, N., Onus, N. O. (2003). The effects of bumblebees on fruit quality and yield of pepper grown in an unheated greenhouse. *Israel Journal of Plant Sciences, 51*, 275-283.

Fewell, J. H., & Winston, M. L. (1992). Colony state and regulation of pollen foraging in the honey bee, *Apis mellifera* L. *Behavioral Ecology and Sociobiology, 30,* 387-393.

Fisher, R. M., & Pomeroy, N. (1989). Incipient colony manipulation, Nosema incidence and colony productivity of the bumblebee *Bombus terrestris* (Hymenoptera: Apidea), *Journal of the Kansas Entomological Society, 62*, 581-589.

Francis, B., Green, M., & Payne, C. (1993). *GLIM: The Statistical System for Generalized Linear Interactive Modelling. Version 4.0.* Oxford University Press; New York.

Free, J. B. (1955b). The division of labour within bumblebee colonies. *Insectes Sociaux, 3*, 195-212.

Free, J. B. (1993). *Insect pollination of crops*. London: Academic Press.

Free, J. B., & Butler, C. G. (1959). *Bumblebees*. London: Collins.

Goulson, D. (2003). *Bumblebees; their behaviour and ecology*. Oxford: Oxford University Press.

Goulson, D., Peat, J., Stout, J. C., Tucker, J., Darvill, B., Derwent, L. C., Hughes, W. O. H. (2002). Can alleothism in workers of the bumblebee *Bombus terrestris* be explained in terms of foraging efficiency? *Animal Behaviour, 64*, 123-130.

Harder, L. D. (1990). Behavioral responses by bumblebees to variation in pollen availability. *Oecologia, 85*, 41-47.

Heinrich, B. (1976). The foraging specialization of individual bumblebees. *Ecological Monographs, 46*, 105-128.

Heinrich, B. (1976). Bumblebee foraging and the economics of sociality. *American Scientist, 64*, 384-395.

Heinrich, B. (1979). *Bumblebee economics*. Harvard University Press, Cambridge, Massachusetts, USA.

Hodges, C. M. (1981). Optimal foraging in bumblebees: Hunting by expectation. *Animal Behaviour, 29(4)*, 1166- 1171.

Hodges, C. M. (1985). Bumblebee foraging: The threshold departure rule. *Ecology, 66*, 179-187.

Jones, K. N., Reithel, J. S., & Irwin, R. E. (1998). A trade-off between the frequency and duration of bumblebee visit to flowers. *Oecologia 117*, 161-168.

Katamaya, E. (1966). Studies on the devlopement of the broods of *Bumbos diversus* Smith (Hymenoptera: Apidea) II. Brood development and feeding habits. *Kontyu, 34*, 8-17.

Katamaya, E. (1973). Observation on the brood development in *Bombus ignites* (Hymenoptera: Apidae) II. Brood development and feeding habits. *Kontyu, 41*, 203-216.

Katamaya, E. (1975). Egg-laying habits and brood development in *Bombus hypocrita* (Hymenoptera: Apidae) II. Brood development and feeding habits. *Kontyu, 43*, 478-496.

Keasar, T., Rashkovitch, E., Cohen, D., & Shmida, A. (2002). Bees in two-armed bandit situations: foraging choices and possible decision mechanisms. *Behavioral Ecology, 13(6)*, 757- 765.

Kevan, P. G., Gadawski, R. M., Kevan, S. D., & Gadawski, S. E. (1983). Pollination of cranberries, *Vaccinium macrocarpon*, on cultivated marshes in Ontario. *Proceedings of the entomological Society of Ontario, 114*, 45-53.

Kevan, P. G., Straver, W. A., Offer, M. & Laverty, T. W. (1991). Pollination of greenhouse tomatoes by bumblebees in Ontario. *Proceedings of the entomological Society of Ontario, 122,* 15-17.

King, M. J. (1993). Buzz foraging mechanism of bumble bees. *Journal of Apicultural Research 32(1),* 41-19.

Knox, D. A., Shimanuki, H., & Herbert, E. W. (1971). Diet and the longevity of adult honey bees. *Journal of Economic Entomology, 64*, 1415-1416.

Krebs, J. R., Kacelnik, A., & Taylor, P. (1978). Test of optimal sampling by foraging great tits. *Nature, 275*, 27-31.

Landry F., Plowright, C. M. S., & Plowright, R. C. (2000). Behaviour of individual bumblebees (*Bombus impatiens)* on blueberry (*Vaccinium angustifolium*) flowers: The effects of pollen and nectar deprivation. *Journal of Apicultural Research 39*, 29-35.

Laverty, T. M. (1980). The flower visiting behaviour of bumble bees: Floral complexity and learning. *Canadian Journal of Zoology, 58,* 1324-1335.

Laverty, T. M., & Plowright, R. C. (1988). Flower handling by bumblebees: A comparison of specialists and generalists. *Animal Behaviour, 36*, 733-740.

Macevicz, S., & Oster, G. F. (1976). Modeling social insect populations. II: Optima reproduction strategies in annual eusocial insect colonies. *Behavior, Ecology and Sociobiology, 1*, 265-282.

Macfarlane, R. P. (1992). An initial assessment of blueberry pollinators in New Zealand. *New Zealand Journal of Crop and Horticulture Science, 29,* 91-95.

Maurizio, A. (1950). The influence of pollen feeding and brood rearing on the length of life and the physiological condition of bees- preliminary report. *Bee World, 31*, 9-12.

McCullagh, P., & Nelder, J. A. (1989). *Generalized Linear Models*, 2nd ed., Chapman & Hall; New York.

Michener, C. D. (1974). *The social behavior of bees*. Cambridge: Belknap Press, Harvard University Press.

Morandin, L. A., Laverty, T. M., Gegear, R. J., & Kevan, P. G. (2002). Effect of greenhouse polyethylene covering on activity level and photo-response of bumblebees. *Canadian Entomologist, 134*, 539-549.

Moritz, R. F.A., & Southwick, E. E. (1992). *Bees as superorganisms: an evolutionary reality*. Berlin: Springer-Verlag.

Niv, Y., Joel, D., Meilijson, I., & Ruppin, E. (2002). Evolution of reinforcement learning in foraging bees: a simple explanation for risk averse behavior. *Neurocomputing, 44-46,* 951-956.

Niv, Y., Joel, D., Meilijson, I., & Ruppin, E. (2002). Evolution of reinforcement learning in uncertain environments: a simple explanation for complex foraging behaviors. *Adaptive Behavior, 10(1),* 5-24.

Paydas, S., Eti, S., Kaftanoglu, O., Yasa, E., & Derin, K. (2000). Effects of pollination of strawberries grown in plastic greenhouses by honeybees and bumblebees on the yield and quality of the fruits. *Acta Horticulturae, 513*, 443-451.

Peat, J. & Goulson, D. (2005). Effects of experience and weather on foraging rate and pollen versus nectar collection in the bumblebee, *Bombus terrestris*. *Behavioral Ecology and Sociolobiology, 58,* 152-156.

Pendrel, B. A., & Plowright, R. C. (1981). Larval feeding by adult bumblebee workers (Hymenoptera: Apidea). *Behavior, Ecology and Sociobiology, 8*, 71-76.

Plowright R. C. & Jay, S. C. (1968). Caste differentiation in bumblebees. The determination of female size. *Insectes Sociaux 2*, 171-192.

Plowright, R. C., & Jay, S. C. (1966). Rearing bumblebee colonies in captivity. *Journal of Apiculture Research 5*, 155-165.

Plowright, R. C., & Jay, S. C. (1977). On the size determination of bumblebee castes (Hymenoptera: apidae). *Canadian Journal of Zoology, 55*, 1133-1138.

Plowright R. C., & Pendrel, B. A. (1977). Larval growth in bumblebees (Hymenoptera: Apidae). *Canadian Entomologist, 109*, 967-973.

Plowright, C. M. S., & Plowright R. C. (1999). Resource management in bumblebee colonies. *Insect Population Growth and the Environment,* 21-28.

Plowright, C. M. S., & Silverman, A. (2000). Nectar and pollen foraging by bumble bees (*Hymenoptera: Apidea*): Choice and tradeoffs. *The Canadian Entomologist 132*, 677-679.

Plowright, C. M. S., Cohen-Salmon, D., Landry, F., & Simonds, V. (1999). Foraging for nectar and pollen on thistle flowers (*Cirsium vulgare*) and artificial flowers:

How bumblebees (*Bombus impatiens*) respond to colony requirements. *Behaviour 136*, 951-963.

Plowright, R. C., & Laverty, T. M. (1984). The ecology and sociobiology of bumblebees. *Annual Review of Entomology, 29*, 175-199.

Plowright, R. C., & Laverty, T. M. (1987). Bumblebees and crop pollination in Ontario. *Proceedings of the Entomological Society of Ontario 118*, 155-160.

Plowright, R. C., & Plowright, C. M. S. (1988). Elitism in social insects: A positive feedback model. In R. L. Jeanne (Ed.) *Interindividual Behavioral Variability in Social Insects* (pp. 419-431). Westview Press.

Plowright, R. C., Thompson, J. D., Lefkovitch, L. P., & Plowright, C. M. S. (1993). An experimental study of the effect of colony resource level manipulation on foraging for pollen by worker bumblebees (Hymenoptera, Apidea). *Canadian Journal of Zoology, 71*, 1393-1396.

Pomeroy, N. & Plowright R. C. (1982). The relation between worker numbers and the production of males and queens in the bumble bee *Bombus perplexus*. *Canadian Journal of Zoology, 60*, 954-957.

Pomeroy, N. (1979). Brood bionomics of *Bombus ruderatus* in New Zealand (Hymenoptera: Apidea). *Canadian Entomologist, 111*, 865-874.

Rasheed, S. A., & Harder, L. A. (1997a). Economic motivation for plant species preferences of pollen collecting bumblebees. *Ecological Entomology, 22*, 209-219.

Rasheed, S. A., & Harder L. D. (1997b). Foraging currencies for non-energetic resources: pollen collection by bumblebees, *Animal Behaviour, 54*, 911-926.

Ribeiro, M. (1994). Growth in bumblebee larvae: relation between development time, mass, and the amount of pollen ingested. *Canadian Journal of Zoology, 72*, 1978-1985.

Ribeiro, M. (1997). The frequency with which *Bombus terrestris* larvae are fed during their development. *Larval nutrition in the bumblebee Bombus terrestris and its influence in caste differentiation.* Ph. D. Thesis: University of Utrecht, Netherlands.

Ribeiro, M., Duchateau, M. J., & Velthuis, H. (1996). Comparison of the effects of two kinds of pollen available commercially on colony development and queen production in the bumblebee *Bombus terrestris. Apidologie, 27*, 133-144.

Richards, K. W., & Kevan, P. G. (2002). Aspects of bee biodiversity, crop pollination, and conservation in Canada. In P. G. Kevan & V. L. Imperatriz – Fonesca (Eds), *Pollinating bees: the conservation link between agriculture and nature*. Brazilia: Ministry of Environment.

Roberstone, A. W., Mountjoy, C., Faulkner, B. E., Roberts, M. V., & Macnair, M. R. (1999). Bumblebee selection of *Mimulus guttatus* flowers: the effects of pollen quality and reward depletion. *Ecology, 80(8),* 2594-2606.

Sampson, B. J., & Spiers, J. M. (2002). Pollination, disease management and performance- evaluating bumblebees as pollinators of 'Misty' southern highbush blueberry growing inside plastic tunnels. *Acta Horticulturae, 574*, 53-61.

Seeley, T. D. (1995). *The wisdom of the hive: the social physiology of honey bee colonies*. Cambridge, MA: Harvard University Press.

Seeley, T., & Heinrich, B. (1981). Regulation of temperature in the nest of social insects. In B, Heinrich (Ed.), *Insect Thermoregulation*, pp. 159-234, New York: Wiley.

Shettleworth, S. J. (1988). Foraging as operant behavior and operant behavior as foraging: what have we learned? *Psychology of Learning and Motivation, 22,* 1-49.

Shettleworth, S. J. (1989). Animals foraging in the lab: problems and promises. *Journal of Experimental Psychology: Animal Behavior Processes, 15*, 81-87.

Shettleworth, S. J. (1998). *Cognition, evolution, and behavior*. New York: Oxford University Press.

Shykoff, J. A., & Muller, C. B. (1995). Reproductive decisions in bumble-bee colonies: the influence of worker mortality in *Bombus terrestris* (Hymenoptera, Apidae). *Functional Ecology, 9,* 106-112.

Sladen, F. W. L. (1912). *The humble bee*. London: Macmillan.

Spaethe, J., & Weidenmuller, A. (2002). Size variation and foraging rate in bumblebees (*Bombus terrestris*). *Insectes Sociaux, 49*, 142-146.

Standifer, L. N. (1967). A comparison of protein quality of pollen for growth stimulation of the hypopharyngeal glands and longevity of honey bees. *Insectes Sociaux, 14*,

Stephens, D. W. (1981). The logic of risk-sensitive foraging preferences. *Animal Behaviour, 29,* 628-629.

Stephens, D. W., & Krebs, J. R. (1986). *Foraging Theory*. Princeton University Press; Princeton.

Stout, J. C. (2000). Does size matter? Bumblebee behaviour and the pollination of *Cystisus scoparius* L. (Fabaceae). *Apidologie, 31*, 129-139.

Sutcliffe, G. H. & Plowright, R. C. (1990). The effects of pollen availability on development time in the bumblebee *Bombus terricola* K. (Hymenoptera: Apidae). *Canadian Journal of Zoology, 68*, 1120-1123.

Sutcliffe, G. H., & Plowright, R. C. (1988). The effects of food supply on adult size in the bumblebee *Bombus terricola* Kirby (Hymenoptera: Apidea). *Canadian Entomologist, 120*, 1051- 1058.

Velthuis, H. H. W. & van Doorn, A. (2006). A century of advances in bumblbee domestication and the economic and environmental aspects of its commercialization for pollination. *Apidologie, 37*, 421-451.

Weinberg, D. & Plowright, C. M. S. (2002). Pollen collection in resource manipulated bumblebee colonies. Paper presented in the 12th annual meeting of the Canadian Society for Brain, Behaviour, and Cognitive Science.

Ydenberg, R. C., (1998). Behavioral decisions about foraging and predator avoidance. In R. Dukas (Ed.,), *Cognitive Ecology* (pp. 343-378). Chicago: The University of Chicago Press.

Ydenberg, R. C., Welham, C. V. J., Schmid-Hempel, R., Schmid-Hempel, P. & Beauchamp, G. (1994). *Behavioural Ecology, 5*, 28.

APPENDIX A

Pollen Collection in Resource Manipulated Bumblebee Colonies

Pilot Study

Pollen Collection in Resource Manipulated Bumblebee Colonies

Introduction

Pollen and nectar are two vital resources for bees' growth and reproduction (Free, 1993; Ribeiro, Duchateau, Velthuis, 1996; Seeley & Heinrich, 1981). Given the importance of these resources, bumblebees and honeybees modify their foraging behaviour according to changes in either nectar reserves or pollen reserves in the colony (Cartar & Dill, 1991; Fewell & Winston, 1995). For example, foraging activity increases after further depletion of nectar (Cartar, 1992), and pollen collection increases following depletion of pollen storage (Fewell &Winston, 1992). These findings suggest that colonies collect more of the resource that is in greater immediate demand.

Bumblebees' pollen collection behaviour is sensitive to different levels of pollen reserves in the colony. For example, pollen-deprived colonies collect more pollen in comparison to pollen-enhanced colonies (Plowright, Thomson, Lefkovitch, & Plowright, 1993). In contrast, pollen collection decreases when the colony is supplemented with pollen and deprived of nectar (Landry, Plowright, & Plowright, 2000). While it is clear how colonies change their foraging strategies following changes in either pollen or nectar storage, it is not clear how colonies change their foraging behaviour when deprived of both resources. Interestingly, despite the occurrence of dual-resource deprivation in colonies in nature, this topic has not received much recognition in the literature (see Plowright & Plowright, 1999).

Two studies compared pollen collection behaviour in pollen-deprived colonies and pollen-and nectar-deprived colonies (Landry, Plowright, & Plowright, 2000; Plowright and Silverman, 2000). Both studies have found significant effects on type of deprivation,

however resulted in unsettled findings. In Plowright and Silverman (2000), colonies that were deprived of both pollen and nectar, collected less pollen compared to when it was deprived only of pollen. In contrast, Landry et al., (2000) found that selected workers collected more pollen when the colony was deprived of both resources.

The study of dual resource deprivation has also practical implications as bumblebee colonies are successfully used to pollinate greenhouse crops (Abak et al., 1997; Dogterom, Matteoni, & Plowright, 1998; Kevan, Straver, Offer, & Laverty, 1991; Macfarlane, 1992; Plowright & Laverty, 1987). In greenhouses, bumblebees collect pollen from flowers, but are provided with a supply of nectar within the colony. We assume that the purpose for supplementing the colonies is implicit and two-fold. First, growers purchase colonies with a "built-in" nectar supply inside the colony, which may be more convenient to growers, because this way, colonies do not require constant handling and feeding. Second, implicit in the practice is also the assumption that if colonies were to collect both pollen and nectar outside the colony, then there would be trade-off between pollen and nectar collection (Stephens and Krebs, 1986). In other words, when having to collect both resources outside the colony, bumblebees may collect nectar at the expanse of pollen when foraging in the greenhouse. However, as noted earlier, research findings are not consistent in supporting this assumption.

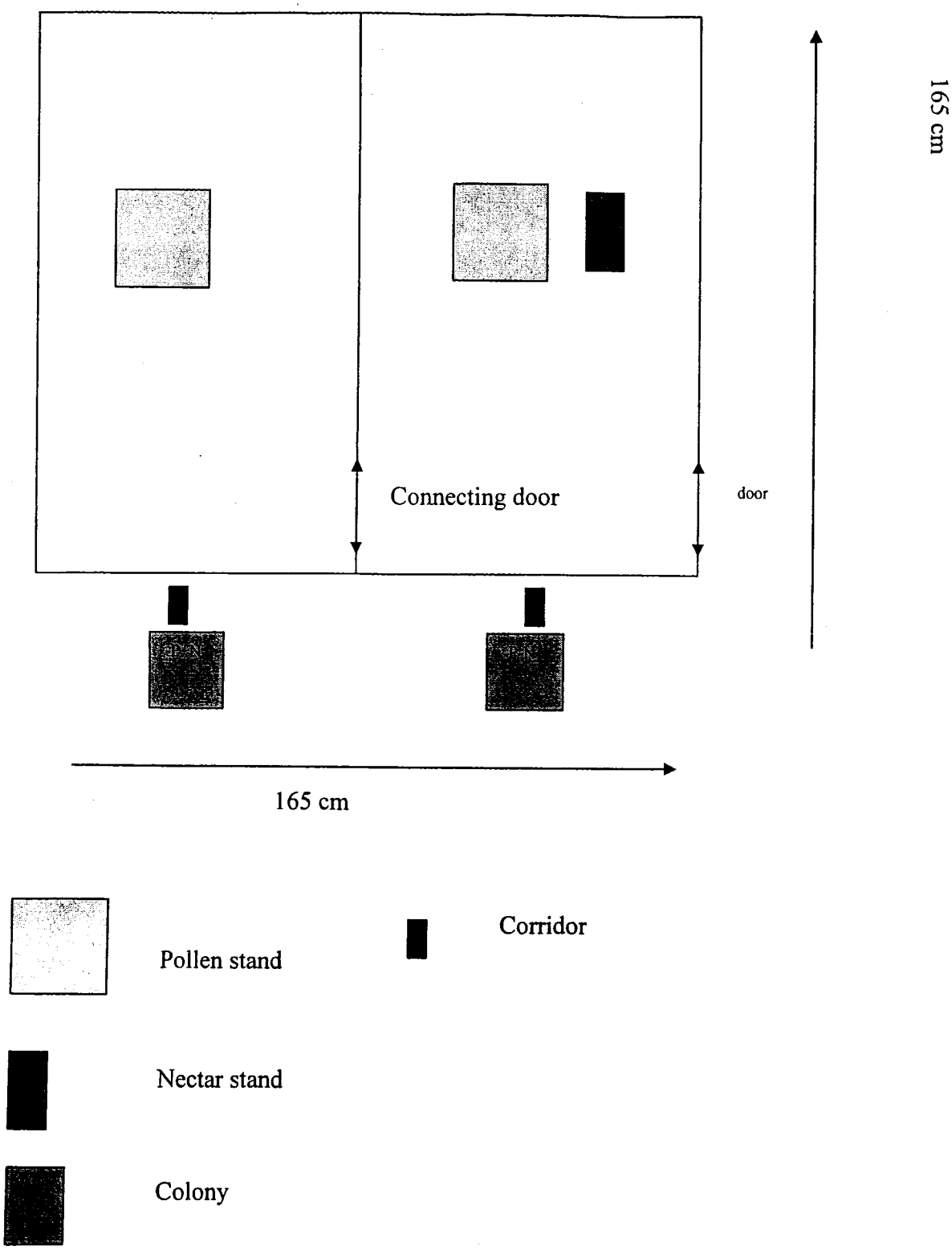
The present study follows on the research by Plowright and Silverman (2000) and Landry et al., (2000) and its main objective is to examine the effect of resource availability on pollen collection in *Bombus impatiens* colonies. We hypothesize that manipulation of resource availability (i.e., inside colony vs. outside colony) will affect foraging behaviour, so that colonies having to forage for resources in need (i.e., pollen and nectar) will differ in

pollen collection from colonies obtaining one resource inside the colony (i.e., nectar). Two opposite predictions are suggested. The first prediction is based on findings in Plowright and Silverman (2000) showing that colonies that had to forage for both resources collected less pollen compared to colonies that had to forage just for pollen and were supplemented with nectar. In contrast, the second prediction states that pollen collection will be increased in colonies foraging for both resources in that colonies will fulfill its nutritional requirements by using pollen as a substitute for nectar, as suggested in Landry et al., (2000). Also, the general foraging activity in colonies collecting both resources may increase (Cartar, 1992), which may be further contributing to an enhanced resource collection (a positive feedback mechanism).

Material and Methods

Subjects Six colonies of *Bombus impatients* were examined in the experiment. Colonies were from wild-caught queens reared in the lab following the methods of Plowright and Jay (1966). Colonies were kept in wooden domiciles (30cm x 15cm x 15cm) in the lab. Colonies were fed with fresh frozen pollen and sugar-and water solution (2:1 by volume). Number of workers in colonies prior to manipulation was 25, 25, 10, 12, 35, and 35 workers. Colonies with more than 15 workers were considered "large" and colonies with less than 15 workers were considered "small".

Materials A 165cm 165cm x 166cm flight cage with wooden frame and screen was divided by a 66x 66.5 cm wooden frame and screen partition into two 165cm x 82.5cm x 166cm flight cages (see Figure 1 for illustration of the flight cage). One flight cage contained 84cm



Appendix A- Figure 1. Illustration of flight cage (Bird's eye view).

wooden stand with pollen and also a140 cm wooden stand with sugar solution. Fresh frozen pollen was ground and changed each day. Pollen and sugar solution stands were placed for colonies foraging for pollen and nectar in the flight cage, and a pollen stand was placed for colonies foraging for pollen in the flight cage and supplemented with nectar inside the colony. An additional fluorescent lightening fixture was assembled on the top of the flight cage.

Procedure The study was conducted in July 2000. Six colonies were paired according to size in the beginning of the experiment. Colonies in pairs were randomly assigned by coin flipping into one of two treatment conditions: Pollen and nectar available in the flight cage (-P-N) vs. nectar available from feeder in the colony and pollen available in the flight cage (-P+N). The treatment began the afternoon before data collection and ended after 20 days. Each day, -P+N colonies received an *ad libitum* supply of nectar, and a 3x3x3mm of pollen for small colonies and 5x5x5mm of pollen for large colonies. -P-N colonies received 3x3x3mm of pollen for small colonies and 5x5x5mm of pollen for large colonies. Also, -P-N colonies also received 4cc of sugar solution or less when the colony had two honey-puts full with nectar to prevent larvae from being expelled.

Workers in each colony were counted every other day. In a -P-N colony of pair number three, half of the workers' population died between days 8 and 9, and hence 20 workers from a naïve colony were added to that colony.

For 20 days colonies in each pair were let to forage side by side for 40 minutes in the flight cage. Forty minutes of foraging was assumed to be a sufficient time for foraging based on previous findings on intensity of foraging activity (16 bees per 5 minutes) in previous research (Dogterom, Matteoni, & Plowright, 1988). Using stopwatches, the

researchers counted number of bees on the pollen stand in each condition to the nearest minute. The behaviour was recorded only in the presence of actual pollen collection, that is, pollen loading in the case of bee landing on the pollen stand (adapted from Plowright & Silverman, 2000).

Data analysis To measure pollen collection, we calculated the proportion of workers on pollen in order to control for difference in colony sizes. The data were treated as binominal proportions (number of bees on pollen/total number of workers in colony) and so a logistic model was used. The data were analyzed using GLIM (Generalized Linear Interactive Modeling, Baker & Nedler, 1994). The tests of significance are χ^2.

Results

The data set consisted of 4800 data points (6 colonies x 40 minutes x 20 days). Overall, an effect for type of treatment deprivation on the proportion of bees collecting pollen was found with -P+N colonies collecting more pollen than -P-N colonies ($\chi^2_{(1)}$= 368.7, $P<.001$). This difference was consistent in each pair of colonies and more noticeable in pairs one and three.

In pair number one, proportion of bees on pollen in -P+N colonies was .58 (bees per minute) in comparison to .37 (bees per minute) in -P-N colonies. The same trend, although not as pronounced, appeared in pair number two. In pair number two, percentage of bees on pollen in the -P+N condition was .39 (bees per minute), compared with .33 (bees per minute) in the -P-N condition. Pair number three had a noticeable difference in number of bees on pollen between treatment conditions. In this pair, -P+N colonies had an average of 2.35 bees

on pollen (per minute) while the -P-N colony had only an average of .60 bees on pollen (per minute).

Discussion

A significant effect for availability of resources on pollen collection was found. Colonies foraging for pollen outside the colony and supplied with nectar inside the colony had a higher proportion of bees on pollen than colonies foraging for both resources outside the colony. This difference was evident in all three pairs of colonies. These findings are in accord with previous findings showing that pollen collection was compromised in colonies required to forage for both pollen and nectar (Plowright & Silverman, 2000).

Despite the striking significance of the results, the numerical difference of number of bees on pollen between conditions appears marginal on the surface. Nevertheless, it is important to emphasize that we measured the proportion of bees on pollen per minute for a period of only 40 minutes. Therefore, even such a small difference could have an impact of pollen collection if measured over a whole day.

No support was found to the notion that foraging for nectar activates foraging in general, which would have lead to an increased pollen collection in return. In fact, the reverse was found. Another explanation for poor collection in -P-N colonies may be the allocation of an insufficient foraging time. Perhaps 40 minutes of foraging were not sufficient to collect both resources in need, and therefore the colony collected nectar to meet its immediate energetic demand. Having said this, -P-N colonies may collect more pollen if time allows.

The nectar feeder available inside colonies mimics what is done in greenhouses. Ideally, bees in the -P-N condition would have had 24 hour access to the feeder in the flight cage, but this was not possible, so the best we could do was add four cc of sugar solution. Even if it had been possible, however, the deprivation level in the two conditions would not have been equated.

The interpretation, however, does not detract from the main point, which is that detail of how nectar is placed, collected or stored are not irrelevant: pollen collection, and hence pollination, depends on more than how much a colony is in need for pollen and nectar. In this study, we did not increase pollen collection by alerting the nectar presentation from the way it is done in greenhouses, though we have envisaged ways in which this might be done. Our data should prompt greenhouses growers to at least consider other factors beyond that of convenience in their decision of how best to deliver nectar to the colony.

While supporting previous research, present findings raise new questions for future studies, such as what role does experience play in foraging behaviour. Perhaps with time -P-N colonies will gain experience and become more efficient in pollen collection. The notion that experience is involved in foraging behaviour is supported in the research literature (Cartar, 1992; Rasheed & Harder, 1997). Another future direction for research involves the effect of colony growth on pollen collection. As noted earlier, smaller colonies differed in their magnitude of pollen collection from large colonies. This finding suggests that colonies in different developmental stages may have different nutritional needs and therefore demonstrate different decisions with regard to resource collection. Indeed, daily pollen consumption per colony is correlated with the presence of larvae (Pendrel & Plowright, 1981). To sum the above, pollen collection was not increased in colonies in

which nectar was available in the flight cage. Differences in treatment effect are due either to the different placement of the feeders or to a difference in amount of nectar stored in the hive. Foraging time may have been a restricting variable compromising pollen collection in colonies required to collect both resource from the flight cage. Future avenues for research on pollen collection in bumblebee colonies may include variables such as foraging experience and colony growth.

References

Abak, K., Dasgan, H. Y., Ikiz, O., Uygun, N., Sayalan, M., Kaftanoglu, O., & Yeninar, H. (1997). Pollen production and quality of pepper grown in unheated greenhouse during winter and the effects of bumblebees (*Bombus terrestris*) pollination on fruit yield and quality. *Acta Horticulturae 437*, 303-307.

Baker, R. J., & Nelder, R. A. (1994). *The GLIM system, release 4: Generalized linear interactive modeling.* Numerical Algorithms Group, Oxford, UK.

Cartar, R. V. (1992). Adjustment of foraging effort and task switching in energy-manipulated wild bumblebee colonies. *Animal Behaviour, 44*, 75-87.

Cartar, R. V., & Dill, L. M. (1991). Costs of energy shortfall for bumblebee colonies: predation, social parasitism, and brood development. *Canadian Entomologist, 123*, 283-293.

Dogterom, M. H., Matteoni, J. A., & Plowright, R. C. (1998). Pollination of greenhouse tomatoes by the North American *Bombus vosnesenskii* (Hymenoptera: Apidae). *Journal of Economic Entomology, 91*, 71-75.

Fewell, J. H., & Winston, M. L. (1992). Colony state and regulation of pollen foraging in the honey bee, *Apis mellifera* L. *Behavioral Ecology and Sociobiology, 30*, 387-393.

Fewell, J. H., & Winston, M. L. (1995). Regulation of nectar collection in relation to honey storage levels by honey bees, *Apis mellifera. Behavioral Ecology, 7(3)*, 286-291

Free, J. B. (1993). *Insect pollination of crops*. London: Academic Press.

Kevan, P. G., Straver, W. A., Offer, M. & Laverty, T. W. (1991). Pollination of greenhouse tomatoes by bumblebees in Ontario. *Proceedings of the entomological Society of Ontario, 122,* 15-17.

Landry F., Plowright, C. M. S., & Plowright, R. C. (2000). Behaviour of individual bumblebees (*Bombus impatiens)* on blueberry (*Vaccinium angustifolium*) flowers: The effects of pollen and nectar deprivation. *Journal of Apicultural Research 39,* 29-35.

Macfarlane, R. P. (1992). An initial assessment of blueberry pollinators in New Zealand. *New Zealand Journal of Crop and Horticulture Science, 29,* 91-95.

Pendrel, B. A., & Plowright, R. C. (1981). Larval feeding by adult bumblebee workers (Hymenoptera: Apidea). *Behavior, Ecology and Sociobiology, 8,* 71-76.

Plowright, C. M. S., & Plowright, R. C., (1999). Resource management in bumblebee colonies. In C. A. Barlow (Ed.), *Insect Population Growth and the Environment* (pp 21-28). Research Signpost; Trivandrum, India.

Plowright, C. M. S., & Silverman, A. (2000). Nectar and pollen foraging by bumblebees (Hymenoptera: Apidae): Choice and tradeoffs. *The Canadian Entomologist, 132,* 677-679.

Plowright, R. C., & Laverty, T. M. (1987). Bumblebees and crop pollination in Ontario. *Proceedings of the Entomological Society of Ontario 118,* 155-160.

Plowright, R. C., Thomson, J. D., Lefkovitch, L. P., & Plowright, C. M. S. (1993). An experimental study of the effect of colony resource level manipulation on foraging for pollen by worker bumblebees (Hymenoptera, Apidea). *Canadian Journal of Zoology, 71,* 1393-1396.

Rasheed, S. A., & Harder, L. A. (1997a). Economic motivation for plant species preferences of pollen collecting bumblebees. *Ecological Entomology, 22*, 209-219.

Rasheed, S. A., & Harder L. D. (1997b). Foraging currencies for non-energetic resources: pollen collection by bumblebees, *Animal Behaviour, 54*, 911-926.

Ribeiro, M., Duchateau, M. J., & Velthuis, H. (1996). Comparison of the effects of two kinds of pollen available commercially on colony development and queen production in the bumblebee *Bombus terrestris. Apidologie, 27*, 133-144.

Seeley, T., & Heinrich, B. (1981). Regulation of temperature in the nest of social insects. In B, Heinrich (Ed.), *Insect Thermoregulation*, pp. 159-234, New York: Wiley.

Stephens, D. W., & Krebs, J. R. (1986). *Foraging Theory*. Princeton University Press; Princeton.

APPENDIX B

Sample of Statistical Output

Data Analysis in Study 2, Experiment 2:

Comparison of pollen foraging in small colonies

$data col day cond csize nwork lsize freq plsize su
$c propfreq is the frequency of all load sizes divi
$c prolsize is the frequency of each load size divi
$c csize 1 big colony, 2 small colony$
$c condition 1 pollen deprived, 2 pollen and nectar
$dinput 11$
File name? ex4length.prn

1	1	1	1	59	1	0	0	0	0
1	1	1	1	59	2	0	0	0	0
1	1	1	1	59	3	0	0	0	0
1	1	1	1	59	4	0	0	0	0
1	2	1	1	56	1	2	4	2	4
1	2	1	1	56	2	0	0	2	4
1	2	1	1	56	3	0	0	2	4
1	2	1	1	56	4	0	0	2	4
1	3	1	1	63	1	0	0	0	0
1	3	1	1	63	2	0	0	0	0
1	3	1	1	63	3	0	0	0	0
1	3	1	1	63	4	0	0	0	0
1	4	1	1	65	1	0	0	0	0
1	4	1	1	65	2	0	0	0	0
1	4	1	1	65	3	0	0	0	0
1	4	1	1	65	4	0	0	0	0
1	5	1	1	54	1	2	4	4	7
1	5	1	1	54	2	2	4	4	7
1	5	1	1	54	3	0	0	4	7
1	5	1	1	54	4	0	0	4	7
1	6	1	1	68	1	2	3	2	3
1	6	1	1	68	2	0	0	2	3
1	6	1	1	68	3	0	0	2	3
1	6	1	1	68	4	0	0	2	3
1	7	1	1	68	1	0	0	0	0
1	7	1	1	68	2	0	0	0	0
1	7	1	1	68	3	0	0	0	0
1	7	1	1	68	4	0	0	0	0
1	8	1	1	68	1	1	1	6	9
1	8	1	1	68	2	3	4	6	9
1	8	1	1	68	3	1	1	6	9
1	8	1	1	68	4	0	0	6	9
1	9	1	1	60	1	0	0	0	0
1	9	1	1	60	2	0	0	0	0
1	9	1	1	60	3	0	0	0	0
1	9	1	1	60	4	0	0	0	0
1	10	1	1	65	1	2	3	2	3
1	10	1	1	65	2	0	0	2	3
1	10	1	1	65	3	0	0	2	3
1	10	1	1	65	4	0	0	2	3

1	11	1	1	65	1	1	2	1	2
1	11	1	1	65	2	0	0	1	2
1	11	1	1	65	3	0	0	1	2
1	11	1	1	65	4	0	0	1	2
1	12	1	1	74	1	4	5	7	9
1	12	1	1	74	2	3	4	7	9
1	12	1	1	74	3	0	0	7	9
1	12	1	1	74	4	0	0	7	9
1	13	1	1	74	1	2	3	2	3
1	13	1	1	74	2	0	0	2	3
1	13	1	1	74	3	0	0	2	3
1	13	1	1	74	4	0	0	2	3
1	14	1	1	77	1	1	1	2	3
1	14	1	1	77	2	0	0	2	3
1	14	1	1	77	3	1	1	2	3
1	14	1	1	77	4	0	0	2	3
2	1	1	1	58	1	0	0	0	0
2	1	1	1	58	2	0	0	0	0
2	1	1	1	58	3	0	0	0	0
2	1	1	1	58	4	0	0	0	0
2	2	1	1	56	1	2	4	5	9
2	2	1	1	56	2	2	4	5	9
2	2	1	1	56	3	1	2	5	9
2	2	1	1	56	4	0	0	5	9
2	3	1	1	60	1	1	2	10	17
2	3	1	1	60	2	3	5	10	17
2	3	1	1	60	3	6	10	10	17
2	3	1	1	60	4	0	0	10	17
2	4	1	1	60	1	3	5	16	27
2	4	1	1	60	2	12	20	16	27
2	4	1	1	60	3	1	2	16	27
2	4	1	1	60	4	0	0	16	27
2	5	1	1	57	1	1	2	5	9
2	5	1	1	57	2	3	5	5	9
2	5	1	1	57	3	1	2	5	9
2	5	1	1	57	4	0	0	5	9
2	6	1	1	62	1	3	5	3	5
2	6	1	1	62	2	0	0	3	5
2	6	1	1	62	3	0	0	3	5
2	6	1	1	62	4	0	0	3	5
2	7	1	1	73	1	6	8	13	18
2	7	1	1	73	2	5	7	13	18
2	7	1	1	73	3	2	3	13	18
2	7	1	1	73	4	0	0	13	18
2	8	1	1	73	1	3	4	4	5
2	8	1	1	73	2	1	1	4	5
2	8	1	1	73	3	0	0	4	5

2	8	1	1	73	4	0	0	4	5
2	9	1	1	85	1	3	4	12	14
2	9	1	1	85	2	8	9	12	14
2	9	1	1	85	3	1	1	12	14
2	9	1	1	85	4	0	0	12	14
2	10	1	1	80	1	1	1	0	0
2	10	1	1	80	2	0	0	0	0
2	10	1	1	80	3	0	0	0	0
2	10	1	1	80	4	0	0	0	0
2	11	1	1	79	1	9	11	18	23
2	11	1	1	79	2	3	4	18	23
2	11	1	1	79	3	6	8	18	23
2	11	1	1	79	4	0	0	18	23
2	12	1	1	79	1	0	0	3	4
2	12	1	1	79	2	0	0	3	4
2	12	1	1	79	3	1	1	3	4
2	12	1	1	79	4	2	3	3	4
2	13	1	1	79	1	5	6	21	27
2	13	1	1	79	2	8	10	21	27
2	13	1	1	79	3	6	8	21	27
2	13	1	1	79	4	2	3	21	27
2	14	1	1	79	1	2	3	3	4
2	14	1	1	79	2	1	1	3	4
2	14	1	1	79	3	0	0	3	4
2	14	1	1	79	4	0	0	3	4
3	1	2	1	37	1	0	0	0	0
3	1	2	1	37	2	0	0	0	0
3	1	2	1	37	3	0	0	0	0
3	1	2	1	37	4	0	0	0	0
3	2	2	1	42	1	0	0	0	0
3	2	2	1	42	2	0	0	0	0
3	2	2	1	42	3	0	0	0	0
3	2	2	1	42	4	0	0	0	0
3	3	2	1	45	1	0	0	1	2
3	3	2	1	45	2	1	2	1	2
3	3	2	1	45	3	0	0	1	2
3	3	2	1	45	4	0	0	1	2
3	4	2	1	53	1	12	23	17	32
3	4	2	1	53	2	5	9	17	32
3	4	2	1	53	3	0	0	17	32
3	4	2	1	53	4	0	0	17	32
3	5	2	1	60	1	3	5	4	7
3	5	2	1	60	2	1	2	4	7
3	5	2	1	60	3	0	0	4	7
3	5	2	1	60	4	0	0	4	7
3	6	2	1	60	1	0	0	0	0
3	6	2	1	60	2	0	0	0	0

3	6	2	1	60	3	0	0	0	0
3	6	2	1	60	4	0	0	0	0
3	7	2	1	50	1	4	8	14	28
3	7	2	1	50	2	0	0	14	28
3	7	2	1	50	3	10	20	14	28
3	7	2	1	50	4	0	0	14	28
3	8	2	1	50	1	6	12	9	18
3	8	2	1	50	2	1	2	9	18
3	8	2	1	50	3	2	4	9	18
3	8	2	1	50	4	0	0	9	18
3	9	2	1	67	1	0	0	0	0
3	9	2	1	67	2	0	0	0	0
3	9	2	1	67	3	0	0	0	0
3	9	2	1	67	4	0	0	0	0
3	10	2	1	63	1	6	10	10	16
3	10	2	1	63	2	2	3	10	16
3	10	2	1	63	3	2	3	10	16
3	10	2	1	63	4	0	0	10	16
3	11	2	1	63	1	1	2	11	17
3	11	2	1	63	2	4	6	11	17
3	11	2	1	63	3	5	8	11	17
3	11	2	1	63	4	1	2	11	17
3	12	2	1	73	1	2	3	7	10
3	12	2	1	73	2	3	4	7	10
3	12	2	1	73	3	2	3	7	10
3	12	2	1	73	4	0	0	7	10
3	13	2	1	73	1	4	5	5	7
3	13	2	1	73	2	0	0	5	7
3	13	2	1	73	3	0	0	5	7
3	13	2	1	73	4	1	1	5	7
3	14	2	1	77	1	5	6	17	22
3	14	2	1	77	2	7	9	17	22
3	14	2	1	77	3	3	4	17	22
3	14	2	1	77	4	2	3	17	22
4	1	2	1	55	1	0	0	0	0
4	1	2	1	55	2	0	0	0	0
4	1	2	1	55	3	0	0	0	0
4	1	2	1	55	4	0	0	0	0
4	2	2	1	72	1	0	0	0	0
4	2	2	1	72	2	0	0	0	0
4	2	2	1	72	3	0	0	0	0
4	2	2	1	72	4	0	0	0	0
4	3	2	1	72	1	0	0	0	0
4	3	2	1	72	2	0	0	0	0
4	3	2	1	72	3	0	0	0	0
4	3	2	1	72	4	0	0	0	0
4	4	2	1	70	1	0	0	0	0

4	4	2	1	70	2	0	0	0	0
4	4	2	1	70	3	0	0	0	0
4	4	2	1	70	4	0	0	0	0
4	5	2	1	70	1	2	3	2	3
4	5	2	1	70	2	0	0	2	3
4	5	2	1	70	3	0	0	2	3
4	5	2	1	70	4	0	0	2	3
4	6	2	1	73	1	0	0	0	0
4	6	2	1	73	2	0	0	0	0
4	6	2	1	73	3	0	0	0	0
4	6	2	1	73	4	0	0	0	0
4	7	2	1	70	1	6	9	10	14
4	7	2	1	70	2	4	6	10	14
4	7	2	1	70	3	0	0	10	14
4	7	2	1	70	4	0	0	10	14
4	8	2	1	70	1	5	7	6	9
4	8	2	1	70	2	1	1	6	9
4	8	2	1	70	3	0	0	6	9
4	8	2	1	70	4	0	0	6	9
4	9	2	1	80	1	5	6	5	6
4	9	2	1	80	2	0	0	5	6
4	9	2	1	80	3	0	0	5	6
4	9	2	1	80	4	0	0	5	6
4	10	2	1	80	1	6	8	10	13
4	10	2	1	80	2	2	3	10	13
4	10	2	1	80	3	2	3	10	13
4	10	2	1	80	4	0	0	10	13
4	11	2	1	80	1	7	9	26	33
4	11	2	1	80	2	3	4	26	33
4	11	2	1	80	3	8	10	26	33
4	11	2	1	80	4	8	10	26	33
4	12	2	1	75	1	2	3	3	4
4	12	2	1	75	2	0	0	3	4
4	12	2	1	75	3	1	1	3	4
4	12	2	1	75	4	0	0	3	4
4	13	2	1	75	1	5	7	17	23
4	13	2	1	75	2	4	5	17	23
4	13	2	1	75	3	5	7	17	23
4	13	2	1	75	4	3	4	17	23
4	14	2	1	77	1	2	3	4	5
4	14	2	1	77	2	1	1	4	5
4	14	2	1	77	3	1	1	4	5
4	14	2	1	77	4	0	0	4	5
5	1	1	2	7	1	0	0	0	0
5	1	1	2	7	2	0	0	0	0
5	1	1	2	7	3	0	0	0	0
5	1	1	2	7	4	0	0	0	0

5	2	1	2	7	1	0	0	0	0
5	2	1	2	7	2	0	0	0	0
5	2	1	2	7	3	0	0	0	0
5	2	1	2	7	4	0	0	0	0
5	3	1	2	7	1	0	0	0	0
5	3	1	2	7	2	0	0	0	0
5	3	1	2	7	3	0	0	0	0
5	3	1	2	7	4	0	0	0	0
5	4	1	2	6	1	0	0	0	0
5	4	1	2	6	2	0	0	0	0
5	4	1	2	6	3	0	0	0	0
5	4	1	2	6	4	0	0	0	0
5	5	1	2	8	1	0	0	0	0
5	5	1	2	8	2	0	0	0	0
5	5	1	2	8	3	0	0	0	0
5	5	1	2	8	4	0	0	0	0
5	6	1	2	9	1	0	0	0	0
5	6	1	2	9	2	0	0	0	0
5	6	1	2	9	3	0	0	0	0
5	6	1	2	9	4	0	0	0	0
5	7	1	2	8	1	0	0	0	0
5	7	1	2	8	2	0	0	0	0
5	7	1	2	8	3	0	0	0	0
5	7	1	2	8	4	0	0	0	0
5	8	1	2	10	1	0	0	0	0
5	8	1	2	10	2	0	0	0	0
5	8	1	2	10	3	0	0	0	0
5	8	1	2	10	4	0	0	0	0
5	9	1	2	9	1	0	0	0	0
5	9	1	2	9	2	0	0	0	0
5	9	1	2	9	3	0	0	0	0
5	9	1	2	9	4	0	0	0	0
5	10	1	2	13	1	1	8	14	108
5	10	1	2	13	2	1	8	14	108
5	10	1	2	13	3	2	15	14	108
5	10	1	2	13	4	10	77	14	108
5	11	1	2	16	1	4	25	12	75
5	11	1	2	16	2	2	13	12	75
5	11	1	2	16	3	3	19	12	75
5	11	1	2	16	4	3	19	12	75
5	12	1	2	17	1	1	6	5	29
5	12	1	2	17	2	1	6	5	29
5	12	1	2	17	3	3	18	5	29
5	12	1	2	17	4	0	0	5	29
5	13	1	2	17	1	4	24	6	35
5	13	1	2	17	2	0	0	6	35
5	13	1	2	17	3	0	0	6	35

5	13	1	2	17	4	2	12	6	35
5	14	1	2	17	1	1	6	7	41
5	14	1	2	17	2	1	6	7	41
5	14	1	2	17	3	3	18	7	41
5	14	1	2	17	4	2	12	7	41
6	1	1	2	8	1	0	0	0	0
6	1	1	2	8	2	0	0	0	0
6	1	1	2	8	3	0	0	0	0
6	1	1	2	8	4	0	0	0	0
6	2	1	2	7	1	0	0	2	29
6	2	1	2	7	2	0	0	2	29
6	2	1	2	7	3	2	29	2	29
6	2	1	2	7	4	0	0	2	29
6	3	1	2	7	1	0	0	1	14
6	3	1	2	7	2	0	0	1	14
6	3	1	2	7	3	1	14	1	14
6	3	1	2	7	4	0	0	1	14
6	4	1	2	7	1	0	0	1	14
6	4	1	2	7	2	0	0	1	14
6	4	1	2	7	3	1	14	1	14
6	4	1	2	7	4	0	0	1	14
6	5	1	2	9	1	0	0	1	11
6	5	1	2	9	2	1	11	1	11
6	5	1	2	9	3	0	0	1	11
6	5	1	2	9	4	0	0	1	11
6	6	1	2	7	1	1	14	2	29
6	6	1	2	7	2	1	14	2	29
6	6	1	2	7	3	0	0	2	29
6	6	1	2	7	4	0	0	2	29
6	7	1	2	7	1	1	14	3	43
6	7	1	2	7	2	2	29	3	43
6	7	1	2	7	3	0	0	3	43
6	7	1	2	7	4	0	0	3	43
6	8	1	2	7	1	0	0	1	14
6	8	1	2	7	2	0	0	1	14
6	8	1	2	7	3	0	0	1	14
6	8	1	2	7	4	1	14	1	14
6	9	1	2	7	1	0	0	0	0
6	9	1	2	7	2	0	0	0	0
6	9	1	2	7	3	0	0	0	0
6	9	1	2	7	4	0	0	0	0
6	10	1	2	9	1	0	0	3	33
6	10	1	2	9	2	1	11	3	33
6	10	1	2	9	3	2	22	3	33
6	10	1	2	9	4	0	0	3	33
6	11	1	2	8	1	0	0	1	13
6	11	1	2	8	2	0	0	1	13

6	11	1	2	8	3	1	13	1	13
6	11	1	2	8	4	0	0	1	13
6	12	1	2	11	1	0	0	0	0
6	12	1	2	11	2	0	0	0	0
6	12	1	2	11	3	0	0	0	0
6	12	1	2	11	4	0	0	0	0
6	13	1	2	20	1	1	5	7	35
6	13	1	2	20	2	2	10	7	35
6	13	1	2	20	3	4	20	7	35
6	13	1	2	20	4	0	0	7	35
6	14	1	2	20	1	1	5	6	30
6	14	1	2	20	2	4	20	6	30
6	14	1	2	20	3	1	5	6	30
6	14	1	2	20	4	0	0	6	30
7	1	2	2	6	1	0	0	0	0
7	1	2	2	6	2	0	0	0	0
7	1	2	2	6	3	0	0	0	0
7	1	2	2	6	4	0	0	0	0
7	2	2	2	8	1	0	0	0	0
7	2	2	2	8	2	0	0	0	0
7	2	2	2	8	3	0	0	0	0
7	2	2	2	8	4	0	0	0	0
7	3	2	2	8	1	0	0	1	13
7	3	2	2	8	2	1	13	1	13
7	3	2	2	8	3	0	0	1	13
7	3	2	2	8	4	0	0	1	13
7	4	2	2	9	1	0	0	0	0
7	4	2	2	9	2	0	0	0	0
7	4	2	2	9	3	0	0	0	0
7	4	2	2	9	4	0	0	0	0
7	5	2	2	9	1	1	11	8	89
7	5	2	2	9	2	3	33	8	89
7	5	2	2	9	3	4	44	8	89
7	5	2	2	9	4	0	0	8	89
7	6	2	2	9	1	11	122	15	167
7	6	2	2	9	2	3	33	15	167
7	6	2	2	9	3	1	11	15	167
7	6	2	2	9	4	0	0	15	167
7	7	2	2	9	1	0	0	6	67
7	7	2	2	9	2	1	11	6	67
7	7	2	2	9	3	5	56	6	67
7	7	2	2	9	4	0	0	6	67
7	8	2	2	9	1	1	11	13	144
7	8	2	2	9	2	9	100	13	144
7	8	2	2	9	3	3	33	13	144
7	8	2	2	9	4	0	0	13	144
7	9	2	2	9	1	1	11	2	22

7	9	2	2	9	2	0	0	2	22
7	9	2	2	9	3	0	0	2	22
7	9	2	2	9	4	1	11	2	22
7	10	2	2	13	1	1	8	10	77
7	10	2	2	13	2	3	23	10	77
7	10	2	2	13	3	4	31	10	77
7	10	2	2	13	4	2	15	10	77
7	11	2	2	14	1	3	21	12	86
7	11	2	2	14	2	2	14	12	86
7	11	2	2	14	3	6	43	12	86
7	11	2	2	14	4	0	0	12	86
7	12	2	2	18	1	0	0	2	11
7	12	2	2	18	2	1	6	2	11
7	12	2	2	18	3	1	6	2	11
7	12	2	2	18	4	0	0	2	11
7	13	2	2	19	1	2	11	10	53
7	13	2	2	19	2	5	26	10	53
7	13	2	2	19	3	3	16	10	53
7	13	2	2	19	4	0	0	10	53
7	14	2	2	19	1	4	21	7	37
7	14	2	2	19	2	1	5	7	37
7	14	2	2	19	3	1	5	7	37
7	14	2	2	19	4	1	5	7	37
8	1	2	2	7	1	0	0	0	0
8	1	2	2	7	2	0	0	0	0
8	1	2	2	7	3	0	0	0	0
8	1	2	2	7	4	0	0	0	0
8	2	2	2	8	1	0	0	0	0
8	2	2	2	8	2	0	0	0	0
8	2	2	2	8	3	0	0	0	0
8	2	2	2	8	4	0	0	0	0
8	3	2	2	9	1	0	0	0	0
8	3	2	2	9	2	0	0	0	0
8	3	2	2	9	3	0	0	0	0
8	3	2	2	9	4	0	0	0	0
8	4	2	2	15	1	0	0	0	0
8	4	2	2	15	2	0	0	0	0
8	4	2	2	15	3	0	0	0	0
8	4	2	2	15	4	0	0	0	0
8	5	2	2	15	1	0	0	0	0
8	5	2	2	15	2	0	0	0	0
8	5	2	2	15	3	0	0	0	0
8	5	2	2	15	4	0	0	0	0
8	6	2	2	18	1	0	0	0	0
8	6	2	2	18	2	0	0	0	0
8	6	2	2	18	3	0	0	0	0
8	6	2	2	18	4	0	0	0	0

8	7	2	2	18	1	0	0	0	0
8	7	2	2	18	2	0	0	0	0
8	7	2	2	18	3	0	0	0	0
8	7	2	2	18	4	0	0	0	0
8	8	2	2	19	1	0	0	1	5
8	8	2	2	19	2	0	0	1	5
8	8	2	2	19	3	1	5	1	5
8	8	2	2	19	4	0	0	1	5
8	9	2	2	19	1	7	37	14	74
8	9	2	2	19	2	2	11	14	74
8	9	2	2	19	3	5	26	14	74
8	9	2	2	19	4	0	0	14	74
8	10	2	2	18	1	2	11	3	17
8	10	2	2	18	2	0	0	3	17
8	10	2	2	18	3	1	6	3	17
8	10	2	2	18	4	0	0	3	17
8	11	2	2	16	1	1	6	1	6
8	11	2	2	16	2	0	0	1	6
8	11	2	2	16	3	0	0	1	6
8	11	2	2	16	4	0	0	1	6
8	12	2	2	17	1	3	18	8	47
8	12	2	2	17	2	3	18	8	47
8	12	2	2	17	3	1	6	8	47
8	12	2	2	17	4	1	6	8	47
8	13	2	2	16	1	1	6	1	6
8	13	2	2	16	2	0	0	1	6
8	13	2	2	16	3	0	0	1	6
8	13	2	2	16	4	0	0	1	6
8	14	2	2	22	1	3	14	8	36
8	14	2	2	22	2	3	14	8	36
8	14	2	2	22	3	2	9	8	36
8	14	2	2	22	4	0	0	8	36
9	1	0	1	31	1	0	0	0	0
9	1	0	1	31	2	0	0	0	0
9	1	0	1	31	3	0	0	0	0
9	1	0	1	31	4	0	0	0	0
9	2	0	1	37	1	0	0	0	0
9	2	0	1	37	2	0	0	0	0
9	2	0	1	37	3	0	0	0	0
9	2	0	1	37	4	0	0	0	0
9	3	0	1	40	1	0	0	0	0
9	3	0	1	40	2	0	0	0	0
9	3	0	1	40	3	0	0	0	0
9	3	0	1	40	4	0	0	0	0
9	4	0	1	50	1	0	0	0	0
9	4	0	1	50	2	0	0	0	0
9	4	0	1	50	3	0	0	0	0

9	4	0	1	50	4	0	0	0	0
9	5	0	1	50	1	0	0	0	0
9	5	0	1	50	2	0	0	0	0
9	5	0	1	50	3	0	0	0	0
9	5	0	1	50	4	0	0	0	0
9	6	0	1	48	1	0	0	0	0
9	6	0	1	48	2	0	0	0	0
9	6	0	1	48	3	0	0	0	0
9	6	0	1	48	4	0	0	0	0
9	7	0	1	49	1	0	0	0	0
9	7	0	1	49	2	0	0	0	0
9	7	0	1	49	3	0	0	0	0
9	7	0	1	49	4	0	0	0	0
9	8	0	1	60	1	0	0	0	0
9	8	0	1	60	2	0	0	0	0
9	8	0	1	60	3	0	0	0	0
9	8	0	1	60	4	0	0	0	0
9	9	0	1	60	1	0	0	0	0
9	9	0	1	60	2	0	0	0	0
9	9	0	1	60	3	0	0	0	0
9	9	0	1	60	4	0	0	0	0
9	10	0	1	65	1	0	0	0	0
9	10	0	1	65	2	0	0	0	0
9	10	0	1	65	3	0	0	0	0
9	10	0	1	65	4	0	0	0	0
9	11	0	1	60	1	0	0	0	0
9	11	0	1	60	2	0	0	0	0
9	11	0	1	60	3	0	0	0	0
9	11	0	1	60	4	0	0	0	0
9	12	0	1	75	1	0	0	0	0
9	12	0	1	75	2	0	0	0	0
9	12	0	1	75	3	0	0	0	0
9	12	0	1	75	4	0	0	0	0
9	13	0	1	90	1	0	0	0	0
9	13	0	1	90	2	0	0	0	0
9	13	0	1	90	3	0	0	0	0
9	13	0	1	90	4	0	0	0	0
9	14	0	1	100	1	0	0	0	0
9	14	0	1	100	2	0	0	0	0
9	14	0	1	100	3	0	0	0	0
9	14	0	1	100	4	0	0	0	0
10	1	0	1	36	1	0	0	0	0
10	1	0	1	36	2	0	0	0	0
10	1	0	1	36	3	0	0	0	0
10	1	0	1	36	4	0	0	0	0
10	2	0	1	50	1	0	0	0	0
10	2	0	1	50	2	0	0	0	0

10	2	0	1	50	3	0	0	0	0
10	2	0	1	50	4	0	0	0	0
10	3	0	1	59	1	0	0	0	0
10	3	0	1	59	2	0	0	0	0
10	3	0	1	59	3	0	0	0	0
10	3	0	1	59	4	0	0	0	0
10	4	0	1	60	1	0	0	0	0
10	4	0	1	60	2	0	0	0	0
10	4	0	1	60	3	0	0	0	0
10	4	0	1	60	4	0	0	0	0
10	5	0	1	68	1	0	0	0	0
10	5	0	1	68	2	0	0	0	0
10	5	0	1	68	3	0	0	0	0
10	5	0	1	68	4	0	0	0	0
10	6	0	1	65	1	0	0	0	0
10	6	0	1	65	2	0	0	0	0
10	6	0	1	65	3	0	0	0	0
10	6	0	1	65	4	0	0	0	0
10	7	0	1	65	1	0	0	0	0
10	7	0	1	65	2	0	0	0	0
10	7	0	1	65	3	0	0	0	0
10	7	0	1	65	4	0	0	0	0
10	8	0	1	75	1	0	0	0	0
10	8	0	1	75	2	0	0	0	0
10	8	0	1	75	3	0	0	0	0
10	8	0	1	75	4	0	0	0	0
10	9	0	1	80	1	0	0	0	0
10	9	0	1	80	2	0	0	0	0
10	9	0	1	80	3	0	0	0	0
10	9	0	1	80	4	0	0	0	0
10	10	0	1	80	1	0	0	0	0
10	10	0	1	80	2	0	0	0	0
10	10	0	1	80	3	0	0	0	0
10	10	0	1	80	4	0	0	0	0
10	11	0	1	80	1	0	0	0	0
10	11	0	1	80	2	0	0	0	0
10	11	0	1	80	3	0	0	0	0
10	11	0	1	80	4	0	0	0	0
10	12	0	1	90	1	0	0	0	0
10	12	0	1	90	2	0	0	0	0
10	12	0	1	90	3	0	0	0	0
10	12	0	1	90	4	0	0	0	0
10	13	0	1	100	1	0	0	0	0
10	13	0	1	100	2	0	0	0	0
10	13	0	1	100	3	0	0	0	0
10	13	0	1	100	4	0	0	0	0
10	14	0	1	100	1	0	0	0	0

10	14	0	1	100	2	0	0	0	0
10	14	0	1	100	3	0	0	0	0
10	14	0	1	100	4	0	0	0	0
11	1	0	2	13	1	0	0	0	0
11	1	0	2	13	2	0	0	0	0
11	1	0	2	13	3	0	0	0	0
11	1	0	2	13	4	0	0	0	0
11	2	0	2	15	1	0	0	0	0
11	2	0	2	15	2	0	0	0	0
11	2	0	2	15	3	0	0	0	0
11	2	0	2	15	4	0	0	0	0
11	3	0	2	19	1	0	0	0	0
11	3	0	2	19	2	0	0	0	0
11	3	0	2	19	3	0	0	0	0
11	3	0	2	19	4	0	0	0	0
11	4	0	2	17	1	0	0	0	0
11	4	0	2	17	2	0	0	0	0
11	4	0	2	17	3	0	0	0	0
11	4	0	2	17	4	0	0	0	0
11	5	0	2	20	1	0	0	0	0
11	5	0	2	20	2	0	0	0	0
11	5	0	2	20	3	0	0	0	0
11	5	0	2	20	4	0	0	0	0
11	6	0	2	21	1	0	0	0	0
11	6	0	2	21	2	0	0	0	0
11	6	0	2	21	3	0	0	0	0
11	6	0	2	21	4	0	0	0	0
11	7	0	2	36	1	0	0	0	0
11	7	0	2	36	2	0	0	0	0
11	7	0	2	36	3	0	0	0	0
11	7	0	2	36	4	0	0	0	0
11	8	0	2	38	1	0	0	0	0
11	8	0	2	38	2	0	0	0	0
11	8	0	2	38	3	0	0	0	0
11	8	0	2	38	4	0	0	0	0
11	9	0	2	38	1	0	0	0	0
11	9	0	2	38	2	0	0	0	0
11	9	0	2	38	3	0	0	0	0
11	9	0	2	38	4	0	0	0	0
11	10	0	2	35	1	0	0	0	0
11	10	0	2	35	2	0	0	0	0
11	10	0	2	35	3	0	0	0	0
11	10	0	2	35	4	0	0	0	0
11	11	0	2	45	1	0	0	0	0
11	11	0	2	45	2	0	0	0	0
11	11	0	2	45	3	0	0	0	0
11	11	0	2	45	4	0	0	0	0

11	12	0	2	55	1	0	0	0	0
11	12	0	2	55	2	0	0	0	0
11	12	0	2	55	3	0	0	0	0
11	12	0	2	55	4	0	0	0	0
11	13	0	2	60	1	0	0	0	0
11	13	0	2	60	2	0	0	0	0
11	13	0	2	60	3	0	0	0	0
11	13	0	2	60	4	0	0	0	0
11	14	0	2	60	1	0	0	0	0
11	14	0	2	60	2	0	0	0	0
11	14	0	2	60	3	0	0	0	0
11	14	0	2	60	4	0	0	0	0
12	1	0	2	9	1	0	0	0	0
12	1	0	2	9	2	0	0	0	0
12	1	0	2	9	3	0	0	0	0
12	1	0	2	9	4	0	0	0	0
12	2	0	2	18	1	0	0	0	0
12	2	0	2	18	2	0	0	0	0
12	2	0	2	18	3	0	0	0	0
12	2	0	2	18	4	0	0	0	0
12	3	0	2	19	1	0	0	0	0
12	3	0	2	19	2	0	0	0	0
12	3	0	2	19	3	0	0	0	0
12	3	0	2	19	4	0	0	0	0
12	4	0	2	20	1	0	0	0	0
12	4	0	2	20	2	0	0	0	0
12	4	0	2	20	3	0	0	0	0
12	4	0	2	20	4	0	0	0	0
12	5	0	2	23	1	0	0	0	0
12	5	0	2	23	2	0	0	0	0
12	5	0	2	23	3	0	0	0	0
12	5	0	2	23	4	0	0	0	0
12	6	0	2	28	1	0	0	0	0
12	6	0	2	28	2	0	0	0	0
12	6	0	2	28	3	0	0	0	0
12	6	0	2	28	4	0	0	0	0
12	7	0	2	45	1	0	0	0	0
12	7	0	2	45	2	0	0	0	0
12	7	0	2	45	3	0	0	0	0
12	7	0	2	45	4	0	0	0	0
12	8	0	2	45	1	0	0	0	0
12	8	0	2	45	2	0	0	0	0
12	8	0	2	45	3	0	0	0	0
12	8	0	2	45	4	0	0	0	0
12	9	0	2	48	1	0	0	0	0
12	9	0	2	48	2	0	0	0	0
12	9	0	2	48	3	0	0	0	0

```
 12     9     0     2     48     4     0     0     0     0
 12     10    0     2     55     1     0     0     0     0
 12     10    0     2     55     2     0     0     0     0
 12     10    0     2     55     3     0     0     0     0
 12     10    0     2     55     4     0     0     0     0
 12     11    0     2     55     1     0     0     0     0
 12     11    0     2     55     2     0     0     0     0
 12     11    0     2     55     3     0     0     0     0
 12     11    0     2     55     4     0     0     0     0
 12     12    0     2     60     1     0     0     0     0
 12     12    0     2     60     2     0     0     0     0
 12     12    0     2     60     3     0     0     0     0
 12     12    0     2     60     4     0     0     0     0
 12     13    0     2     67     1     0     0     0     0
 12     13    0     2     67     2     0     0     0     0
 12     13    0     2     67     3     0     0     0     0
 12     13    0     2     67     4     0     0     0     0
 12     14    0     2     80     1     0     0     0     0
 12     14    0     2     80     2     0     0     0     0
 12     14    0     2     80     3     0     0     0     0
 12     14    0     2     80     4     0     0     0     0
$cal nc=cond+1$
$c nc is just renaming condition so that it lies be
$cal w=%if(%eq(nc,1),0,1)$
$cal a=%if(%eq(csize,1),0,1)$
$cal g=a*w$
$weight g$
$fac nc 3 csize 2 lsize 4$
$cal sq=2*%sqrt (freq+1)$
$yvar sq$
$err n$
$link l$
$tab the freq mean for nc$
  NC    1      2      3
 MEAN  0.0000  0.9509  1.3348
$tab the propfreq mean for nc$
  NC    1      2      3
 MEAN   0.00  13.54  21.89
$tab the freq mean for nc;csize;lsize into wmean by
$tprint(s=1) wmean wnc;wcsize;wlsize$
          +-----------------------------------+
     WLSIZE|    1       2       3       4   |
  WNC WCSIZE|                                |
+------------+-----------------------------------+
|  1     1 | 0.0000  0.0000  0.0000  0.0000 |
|        2 | 0.0000  0.0000  0.0000  0.0000 |
-------------------------------------------------
```

```
|  2     1 | 2.0000   1.9286   0.9643   0.1429 |
|        2 | 0.5357   0.5714   0.8214   0.6429 |
-------------------------------------------------
|  3     1 | 2.9643   1.3929   1.4643   0.5357 |
|        2 | 1.4643   1.3214   1.3571   0.1786 |
+------------+----------------------------------+
$fit :+nwork:+nc:+nwork.nc$
  deviance =  197.20 at cycle 4
residual df =  223    from 224 observations

  deviance =  175.23 (change =   -21.97) at cycle
4
residual df =  222    (change =    -1    ) from 224
observations

  deviance =  175.23 (change =  -0.002224) at
cycle 4
residual df =  221    (change =  -1       ) from
224 observations

  deviance =  169.90 (change =   -5.325) at cycle
4
residual df =  220    (change =   -1    ) from 224
observations

$stop$
```