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Title: TOWARD A CONTINUOUS CULTURE OF BUMBLE BEES (BOMBUS LATR.)

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Out of 53 queens captured for initiating colonies in the laboratory, 33 laid eggs (62 percent), 29 of these produced colonies (55 percent), and 14 produced queens (26 percent). A behavioral model depicts the activities of queens from spring emergence through colony development. A discussion of its application to bumble bee domestication is made. New queens entered a diapause under laboratory conditions, and their dependence on a "proper" colony environment for doing so is suggested. The changes in fat body and ovary development support the concept of a fat body timer responsible for maintaining diapause. Eggs laid by new queens of B. occidentalis were largely nonviable and those which hatched died. A discussion of the problem in terms of queen motivation and inadequate releasers for maternal behaviors is presented. Artificial insemination of bumble bee queens is feasible. Sperm collection, insemination techniques, the female reproductive anatomy, and suggestions for improvements are included.

Toward A Continuous Culture of Bumble Bees (*Bombus* Latr.)

by

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

TOWARD A CONTINUOUS CULTURE OF BUMBLE BEES (BOMBUS LATR.)

I. INTRODUCTION

Bumble bees (Bombus Latreille) belong to the tribe Bombini, family Apidae and the superfamily Apoidea (Michener, 1974). There are approximately 200 species of bumble bees found mostly in the temperate zones of North America and Eurasia but some are endemic to South America, the Arctic and the northern fringe of Africa (Alford, 1975). A few species were successfully introduced into New Zealand in the late 1800's (Cumber, 1954).

All species of Bombus studied to date are eusocial (Wilson, 1971). Michener (1974) considers them primitively eusocial because bumble bee colonies are for the most part annual, initiated by solitary fertilized queens, rather than perennial like those of the honey bee (Apis sp.).

The life cycle and colony development of the bumble bee is strikingly similar in all species. A fertile, solitary, overwintered queen emerges from her hibernaculum in spring to early summer to feed and search for a nesting site. The site is usually an abandoned nest of a mouse, vole, or shrew, or other similarly protected site in open and relatively undisturbed habitats.

The queen builds a small waxen pot in the nest which is constructed from wax secreted by intersegmental glands on the abdomen. She then provisions the pot with nectar following which she collects pollen and forms it into a small rounded mass. Prior to egg laying the queen becomes "broody," flattening her body against the nest floor and expanding and contracting her abdominal muscles (Plowright and Jay, 1966). This behavior is presumably associated with ovary development. Nest preparation and broodiness culminate with the laying of the first batch of eggs. The queen incubates the brood, elevating her abdominal temperature via a heat exchange system (Heinrich, 1972). Upon hatching, the larvae are fed by the queen. The waxen cells enclosing the larvae are expanded to accommodate the larvae's increasing size. Development from egg to adult requires approximately three to five weeks (Alford, 1975). Once the first brood emerges, all female workers, it shares the

responsibility of incubating and feeding the brood cohorts which are being produced at staggered intervals. The workers assume foraging duties while the queen remains in the colony to lay eggs. The colony grows in size until at some point males and queens are produced. The reason for the switch from workers to reproductives is unclear although it is believed to be linked to increased care and food afforded the developing larvae by the greater worker to brood ratio (Cumber, 1949; Katayama, 1975) or food quality (Röseler, 1976). The conversion from worker to male and queen production is irreversible, workers never again being produced in the life of the colony.

Newly emerged queens remain in the colony, feeding and developing their fat body in preparation for hibernation. Soon afterward they mate. Mating strategies vary for different bumble bee species. Some queens are met by males at nest entrances; other males choose a prominent landmark and stand or hover about the spot until a queen flies by; still other males mark a mating lane with a mandibular gland secretion, flying the path in search of queens. Once mated the queen searches for a hibernation site. The site is usually a north to northwest facing slope not warmed by winter sunshine (Sladen, 1912). Pouvreau (1970) experimentally demonstrated that bumble bees preferred hibernating in soil with humidity above five percent, covered with a layer of litter. The queen digs into the duff or into the soil layer beneath, to form her hibernaculum. Different species prefer different levels in the soil-litter interface in which to hibernate (Alford, 1975). Pouvreau (1970, 1976) considers the depth of hibernation to be correlated to the microclimate of the site and ecological requirements of the species.

Life history information on many species is well documented and continually updated because of interest in bumble bees as crop pollinators and the fact that they are a conspicuous and attractive insect. Holm (1966) summarized the areas of research devoted to the "domestication" of the bumble bee and the reasons behind such work. The following is a brief summary of his review into which some more recent information has been incorporated.

The bumble bee, an effective pollinator, is notorious for its fluctuations in population size both locally and on an annual basis. This has stimulated interest in "domesticating" the bee. In addition,

the wide scale use of pesticides has had a severe effect on non-target insects including bees (Johansen, 1977; Kevan, 1975), the latter perhaps being more susceptible than most, because of their collection of contaminated pollen for larval food. This fact plus the ever increasing size of agricultural operations and the subsequent loss of nesting and overwintering sites has aroused further interest in augmenting and protecting native pollinator populations. Bohart (1958) has suggested leaving open land for nesting and hibernation sites and as a source of spring forage to counter diminishing bee numbers.

Research on the domestication of bumble bees has taken three primary avenues. The first approach has been to encourage natural populations of bumble bee queens to nest by placing nesting boxes where they will attract queens. Once the colony is established it can be repositioned in a desired location. The second approach has been to initiate colonies in the laboratory from field captured spring queens. These could be used to augment field populations where desired and alleviate some of the problem of fluctuating populations. Finally, in order to effectively utilize laboratory reared colonies in the field, it is necessary to begin colonies at a predetermined time so that they would be at peak development when the crop is in full bloom. This is not usually possible with queens caught each spring, as the colony may develop too late for pollination purposes. Also, it is often difficult to collect enough spring queens to make the effort practical. Consequently much interest has been shown in artificially hibernating queens which can be obtained in great numbers at the time of male and queen production. The results in all three areas of research have shown some success, but each has its inherent limitations making commercial "domestication" impractical at the moment.

The obvious problem with attempting to lure natural populations of queens to specific areas for nest establishment depends on the local or seasonal occurrence of the species sought. The approach may be practical where nesting sites are limiting and has been used with some success in Canada (Arnason, 1966).

Most domestication research is concentrated in the laboratory. Colonies being started in portable domiciles in the laboratory can be transported to any desired location in the field. Medler (1958)

outlined four main techniques for initiating colonies in the laboratory: a) confining a queen alone; b) confining two queens together; c) confining one queen with the brood of another nest, and d) confining a queen with two or three workers. Free and Butler (1959) reported eggs were laid by 32 percent of the queens when confined alone, 39 percent when two queens were confined together, and 87 percent when a queen was confined together with two or three workers. Plath (1934) reported improved colony establishment results when one queen was confined with brood from another colony.

Holm (1966) found these results of biological interest but saw no practical value to the phenomena as brood or workers are not available when colony establishment is needed. This problem could be remedied should effective means of rearing bumble bees in continuous culture become feasible. Workers and brood would then be available at all times of the year including early spring. Horber (1961) suggests the combined use of continuous laboratory cultures with the cold storage (artificial hibernation) of surplus queens. His work rearing B. hypnorum for five generations demonstrated that diapause is not obligatory for new queens prior to nest establishment.

Plowright and Jay (1966) confined new queens in cartons. Many of these laid eggs suggesting the possibility of rearing colonies from queens that have not been overwintered. Field observations indicate that several species of bumble bees may be double-brooded, a second generation of queens developing colonies without overwintering (Sladen, 1912; Alfken, 1913; Hobbs, 1967; Meidoll, 1968; Alford, 1975).

One of the problems needed to be resolved before large scale rearing of all bumble bees becomes economically feasible, is the control of mating (Plowright and Jay, 1966). Alford (1975) briefly mentioned controlled fertilization saying that,

> "mating bumble bees in captivity is usually no problem and can be achieved either in nest-boxes or by allowing males and females to contact one another more naturally in larger enclosures."

However, unless individual queens can be constantly monitored following emergence, there is no way to determine if the specimens selected for

subsequent study have been inseminated. It would be advantageous if bumble bee queens could be artificially inseminated. Additionally, the need for the space and supplementary care a large mating room would require is removed as is the wear and tear (stress) queens suffer in a mating room due to the time required to assure mating. The handling time from emergence to the next step in the rearing process would be reduced. Finally, research on controlled cross-matings from different populations of bumble bees would be facilitated. The latter would be true even if phenologies did not overlap should a viable method of storage for bumble bee sperm be developed.

The purpose of this research was to apply and evaluate techniques currently used for rearing bumble bees in the laboratory, to establish queens which have not overwintered for continuous laboratory cultures, and to devise a means of artificially inseminating Bombus queens to assist in the overall scheme of domestication.

Finally, a miscellaneous section has been appended in order to report and discuss some observations which may be of interest to the student of bumble bee biology and behavior.

II. MATERIALS AND METHODS

Laboratory Rearing of Bumble Bees

Queen bumble bees were collected from late April through June 1976 and 1977, as they foraged for nectar and pollen. They were captured with a sweep net, placed in a vial, and kept in a cooler until they could be put in artificial rearing chambers or laboratory domiciles.

The rearing methods, i.e., domiciles and care instructions, were patterned after those used by Plowright and Jay (1966) (Series III design) and modified by W.P. Stephen (personal communication). The domiciles consisted of two chambers constructed of 3/8-inch plywood. The larger flight box was 18cm x 13cm x 10cm, and the smaller nesting box was 8cm x 8cm x 5cm (interior dimensions). The two boxes were connected by a 1.5cm diameter hole. Plate glass covers were used for easy observation of colony development and behavior. Both the nest box and the flight box had balsa wood floors. No nesting material was provided. Both chambers rested on a larger plywood board to facilitate handling.

Prior to placing a queen in a domicile, a 12mm diameter by 6mm high lump of pollen mixture was placed in the middle of the nest box. The pollen mixture was prepared by grinding honey bee collected pollen using a mortar and pestle, then moistening it with equal parts of a 50:50 solution of honey-water and mineral oil until a soft dough consistency was achieved. The mineral oil kept the pollen moist for a longer period than honey-water alone.

A nectar solution was provided in the flight box by gravity fed 1cm diameter glass tubes, 25cm long. The tubes were closed at one end and had a small hole for access by the bees at the other end. The nectar solution was prepared by mixing 1000gm sugar, 1000ml water, 100ml honey, and 50mgm of p-hydroxybenzoic acid to retard fermentation. Both the pollen mixture and nectar solution were refrigerated when not in use. The nectar tube(s) was replaced every third day as was the pollen mass until it was accepted. The tubes were cleaned by shaking out excess nectar, refilling them with water and boiling for 1/2 hour to kill honey fermenting yeasts.

Newly installed queens received approximately one, half-full tube

of nectar. Nectar demands changed with the queen's activity and the size of the colony. Large colonies were given four full tubes. As the first brood began to develop, additional pollen mixture was supplied in small amounts for larval food. Care was taken not to provide surplus pollen, for too much pollen can produce queens in the first brood (Stephen, personal communication). The amount of pollen given varied greatly with each queen and colony, depending on the quality of care given to each larva, and the instar and number of larvae to be fed. Excess pollen was supplied after the first brood emerged to provide food for new brood production.

As some colonies developed, they became too large for the small nest box. At this time they were transferred to a second nest box equal in size to the flight box.

All domiciles were kept in a 2.4m x 3.3m x 2.15m controlled environment room on a 15:9 daylight cycle at 27°C.

Variations in colony initiating techniques were made to evaluate those conditions best for stimulating a queen to lay eggs. In addition to confining a queen alone, a queen was confined with a batch of developing brood, with workers from an established colony, or with both workers and brood.

Some colonies were allowed to collect their own pollen and nectar. Pollen was finely ground and spread out on cheesecloth suspended in a glass bowl. Nectar solution was provided by a gravity fed feeding dish (von Frisch, 1967). The controlled environment room served not only as a foraging universe for the bees but also as a mating arena for Bombus griseocollis queens and males.

Four colonies were allowed to forage from my fourth floor office window in which no supplemental care was provided. Differences between these colonies and controlled environment colonies were noted.

Rearing Nonoverwintered Queens

Nonoverwintered queens were introduced into laboratory domiciles. Rearing conditions were the same for these queens as they had been for spring, field captured queens (above). Workers and brood were no longer available at the time they were needed, thus pairing queens with

brood or workers and brood was not attempted. Two new techniques were used for encouraging new queens to nest. The first involved grouping ten queens together in hopes of stimulating egg development (Free, 1957). The second technique involved the application of juvenile hormone (III), methyl (2E, 6E)-(10R)-10,11-epoxy-3,7,11-trimethyl-2, 6-dodecadienoate, in an attempt to initiate ovary development (Chapman, 1969). According to Trautman, et. al. (1973) JH(III) is found in Hymenoptera.

In 1976, juvenile hormone applications were made by topically applying a mixture of 100μg JH(III) in 5μl of acetone. In 1977, acetone was replaced by olive oil as the solvent because some question was raised concerning the possibility that acetone mimics the results expected from juvenile hormone (V. Brookes, personal communication).

Finally, dissections of queens were made to determine the extent of ovary and fat body development associated with specific stages in queen maturation and behavior outlined in Section III B.

Artificial Insemination

Dissections of bumble bee queens and males were made to determine the arrangement of the reproductive organs and the feasibility of applying honey bee insemination technology to Bombus.

Males varying in age from late pupa to day 28 adults were dissected to determine the age at which sperm were available for collection.

The following methods were attempted for the collection of sperm: 1) grasping a male between the thumb and index finger, and squeezing the abdomen while rolling the fingers towards the posterior end of the body; 2) dissection and removal of the sperm containing seminal vesicles; 3) eversion of the genital capsule and penis through slight abdominal pressure, then grasping the base of the genital capsule and pulling it gently from the male's body so as not to tear off the accessory glands and seminal vesicles attached to the capsule.

Once seminal vesicles were collected, rendering the sperm in a form suitable for collection by the insemination syringe was studied. Methods included: 1) masceration of seminal vesicles in saline,

followed by centrifugation to separate and concentrate the sperm; 2) sonication of the seminal vesicles in saline followed by centrifugation; and 3) crushing the seminal vesicles in a 5μl drop of saline below a cover slip and gradually lifting the cover slip from one side to form a small drop containing the sperm at the opposite side for collection.

Standard honey bee insemination equipment was used. This consisted of a commercial Roberts and Mackensen apparatus for securing a queen holder, ventral hook, sting hook and syringe in a movable fashion, a pair of forceps, a tank of carbon dioxide gas, and a dissecting scope with a light source. A detailed description of the equipment and its operation is given by Mackensen and Tucker (1970). The queen holder in this study was modified from a 3cc syringe, because bumble bee queens were larger than could be accommodated by the standard holder for honey bee queens. Sanitation procedures included dipping the syringe tip in clorox, and wiping the hooks and forceps with alcohol between insemination (Mackensen and Tucker, 1970).

The insemination procedure consisted of anesthetizing a queen with carbon dioxide and placing her, abdomen first, into the queen holder. A hose connected to the carbon dioxide was used to hold the queen in place and to re-anesthetize her should she require it. The sting was teased out using forceps and the hooks placed to keep the queen's sting chamber open for the placement of the insemination syringe tip. The sperm was injected directly into the bursa copulatrix.

After insemination the queen was removed from the holder, marked and returned to a domicile. The queens were dissected when they prematurely died or showed no signs of egg laying behavior after several weeks of confinement. Their spermathecae were removed and crushed beneath a cover slip, and observed for the presence and viability of the sperm.

All males were removed from colonies soon after their emergence so that they could not inseminate the queens.

III. RESULTS AND DISCUSSION

A. Laboratory Rearing Spring Queens

The successful establishment of field captured queens in the laboratory domiciles varied greatly among species (Table 1). Bombus griseocollis was very easy to start. Three of the four B. griseocollis queens laid eggs within two weeks of their confinement and the fourth began egg cell construction the day after a larval cell from an established colony was introduced.

Bombus vosnesenskii was a much slower starter, the first queen laying eggs 17 days after confinement with the other four following within three weeks. The remaining five B. vosnesenskii queens which laid eggs did so after larvae from other colonies, and in one case both larvae and a worker were introduced.

Bombus melanopygus readily established colonies. Three of four queens started 4, 5 and 10 days after confinement. The fourth queen was sluggish from the beginning and never showed interest in the pollen.

Six Bombus occidentalis queens which laid eggs within the first two weeks did so without supplementary stimulation from workers or brood. Three additional queens produced offspring after being introduced into colonies where the original queen had died, and three queens developed a colony after being given some larval brood from another colony.

Bombus sitkensis queens laid eggs 21 and 35 days after confinement. The earlier laying queen destroyed her first egg cluster three days after laying them and laid a second batch 20 days later. These she also destroyed. She died shortly thereafter. The other queen produced only one larva in her first cohort, this developing into a queen, presumably due to the excessive provisions afforded it in the form of food and brooding. The foundress then reared seven more workers before she died.

Bombus nevadensis was difficult to establish. The queens showed no interest in the pollen. One queen started a colony a month after confinement but produced only a few workers and a male.

Table I. Results of rearing attempts in the laboratory using spring captured queens. SQ = solitary confined queens, Q+B = queen confined with brood, Q+B+W = queen confined with workers and brood, E = eggs, L = larvae, P = pupae, W = workers, QC = queenless colony, + = yes, - = no, ♀ = workers, ♂ = males, ♀♀ = queens.

Species	SQ	Q+B	Q+B+W	Eggs	Colony Produced
B. centralis Cresson	X			-	
B. fervidus Fabr.	X			+	♀, ♂
B. fervidus Fabr.	X			-	
B. fervidus Fabr.	X			-	
B. fervidus Fabr.	X			-	
B. fervidus Fabr.	X			-	
B. griseocollis De Geer	X			+	♀, ♂, ♀♀
B. griseocollis De Geer	X			+	♀. ♂, ♀♀
B. griseocollis De Geer	X			+	♀, ♂
B. griseocollis De Geer	X	+E +L		--+	♀, ♂, ♀♀
B. melanopygus Nylander	X			+	♀, ♂, ♀♀
B. melanopygus Nylander	X			+	♀, ♂
B. melanopygus Nylander	X	+L		-+	♀
B. melanopygus Nylander	X			-	
B. mixtus Cresson	X			+	♀, ♂
B. mixtus Cresson	X			-	
B. nevadensis Cresson	X			+	♀, ♂
B. nevadensis Cresson	X			-	
B. nevadensis Cresson	X			-	
B. occidentalis Greene	X			+	♀, ♂, ♀♀
B. occidentalis Greene	X			+	♀
B. occidentalis Greene	X			+	♀, ♂, ♀♀
B. occidentalis Greene	X			+	♀
B. occidentalis Greene	X			+	♀
B. occidentalis Greene	X			+	
B. occidentalis Greene	X	+P +L		--+	♀
B. occidentalis Greene	X	+L	+W	--+	♀
B. occidentalis Greene	X	+L		-+	
B. occidentalis Greene	X	+L		--	
B. occidentalis Greene	X	+L		--	
B. occidentalis Greene	X	+E +L		--+	♀, ♂
B. occidentalis Greene	X	+QC		-+	♀, ♂, ♀♀
B. occidentalis Greene	X	+QC		-+	♀, ♂, ♀♀
B. occidentalis Greene	X	+QC		-+	♀, ♂, ♀♀
B. occidentalis Greene	X			-	
B. occidentalis Greene	X			-	
B. sitkensis Nylander	X			+	♀, ♀♀
B. sitkensis Nylander	X			+	
B. vosnesenskii Rad	X			+	♀, ♂, ♀♀
B. vosnesenskii Rad	X			+	♀, ♂, ♀♀
B. vosnesenskii Rad	X			+	♀, ♂
B. vosnesenskii Rad	X			+	♀, ♂

Table I. Continued.

Species	SQ	Q+B	Q+B+W	Eggs	Colony Produced
B. vosnesenskii Rad	X	+L		-+	♀
B. vosnesenskii Rad	X	+E +L		--+	♀, ♂, ♀♀
B. vosnesenskii Rad	X	+E +L		---	
B. vosnesenskii Rad	X	+L	+W	--+	
B. vosnesenskii Rad	X	+L		-+	
B. vosnesenskii Rad	X	+P +L		--+	♀, ♂
B. vosnesenskii Rad	X			-	
B. vosnesenskii Rad	X			-	
B. vosnesenskii Rad	X			-	
B. vosnesenskii Rad	X			-	

B. ferridus was very aggressive. The queens attempted to sting if they were even slightly disturbed. One succeeded in producing a few workers and a male. The remaining queens never showed any signs of beginning.

One B. mixtus queen started a colony 13 days after confinement. She produced a few workers and males.

Queens that never laid eggs eventually died. It is not known if they did not start because they could not adjust to colony conditions, they were parasitized by the nematode, Sphaerularia bombi, as is often the case (Alford, 1975), or if some other factor was involved. Out of 53 queens captured for initiating colonies in the laboratory, 33 laid eggs (62 percent), 29 of these produced colonies (55 percent), and 14 produced queens (26 percent).

The variation in colony initiation results was not confined to interspecific differences. Within a given species there were some queens which initiated colonies soon after confinement, while others took two to five weeks longer or did not start at all.

The size of a colony to a large extent depended upon the behavior of the queen. Active, vigorous, queens produced the largest colonies, usually brooded and fed their first brood cohort well and maintained colony cohesion until sexuals were produced or they died. The maternal care given to the brood varied greatly between queens and the developmental rate of the brood reflected the quality of care given them. In B. occidentalis the brood of some queens emerged in 21 days while brood from other queens took a month or more.

In all cases where a queen began incubating donated brood cells, she did so either immediately upon contacting the brood cell or within a day. The queen's response, if receptive, was to rest the brood cell with her antennae and begin incubating it with her abdomen wrapped around it. In five of 22 cases when brood was introduced, the queen opened the cell and removed the contents, usually eggs, and did not respond further. One B. occidentalis queen, one B. griseocollis queen and two B. vosnesenskii queens rejected eggs, but all accepted larvae containing cells at a later time. The queen usually laid eggs several days after acceptance of the donated brood. In two cases, eggs were laid on the following day indicating mature eggs were already developed.

Combined worker-brood introductions were successful in some cases. Where both workers and brood were introduced, the worker was placed in the domicile only after the queen failed to respond to the brood alone. The worker usually began incubating the brood soon after adjusting to the new domicile. Queens which became broody with workers present did so more slowly than if they had responded to the brood, taking several days to do so.

In three cases, B. occidentalis queens died after establishing colonies. Each time, I introduced another queen which had been non-responsive during solitary confinement into the queenless colonies. Their response to the new colony environment was notable. After an initial period of adjustment, each queen hurried around their colony antennating workers and brood. All three began laying eggs within a week and assumed the role of colony queen without apparent hesitation.

From the literature (Alford, 1975; Plowright and Jay, 1966) and my results, it appears that queens must be stimulated to lay eggs as soon as possible after confinement because the probabilities of rearing strong colonies decrease with time. The largest colonies produced by queens of B. griseocollis, B. melanopygus, B. vosnesenskii, and B. occidentalis, were produced soon after queen confinement. Comparisons were made only among queens of the same species.

Alford (1975) suggests that queens which fail to become broody after a week or two in confinement, should be discarded. This would prove unwise for some species, particularly B. vosnesenskii, because colony initiation took much longer than two weeks.

When compared with work of other investigators, my rearing success was average, and does not support rearing on a commercial scale. However, one technique of particular interest and deserving of more consideration than given in the past is that of encouraging "uncooperative" queens to begin colonies using introduced brood and/or workers. Holm (1966) recognized the technique of pairing one queen with workers as the most successful method for rearing queens in the laboratory.

Techniques such as the latter are usually only casually discussed and neither answer the why behind the phenomenon nor ask questions which might contribute to their clarification. To achieve more consistent and useable techniques for rearing bumble bees, behavior

analysis beyond a general description are necessary.

By systematically examining the behavior of bumble bees in the wild and comparing it with the behavior of queens in the laboratory, the components of the environment necessary for normalizing laboratory behavior might be understood and applied.

The results of my research as well as information from the literature on field and laboratory reared queens are utilized to discuss queen behavior during the period from spring emergence to first brood eclosion. This is the most critical time for queens in the laboratory. Examining these behavior patterns and placing them into units which can be dealt with in an orderly fashion is facilitated by using components of behavior described by Eibl-Eibesfeldt (1970) and Markl (1973).

The behavior leading up to and including colony establishment in Bombus sp. must be regarded as genetically fixed. Each healthy queen emerging in the spring follows the same behavioral sequence (Alford, 1975) with limited infraspecific variability. These behaviors (fixed action patterns) are charted in chronological order in Figure 1 along with the stimuli which appear necessary for triggering each in wild queens. In order for each fixed action pattern to be elicited the queen must be motivated and the proper releaser (stimulus) must be provided. In this heirarchical organization of behavior each activity pattern is modified by the previous experience as the queen's internal readiness to act (motivation) and the proper environmental conditions (stimuli) interact to release the next behavior in the sequence. Motivation is a complex condition involving the interaction of environment and physiology.

Herein lies the problem of rearing laboratory queens. By confining field queens in artificial domiciles their behavioral sequence is interrupted and further activity is dependent upon the queen's current motivational state and its response to the limited stimuli of the altered environment. The point at which the behavioral chain of field captured queens is interrupted is unknown. It could conceivably be anywhere from reactivation (R), to foraging for supplies during maternal care (M) as outlined in the simplified ethogram.

The queens are placed in their domiciles with only nectar and a pollen mass to stimulate them to continue behavioral development. Some

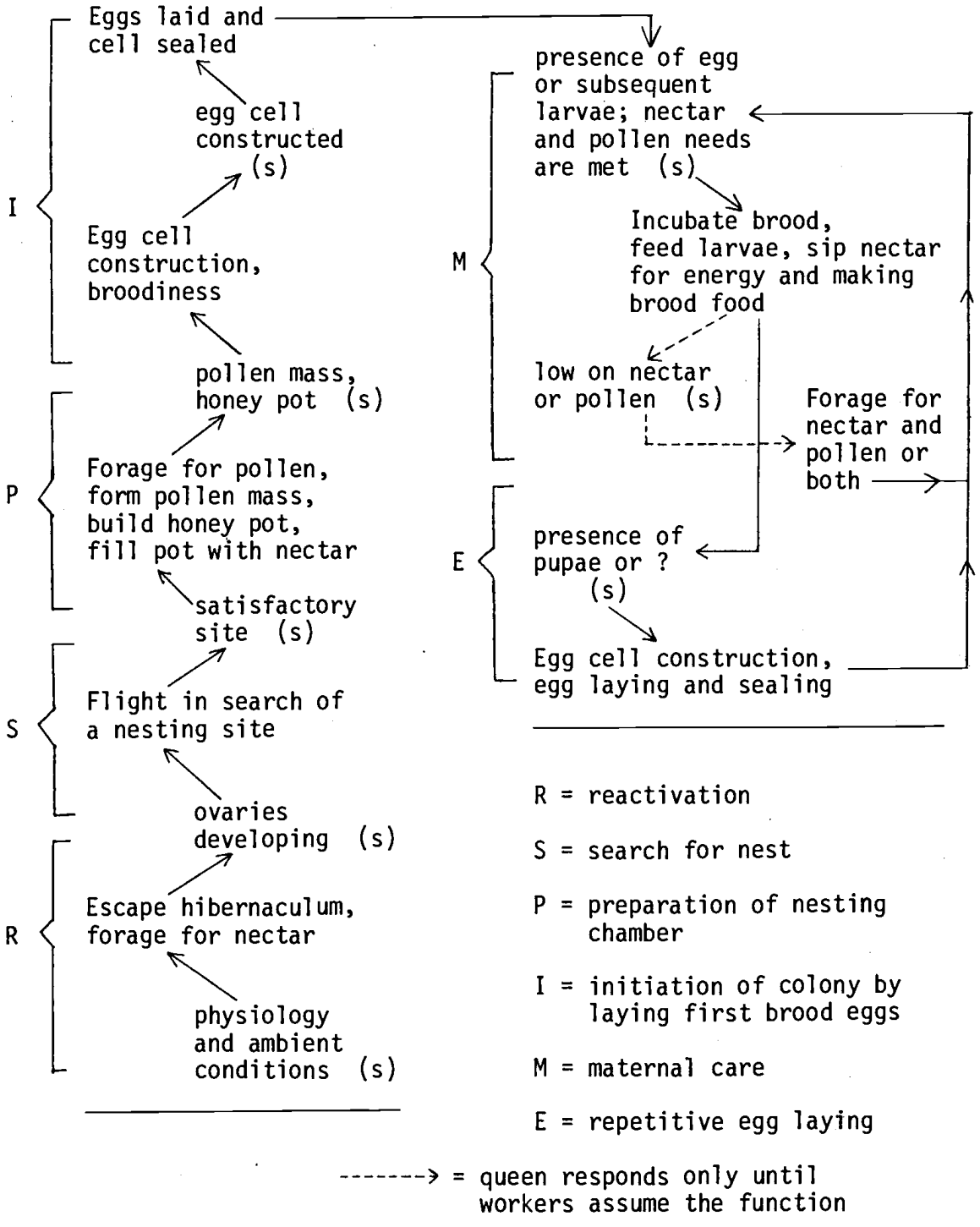

Figure 1. A simplified ethogram depicting the behaviors of queens following spring emergence. The (s) denotes presumed stimuli. Behavioral formula: RSPI $(M^nE)^x$. (Patterned after Evans, 1966).

queens respond to these conditions by continuing in the normal behavioral sequence; becoming broody, building a honey pot, laying eggs, etc. Other queens show no response. The fact that some species are easier to rear than others suggests that species specific response thresholds or releasing stimuli vary considerably among species. Additionally, it seems that the response threshold and stimulus requirements are reduced with time, as many queens, if confined for long periods, will develop colonies. Ethologically this can be explained as an increase in the specific drive state or readiness to act, which lowers the threshold for release of a fixed action pattern. Said another way, the queen has been prevented from performing the behaviors to which she has been genetically programmed and physiologically motivated to perform due to inadequate releasers. Consequently her search for a releaser (appetitive behavior) is satisfied by a weak stimulus as a result of a lowered response threshold. The increase in drive is probably due to a complex interaction of physiology and environment. In the case of bumble bee queens it appears to be linked to the development of their ovaries (see Section III B). Queens which begin maternal care and egg laying after the addition of brood do so in response to sign stimuli associated with the brood. This release of care giving behavior has been termed epimeletic behavior by Scott (1956). The associated care soliciting, et-epimeletic behavior, has not been demonstrated for Bombus larvae although it has been for some wasps (Ishay, et. al., 1972).

Heinrich (1974) provides evidence that queens deposit a pheromone at the time eggs are laid. This pheromone, he argues, solicits the queen's own epimeletic behavior as well as that of subsequent workers, and is species specific. The repetitive epimeletic behavior M^n of the behavioral formula (Figure 1) requires a stimulus. If the stimulus is a chemical as Heinrich suggests, it would be valuable to identify and produce it. As already discussed, the technique of using bumble bee brood for care soliciting has been shown quite effective, and should a pheromone help release the appropriate behavior, its use would contribute greatly in bumble bee domestication programs. Additionally, a species specific chemical soliciting epimeletic behaviors carries some interesting implications regarding the evolution of species specific

parasitization by the social parasite, Psithyrus sp.

Free and Butler (1959) report great success establishing colonies using workers confined with queens. The presence of workers and/or another queen (Alford, 1975) may facilitate the queen's onset of broodiness and associated colony initiation behaviors (P, Figure 1). The approach is supported by the results in Section III B.

Lastly, many queens showed signs of developing colonies but never did. Many became broody and a few even built honey pots and laid eggs. The eggs never hatched and the queens never showed strong rearing qualities. To account for this at the level of interaction between environment and queen one must consider why the behavior of the queen was incompletely expressed. The behaviors of laying eggs and care giving have been presented as fixed action patterns. The variability of these motor patterns are modulated to some extent by the external stimuli as well as the level of motivation (Markl, 1973). When the level of the motivating and/or releasing factors are varied then a behavior pattern can occur in all degrees of intensity, from trace-like to complete responses (Markl, 1973). This is possibly an explanation why many of the queens incompletely started or did not start colonies. Their level of motivation in captivity depended upon the interaction of the level of motivation when they were caught and the effect confinement had on it. Finally, the stimulus provided by the domicile is undoubtedly infinitely weaker than those in the natural environment.

The physiological (i.e., endocrine, neural, biochemical) activity of the queen ultimately determines her level of motivation. The following section (B) discusses some of the physiological aspects of motivation in more detail.

B. Rearing Nonoverwintered Queens

Results

1976

New queens went into a period of dormancy within a few weeks of eclosion. They eventually became active again and queens which had been in a laboratory free flight colony began foraging and incubating eggs like a worker. Aggression in queens was often seen at this time and dead queens were common outside the nest entrance.

Ten new queens were treated with JH(III) approximately three weeks after the dormant stage. All but four died within a few days. Mortality was not restricted to JH(III) treated queens for four of five control queens died as did most of the queens in the flight room from which the stock was drawn. The surviving control queen did not become broody. It is possible that the queens died from stress effects of the flight room. All of them were worn in appearance, possibly due to the continued harassment they received from persistent males. It is also possible that the queens were diseased. B. vosnesenskii queens suffered a high mortality earlier in the summer and an unidentified bacteria was believed to be the cause.

The effect of JH(III) on the B. griseocollis queens is impossible to interpret because of the high mortality suffered by both treated and control queens. However, three queens did initiate colonies and rear sexuals in two of the cases. The results were sufficiently encouraging to try similar studies in 1977.

1977

New queens of Bombus occidentalis were confined to their colony after eclosion. The queens were observed feeding and dissections revealed the development of large white fat bodies as is observed in field queens preparing for diapause. No matings were observed. In three colonies males were removed so as not to interfere with insemination studies. The queens do not need to mate in order to develop their

fat body as the fat body normally develops prior to mating (Alford, 1975).

After the fat bodies were developed the queens positioned themselves out of the path of hive activity, usually at the sides of the nest chamber and became dormant. The queens barely moved, nor were they observed to feed for several weeks. A few queens of B. nevadensis and B. griseocollis were also reared in laboratory colonies, and with one exception (below) the behavior of the new queens was similar to B. occidentalis. The length of time queens of different species remained dormant was characteristic. Queens of B. occidentalis became active one to three weeks before queens of B. griseocollis (13 individuals). B. griseocollis became active two to six weeks before queens of B. nevadensis (3 individuals). B. nevadensis was inactive for almost two months while some queens of B. occidentalis became active after only a week in the dormant condition.

Ovary and fat body development changed markedly with the distinct phases of new queen development. Queens newly emerged had undeveloped, unpigmented fat bodies and undeveloped ovaries. Dormant queens showed a white well-developed fat body which filled the abdomen. Their ovaries appeared stringy and undeveloped. Queens out of torpor but not yet broody showed varying degrees of fat body and ovary development. It can be said that the fat body, ranging from well developed (white and fluffy) to reduced (yellowish and foamy to brown and inconspicuous), was inversely proportional to the development of the ovaries. Those queens with a well developed fat body had poorly developed ovaries. Those queens with a reduced fat body had developing or often completely developed ovaries.

The fat body consists of two cell types, the fat cells or trophocytes and the oenocytes (Palm, 1948; Alford, 1969a). When fat body enlarges it is due to storage of lipid and glycogen in trophocytes and as the fat body diminishes the brown pigment that develops is attributed to waste product accumulation in the oenocytes (Alford, 1969a). The waste products are presumably produced by the fat body, as a result of intermediary metabolism (Kilby, 1963; Wigglesworth, 1965).

The results of attempts at establishing colonies using new queens of B. occidentalis are summarized in Table 2. All queens had passed

Table II. Summarized results for rearing new queens. All queens had exited the dormant state except TDQ. TDQ = treated dormant queens, SQ = single queens, PQ = paired queens, GQ = queens confined in a group, CQ = colony confined queens, TSQ = treated single queens, TPQ = paired queens (1 treated and 1 control), OTSQ = older treated single queens.

Treatment	TDQ	SQ	PQ	GQ	CQ	TSQ	TPQ	OTSQ
No. of queens	10	22	16 (8 pair)	12	15+	4	8 (4 pair)	6
Never broody	10	8	4	1	?	1	2	1
Broody	0	14	12 at least one/pair	11	most became broody	3 (2-3 wks)	4T (5-7 days) 2C (2-3 wks)	5
Nonviable eggs laid	0	4	3	? egg eating	? egg eating	0	3	5
Viable eggs laid	0	2	2	?	?	0	1	0

through the dormant phase and were again feeding with the exception of ten queens which received a juvenile hormone application while they were still dormant.

Most queens became broody regardless of the rearing technique. Treated torpid queens were an exception, but it is presumed that they too would have become broody as their individually confined sisters after becoming active and feeding. The effect of JH treated and paired queens was seen in the time elapsed before the onset of broodiness. Queens which were treated and paired became broody before queens which were paired but not treated. Paired, untreated queens became broody before treated, single queens, and treated, single queens became broody before queens which were single and not treated.

Nontreated, paired queens became broody within a few days of one another as did all but one queen in the group of ten queens. Queens which were treated and paired each had one nontreated member acting as a control. The control queen in the pairs became broody after the treated queens, but prior to single treated queens in a parallel experiment.

A later experiment, using older queens of the same stock from which previous queens were drawn had treated queens becoming broody within a week of treatment and laying eggs shortly thereafter.

One interesting exception to the pattern of prediapause queen development described for B. occidentalis was seen in a B. griseocollis queen. A number of what were believed to be B. griseocollis males were collected from the field for the purpose of comparing their anatomy with that of other Bombus species. By accident a number of workers and apparently one abnormally small queen were also collected. These were all placed in a nest box and pollen was supplied. Shortly after confinement a number of egg cells began appearing scattered about the domicile and tended by several different workers and the small queen. Several queens developed from two egg clusters. Eight days after the emergence of the first queen she became noticeably broody. The remaining queens emerged within a week of their sister but never became broody. These queens followed the more typical pattern of new queen development seen in the B. occidentalis queens.

Fourteen days after this unique queen emerged she was placed into

a domicile by herself and given pollen and nectar. Within two days she laid eggs, all of which matured to adult males. She laid a second batch of eggs which also developed into males. Workers were not expected as she was never inseminated.

One additional note on new queen rearing concerns B. nevadensis queens reared in my office. Five queens were removed several days after emergence to the controlled environment room for comparison with some of their sister queens. Two to three months after their confinement three of the queens in the environment controlled room became broody. Their sisters in my cooler office did not become broody even a month later. One sacrificed queen from both environments showed the fat body reduced and ovaries developed in the queen which became broody, while her sister was the reverse, with an abundant fat body and small ovaries.

Discussion

Queen Establishment

The changes new laboratory reared queens undergo appear comparable to those seen in naturally reared queens from eclosion to egg laying.

In order to better evaluate the similarities between laboratory and field reared queens it would be helpful to examine the physiological development of each event.

Reproductive diapause in adult insects finds its immediate cause in the inactivation of the brain neurosecretory cells controlling the corpora allata (CA) (Saunders, 1976; Williams, 1976). The CA produces juvenile hormone which mediates the development of the oocytes (Chapman, 1968) by regulating the production of vitellogenins. Vitellogenins are the basic constituent of the oocyte (Engels, 1974) and have been shown to be lipo-proteins (Steele, 1976). The site of synthesis of vitellogenins is known to be the fat body in a number of insects (Steele, 1976).

In many species preparing for diapause the CA is inactive and juvenile hormone is not produced. At this time little or no growth of the oocytes occurs and a large fat body develops in response to

feeding. This condition is known as gonotrophic dissociation (Wigglesworth, 1970). Steele (1976) offers an explanation for this phenomenon. He says that in the absence of juvenile hormone a decrease in protein synthesis occurs which is accompanied by an increase in lipid and glycogen levels in the fat body. This suggests that amino acids and carbohydrates are converted into lipid rather than being used in the process of protein synthesis. At the same time lipid reserves accumulate in the fat body because proteins are not available to conjugate with them for transport to the ovaries and incorporation into the oocytes.

Bumble bees preparing for diapause do so via the process of gonotrophic dissociation just defined. When a queen emerges from her puparium her CA is small and remains so until late in her winter torpor (Palm, 1948). Likewise fat body development occurs in response to fall feeding, and ovaries enlarge very little (Palm, 1948; Alford 1969a; Millareau, et. al., 1974; Pouvreau, 1976).

After a fat body is developed bumble bee queens in the field enter a period of diapause (Alford, 1975), and it appears that queens in the laboratory do this also. Laboratory reared queens will mate, construct a hibernaculum and enter diapause just as the queens do in the wild, if given the opportunity (Frison, 1927). Consequently, one must surmise that the B. occidentalis queens in this study, although not allowed to mate and construct a hibernaculum, also entered diapause when they became dormant.

Diapause must not be confused with quiescence. Both are a form of dormancy. Quiescence is distinguished from diapause in that the state of dormancy is directly imposed by adverse conditions. Diapause on the other hand is an actively induced state often involving a decrease in neuroendocrine activity usually at a specific time in the insects life cycle. It is brought on by conditions which signal the onset of adverse environmental conditions rather than being adverse in themselves (Saunders, 1976). Bumble bees can become quiescent under unfavorable conditions during the summer when poor weather prevents the collection of nectar (personal observations on colonies allowed to forage from a window in my office). The workers and males, as well as the queens become dormant under these conditions. However, a return to

normal activity, including egg laying by queens occurs with the addition of nectar.

Queens in this study were maintained at a constant 27°C with a 15 hour photoperiod and were always given nectar in excess. Their diapause was imposed by other than abiotic conditions. Either, diapause is obligatory, operating at a species-specific stage without apparent environmental conditioning, or the one factor I had no control over, the interrelation among colony members was influencing the queens.

Röseler (1977) demonstrated that the presence or absence of the queen is the factor controlling the ovarian development and subsequent behaviors of worker bumble bees. He demonstrated that in the presence of the queen, the worker CA was small, juvenile hormone production was minimal and the ovaries remained undeveloped. However, in the absence of the queen, the worker's CA enlarged, juvenile hormone production increased, the ovaries developed and eggs were laid. In view of this it seems likely that the presence or absence of a queen, other colony members, or some combination of these conditions, newly emerged queens may be influenced in much the same way as workers. Normal colony conditions suppress CA activity, while in the absence of the normal colony stimuli the new queen's CA becomes active, increasing juvenile hormone titers and developing the ovaries. If this proves to be true, diapause could not be considered obligatory but dependent on a particular stimulus.

Supporting this "colonial stimulus" hypothesis is the unique case of the B. griseocollis queen which developed ovaries and produced adult bees without entering diapause. The colony in which she was reared was clearly atypical. There was no aggression displayed between the small mother queen and the workers even though the workers were laying eggs. The small mother queen of the prematurely, reproductively active queen was behaving more as a worker than a queen.

The conditions that this atypical new queen was reared in deserve further investigation. It may be that isolating a queen on or before eclosion would prove a successful way in which to influence the immediate development of ovaries in new queens thereby preventing fat body deposition and diapause.

My interpretation of the physiological response of the atypical

queen is supported by the results of Röseler (1976). He increased juvenile hormone titer in newly emerged queens using exogenous hormone and found that new queens developed their ovaries rather than their fat body. However, he believed that the control of juvenile hormone production in new queens was fixed during the larval stage, and the CA would remain inactive until diapause was complete. We differ here, in that Röseler did not consider the role colony conditions can play in influencing the queen's "decision" to develop her ovaries or fat body. Nevertheless, we agree that new queens which do not enter diapause behave just like spring queens.

One further explanation for the atypical queen's behavior could be that she was an expression of a nondiapausing gene(s) which occur at low frequencies in bumble bee populations. The nondiapausing gene concept has been demonstrated in several insects (Hoy, 1978).

The length of diapause in the laboratory queens is obviously much shorter than that of field queens. This in itself suggests that a physiological timer exists which controls diapause and can be accelerated by higher temperatures.

I indicate a timer must be responsible for diapause termination because no stimuli in the form of specific temperature thresholds or changes in photoperiod were possible in the controlled environment chamber. Additionally, queens in nature hibernate beneath the soil-litter interface where they are well shielded from light, making photoperiodic control of diapause unlikely. The visible change of the fat body preceding, during and following diapause and its role in intermediary metabolism make it an obvious candidate for playing a role in this intrinsic, diapause maintaining timer.

The dissections of laboratory queens from early diapause to the onset of broodiness showed a visible decrease in fat body volume with time. Alford (1969a, 1969b), Marilleau, et. al. (1974) and Pouvreau (1976) support these observations.

Alford (1969a, 1969b) conducted his research on queens in the wild and found that 80 percent of the fat stored in queens is utilized during hibernation, most of which was used during the first half of that period. The metabolism of the greater part of the fat body during the first half of hibernation may be due to the fact that queens go into

diapause during the summer or fall when ambient temperatures are still quite warm and a high basal metabolic rate is maintained (Chapman, 1968; Tauber and Tauber, 1976).

Marilleau et. al. (1974) and Pouvreau (1976) in their laboratory studies report slight changes in lipid content of queens during the first part of hibernation with a decrease in storage lipids during the second part and a steeper decline during post hibernation. The sharper decline in lipid consumption during reactivation was attributed to the increased temperature to which the queens were subjected. The artificial hibernation temperature was held at 4°C and 0°C, while post hibernation (reactivation) temperatures were increased stepwise to room temperature.

The observation that diapause length can be shortened in some insects with increasing temperature (Andrewartha, 1953; Tauber and Tauber, 1976), and the observations on accelerated fat body depletion in Bombus with increasing temperatures, suggests that the timer mechanism is associated with the fat body. Still the possibility exists that the fat body may merely reflect diapause control at some other site. The short period of time required for diapause in Bombus in this study appears to be a function of the higher ambient temperature conditions to which the queens were confined. Additionally, B. nevadensis queens confined at 27°C became active while those held at approximately 20°C did not, further supporting the other temperature controlled, diapause rate arguments above.

More circumstantial evidence for a fat body linked timer which "counts" the degree days is found in observations by (Cumber, 1953; Horber, 1961; Skou et. al., 1963; Alford, 1969b; Holm, 1972; and Bols, 1973).

Horber (1961) and Holm (1972) have shown that heavier queens, those with larger fat bodies, live longer under artificial hibernation conditions than lighter queens, those with smaller fat bodies, presumably because of increased food reserve in the form of fat. Additionally, Skou, et. al., (1963) found that queens which died soon after emergence were those with small fat bodies. The rational is simple, the more reserve for hibernation, the longer the queen survives adverse conditions.

The utilization of this reserve depends on the ambient temperature conditions. The ambient conditions can be modified by the site of the hibernaculum, and so it is seen that queens hibernating at greater depths are likely to emerge later than those hibernating at more shallow depths (Bols, 1973). Furthermore, queens overwintering in chambers immediately below the soil-litter interface and subjected to higher daily maximum temperatures in spring, emerge before queens in more protected banks or slopes (Alford, 1969b). Alford feels that Bombus spp. are programmed to select hibernation sites on the basis of the environmental conditions they are subjected to. Fat body supply and the behavior associated with hibernaculum site selection, including aspect, soil type and hibernaculum depth are undoubtedly finely tuned through evolution. An upset in this fine tuning may result in an upset in expected patterns of development. Confinement of queens in the laboratory was one example of this, and the response of B. terrestris in New Zealand (below) illustrates another.

B. terrestris, introduced from England into New Zealand displays a considerably modified perennial life cycle (Cumber, 1954; 1962). Queens can be found at all seasons of the year. It is possible that the higher seasonal temperatures in New Zealand provide for an acceleration in fat body metabolism terminating diapause earlier than normal. This would fit in with my laboratory observations. However, some queens may have never entered diapause, responding instead to the new environment in the colony created by those queens which broke diapause early. My observations on the B. griseocollis queen above support the concept. This seems feasible as the perennial nature of these colonies must find colony cohesion in a continuous state of flux.

I also suggest that the variability in fat body size is an adaptive mechanism for "spreading the risk" of emerging into an unpredictable environment (den Bohr, 1968, 1970). An early emergent queen of a species (smaller fat body) can exploit an early spring bloom and thus escape both intraspecific and interspecific competition as well as parasitization by Psithyrus and other species. This variability benefits the species as a whole.

How could the fat body act as a timer? It is difficult to say due to the paucity of information on the fat body itself, but its role in

intermediary metabolism suggests many possibilities. As already mentioned, it is believed that the inactivity of the pars intercerebralis is immediately responsible for the maintenance of diapause in adult insects because of its control over CA secretory activity. The fat body, capable of breaking down juvenile hormone (Röseler, 1977) may function in the half life of other hormones also. The fat body acting as a regulator of hormone half life could, when superabundant as during diapause, act as a sink, continually removing hormones produced by the brain or elsewhere via diffusion and breakdown into secondary compounds. Without the necessary hormone titer, homeostatic mechanisms such as feedback or target organ activity, would not operate and the queen would enter diapause. As the fat body was metabolized for energy the "sink" would decrease in size and the hormone titers could return to an active level.

The possibility of products of fat body metabolism acting as a switch to stimulate brain activity and end diapause has also been suggested (Andrewartha, 1953). The concept appears viable and deserves further study.

Post diapause development (reactivation) to the onset of egg laying and beyond are discussed in behavioral terms in Section II. Some interesting physiological changes are associated with these behaviors.

As diapause is ending there is an associated increase in the size of the corpora allata (Palm, 1948) and presumably the secretion of juvenile hormone begins. This is in contrast to a queen preparing for diapause whose CA is small, produces no juvenile hormone and upon feeding commences gonotrophic dissociation. In the presence of juvenile hormone protein synthesis begins; vitellogenins are synthesized and transported to the ovaries, and the eggs develop.

When the first eggs are laid, Palm (1948) reports that the corpora allata displays "exhaustion symptoms." I take this to mean a reduction in juvenile hormone secretion, but he does not elaborate. Most likely the CA is reducing its secretion rate to allow the ovaries to "rest" i.e., the queen does not lay eggs again until the first brood is well developed, usually pupae. Whether the reduction in secretion rate is a response to an ovarial hormone or environmental feedback is unknown.

The fact that a queen's egg laying rate in a developed colony depends on cocoons (egg laying sites) suggests the latter may be more important. Figure 2 summarizes the physiological condition of a new queen from eclosion until the routine of egg laying begins.

Problems With Egg Development, Viability and Maturation

The majority of B. occidentalis queens which became broody did not lay eggs. Dissections revealed developed ovaries with an accumulation of disintegrating eggs and trophocytes at the end of each ovariole just before they join at the lateral oviduct. The color of the ovaries was a soft white, while the site of egg tissue breakdown was pale yellow. This material has been referred to as a corpus luteum (Palm, 1948). The process of oocyte breakdown and absorption into the ovariole tissue is termed oosorption (Bell, 1975).

The corpus luteum did not block the ovariole passage into the lateral oviduct permanently. In some queens terminally positioned chorionated eggs were found within the degenerating tissue. Because the eggs were chorionated and appeared normal in all other respects I assumed that it was not at that time contributing to the corpus luteum surrounding it. Palm (1948) found chorionated eggs of Bombus sp. which had been ruptured and the contents resorbed. If these eggs were not to be laid soon they too may have contributed to the corpus luteum.

Flanders (1942) observed that parasitic hymenoptera apparently show restraint in oviposition when suitable hosts are not present and that the processes of oosorption and oogenesis occur synchronously so that the female can deposit viable eggs after a period of inhibited oviposition. Medler (1962) observed that oosorption also occurred in spring captured bumble bee queens. He interpreted his results using Flander's (1942) hypothesis. In other words mature eggs had developed in Medler's laboratory confined bumble bee queens, but due to inappropriate conditions the queens had shown "restraint" in depositing their eggs and subsequently resorbed them. Medler further points out that Bombus ovaries are polytrophic. A succession of eggs probably continued to develop so that when conditions were "right" eggs would be

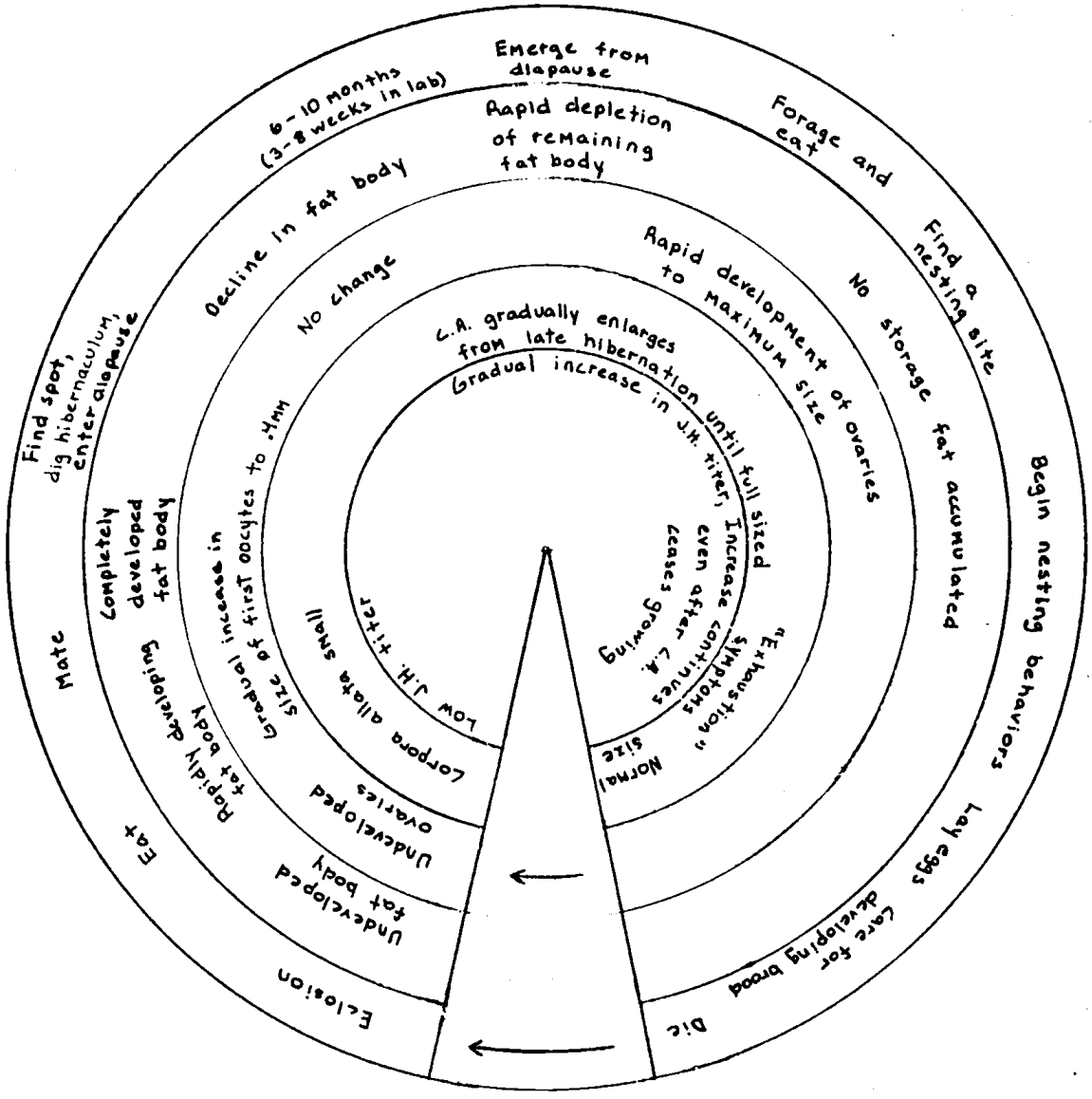

Figure 2. Correlation between observable life history events of bumble bee queens and associated physiological conditions.

ready to be laid. Medler's (1962) observations on bumble bee oosorption have been further substantiated by several other authors (Fridén, 1966; Plowright and Jay, 1966; Alford, 1969). These observations correspond to my own, and Cumber's account fits the pattern well.

Sixteen confined B. occidentalis queens laid eggs as a kind of last desperate effort. Plowright and Jay (1966) report a similar situation. Egg laying in these cases may be due to a breakdown of normal restraints as a result of age, manifested in the form of a lowering of stimulus thresholds or a gradual increase in the level of motivation linked to ovary development. It is probably an interaction of the two.

Eggs resulting from such queens were mostly nonviable. Their failure to develop may have indicated the eggs had passed a viability time limit. If this were the case, eggs were laid prior to the breakdown of the chorion. Labeyries (1959) reports wasp eggs which appeared partially resorbed. The eggs were nonviable. Bell, et. al., (1975) suggest the mechanism of oosorption must begin within the egg. This explanation would account for my observations on the B. occidentalis eggs. The eggs would have begun their demise via an internal mechanism of autolysis. The autolysis would begin only after the egg had not been laid and placed under the proper conditions for the induction of embryogenesis. Through resorption the nutrients of the egg could be retained in the queen. She could recycle the products from oosorption using them for the continually developing oocytes of her polytrophic ovaries. This capability would serve the queen well during periods of adverse colony conditions.

Many insects presumably can retain an egg for long periods without affecting viability because embryogenesis commences only after fertilization (Chapman, 1968). In Hymenoptera this does not appear to be the case as unfertilized eggs can develop into males. There may be other mechanisms for inducing embryogenesis in Hymenoptera, but the assumption that the egg has a short half life (as supported above), after which it is resorbed and replaced by a new egg, is a more logical explanation of the observations in bumble bees.

The laying of a chorionated egg in the process of autolysis must be due to either an increase in motivation or a lowering of the egg laying stimulus threshold prior to chorion decay.

The nonviable eggs laid varied in appearance from almost an undetectable to a heavy mottling of the normally homogenous white contents. An extreme case is seen in Figure 3.

There is another explanation for the mottling in nonviable eggs. Autolysis may not be occurring, but rather simply as an artifact of the age of the egg the yolk bodies may be coagulating.

In one experiment using older queens (Table 2, column 8) treatments with juvenile hormone resulted in five of six treated queens laying eggs within a few days of treatment. This could be explained in two ways. To begin with, since juvenile hormone controls vitellogenin synthesis and for this reason oogenesis, it seems logical that a juvenile hormone deficiency would result in a slower rate of oogenesis than if the titer were higher. This decreased rate of oogenesis could conceivably be the lack of motivation necessary for the onset of egg laying behavior. This may be what Evans (1966) referred to as "positive egg pressure" as a source of motivation in wasps. In this experiment when the JH titer was boosted with exogenous hormone, the rate of oogenesis may have increased and this "positive egg pressure" released the oviposition behavior. Secondly, the hormone titer may be the factor which directly stimulates motivation. It is interesting that the juvenile hormone treatment given to younger queens did not ellicit egg laying. The indication is that the endogenous juvenile hormone titer may have been higher in older queens because of the lower stimulus threshold responsible for juvenile hormone secretion as discussed above. A lower endogenous juvenile hormone titer in younger queens, even in combination with exogenous hormone could account for an inadequate titer present for the period of time necessary to achieve the egg development rate (positive pressure) responsible for egg laying.

Finally, even when eggs laid by long-confined B. occidentalis queens were viable they did not develop to maturity. It is impossible to say whether it was due to insufficient care provided by the queens, or to some developmental defect. However, the maternal care afforded the larvae, incubation and feeding, was minimal. Most often, the queens brooded the space next to the larval cell. I never observed queens opening cells or giving any indication they were feeding the larvae. The queens were continually broody much as if they were

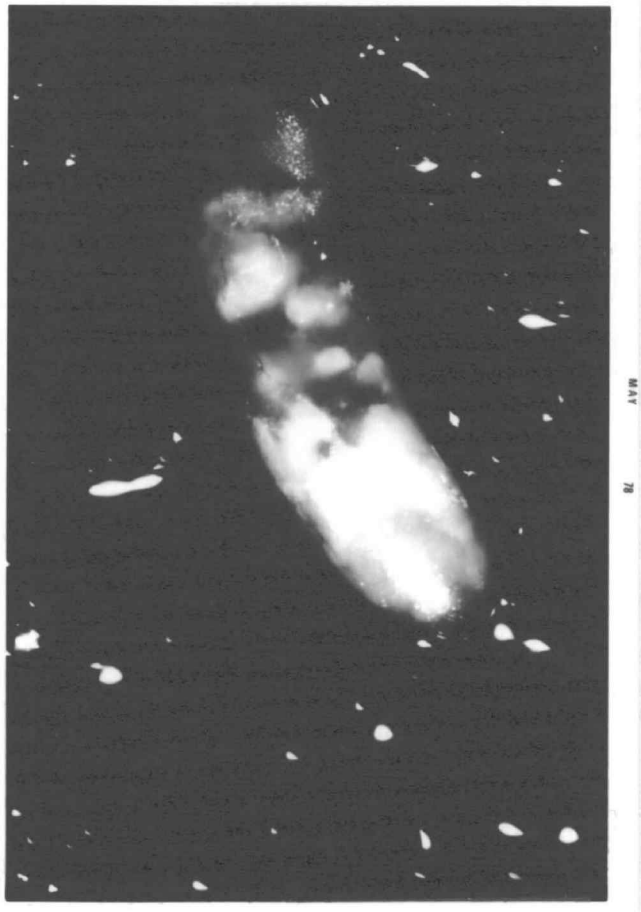

Figure 3. Nonviable egg showing heavy mottling.

preparing to lay eggs but had not done so. This lack of maternal care may have been associated with a juvenile hormone deficiency although overt brooding behavior seemingly associated with the presence of juvenile hormone was always evident.

C. Artificial Insemination

The Female

The reproductive organs of the queen bumble bee are illustrated in Figure 4. Anatomical designations are after Snodgrass (1925). Two ovaries, consisting of four ovarioles each, lead into their respective lateral oviducts, these uniting at the median oviduct. The median oviduct is slightly constricted where it joins the cephalic margin of the vagina.

The vagina is expanded dorso-laterally forming two bulbous pouches. The spermatheca is located just above and behind the vagina resting next to the muscles of the genital capsule. The spermatheca has two accessory glands at its dorso-cephalic margin and is connected to the vagina by the spermathecal duct. The spermathecal duct enters the vagina at its dorso-cephalic margin after passing between the constriction dividing the vaginal pouches. The vagina narrows posteriorally forming the vaginal orifice between the vagina and the bursa copulatrix. The bursa copulatrix enlarges just after the vaginal orifice and envelopes the muscles and sclerotized arms of the base of the sting. The opening into the bursa is a membranous pouch as viewed from the sting chamber and can be found just below the basal arms of the sting with the sting extended (Figure 5).

The easy accessibility of the opening to the bursa copulatrix and the general structure of the vagina are well suited to the insemination of the queen using the Mackensen syringe. The tip of the syringe slides easily into the opening of the bursa. The Roberts and Mackensen manipulating apparatus can also be adapted satisfactorily for use with Bombus queens. The modified sting holder needs to be taped into the clamp as it is too large to be held securely. The ventral hook can be improved by increasing the length of the tip which fits over and pulls out the ventral plate. The sting hook fits well between the arms of the sting anchoring securely on two posteriorly directed sclerotized invaginations (Figure 5).

The bumble bee lacks a valuefold and thus the need for a valuefold probe necessary for honeybee insemination. This frees one hand of the

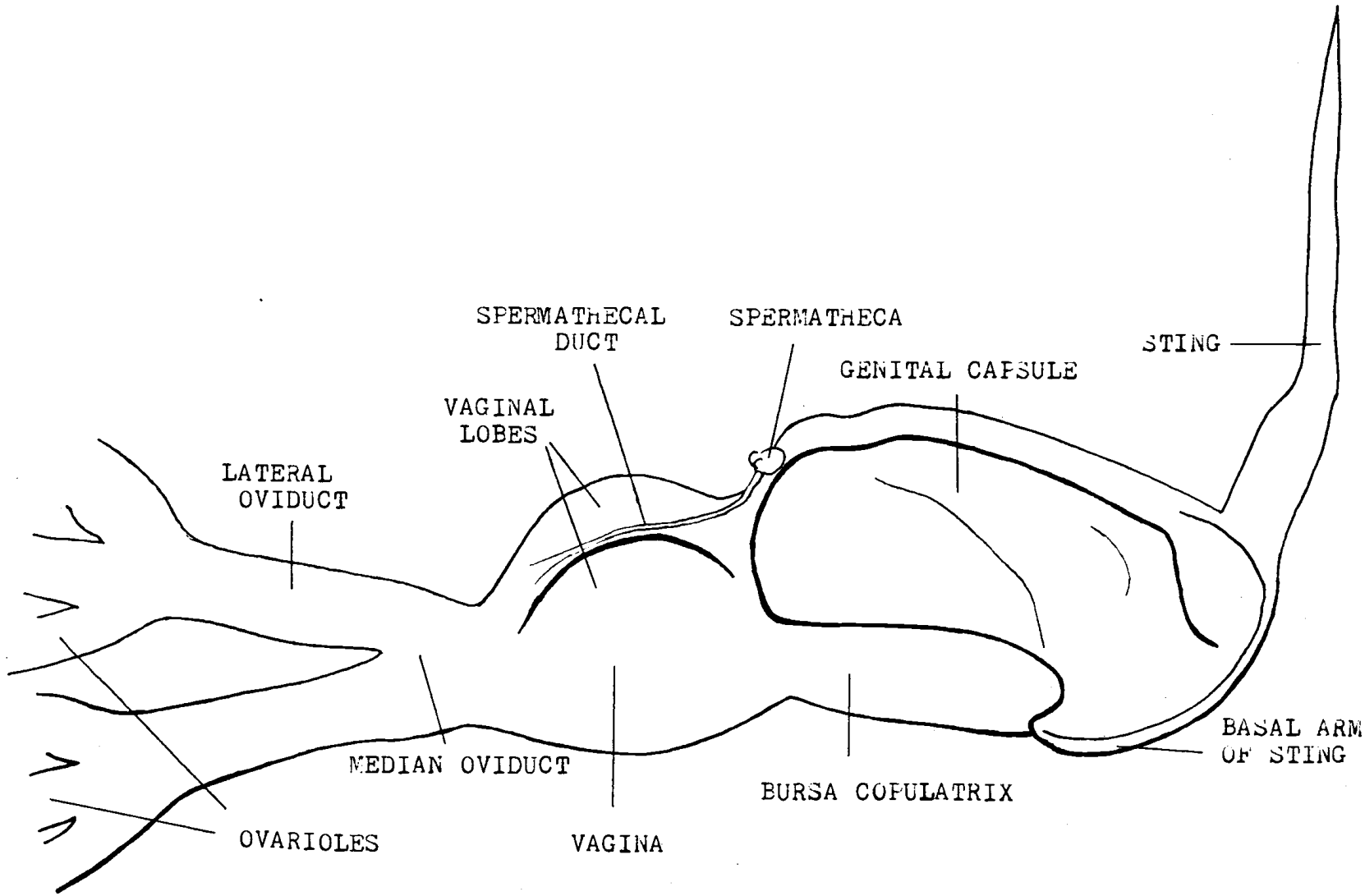

Figure 4. Reproductive organs of the bumble bee queen.

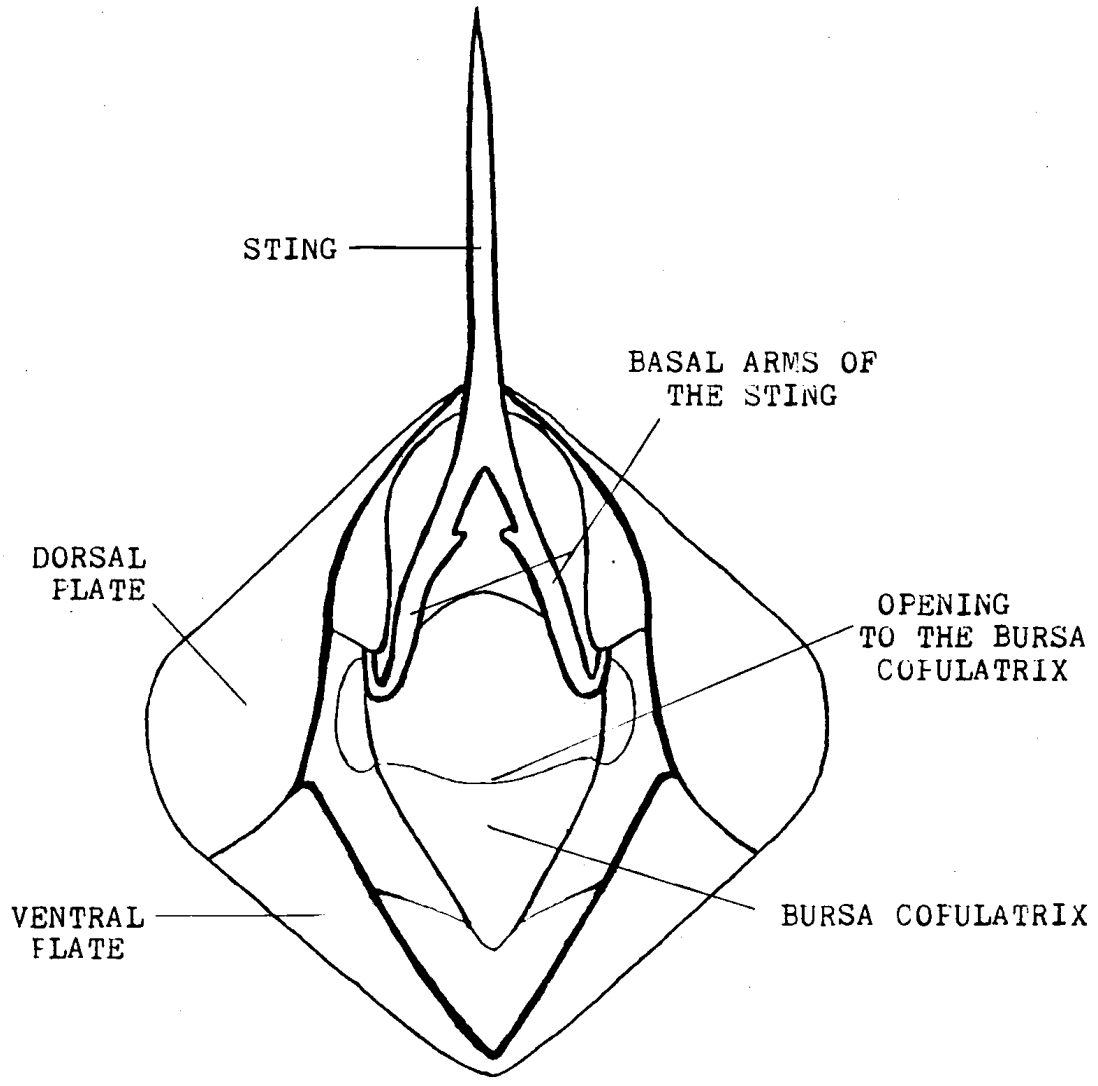

Figure 5. View of sting chamber opened for insemination.

operator making it possible to pull the sting out with a pair of forceps using the free hand, and to insert the syringe tip and inject the sperm homogenate with the other. The need for the sting hook is removed. In addition, tissue disturbance from the sting hook is eliminated and there is a reduction in the handling time necessary for each queen. The use of the Roberts and Mackensen manipulation apparatus requires the passing of one arm in front of the body and is somewhat awkward. It might be easier to use the Laidlaw apparatus (Mackensen and Tucker, 1970) which would allow manipulation of the sting and Mackensen syringe with the operator's arms positioned normally.

The Male

The reproductive anatomy of the male bumble bee is very similar to that of the male honeybee but on a much smaller scale relative to body size. Details on the drone honeybee are provided by Snodgrass (1925) and Dade (1962). The parts of the male bumble bee's reproductive anatomy examined include the testes, vas deferentia, seminal vesicles and accessory glands.

The testes in the male bumble bee decreased in size from day one to day two of adult life and remained the same from that day on. Viable sperm were found in the seminal vesicles beginning the second day after eclosion. This indicates that sperm from the testes begin to migrate to the seminal vesicles during the first day of the male's adult life as no sperm are found in the seminal vesicles when the male emerges. Males are known to copulate repeatedly (Roseler, 1973).

Sperm counts were made using a haemocytometer with the mean number of sperm per seminal vesicle being 1.62×10^6 and a standard deviation of 2.04×10^5. The sperm were difficult to count as the length of one crossed many grid lines and they were difficult to see. Phase-contrast could not be used due to the thickness of the haemocytometer. Errors in the estimates can be largely attributed to these problems.

Sperm: Collection and Handling

Sperm collection could be made only by removing the seminal

vesicles. This was accomplished in two ways. First, the seminal vesicles could be dissected out of the male abdomen, but the time spent collecting enough seminal vesicles for one insemination (average 8) was prohibitive. The second and most expedient technique suggested and demonstrated by S. Taber III (personal communication) involved the gentle extrusion and removal of the genital capsule to which the accessory glands and seminal vesicles were attached. I had approximately an 80 percent success rate at getting both seminal vesicles using this method.

There was a question as to whether insemination with sperm taken directly from the seminal vesicles was as efficient as insemination with seminal ejaculate, i.e., sperm plus accessory gland products and fluid from the penis bulb. Mackensen and Roberts (1948) have stated that insemination with sperm from the seminal vesicles of male honeybees can be used to fertilize queen honeybees, but that considerably more sperm reach the spermatheca when using male ejaculate. This may be due to the sperm activating qualities of the accessory glands (Taber, 1977).

Poole and Edwards (1972) demonstrated the importance of sugars to honeybee sperm motility. Sugar may increase the motility of the honeybee sperm by acting as an exogenous energy source even though endogenous energy may be available in the form of fatty acids (Taber, 1977).

On the assumption that Bombus sperm is similar to that of Apis the seminal vesicles were crushed in a saline-sugar solution in order to mobilize the sperm. The dilution of the sperm itself may result in an increase in sperm motility (Taber, 1977). The sugar-saline solution was prepared by mixing .85g NaCl, .35g glucose and .2g fructose in 100ml water (Poole and Edwards, 1972; Taber, 1977). Glucose and fructose were both ranked among the best sugars to maximize honeybee sperm motility in saline solution (Poole and Edwards, 1972).

The seminal vesicles were placed into the drop of saline-sugar, crushed and homogenized. The activated sperm were then sucked into the syringe tip.

Insemination

The purpose of the insemination process was to place sperm into the reproductive tract of the queen and evaluate the success of the technique by examining the spermatheca for the presence of sperm. This was not a measure of fertility for in order for the insemination to have been completely successful queens would have to lay viable fertilized (female) eggs (Taber, 1977). The sex of the developing larvae can be determined only in the third or fourth instar (W.P. Stephen, personal communication) or upon the emergence of the adults.

It was not possible to determine the ultimate success of the insemination for the eggs of B. occidentalis were usually nonviable and the few larvae which developed never matured to an instar in which sexing was possible.

Of 45 queens inseminated, 10 (22 percent) contained viable sperm in their spermathecae. Four of these died prior to dissection. In 29 queens, spermathecae were not recovered. Of these, six had "rubberized" abdominal organs, two had growths which obscured the spermatheca, two had mycelial mats in the body cavity, 12 queens had putrified, and in seven the spermathecae were not recovered due to poor dissections. All but four of these queens died prior to dissection. The remaining six queens, which appeared in good internal condition and from which the spermathecae were recovered contained no sperm. Three of these died prior to dissection. The high failure rate and mortality in these tests is believed to be the result of the procedure used in sperm injection. The abdominal cavity was probably contaminated at the time of insemination by inserting the syringe tip too deeply or at the wrong angle, puncturing the wall of the bursa or vagina; or injecting too large a volume of fluid thereby rupturing the delicate membranes of the reproductive tract and contaminating the body cavity. In either case, the sperm would have been lost and would account for the empty spermathecae.

In three cases when the insemination was successful the syringe tip was inserted only part way into the bursa copulatrix and sperm oozed back out of the opening. When this occurred the pouches of the vagina were probably filled to their capacity and the excess

sperm-homogenate was being discharged the only way possible without rupturing the membranes lining the organ.

It is suggested that in future studies, the syringe tip should not be inserted much beyond the entrance to the bursa. In addition, the syringe tip could be shortened and rounded slightly to reduce the chances of puncturing the reproductive tract and to facilitate sperm collection. The small, extended tip oftentimes plugged during sperm uptake.

V. SUMMARY AND CONCLUSIONS

Modified rearing methods have had little impact on the successful domestication of bumble bees in the last few decades. The main problem in rearing bumble bees is the initiation of colonies by queens in the laboratory. Results from this study show that queens can be stimulated to lay eggs and develop colonies with the introduction of brood or worker-brood combinations.

It is suggested that these introductions act as stimuli to release the fixed action patterns of maternal care. Subsequent egg laying is presumably associated with motivation improvement and a change in releasers. An ethogram is presented for queen behavior providing a conceptual framework from which questions regarding specific stimuli and responses might be asked and answered. Normally a queen would lay eggs in response to the environment created by her colony founding behaviors i.e., honey pot and pollen mass construction, etc. Instead when she is removed to the laboratory from the field and paired with brood or workers and brood she is receiving stimuli she would normally realize only after colony initiating has begun. By using this technique the inadequate releasers of the laboratory environment for founding behaviors can be overcome, and a later more "laboratory tolerant" stage in the queen's behavioral heirarchy can be taken advantage of with improved results.

To exploit the advantages of pairing queens with brood or workers and brood, early spring or midwinter colonies would have to be present from which desired worker and brood combinations could be drawn. Obviously, queens would also have to be available to begin rearing procedures.

The problem of brood availability for any desired time could be resolved by using queens of those species which do not enter diapause. These queens could produce colonies in a seemingly endless sequence. The nondiapausing phenomenon was shown to occur when a newly emerged queen was kept under atypical colony conditions. It was interpreted as being a result of the inadequate stimulus the loosely defined colony provided for the inhibition of juvenile hormone production in the new queen or the rare expression of a nondiapausing gene(s). The juvenile

hormone, when present, is responsible for ovary development and when absent, for fat body accumulation and prediapause behavior. The possibility of obtaining queens in identical physiological states by using nondiapausing new queens, makes possible a quantitative and comparative approach to bumble bee rearing.

Queens necessary for beginning new colonies could come from new, nondiapausing queens or from hibernated queens whose diapause length has been manipulated by temperature. The results show that diapause in Bombus is temperature dependent and the length of diapause can conceivably be controlled by a combination of different time-temperature regimes. The fat body appears to be a part of the mechanism behind a hypothetical temperature dependent diapause timer. The visible change in the fat body from prediapause to egg laying, its role in intermediary metabolism, and the fact that the fat body provides the only obvious difference between diapausing and nondiapausing queens provide the impetus for the idea.

Artificial insemination of queens was shown to be possible although further work needs to be done to improve the success of the techniques. The main problem appears to have been the contamination of the haemocoel at the time of insemination by rupturing the reproductive tract with the insemination syringe tip or the injection of too much sperm homogenate. Clearly, more needs to be done to determine the value of artificial insemination, especially as to the quality and quantity of the sperm which reaches the spermatheca.

On the basis of several techniques outlined in this thesis, an approach to the rearing of bumble bees for use in pollination and conservation purposes is conceptualized (Figure 6). Although many of the techniques are inadequately supported by experiment, the management model points to those areas in most need of attention.

The practicality of the effort to domesticate bumble bees for pollination and conservation purposes must be qualified. In areas where monocultures are the dominant agricultural practice, all economically and biologically feasible efforts at bumble bee reintroduction and population enrichment would fail. This is obvious when one considers the lack of nesting and hibernation sites available for bumble bee populations as well as the virtual absence of secondary plant forage

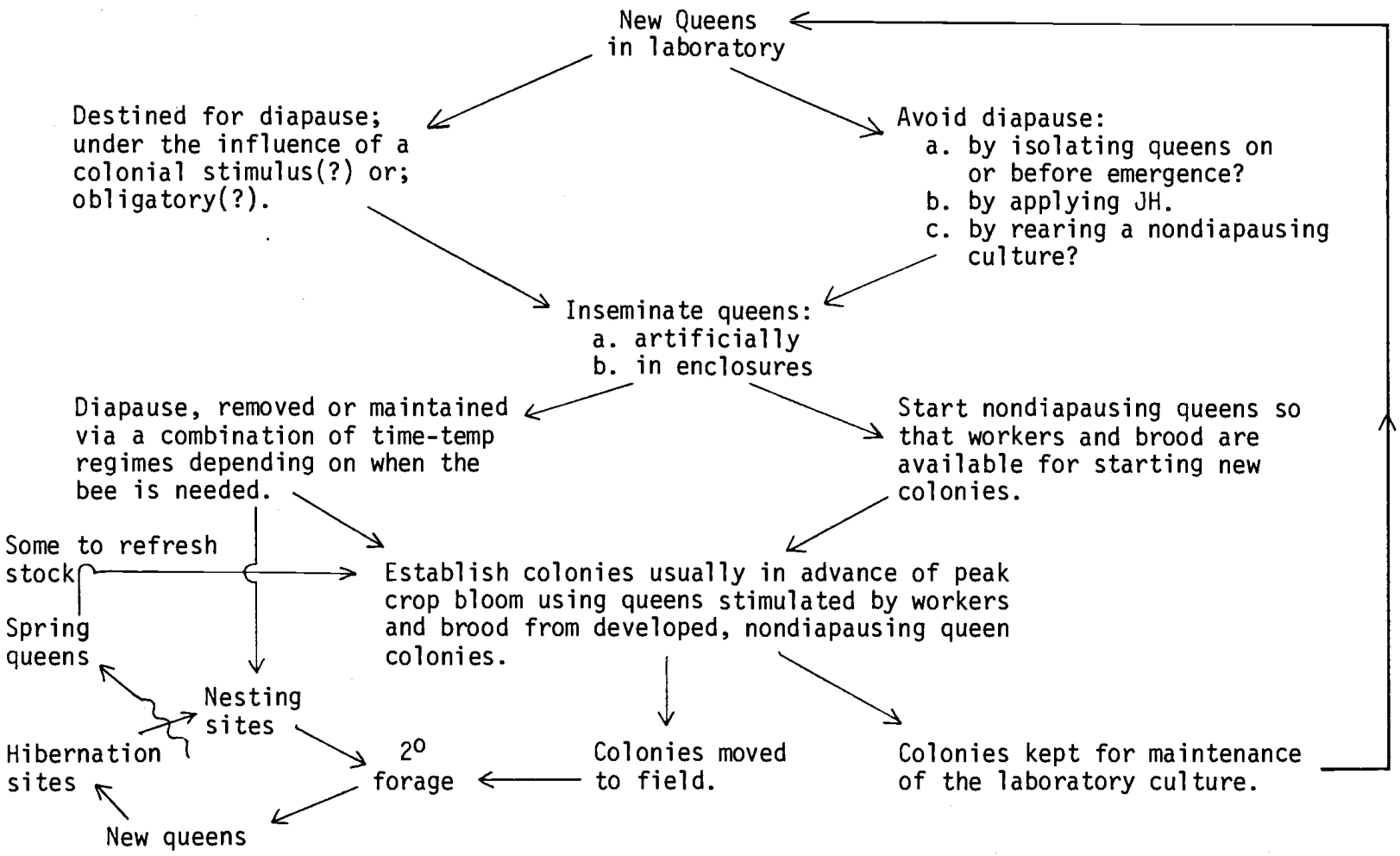

Figure 6. Bumble bee management model.

needed to support bumble bees when the primary forage source is gone. There is currently no reason to believe these conditions will change. Portable domiciles may appear to provide a solution to these problems, but the repeated movement of colonies has not been shown to be practical, and the economics would be prohibitive. Only bees amenable to intensive management such as the honeybee or leaf cutter bee (Megachile pacifica Panzer), can be expected to meet the demands of the diversity depauperate lands of large scale agriculture. The indescriminate use of pesticides also limits hopes of success. However, bumble bee domestication can be expected to be a major factor in agricultural communities where land for nesting sites and secondary forage is available and the use of pesticides is minimal. In these situations laboratory domestication of bumble bees could realize its real potential in supplementing locally fluctuating bee populations.

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APPENDICES

APPENDIX 1.

OBSERVATIONS ON THE USURPATION OF TWO B. OCCIDENTALIS LABORATORY COLONIES BY INTRODUCED PSITHYRUS SUCKLEYI (GREENE) QUEENS

On 19 June 1977 at 1400 hours I introduced a P. suckleyi queen into a declining colony of B. occidentalis, one in which only males were being produced and the queen was losing control. P. suckleyi is reported to be a social parasite of B. occidentalis (Hobbs, 1968).

The Psithyrus queen was placed directly into the brood chamber. She immediately crawled up the side of the box and became still. She remained in this position for three hours. At 17:07 I observed her mingling with the colony. As she contacted the first workers she presented a nonaggressive defense posture, momentarily leaning to one side and raising one or two legs. This behavior kept the aggressive workers from attacking her. The Psithyrus queen followed immediately with an aggressive, offensive behavior by which she dominated all workers. She grabbed the nearest worker and furiously thrust the tip of her abdomen in towards it, "mock-stinging" her. Upon release, the worker walked slowly over to the side of the domicile and became very still. The following day, this worker was still alive, indicating she was not fatally stung even though the Psithyrus queen was capable of doing so. In addition to mock stinging workers the queen also nipped their legs and wings with her mandibles.

The Psithyrus queen continued to plunder the colony, incessently antennating each worker upon contact. They appeared to retreat after the experience. One worker in particular, which I had observed to be broody, challenged the Psithyrus queen and was mock stung. She revived later and showed no further signs of broodiness. The failing queen of the colony remained beneath the brood, but was found the following day dead in the flight box.

By 17:25 of the same day the queen was opening larval cells and removing larvae. No workers challenged her, but she would still antennate and nip at them at each encounter. The removed larvae had not yet separated themselves into cells. Later, the queen also removed last instar individuals. The workers followed the Psithyrus queen,

removing extracted larvae to the outer flight box and repairing torn cells. She had taken nectar from the honey pots once by this time and at 17:40 was seen consuming pollen. She continued to open larval cells and destroy egg clusters the remainder of the day.

On 20 June, the Psithyrus queen was still harassing workers. Most of the larvae (second to fifth instar) were dead, scattered about the flight box. At 16:45 she was observed building an egg cell using old wax from the surface of brood cells. On the following day, 21 June, eggs had been laid and the Psithyrus queen was in complete control. She was still aggressive towards certain workers, but none were showing signs of broodiness.

The queen continued dominating workers at a reduced level until her males and queens were produced and she began losing control. Males were produced before queens, and when the queens began emerging, the brood was of mixed sex with both males and queens present in the same cohort.

A second P. suckleyi queen was introduced into another B. occidentalis colony on 11 July after the colony queen had died. Only a few egg clusters had been produced in the two weeks prior to the queen's death, and workers were broody. The Psithyrus queen I introduced was weak and sluggish. In order to maximize the possibility of her acquiring a colony odor without conflict, the colony was anesthetized with carbon dioxide before placing her in the nesting box. In the absence of confrontations from the workers she began running over and beneath the brood antennating the cells as she went. When workers started recovering from their anesthetized state the queen began rapidly tapping them with her antennae and nipping a few. As the workers returned to normal activity levels the Psithyrus queen began mock-stinging any of them that were nearby. She passed over workers which did not acknowledge her presence as they kept feeding or incubating brood. Occasionally, a very aggressive worker challenged the queen, at which time she assumed the same defensive posture as the previous Psithyrus queen. She was never attacked.

The next day all nine of the colony's remaining larvae were torn from their cells. None of the pupae were disturbed. On 12 July, the Psithyrus queen was still mock-stinging and nipping workers, but she

had displayed no signs of egg cell construction. At this time a second Psithyrus queen was introduced to observe the worker and established queen's reaction. The workers of the colony behaved normally, not even acknowledging the presence of the new Psithyrus queen. However, the established Psithyrus queen went wild and buzzed madly over to the new Psithyrus queen where they went into a stinging embrace. Both queens broke the embrace in a stunned condition. The established queen attacked the second queen again, killing her.

The following day, 13 July, the Psithyrus queen laid eggs using old wax to construct the cell. Males were once again produced first, followed by mixed cohorts of males and queens.

Psithyrus suckleyi queens exercised control over B. occidentalis workers by dominating them with physically aggressive actions in the form of mock-stinging and nipping. It appears that strong antennation may also contain workers without the Psithyrus queen being otherwise abusive.

Some of the workers showing signs of broodiness, were seen to return to more typical worker behaviors after being dominated by the Psithyrus queen. This suppression can also occur intraspecifically as Röseler's (1977) work demonstrated. He showed that queenless workers which had developed their ovaries were later suppressed when they were placed with an active, healthy queen.

The Psithyrus queen's removal of larvae might be explained as an intrinsic mechanism for increasing the ratio of Bombus workers to Psithyrus brood. This increased ratio is known to be important for the production of Bombus queens. It has been shown that the quantity of food a female bumble bee larva receives will influence both its size and caste (Roseler, 1976). Although Psithyrus have no worker caste it is clear that the size of the new Psithyrus queens will be dependent on the quantity of food they receive. More workers in a colony result in a faster rate of larval development, food not limiting, and assure size optimization in a developing Psithyrus larvae. Size may be particularly important for the Psithyrus queens because of their aggressive strategy in colony usurpation.

The fact that the Psithyrus queen was removing only larvae and not pupae indicates that she may be able to differentiate among brood which

would attract worker maternal care away from her larvae. The pupae no longer requiring feeding would be an asset if they were workers as they would contribute to the future worker force. It is also possible that Psithyrus queens may have been unable to tear open the tough cocoons with their mandibles and did not disturb them for this reason. The ease with which new adults chew out of the cocoon, however, weakens the latter explanation.

Finally, one must ask if female B. occidentalis larvae would also have been removed from their cells had they been present. At the time of the Psithyrus introductions only males were being produced. The ability of the Psithyrus queen to identify the sex of the larvae would be highly advantageous. All host larvae would detract from the attention given Psithyrus brood for a time, but host female larvae would soon emerge as adults in the worker force. This would only strengthen the chances for the successful rearing of many Psithyrus sexuals. The sexes of the larvae may indeed be distinguished by the Psithyrus queen on the basis of odor, as suggested by her extensive antennation of the brood.

APPENDIX 2.

NECTAR TONGUING AND DORMANCY IN BUMBLE BEES

Alford (1975) reports that bumble bees do not tongue nectar. Tonguing nectar as seen in honeybees involves the regurgitation and manipulation of nectar by the bee's tongue in such a way that a thin film of nectar is exposed to the air. Water evaporates from the large nectar surface area formed, concentrating the solution.

Contrary to Alford's (1975) report, I have observed workers, males and queens tonguing nectar. The manipulation of nectar was not associated with honey storage as is seen in honeybees, but rather was associated with a behavior not usually given attention by bumble bee biologists. All individuals observed tonguing retained the nectar concentrate, presumably in their crop.

The first observation was made on a colony of B. melanopygus foraging from a window box in my office. The weather was unseasonably wet and workers were kept from foraging by the incessant rain. The first worker was observed tonguing nectar when only one full honey pot remained in the colony. She and her sisters had been feeding and brooding the larvae up to this point. Shortly after I observed the first worker tonguing, three of her four sisters were seen to follow suit. They too removed nectar from the honey pot and began tonguing it. When they were next observed, several hours later, they were all quiescent, away from the brood in a corner alone. They remained this way for the remainder of the day and through the following morning until the rain stopped. When the sun came out, they again began foraging for nectar and pollen and commenced normal brood care behavior.

Only one of three cohorts or larval clusters were cared for during the inclement weather. Two of the clusters were not seen to be fed or incubated for 12 consecutive days after which they were removed for examination. Most of the larvae were still alive indicating their ability to survive for comparatively long periods on metabolic reserves. The workers incubating the first cohort did so by packing very closely together presumably to conserve available heat energy. Concentrating their efforts on one portion of the brood during

inclement weather conditions would appear to be the wisest strategy for heat energy conservation and insurance that at least one brood cohort would survive.

Workers were seen tonguing nectar on another occasion when I had inadvertently let a laboratory colony's nectar tubes go dry. A dormant phase followed.

Two new queens of B. griseocollis were seen tonguing nectar while resting on the walls of the flight room in which they were allowed to freely fly. Fresh nectar was available at all times. The queens were later found in diapause as described in Section III B. W.P. Stephen, (personal communication) supports this observation on queen tonguing of nectar with observations of his own.

The last observations on tonguing were made in the field on a male B. griseocollis. It was approximately 17:00 and the temperature was 32 to 37°C. The mating aggregation being observed was no longer active and the males were on and around a patch of thistle (Circium sp.). Males were found in three distinct activity states: on the thistle heads collecting nectar; on surrounding vegetation tonguing nectar; and on the undersides of leaves of the surrounding vegetation in a quiescent condition, i.e., when disturbed they would buzz their wings but would not fly.

Workers, queens and males exhibited nectar tonguing and the subsequent storage of the concentrated nectar in their crop for personal use. All three castes would benefit from the concentration and internal storage of nectar as an energy source for unfavorable periods. The bees must be programmed to sense or "anticipate" the presence of adverse conditions and respond with the tonguing behavior. Workers know to reserve the last nectar holds for their own survival. Prehibernating queens have long been known to store nectar in their crop, presumably as an energy source in the fall and spring. It is interesting that tonguing by queens has not been reported in the literature to date. Males do not have nectar available throughout the night, as do bees in a colony with the previous day's nectar cache. Therefore, on warm nights in particular, when the male's basal metabolic rate is higher than on cooler nights, a store of nectar in the crop would be beneficial.

The observation that tonguing incidents are followed by a period of dormancy lends credibility to the hypothesis that nectar is concentrated for personal use in response to and/or "anticipation" of adverse conditions.