

Brood cycles in queenless colonies of
Apis mellifera capensis

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By
Kendall Lauren Crous
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ABSTRACT

In a honeybee colony, the loss of a queen is considered to be a serious occurrence and, if a new queen is not produced, ultimately doomed. However, in colonies of *Apis mellifera capensis* (Cape honeybees), numerous pathways are available for a colony which unexpectedly losses a queen.

At the onset of this experiment, four colonies of *A. m. capensis* were dequeened. Following this photographs of all brood frames in each colony were taken and the contents of the cells analysed. Cells were chosen at random but once selected were repeatedly analysed for the duration of the experiment. The contents of a total of 44 888 individual cells were analysed. Any queen cells constructed during the sampling period were removed, maintaining a queenless state.

In each colony, as predicted, the removal of the queen evoked a variety of responses in an attempt to rectify the sudden loss. However, ultimately three of the four colonies absconded, leaving little by way of stores. Three of the four colonies initially attempted to rear a new queen while one colony was immediately invaded by a presumed foreign queen and hence any attempt at queen cell construction ceased. An increased number of queen cells in the swarm position were recorded in all colonies. The invasion of a colony by a foreign queen was considered to be a new pathway available for queenless colonies of *A.m capensis*.

Worker policing and suspected brood cannibalism was prevalent in all sampled colonies yet in addition, the transfer of eggs and larvae from cell to cell was also observed which may have increased the suspected cases of policing and cannibalism. It was unclear whether an egg or larva had been consumed or simply moved to another cell on the brood frame. All colonies contained eggs from laying workers to varying degrees, based on the length of each individual sampling period which varied between colonies due to differences in absconding dates.

A steady breakdown in the effectiveness of the division of labour amongst the worker bees was observed in each of the colonies highlighting the vital role of a queen. This breakdown was clearly seen in the reduction in general housekeeping within a colony. An increase in stores indicated a possible increase in the number of forager bees, thereby reducing the number of worker bees available for other duties.

Pathways available to broodright colonies and strategies used following sudden queen loss are discussed.

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Declaration

The following thesis has not been submitted to any university other than Rhodes University, Grahamstown, South Africa. The work presented here is that of the author.

_____ Date _____

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*“To men, time is money; to bees, it is honey;
and all the arrangements of the hive should
be such as to economize it to the utmost.”*

L.L. Langstroth

I

INTRODUCTION

1.1 Honeybee queens

In a honeybee colony, the queen is generally the sole reproductive (Ribbands 1953), pheromonally suppressing ovarian development in workers (Butler 1954; Free 1977; Hepburn *et al.* 1988; Hepburn & Radloff 1998; Winston & Slessor 1998; Hepburn & Radloff 2002; Moritz *et al.* 2002). The queen, through the production of a cocktail of inhibitory pheromones secreted by her mandibular glands, also inhibits any queen cell construction (Butler 1956; Anderson 1965; Hepburn & Radloff 1998; Tribe & Allsopp 2001a).

The loss of a colony's queen creates apparent chaos and is considered to be a very serious occurrence within a colony (Laidlaw & Eckert 1950; Butler 1954; Sakagami 1958; Free 1977; Fell & Morse 1984; Skirkevičius 2004). On the loss of their queen and in the absence of queen pheromone production, workers will initially attempt to rear a new queen from her existing brood (Ribbands 1953; Hepburn *et al.* 1988). If this is unsuccessful, the workers themselves will begin ovipositing (Onions 1912; Anderson 1963; Hepburn *et al.* 1998; Miller & Ratnieks 2001). In most races of *Apis mellifera* Linnaeus, workers in queenless colonies will construct drone cells and lay unfertilized haploid eggs in them. However, in queenless *Apis mellifera capensis* Escholtz colonies, some laying workers will begin ovipositing unfertilized diploid worker eggs, others haploid, drone eggs (Anderson 1963; Hepburn & Radloff 1998; Lattorff *et al.* 2005; Lattorff *et al.* 2007). In addition, *A. m. capensis* workers are able to reproduce via thelytokous parthenogenesis (Hepburn & Crewe 1991; Hepburn *et al.* 1999; Hepburn 2001) under both queenright and queenless

conditions (Moritz *et al.* 1996; Moritz *et al.* 1999; Hepburn 2001; Martin *et al.* 2002). These workers have well developed ovaries compared with other races, enabling rapid egg production following queen loss (Anderson 1963; Hepburn 1992; Greeff 1996).

On queen loss, and in the absence of young larvae, in all races of *A. mellifera* except *A. m. capensis*, colonies are doomed and the only available reproductive strategy open to them is the export of colony genes through the production of drones (Moritz & Southwick 1992; Hepburn 1994; Hepburn & Radloff 1998; Hepburn 2001). *Apis mellifera capensis* has several reproductive strategies on queen loss (Hepburn 2001). Many pathways may be taken by an *A. m. capensis* colony in an attempt to raise a new queen, either from queen-derived brood or from laying worker brood (Hepburn 2001). On the loss of a queen, particularly when this is a sudden event, workers will begin to construct emergency queen cells around existing queen brood (Ribbands 1953; Butler 1957; Seeley 1985; Hatch *et al.* 1999) either with eggs or larvae no more than three days old (Ribbands 1953; Hepburn *et al.* 1988). However, Fell and Morse (1984) observed queen cells being constructed over larvae of up to four days old in bees of mixed European races. Hatch *et al.* (1999) found that on dequeening the construction of queen cells was rapid, occurring within 24 hours, while Fell and Morse (1984) maintained that the construction of queen cells could be initiated as early 12 hours following queen loss.

Apis mellifera capensis workers will rapidly begin developing queen-like pheromones on the loss of their queen (Hepburn 1992; Hepburn 1994; Moritz *et al.* 2000; Hepburn 2001; Moritz *et al.* 2004). Katzav-Gozansky *et al.* (2003) found that *Apis mellifera ligustica* Spinola laying workers developed a queen-like pheromone that was attractive to other nestmates, however, non-reproductive workers had no such pheromone. However, in *A. m. capensis* laying workers may either develop a queen-like pheromonal bouquet or retain one that is worker-like (Hepburn 1994). It has also been observed that some non-laying workers may begin to develop a queen-like pheromone (Hepburn 1994).

Individual *A. m. capensis* workers have the genetic ability to produce either diploid eggs via thelytokous parthenogenesis, or haploid eggs via arrhenotokous parthenogenesis (Lattorff *et*

al. 2005). In experiments conducted by Lattorff *et al.* (2005) it was found that laying workers could only lay either female or male eggs and in no cases did an individual show amphitoky.

1.2 Worker policing

Conflict in eusocial insects is commonplace due to the genetic differences occurring within colonies (Ratnieks & Visscher 1989; Visscher 1996), and worker policing, one source of conflict, is thought to be a textbook example of kin selection (Beekman & Oldroyd 2005). *Apis mellifera* queens are polyandrous and mate with as many as 7 – 20 drones (Roberts 1944; Estoup *et al.* 1993; Baudry *et al.* 1998) although mating with up to 45 drones has been recorded (Moritz *et al.* 1996; Kraus *et al.* 2005). Polyandry results in workers being more closely related to their male offspring than the queen's sons/their brothers (Ratnieks 1988; Ratnieks & Visscher 1989; Visscher 1996; Barron *et al.* 2001; Foster *et al.* 2002; Martin *et al.* 2002). In addition, the increased number of patriline lowers the mean relatedness between workers and their sisters (Foster & Ratnieks 2001b).

It has been observed that honeybee workers will readily eat worker-laid eggs but not those laid by the queen in a queenright colony (Ratnieks & Visscher 1989; Ratnieks 1993; Foster & Ratnieks 2001a; Pirk *et al.* 2003; Pirk *et al.* 2002; Pirk *et al.* 2004; Beekman & Oldroyd 2005). In the European hornet, *Vespa crabro* Linnaeus, an increased rate of policing of worker-laid eggs was observed in queenright colonies however, in queenless colonies the removal rate of queen laid eggs over worker laid eggs, increased (Foster *et al.* 2002). In queenright colonies of a ponerine ant, *Pachycondyla inversa* Mayr, worker-laid eggs were policed, and subsequently eaten; however, in queenless colonies, the majority of worker-laid eggs remained untouched (D'Etorre *et al.* 2004).

Worker policing is thought to be favoured when it has no cost effect on the colony, and is successful in decreasing the number of individual eggs produced by laying workers (Ratnieks 1993). In *A. mellifera*, workers are able to differentiate between eggs laid by the queen and those of laying workers, and will discriminate against those laid by workers

(Ratnieks & Visscher 1989; Ratnieks 1993). A similar result is observed in the dwarf honeybee, *Apis florea* Fabricius, where worker laid eggs are distinguished from queen laid eggs, and consequently eaten (Halling *et al.* 2001).

Apis mellifera capensis displays thelytokous parthenogenesis, so that unfertilized diploid eggs are laid, resulting in female worker bees (Onions 1912; Ruttner 1988; Hepburn & Crewe 1991; Beekman *et al.* 2002). *Apis mellifera capensis* workers however either evade worker policing (Moritz *et al.* 1999, Martin *et al.* 2002), or policing is thought to be significantly reduced as workers are equally related to eggs laid by the mother queen or those laid by a worker (Beekman *et al.* 2002).

The concept of worker policing is, however, controversial with opposing arguments being put forward (Pirk *et al.* 2003; Pirk *et al.* 2004; Beekman & Oldroyd 2005). Pirk *et al.* (2004) showed that egg viability was an important factor in determining worker policing. Beekman and Oldroyd (2005) countered this by suggesting that the policing of eggs was based on egg maternity regardless of its viability. Both, however, do not deny that worker policing is prevalent in colonies of *A. mellifera* despite having differing conclusions on the reasons for policing (Pirk *et al.* 2003; Beekman & Oldroyd 2005).

1.3 Brood cannibalism

It is not uncommon for young social hymenopteran larvae to be cannibalized. Schmickl and Crailsheim (2001) observed that larvae of *Apis mellifera carnica* Pollman were eaten following shortages of pollen in the colony during a dearth. Similarly, in many other races of honeybees, young bees may resort to brood cannibalism following bad weather and the consequent reduction in pollen availability (Johannsmeier 2001). However, Nakamura (1993) reported that in colonies of *Apis cerana* Fabricius, brood cannibalism was prevalent even in the presence of excess pollen stores suggesting that pollen availability is not the sole determining factor with regard to cannibalism (Weiss 1984). Brood cannibalism may be a result of nurse bee feeding capability, with larvae on the edges of brood frames being

cannibalized first (Nakamura 1993; 1995) and maintaining brood in the centre of each frame thereby forming the natural concentric brood pattern (Camazine 1991).

Roubik (1982) found that the stingless bees, *Melipona favosa* Fabricius and *M. fulva* Lepeletier, showed signs of brood cannibalism towards uncapped brood but noted that pupae were never eaten or removed from cells. The eating of older larvae has been observed in colonies of *A. cerana* in an attempt to increase protein intake and avoid absconding (Hepburn 2006). Before absconding, colonies will usually engorge themselves on honey stores and eggs. In some cases the eating of recently capped brood has also been recorded (Nakamura 1995; Hepburn 2006).

1.4 Absconding behaviour

Absconding behaviour is frequently observed in colonies of African races of *A. mellifera* (Hepburn & Radloff 1998; Spiewok *et al.* 2006). Absconding may be in the form of seasonal migration and is usually as a result of resource depletion and a form of prepared absconding (Nakamura 1993; Swart 2001; Hepburn 2006). Alternatively, absconding may be induced and fall into two categories: acute or chronic (Nakamura 1993; Hepburn 2006). Acute disturbance absconding is an immediate form of absconding and is a reaction to some external catastrophe, while chronic absconding is prepared absconding where no brood and little or no stores are left behind (Hepburn 2006).

When preparing to abscond in the case of either seasonal migration or chronic absconding, honeybee colonies refrain from rearing brood and begin to eat available stores, but when pressure to leave intensifies, the colony may abandon its capped brood (Nakamura 1993; Tribe & Allsopp 2001a; Hepburn 2006; Spiewok *et al.* 2006). However, given sufficient time to prepare, *A. m. capensis* colonies will allow for brood to eclose before absconding, thereby reducing the amount of brood being abandoned (Spiewok *et al.* 2006).

During absconding, the amalgamation of two small *A. m. capensis* colonies has been observed which involves the merger of two queens (Hepburn 2006). Such mergers would

increase the number of patriline of the colony as a whole and the chance of survival of small colonies (Hepburn 2006).

1.5 Cell usage

Honeybee combs are comprised of three different sized cells: queen cells, drone cells and worker cells. Queen cells hang vertically from the comb and are larger than any of the other cells (Tribe & Allsopp 2001b). Drone cells are bigger than worker cells, and therefore are able to house and develop the larger drones (Grout 1964). Drones raised in smaller worker cells have been observed; this is usually attributed to a queenless colony with laying workers and will produce smaller sized drones (Grout 1964). Conversely, if a worker emerges from a drone brood cell, a larger adult worker will be produced (Grout 1937).

It has been observed that cells used for storing resources are not used for brood rearing, indicating that there is perhaps a scent-marking cue used for oviposition (Free 1977; Free 1987). Contrary to this, it has often been noted that brood cells can be utilised as storage cells (nectar, honey and pollen) (Camazine 1991), and indeed it has been recorded that honeybees prefer to utilise older comb for nectar storage (Johnson & Baker 2007), though it is unlikely that storage cells will later be used again as brood cells (Fig. 1.1) (Free 1977). In addition, it is highly unlikely that pollen will be stored in the larger drone cells but is rather found in the smaller worker cells (Grout 1964) while other stores (nectar and honey) may be found in drone cells (Grout 1964).

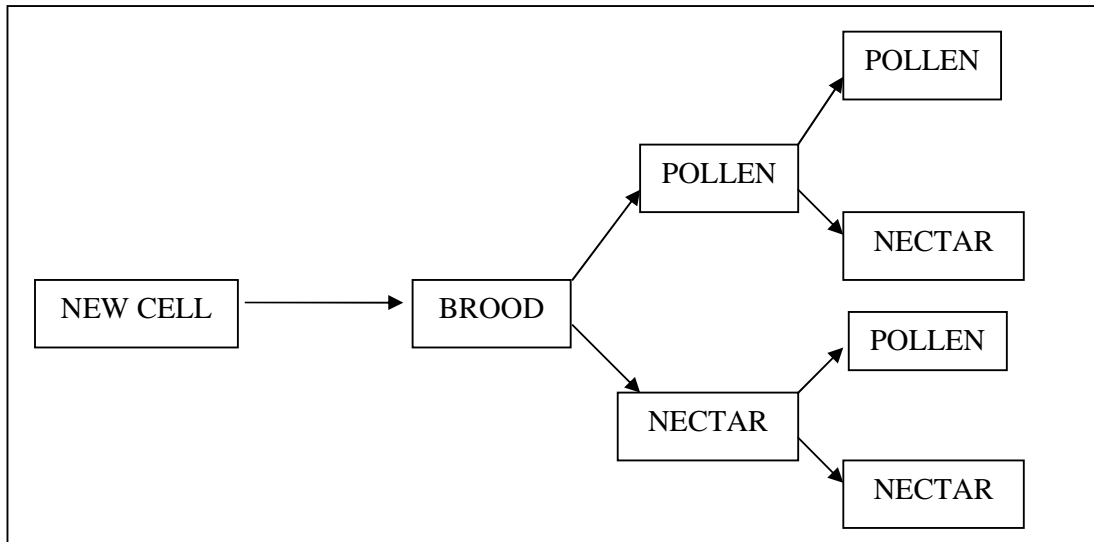


Figure 1.1 Progressive use of cells in a natural honeybee colony (Adapted from Free 1977).

1.6 Developmental cycles of *Apis mellifera capensis*

The developmental time of an *A. m. capensis* worker, from egg to adult, has been recorded at between 18 and 20 days (Tribe & Allsopp 2001b). Eggs hatch after 3 days with a subsequent larval period of between 5 and 6 days (Tribe & Allsopp 2001b). Following this, larvae are capped and remain in this cocoon for 10 to 12 days (Tribe & Allsopp 2001b). Slight variations in the duration of development are recorded in other races of *A. mellifera* (Tribe & Allsopp 2001b).

Camazine (1991) suggested a “self-organization” hypothesis for the repeated patterns observed on brood combs in queenright honeybee colonies. The hypothesis explains the patterns observed in honeybee brood combs and the intermediate patterns observed between brood cycles. This hypothesis is concerned with the distribution, not only of brood, but also the placement of pollen, nectar and honey on the comb. Camazine’s (1991) self organisation hypothesis is concerned with queenright colonies; however, these ideas have never been tested in queenless colonies. In addition, even queenright *A. m. capensis* may not fit the self organisation hypothesis in the same way that queenright *Apis mellifera ligustica*, used in Camazine’s (1991) experiments, did.

Apis mellifera capensis colonies have a variety of pathways available to ensure survival of the colony following the loss of a queen (Hepburn 2001). With the onset of laying by workers, worker policing increases (Pirk *et al.* 2002); and, although not well documented for *A.m. capensis*, there is the potential for brood cannibalism due to colony store depletion and reduced foraging (Schmickl & Crailsheim 2001) following queen loss.

This study aims to determine the pattern of brood cycling used in *A. m. capensis* workers, following queen loss. Such loss is a critical event in a colony and can induce a variety of responses at the comb (or brood) level; ultimately, this may lead to the colony absconding. As laying workers are present in *A.m. capensis* colonies, brood cycling via laying workers may continue for a number of weeks or months while the colony attempts to raise a new queen. In addition, the study aims to see the effects of queen loss on the colony, at a cellular level of a comb, with specific interest in the movement of eggs and larvae, the potential policing of eggs and the cannibalism of larvae and brood.

II

MATERIALS AND METHODS

2.1 Study site

Four colonies of *Apis mellifera capensis* were used throughout the experiment. That these colonies were representative of the natural “wild” population is evidenced by the total lack of any honeybee selection and breeding in the Eastern Cape Province of South Africa (Eardley *et al.* 2001). The experiment was conducted at the Department of Zoology and Entomology, Rhodes University in Grahamstown, Eastern Cape Province, South Africa. Colonies were kept in nucleus boxes with a minimum of two brood frames and three storage frames with honey, nectar and pollen. Colonies were placed approximately one metre apart. The experiment was conducted from February 2007, the end of summer, through autumn to the beginning of May 2007.

2.2 Queen removal

Each colony was dequeened on 25 February 2007, at the onset of the experiment. Throughout the experiment the presence and position of queen cells were recorded and then removed so as to ensure that no requeening of colonies took place. If a queen was found within a colony, it was assumed to be most likely foreign and therefore it was concluded that a merger had probably occurred. Indeed, the invasion of foreign queens has been documented in other experiments in the same area of the Eastern Cape Province, South Africa (Moritz unpubl. obs.).

2.3 Photographical data recording

After the initial removal of the queens from the colonies, the brood situation in each was recorded photographically prior to any possible activity by laying workers present. Photographs of each frame containing brood were taken. Prior to photographing each comb, a perspex and wire frame containing 5cm x 5cm squares labeled from A through Q on the horizontal and 1 through 8 on the vertical (Figs. 2.1 & 2.2), was placed over the brood frame so that a precise point of reference could be obtained. Brood frames and the perspex frame were hung on a wooden stand to ensure the exact positioning of the comb cells with respect to the grid when photographing. Frames were labeled prior to photographing so as to ensure there was no confusion over which frame or side of a frame was used. This was standard procedure throughout the duration of the experiment.

For the first eight days following queen removal, photographs were taken every alternate day. This interval was chosen so as to ensure that eggs found after dequeening were those derived from laying workers and, in addition, that all queen-laid eggs present at the onset would be capped brood by day eight. However, from the eighth day onwards, photographs were taken every third day until colonies either absconded or a merger with a wild colony occurred. A total of twenty-five photographic intervals were conducted throughout the research period.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1																	
2																	
3																	
4																	
5																	
6																	
7																	
8																	

Figure 2.1 Stylized image of the perspex grid placed over the brood comb used to establish precise positioning of squares for each photographic sampling interval.

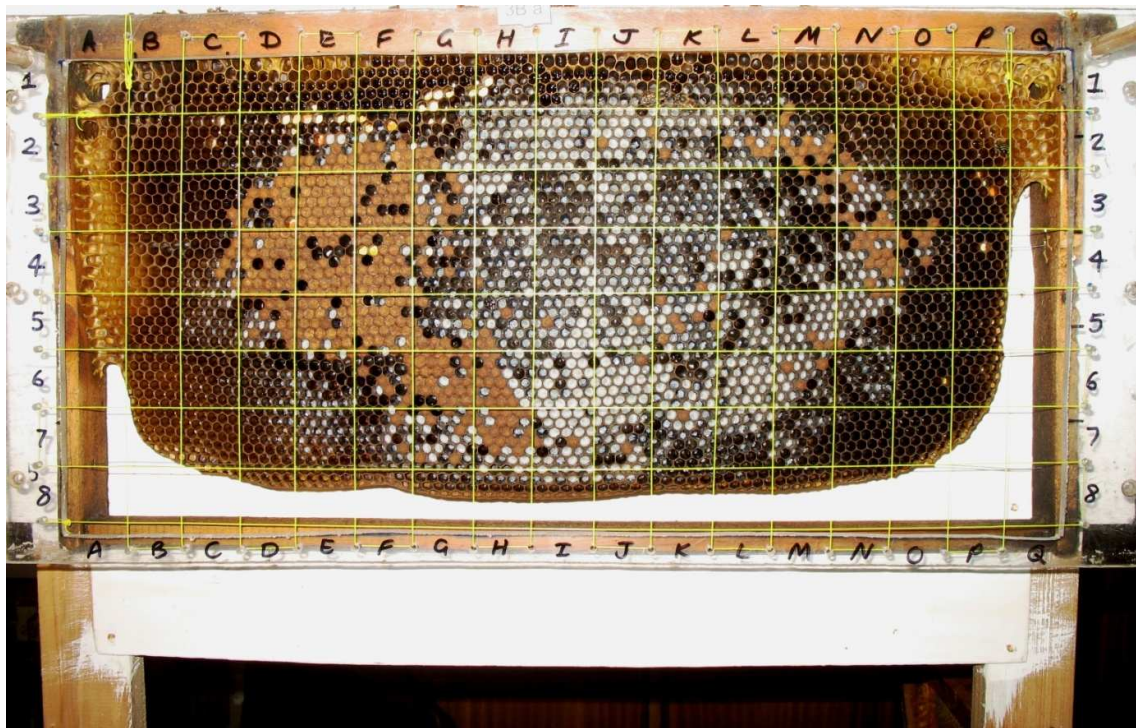


Figure 2.2 Typical data photograph with perspex grid placed over the brood frame.

2.4 Photographic analyses

Since there were 136 squares (17 x 8) on the grid, to determine the required number of squares per frame to be sampled in the study, a random sampling technique for stratified sampling across three time periods (three consecutive time intervals) was used. Randomly generated sample sizes of 1 through 30 squares were used and the mean number of brood cells for each sample size was calculated. Graphs of the mean number of brood cells against sample sizes were plotted for three different frames; namely frame 2Ba (colony B; side “a”), frame 2Bb (colony B; side “b”) and frame 3Da (colony D). Graphs showed that the estimated mean number of brood cells converged to the true mean number of 14 when samples of 25 or more squares (i.e. after 8 squares per frame) were sampled (Figs. 2.3 to 2.5).

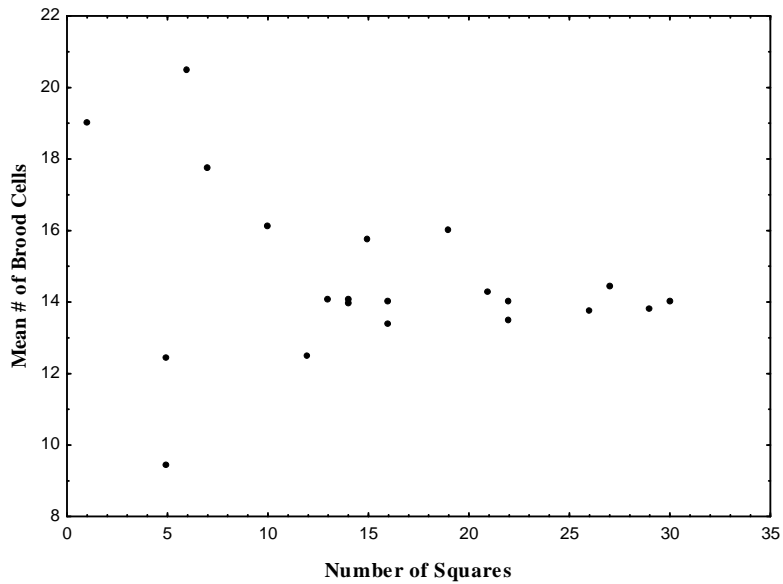


Figure 2.3 Frame 2Ba: Mean number of brood cells (14) per square versus number of squares sampled (n=30) showing convergence to the true mean at 25 squares for three consecutive intervals (i.e. after approximately 8 squares).

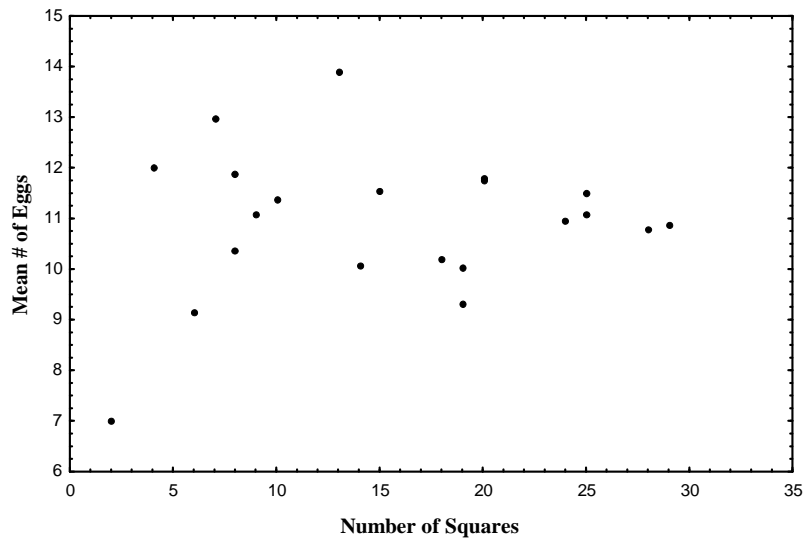


Figure 2.4 Frame 2Bb: Mean number of eggs (11) per square versus number of squares sampled (n=30) showing convergence to the true mean at 24 squares for three consecutive intervals (i.e. after approximately 8 squares).

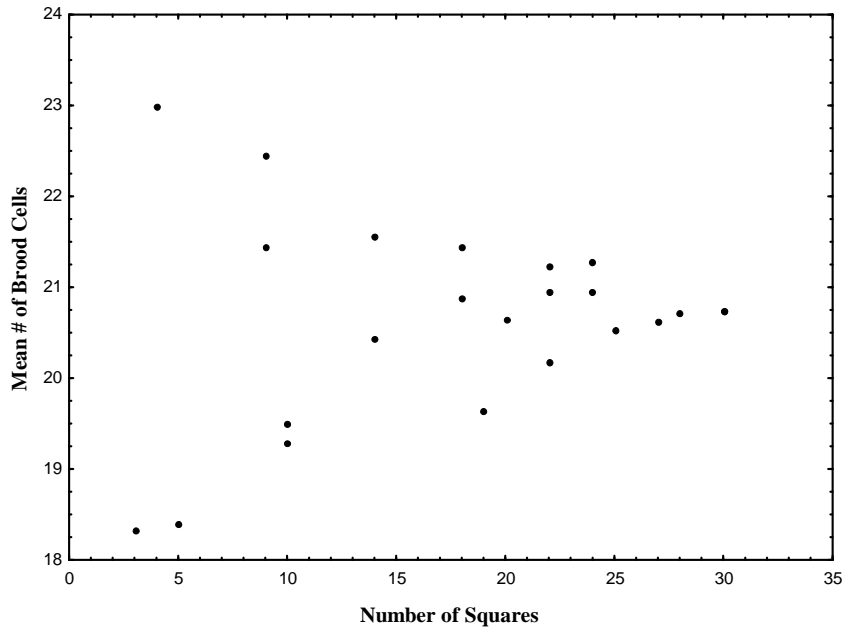


Figure 2.5 Frame 3Da: Mean number of brood cells (20.5) per square versus number of squares sampled (n=30) showing convergence to the true mean at 25 squares for three consecutive intervals (i.e. after approximately 8 squares).

Cells in columns A, B, C, O, P and Q and rows 1, 2, 7 and 8 were excluded from analysis because they lacked brood and concentration was placed on the centre of the frames which contained brood (i.e. 44 squares). This was done to avoid skewed results due to the lack of brood present at the edges of all frames. Focus was therefore placed in the central area of each frame and, from this subsample, squares were chosen randomly, but once chosen, the same squares for each frame were analysed for the duration of the experiment.

Individual cells for each of the squares were then checked and the contents recorded. Brood cell contents were defined into categories, namely: empty, egg, larva, capped worker brood, capped drone brood, queen cells (capped or uncapped), nectar, pollen, honey, uncapped brood and chalk brood. Prior to statistical analyses, these were condensed into eight categories: 1) empty, 2) stores (nectar, pollen and honey), 3) eggs, 4) larvae, 5) capped brood (worker), 6) uncapped brood, 7) chalk brood and 8) other.

Each square was then repeatedly analysed, for each photographic interval, until absconding of the colony occurred or a merging event with a foreign queen took place, in which case the colony was considered to be new.

2.5 Statistical analyses

A total of 14 frames were analysed, with 8 grid squares per frame and a mean of 27.4 ± 2.6 cells per frame. This was an average of 219.2 cells per frame, or 3068.8 cells in total, per photographic interval (Table 2.1). Due to differing absconding times, the number of photographic intervals varied between colonies from 7 to 25 intervals.

Table 2.1 The average number of cells sampled for each square, frame and in total for each colony, for the duration of the experiment.

<i>Colony</i>	<i>Intervals</i>	<i>Frames sampled</i>	<i>Average number of cells per square</i> \pm <i>standard deviations</i>	<i>Average number of cells per frame</i> \pm <i>standard deviations</i>	<i>Total cells sampled per colony</i>
A	25	3	26.7 ± 2.9	213.7 ± 19.5	16025
B	8	4	27.5 ± 2.3	219.0 ± 15.4	7008
C	7	4	28.2 ± 2.5	224.3 ± 12.1	6279
D	24	3	27.0 ± 2.6	216.3 ± 3.5	15576
Total		14	27.4 ± 2.6	873.3 ± 50.5	44888

To test for differences within the data (i.e. between cell categories), a repeated measures analysis of variance (ANOVA) was performed using STATISTICA Software package (version 8.0) for each colony. Tests were operated at a 5% level of significance. Data generated from each frame were combined into total colony data, and the means used in each analysis.

For each colony, graphical representation of the proportion of cells used for each of the categories, for each consecutive interval, was conducted using a frequency of occurrence graph generated through Microsoft Excel (2007). Similarly, graphs of the average number of queen cells constructed following queen loss, for each colony, for the duration of the experiment were produced as well as a graph of their position on the comb.

III

RESULTS

3.1 Distribution of brood and stores

At the onset of the experiment, colonies lacked large amounts of stores in the cells which were sampled. This is not unusual as most of the stores (nectar, pollen and honey) are located on combs surrounding the central brood combs (Fig. 3.1). In addition, a reduced number of empty cells in the central region were observed. This is not uncommon as queens begin ovipositing from the centre of the comb outwards, in concentric rings, limiting the number of central, empty cells. Cells on the periphery of the comb contained stores of nectar and pollen; however these cells were excluded for the original analysis and focus was placed on the cells in the central comb region.

Following dequeening however, the colonies rapidly changed the use of the central brood combs and changed the function of cells which were previously utilised as brood cells to storage cells containing pollen or nectar. This was typical of all four colonies, with storage cells being interspersed with brood cells (Fig. 3.2). Contrary to that observed by Free's (1977) findings, two of the colonies used in this research projected, colonies A and D, regularly interchanged cell use between storage and brood (Appendices A and D).

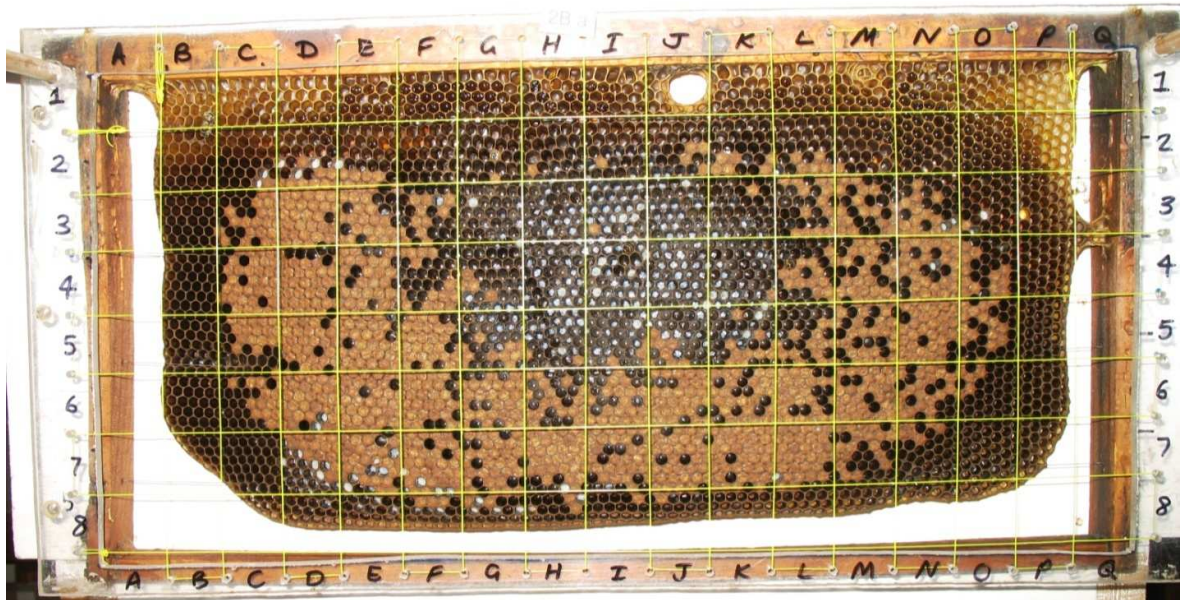


Figure 3.1 Frame 2Ba from colony B immediately following dequeening. Capped brood, larvae and eggs are located in the centre of the frame while nectar and pollen are found in the peripheral cells.

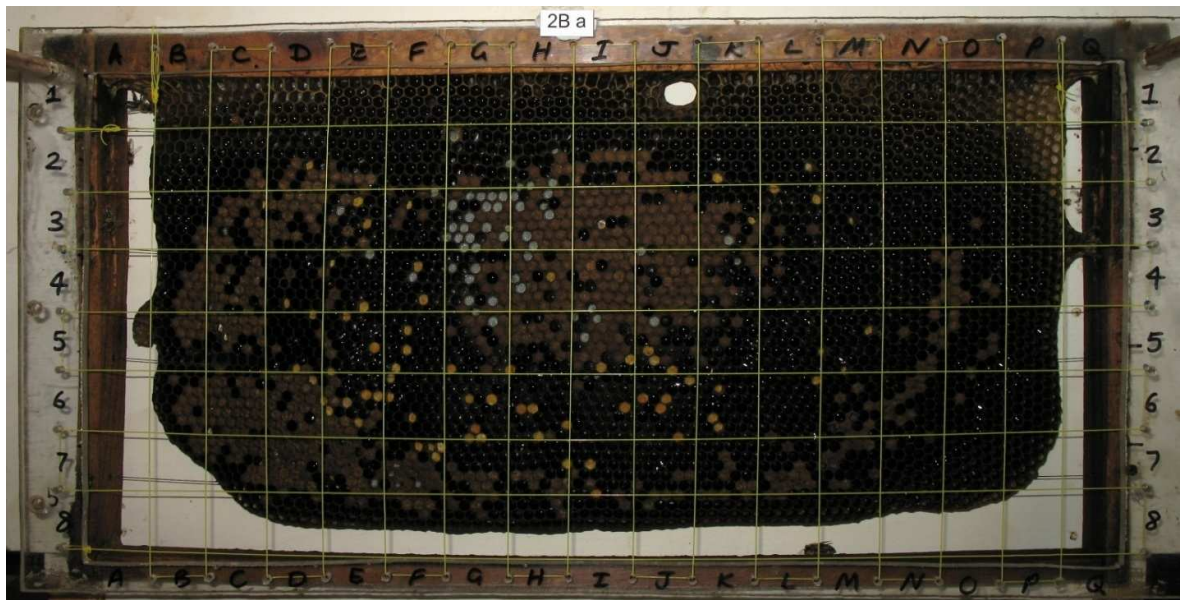


Figure 3.2 Frame 2Ba from colony B three photographic intervals (6 days) after dequeening. After young bees emerge, the cells in the centre of the brood comb are utilised as storage cells. Pollen and nectar stores in the peripheral cells were reduced and relocated to the central cells. (Note: Emergency queen cell on the left, in the swarm position, in square A5).

3.2 Absconding

Colony A was the only of the four colonies to not abscond during the experiment (Fig 3.3). Although colony B did abscond (Fig. 3.4), colony C was the first colony to after only seven photographic intervals (14 days) (Fig. 3.5). Shortly after this, after eight photographic intervals (17 days) colony B absconded. Although not conclusive, it is thought to be highly likely that, following absconding, colony C merged with colony B. Following the absconding of colony C, the number of individuals in colony B increased greatly, indicating an amalgamation, probably with colony C.

No stores or brood remained following absconding of both colonies B and C, suggesting that this was an instance of prepared absconding. Before absconding, both colonies B and C allowed all brood to emerge and any eggs found, derived from the laying workers, were policed.

Colony D absconded after 24 photographic intervals (61 days) leaving behind approximately 10 percent of their stores in the cells which were sampled (Fig. 3.14). Again, absconding appeared to be prepared due to the reduction in the amount of stores left behind and ensuring that all capped brood had eclosed prior to absconding.

3.3 Queen cells

In all four colonies queen cells were constructed; however, in only three of the four colonies (A, B and C) were queen cell constructed as an immediate response to dequeening, and from queen-derived brood.

3.3.1. Queen brood

It is well documented that larvae older than three days are no longer viable for queen rearing (Ribbands 1953; Hepburn *et al.* 1988) except under extreme conditions where larvae of up to four days are reported to have been so used (Fell & Morse 1984). For the three photographic intervals following initial dequeening (six days), any queen cells observed were assumed to be from queen-laid brood (Figs. 3.3-3.6).

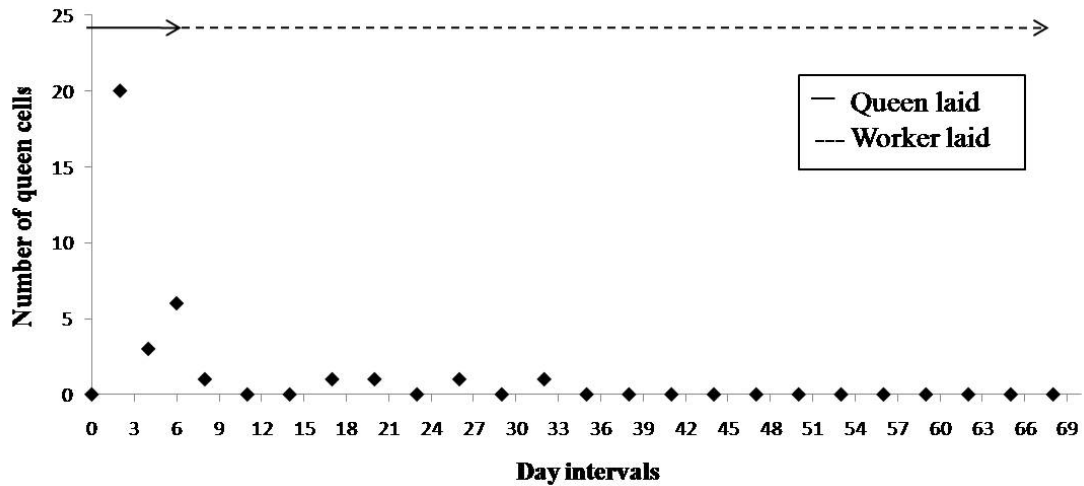


Figure 3.3 Colony A: number of queen cells observed, and subsequently removed. Following dequeening, a large number of queen cells were produced and all were constructed in the swarm position. Queen cells produced at interval 5 (8 days after dequeening) could possibly have been derived from a laying worker egg; or, alternatively using a queen-derived larva which would have to have been older than three days.

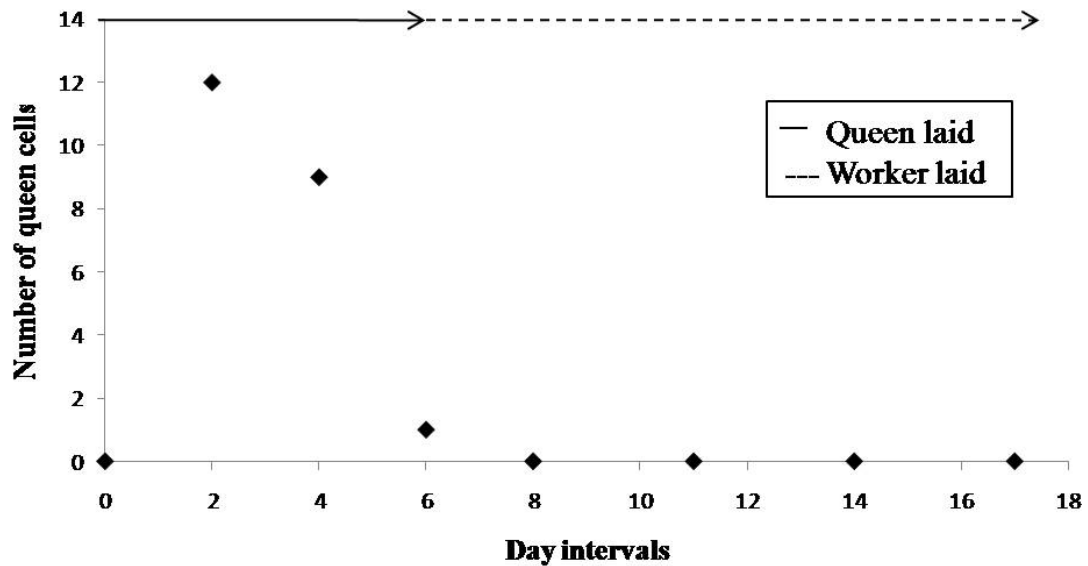


Figure 3.4 Colony B: number of queen cells observed and removed. All larvae or eggs used were assumed to be those of the queen as queen cell production ceased after four photographic intervals (6 days) indicating that queen produced larvae were no longer viable (i.e. were older than three days).

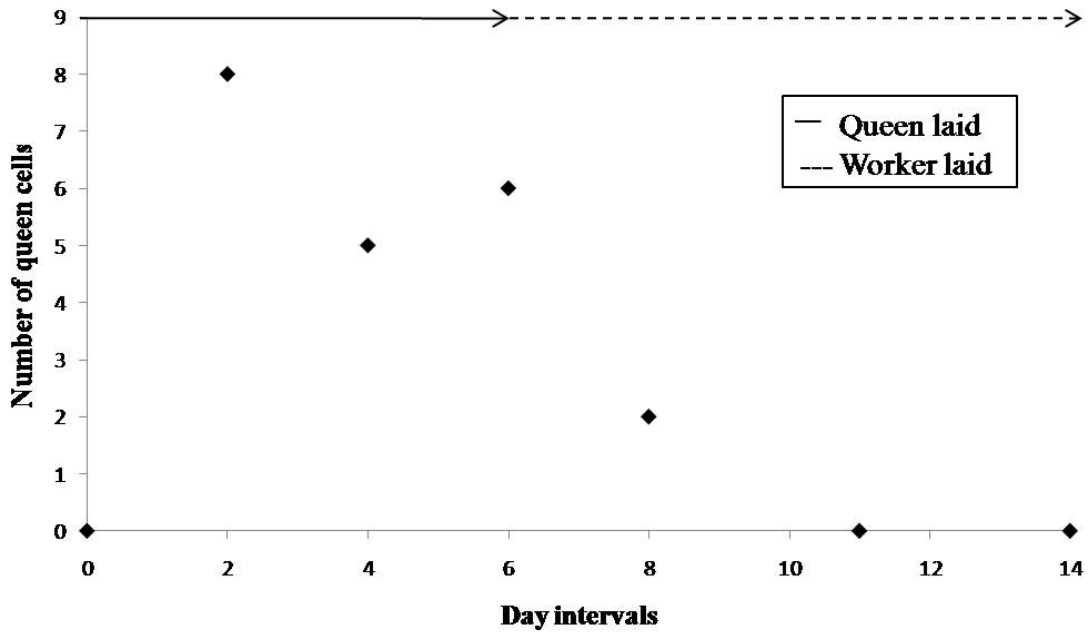


Figure 3.5 Colony C: number of queen cells observed and removed. The queen cells observed at interval 5 (8 days) indicate the possible presence of laying workers or the unlikely utilization of larvae older than three days.

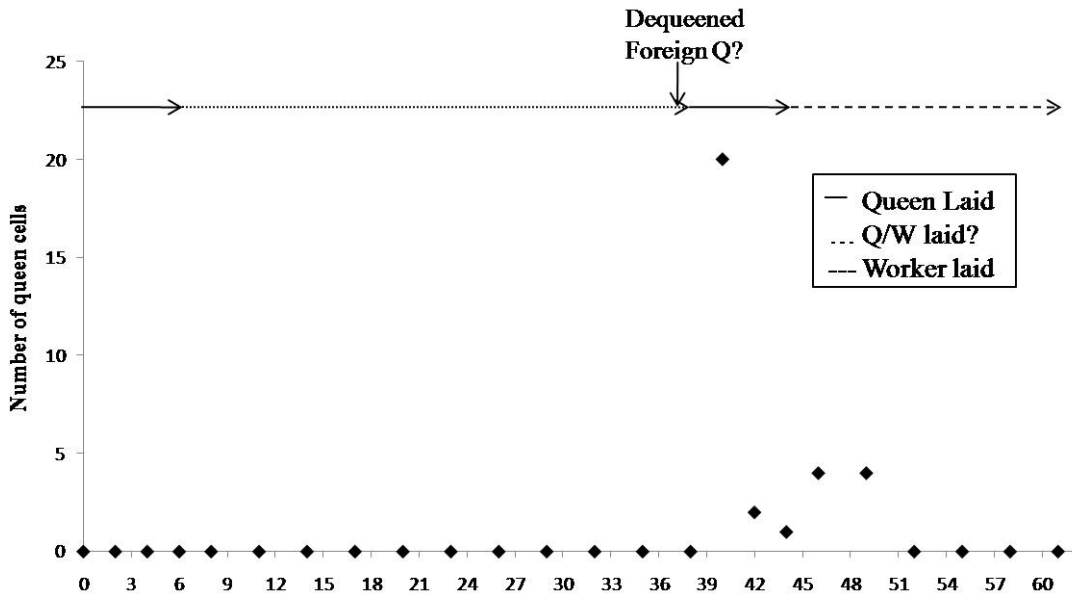


Figure 3.6 Colony D: number of queen cells observed and removed. The lack of queen cell production following initial dequeening indicates that there was possibly an immediate merger with a foreign queen, thereby suppressing the colony from constructing any emergency queen cells. The colony was again dequeened (the queen assumed to be foreign) at day 38 (photographic interval 15) and following this, an increase in queen cell production was observed. Any eggs produced between days 6 and 38 were assumed to be derived from either the foreign queen or laying workers.

3.3.2 Laying worker brood

Queen cells observed after 6 days (i.e. three photographic intervals) were assumed to be those of laying workers, because workers will rarely use larvae older than 3 days for emergency queen rearing (i.e. 3 days for eggs to hatch and maximum 3 day old larvae gives 6 days). Colonies A, C and D potentially used laying worker eggs to produce queen cells (Figs. 3.3, 3.5 & 3.6). Although laying workers were present in colony B (Figs. 3.10 & 3.11) no queen cells were constructed after day 6.

3.3.3 Foreign queen invasion

In colony D, a queen (assumed to be foreign) was removed at day 38 (photographic interval 15). Queens were assumed to be present when the laying pattern became concentric in the brood frames. Following queen removal, a marked increase in the number of queen cells produced was observed (Fig. 3.6). In assessing alternative cells in combs, new eggs were observed between days 2 and 4 (photographic intervals 2 and 3) confirming the suspicion that a foreign queen invaded colony D immediately following initial dequeening.

In colony A, on day 68 (photographic interval 25), a single queen was removed which again was believed to be foreign. The queen was found by chance during a routine search of each comb in the hive; no concentric laying pattern or increase in brood stores were observed to suggest the presence of the foreign queen. From this it was assumed that the queen had recently invaded the colony and had yet to begin ovipositing in cells which were being sampled. The possibility that the removed foreign queen may have been a young, virgin queen cannot be excluded, hence the lack of brood present.

3.3.4 Position of queen cells

In all four colonies substantially more queen cells were constructed in the swarm position (situated along the edge of the comb) than in the emergency queen cell position (Fig. 3.7). Queen cells built in the swarm position requires the transfer of brood from cells in the centre of the comb to cells constructed along the periphery of the brood comb. With the number of queen cells in the swarm position being so high, this may indicate that laying workers start ovipositing in these cells earlier than expected.

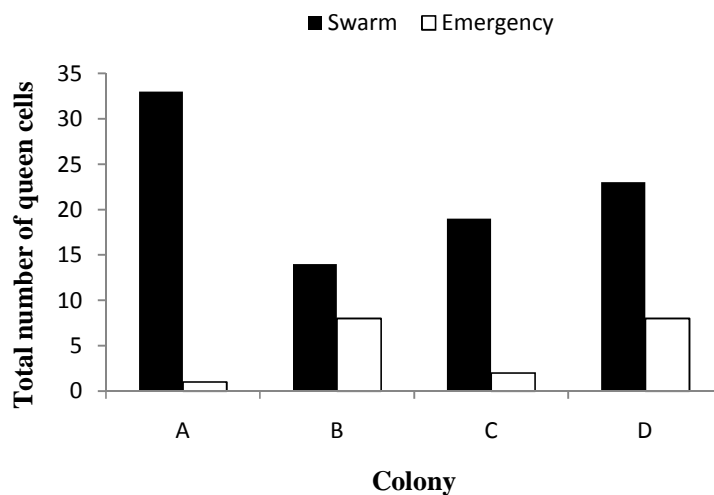


Figure 3.7 Total number of queen cells found in either the swarm position or emergency position for each colony.

Colonies B and D had the highest number of queen cells constructed in the emergency position, both with eight cells in total. Colonies A and C had considerably fewer cells with one and two in total, respectively. However, in relation to swarm cells, colony B had the smallest ratio of swarm cells to emergency cells (1.75:1) with only 14 cells being constructed in the swarm position, while colony D had 23 cells in total in the swarm position (a ratio of 2.88: 1). Colony A had the most queen cells constructed in the swarm position (33 in total) and the highest ratio of swarm to emergency (33:1) with colony C close behind with a ratio of (9.5:1).

3.4 Frequency of occurrence

3.4.1. Colony A

Upon dequeening, approximately 35 percent of the cells sampled in colony A were either empty or contained stores. The other 65 percent were cells containing either eggs, larvae or capped brood.

Eggs were noted in cells at most photographic intervals indicating the presence of laying workers. At photographic interval 25 a single, presumably foreign queen was removed. Based on the reduced amount of eggs, larvae and capped brood at the time of this discovery, it was assumed that the foreign queen had only recently invaded the colony. Over 90 percent of cells sampled on the final day (photographic interval 25 day 68), were either empty or contained stores, with only 5 percent being filled with capped brood (Figs. 3.8 & 3.9). Multiple eggs were present in some cells at photographic interval 25 (day 68), again verifying the presence of laying workers as well as supporting the inference that a foreign queen had recently invaded (Appendix A). Following initial dequeening, the proportion of empty and storage cells versus brood cells shifted dramatically, with the percentage of storage and empty cells increasing radically. The number of brood cells initially decreased significantly, and continued to do so throughout the experiment although at a lower rate, due to the ovipositing by laying workers (Figs. 3.8 & 3.9).

Chalk brood was observed in colony A, at a low level, and at times was not immediately removed, with the most extreme case remaining in a cell for 6 photographic sampling intervals (approximately 18 days) (Fig. 3.8). At photographic intervals 20 and 21 (day 53 and 56) a dead pupa was observed in a cell and was not removed until photographic interval 22 (approximately 5 or 6 days) indicating an apparent reduction in the thoroughness of cleaning from workers undertaking housekeeping tasks.

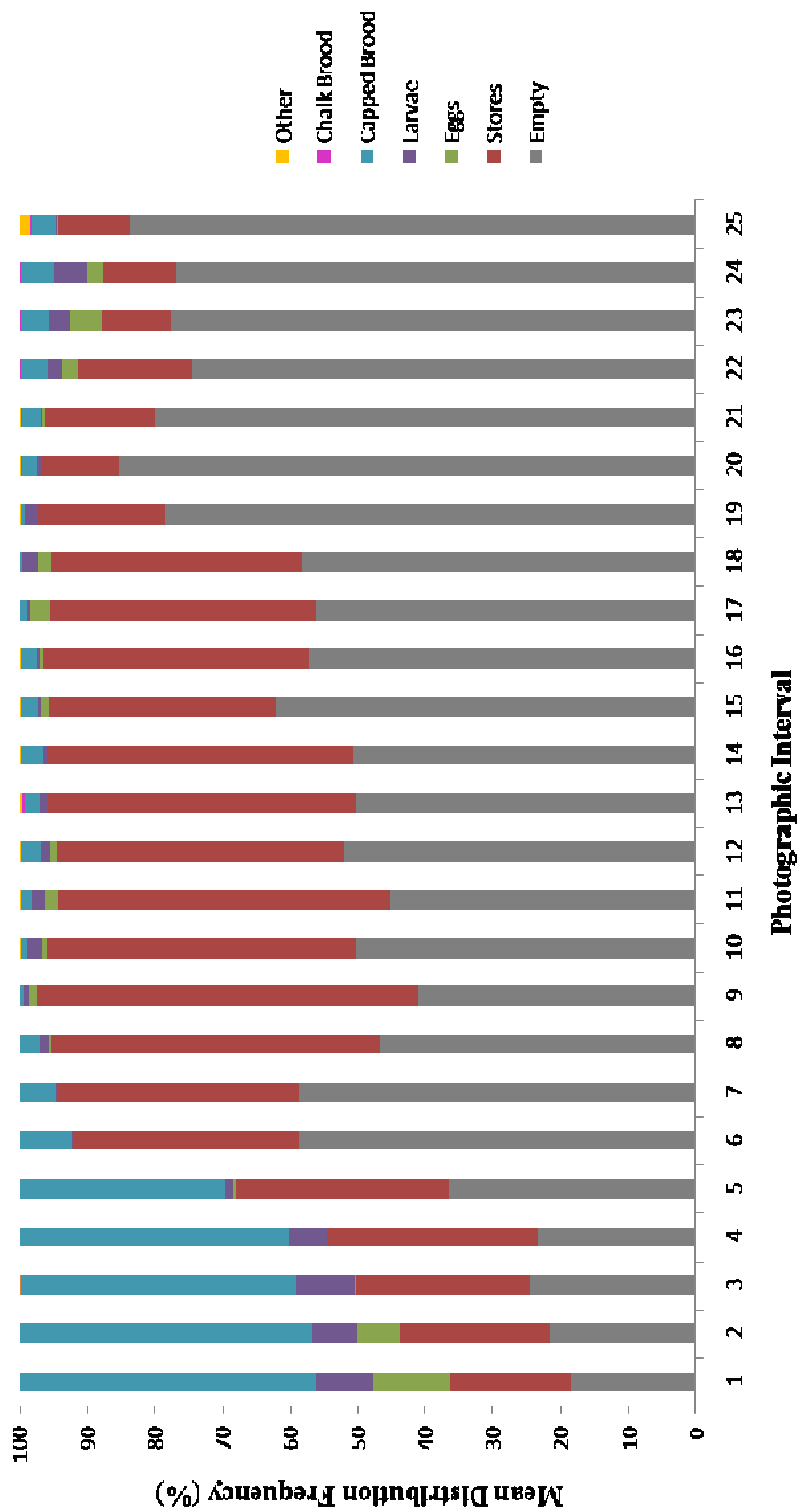


Figure 3.8 Colony A: Mean frequency of occurrence for each category for the duration of the experiment, for all sampled frames.

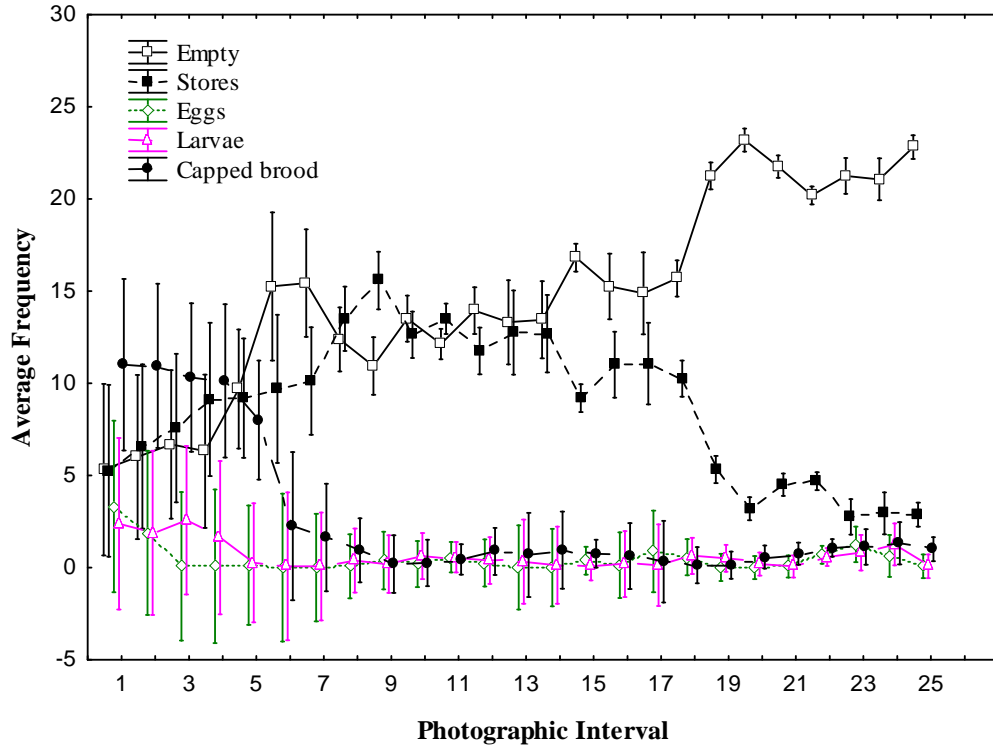


Figure 3.9 Colony A: Repeated Measures ANOVA with the dependent variable plotted against photographic intervals. Vertical bars denote \pm standard error (Category: $F_{4,5} = 10.7$, $p < 0.0115$; category and photographic interval interaction: $F_{96,120} = 2.6$, $p < 0.0001$). The empty and storage cell categories were significantly different from the other categories (Tukey: $p < 0.05$).

The large standard error bars denote the differences observed not only within combs (i.e. between individual cells) but the differences observed between different frames within the same colony (Fig. 3.9). The error bars were reduced after photographic interval 8, when the number of empty and storage cells increased and in even proportions, covering over 90 percent of those cells sampled (Fig. 3.8).

3.4.2 Colony B

At the onset of the experiment, colony B had a healthy brood situation consisting of capped brood, larvae and eggs accounting for approximately 90 percent of total cells sampled, with empty or storage cells found in the centre of the comb, an estimated 10 percent. Further, after dequeening, as brood eclosed, the amount of storage and empty cells steadily increased while the number of capped brood, larvae and eggs decreased (Figs. 3.10 & 3.11).

The presence of laying workers was observed at photographic intervals 5 and 7 (Fig. 3.10); however, in accordance with laying workers, not many eggs were observed. Due to the erratic laying pattern of laying workers, it is possible that this number does not accurately depict the number of eggs present due to only eight, randomly selected squares being sampled. Eggs found at photographic interval 5 (day 9) were no longer visible at photographic interval 6 (day 12), nor were any larvae found in the cells, indicating that the eggs had either been policed prior to photographic interval 6, or the larvae cannibalized or moved. Eggs found at photographic interval 7 (day 15) were not found at photographic interval 8 (day 18) due to the subsequent absconding of colony B; it was assumed that these eggs had been policed.

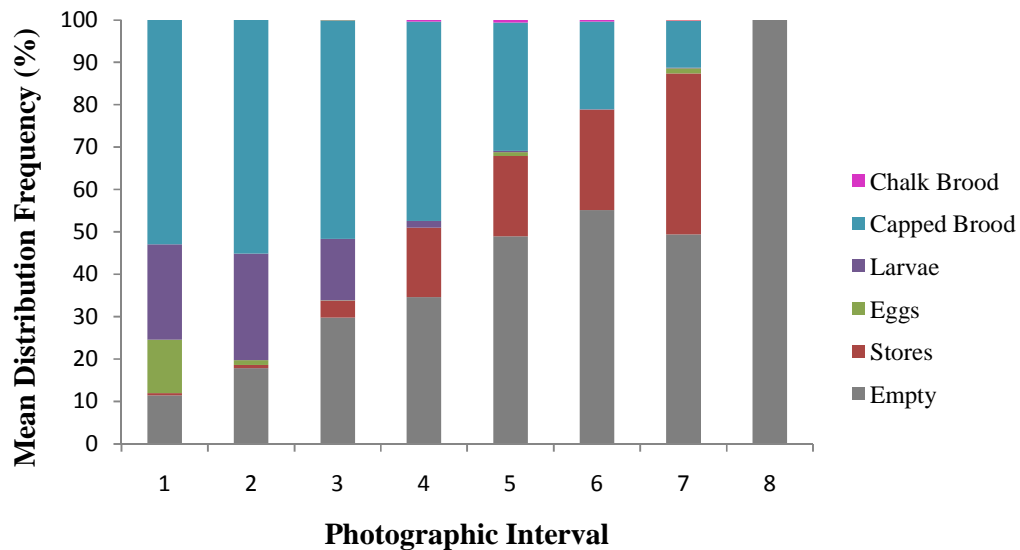


Figure 3.10 Colony B: Mean frequency of occurrence of categories plotted against photographic intervals before absconding.

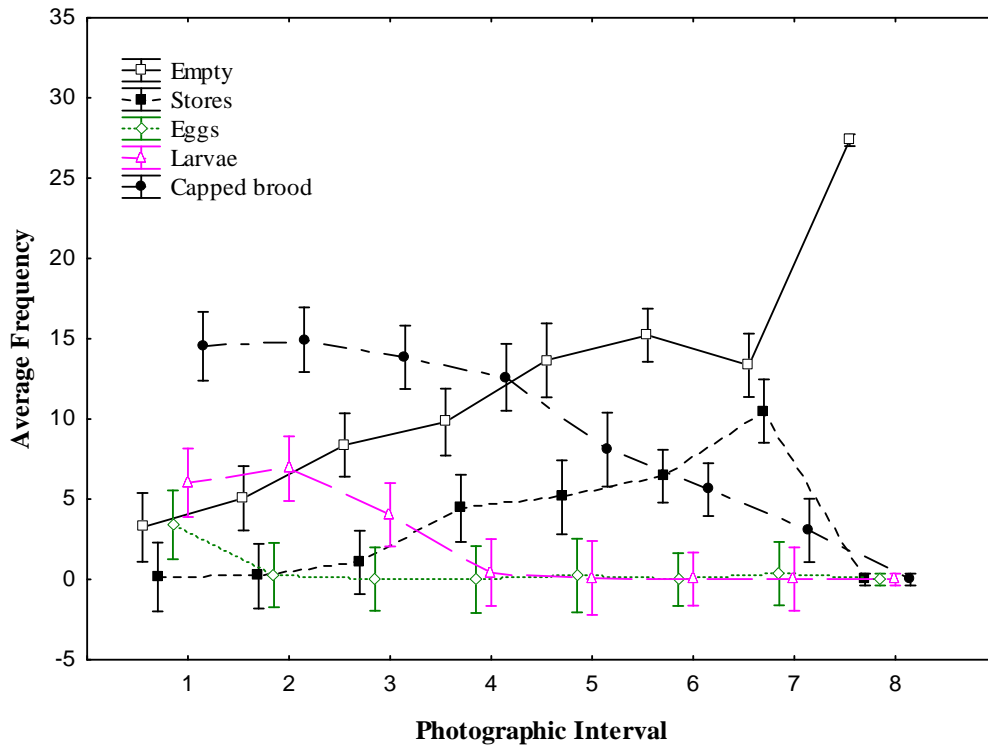


Figure 3.11 Colony B: Repeated Measures ANOVA with the dependent variable plotted against number of photographic intervals. Vertical bars denote \pm standard error (Category: $F_{4,15} = 15.1$, $p < 0.0001$; category and photographic interval interaction: $F_{28,105} = 7.2$, $p < 0.0001$). The empty and capped brood cell categories were significantly different from the other categories (Tukey: $p < 0.05$).

3.4.3 Colony C

The brood situation in the colony at the onset of the experiment was healthy totaling 80 percent, while storage and empty cells accounted for 20 percent. As brood began to eclose, the empty cells were either replaced with stores or remained empty. Following dequeening, no additional eggs were found in worker cells in the colony, indicating a lack of laying workers, and consequently larvae and brood declined rapidly (Figs. 3.12 & 3.13). Laying workers were thought to be present during queen cell construction however no evidence of workers ovipositing in any other brood cells was observed. Due to the random sampling of squares and cells during the experiment, it is possible that eggs may have been oviposited in cells which were not part of those sampled.

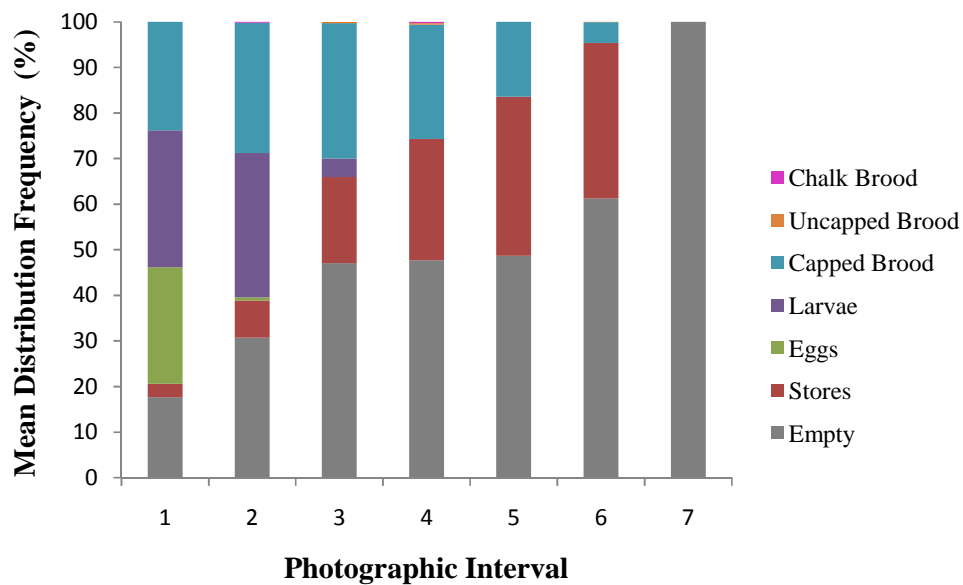


Figure 3.12 Colony C: Mean frequency of occurrence of each category, plotted against the total number of photographic intervals before absconding.

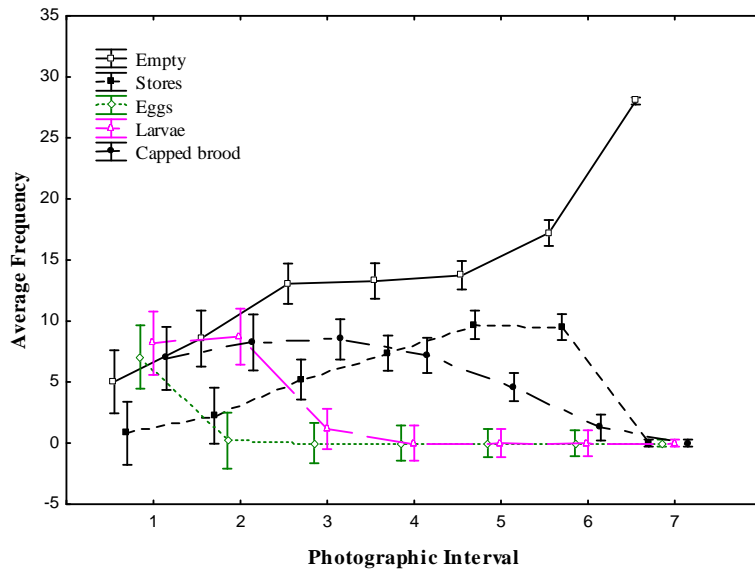


Figure 3.13 Colony C: Repeated Measures ANOVA with the dependent variable plotted against number of photographic intervals. Vertical bars denote \pm standard error (Category: $F_{4,15} = 17.7$, $p < 0.0001$; category and photographic interval interaction: $F_{24,90} = 9.3$, $p < 0.0001$). The empty cell category was significantly different from the other categories (Tukey: $p < 0.05$).

3.4.4 Colony D

The brood situation at the onset of the experiment was dominated by larvae but comprised capped brood and eggs as well. The number of empty and storage cells in the centre of the comb was less than 20 percent. Following dequeening (i.e. photographic interval 2), the number of empty and storage cells decreased to approximately 12 percent, indicating a high probability that workers were transferring larvae to cells located within the sampling area (Fig. 3.14). In addition, at photographic intervals 9 and 10, larvae were present although at the previous photographic interval no eggs were recorded in these cells, again indicating the movement of larvae from cell to cell.

The relatively steady amount of brood present in the colony, even after dequeening, supports the inference that a foreign queen invaded almost immediately (Figs. 3.14 & 3.15). The significant reduction in the number of eggs being produced following the dequeening at photographic interval 15 indicates that laying by workers was not immediate. The first sign of eggs in the brood comb following the second dequeening event occurred at photographic interval 21 (day 51) and this would most likely indicate the presence of laying workers. These eggs were subsequently policed suggesting that they were laying worker derived.

Chalk brood was seen to be not immediately removed from cells and in an extreme case, remained in a cell for four consecutive photographic intervals (approximately 12 days) before being removed (Fig. 3.14 and Appendix D).

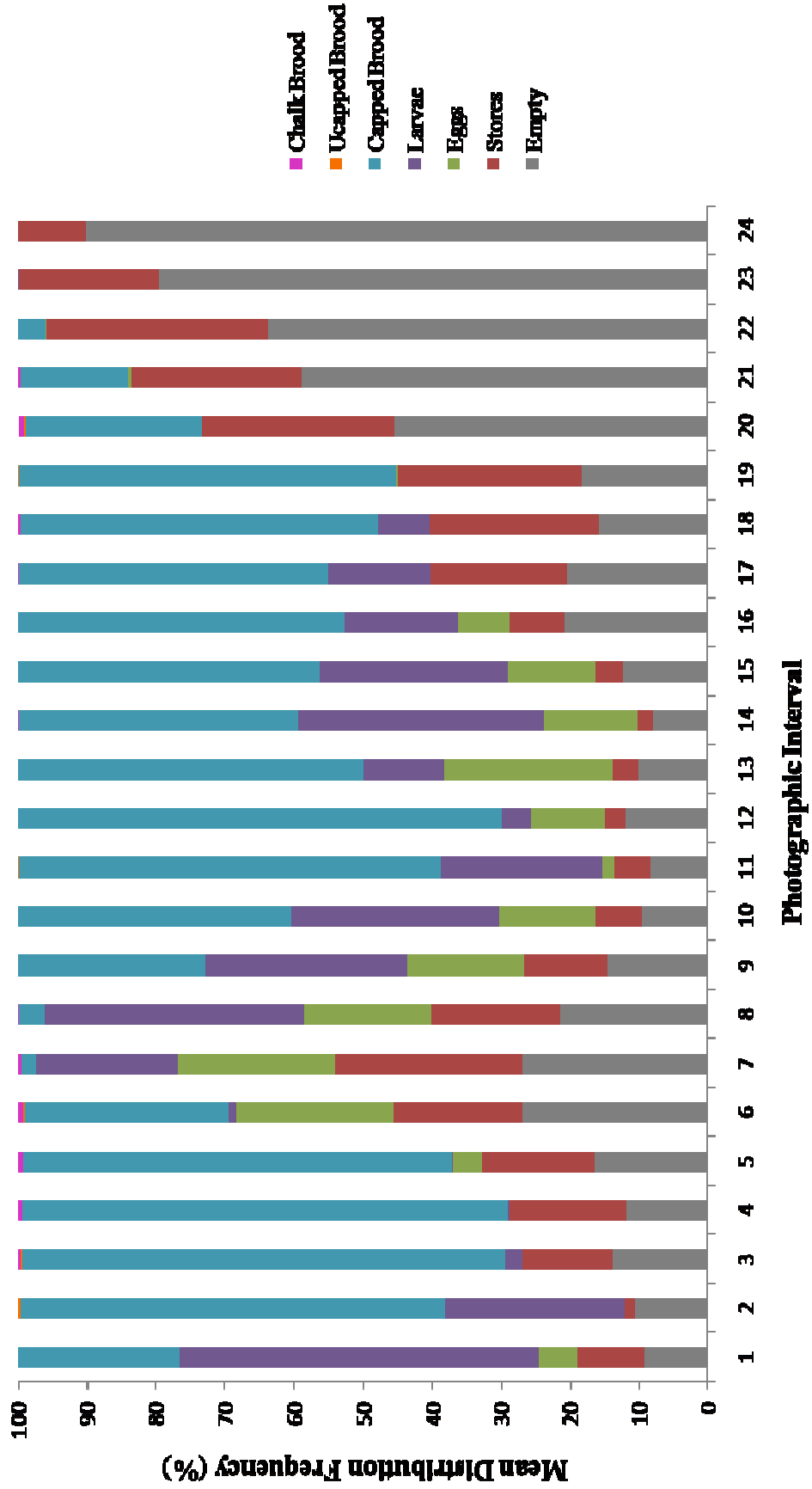


Figure 3.14 Colony D: Mean frequency of occurrence of each category, plotted against the total number of photographic intervals.

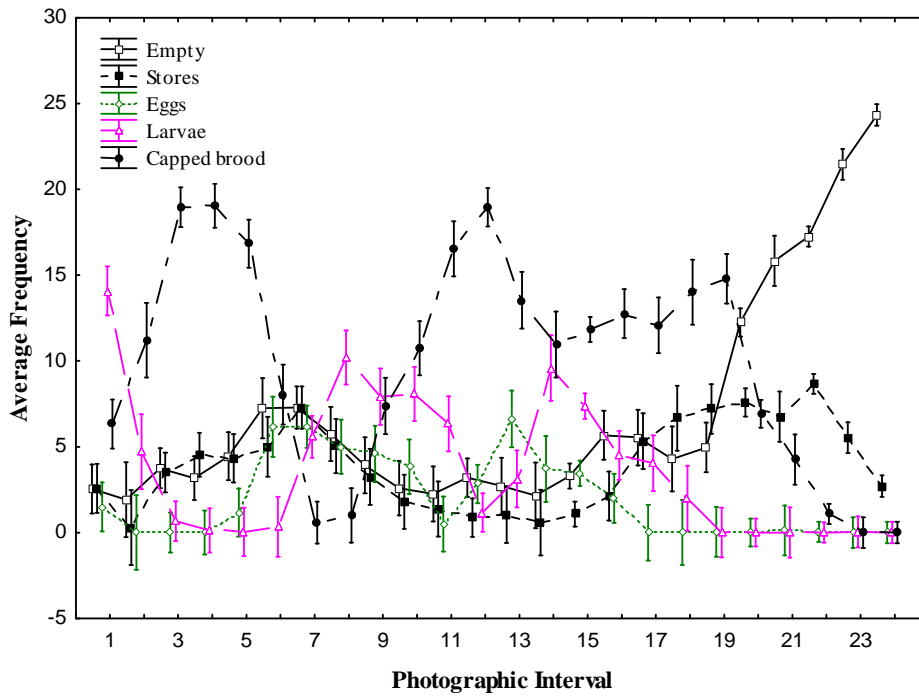


Figure 3.15 Colony D: Repeated Measures ANOVA with the dependent variable plotted against photographic interval. Vertical bars denote \pm standard error (Category: $F_{4,10} = 38.5$, $p < 0001$; category and photographic interval interaction: $F_{92,230} = 8.6$, $p < 0001$). All categories were significantly different to one another except between storage and larvae cells (Tukey: $p < 0.05$).

3.5 Brood

At the onset of the experiment, all colonies had eggs, larvae and capped brood (Figs. 3.8 to 3.15; Appendices A to D). At photographic interval 2 (day 2), immediately following dequeening, the brood situation in all colonies began to change and observations of worker policing, brood cannibalism and the movement of larvae from cell to cell were noted (Appendices A to D). In all colonies, cannibalism of larvae was an immediate reaction to dequeening (Appendices A to D). The movement of larvae from cell to cell was frequently observed in colonies B and D at photographic interval 2, directly after dequeening (Appendices B and D). Worker policing was also regularly observed, in colonies C and D, at photographic interval 2 although the frequency of this observation was limited by the initial number of eggs observed in each colony due to random sampling of cells.

3.5.1. Movement vs. cannibalism

It is commonplace for young honeybee larvae to be cannibalized (Nakamura 1993; 1995); however, the movement of larvae from cell to cell has been less frequently documented. In all four colonies, the movement of larvae from cell to cell was recorded and subsequent cannibalism of moved larvae was also regularly documented (Appendices A to D). In addition, cannibalism or possible movement of older larvae occurred in all colonies and was regularly observed as well as the cannibalism of capped brood in colonies A, B and D (Appendices A, B and D). It would appear that cannibalism of both queen and laying worker-derived larvae occurs.

Although not regularly observed, the movement of older larvae (i.e. between 3 and 5 days) in colonies A and B (Appendices A and B) and of pupae in colony A was also noted (Appendix A). It was assumed that older larvae had been used due to the sudden appearance of capped brood in previously empty cells.

3.5.2. Worker policing

The policing of eggs was observed in all four colonies with varying frequency among colonies. In colony A both queen- and worker-laid eggs were policed. In colony B only worker-laid eggs were policed while in colony C only queen-laid eggs were policed.

In colony D it was not possible to determine whether policing was of queen- or worker-laid eggs due to the removal of a foreign, laying queen during the experiment. It was assumed that the eggs policed were queen-laid based on the timing of policing (between photographic interval 1 and 2; day 0 to 2). The initial queen's eggs were policed and this may be attributed to the subsequent invasion of a new, foreign queen. Following this invasion, policing was greatly reduced and was only observed again at photographic interval 15 and 16 subsequent to the removal of the foreign queen (Fig. 3.14 and Appendix D).

IV

DISCUSSION

4.1 Strategies following queen loss

Apis mellifera capensis colonies utilise different pathways in an attempt to rectify the sudden loss of a queen. Hepburn (1994) described four pathways that queenless, broodright colonies of *A. m. capensis* might use. It was suggested that colonies may either a) rear a new queen from existing queen brood (“classical requeening”), b) initially use queen laid eggs in queen cell construction but abort these and use worker laid eggs in queen rearing, c) persist as a laying worker colony yet probably ultimately dwindle or d) abscond. From this experiment it was seen that, although all above mentioned pathways were observed, they were not necessarily utilised in isolation. In colonies of *A. m. capensis*, a combination of these pathways may be used in an attempt to survive an unanticipated queen loss.

4.1.1 Queen rearing

Butler (1954) recorded that the initial reaction of a colony to queen loss was the construction of emergency queen cells. In addition, he noted the loss triggered some restlessness within the colony. The immediate reaction to queen loss, in three of four colonies, namely colonies A, B and C, was an attempt at raising a new queen from existing queen brood. Fell and Morse (1984) recorded that the commencement of queen cell construction as early as 12 hours but could be initiated as late as 48 hours following loss (Free 1984).

At photographic interval 2 (day 2) these three colonies had all initiated construction of queen cells. It was assumed that queen brood had been used in queen rearing based on the time of construction and the presence of queen laid eggs in all colonies following dequeening. However, with the majority of the queen cells being in the swarm position, the

possibility that the eggs used were worker laid cannot be excluded. Without proof at a genetic level however, it can only be inferred that the cells constructed may have contained eggs from a laying worker.

Queen cells constructed after day 6 (photographic interval 4) were thought to be more likely those of a laying worker. The utilization of larvae older than three days is highly improbable and, although observed by Fell and Morse (1984), was only infrequently noted under extreme conditions.

4.1.2 Laying workers

The presence of laying workers was noted in three colonies, namely colonies A, B and D. The onset of ovipositing via laying workers varied between colonies however, ovipositing may have commenced earlier in each colony but had not yet been detected due to the random selection of squares sampled.

In colony A the onset of ovipositing was almost immediate and what was thought to be worker-laid eggs were detected as early as photographic interval 3 (day 4). Hepburn (1994) reported the onset of laying for dequeened *A. m. capensis* workers at 1 week while Tribe and Allsopp (2001 a) suggested ovary development for a worker, maturation of eggs and then subsequent onset of laying, would be in the region of 15 days following queen loss. Few eggs were observed at this early stage (approximately 1% of cells sampled) (fig 3.8) suggesting that if indeed the eggs were those of a laying worker, the number of workers with active ovaries present at that interval were minimal.

The idea that laying workers were present in the colony prior to the loss of the queen cannot be disregarded. Oldroyd and Osborne (1999) observed laying workers in queenright colonies of *Apis mellifera* and it has been recorded that in any given colony, up to 10% of workers show some signs of ovary development (Jay 1968; Visscher 1996). Ratnieks (1993) recorded that 1 in 10 000 workers had a fully developed egg in their ovaries, suggesting that laying workers, although rare, are found in queenright colonies. This would explain the presence of presumed worker laid eggs so soon after dequeening.

At photographic intervals 5 and 7 (days 11 & 14), laying worker eggs were present in colony B. The subsequent policing of said eggs also indicates a higher probability of the eggs being worker-laid.

Worker-laid eggs were only found in colony D after the second dequeening event and in reduced numbers. Eggs found at one photographic interval were not present at subsequent photographic intervals, nor were young larvae, adding to the notion that they were worker-laid and had therefore been policed.

4.1.3 Absconding

In the event that a queen could not be produced, colonies opted to abscond, leaving behind little, if any, stores. Absconding occurred in three of the four colonies, namely, colonies B, C and D.

In colonies B and C there was it was believed that with a large and sudden reduction in stores, the decline in brood and limited number of laying workers present, the only available pathway for these colonies to utilise would be that of absconding. Initially, colony C attempted to merge with colony B as a way of avoiding immediate absconding. However, given the same reduction in brood and stores observed in colony B, absconding following the presumed merger was immediate.

However, in the case of colony D, the presence of a foreign queen deterred absconding for a number of weeks. Following the loss of their second queen, colony D did not recover and only a limited number of eggs were produced via laying workers, which were subsequently policed. As with colonies B and C, combined with the reduction in stores, lack of laying workers and the steady decline in brood, absconding appeared to be the only pathway available.

Nakamura (1994) suggested that, in *Apis cerana*, two types of absconding could be observed, namely disturbance-induced absconding and seasonal migration. Disturbance-induced absconding could be acute or chronic based on the nature of the disturbance. The same ideas of absconding can be used in understanding absconding in colonies of *Apis mellifera*. The sudden removal of a queen could be considered a chronic disturbance and,

with colonies B and C, be the major motivating factor for said absconding. The absconding of colony D was at the end of autumn (beginning of May) and a combination of seasonal factors could also have contributed. The imminent onset of winter, and subsequent reduction in available stores, combined with a second queen removal, could have assisted in the decision to abscond in search of a new foraging area.

4.1.4 Foreign queen invasion

In addition to the four pathways described by Hepburn (1994), a fifth pathway was discovered in the course of this experiment. This was the invasion of a queenless colony by a foreign queen. In two of the four colonies (namely colonies A and D) a presumed to be foreign queen was removed. Queens which were found in a colony following dequeening at the onset of the experiment were thought to be foreign due to the thorough searching of each comb during each photographic interval and removal of any queen cells during this time.

In colony D, the presence of a foreign queen was initially detected by the concentric laying pattern which was observed on the brood frames. This invasion was thought to be almost immediate due to a lack of queen cells constructed following dequeening. With closer inspection of the photographed combs, the presence of eggs were noted between day 2 and 4 (photographic intervals 2 and 3) and, although possibly those of a laying worker, the placement of single eggs in the centre of each cell (Tribe & Allsopp 2001 a) combined with lack of queen cells, suggested queen-laid.

The presence of a foreign queen in colony A was not detected based on the increased brood and concentric laying pattern but rather by chance when surveying each of the combs in the colony during a photographic interval. Due to the lack of eggs, larvae and capped brood, it was assumed that the queen had recently merged with the colony when she was detected. There was little increase in the size of the colony, indicating the foreign queen invaded with few, if any, of her own workers.

4.2 Brood situation

The brood situation at the onset of the experiment was typical of normal *Apis mellifera capensis* colonies, with brood frames located in the centre of the hive and brood localized to the central region of each comb.

4.2.1 Brood cannibalism

Reports of brood cannibalism in *Apis mellifera* colonies are numerous. The idea that brood cannibalism is a result of poor foraging conditions (Johannsmeier 2001; Schmickl & Crailsheim 2001) do not apply to any of the colonies sampled in this experiment. The percentage of stores fluctuated throughout the experiment, and between colonies, yet never diminished to less than 15% in any one colony. However, it is important to keep in mind that squares samples were restricted to brood frames and samples were randomly taken from the central, brood region, where previously, little by way of stores was observed. Therefore, based on the amount of stores available, it is unlikely that the cannibalism of larvae was due to a lack of nectar, pollen or honey, or a poor foraging area.

Weiss (1984) suggested that the consumption of a combination of larvae, pollen and nectar, not only improved the longevity of individual bees but the additional protein from cannibalizing larvae assisted in the rearing of young larvae to adulthood. Although cannibalism proves to be useful when resources are limited, it is regarded as a short term solution, hindering colony growth (Nakamura 1995). However, when larvae from peripheral brood cells are consumed, this is considered to be a sustainable form of protein, recycled by being fed to larvae in the central cells (Nakamura 1995). Therefore, it is possible that the cannibalism of certain larvae was for their protein content in sustaining a colony.

The movement of a larva from cell to cell was on numerous occasions noted. With this in mind, it is then not known whether larvae were cannibalized or simply moved to another cell.

4.2.2 Movement of brood

The movement of larvae and pupae from cell to cell has not previously been recorded. The presence of larvae in cells that previously did not contain eggs was an indication of such movement. Although possible for the larva to crawl out of or eat its way through one cell into another cell, the idea is highly unlikely as moved larvae were not found in sampled squares which previously had eggs or larvae. Moreover, no holes through cell walls were detected. Therefore, it was assumed that transfer of eggs and larvae were through physical movement by the worker bees themselves.

4.2.3 Worker policing

As an *Apis mellifera capensis* queen is polyandrous (Roberts 1944; Estoup *et al.* 1993; Baudry *et al.* 1998; Neumann *et al.* 1999), numerous different patrilineages are found among the workers in any given colony. Following sudden queen loss, competition to become a reproducer within a colony is observed (Moritz *et al.* 1996; Neuman *et al.* 2000) and the level of conflict between individuals, a result of the polyandrous nature of the queen and their genetic relatedness (Ratnieks 1988; Inbar *et al.* 2008).

Worker policing behaviour reduces the number of eggs seen through to adulthood, hence reducing the reproductive capacity for another nestmate (Ratnieks 1988; Oldroyd & Ratnieks 2000). With the sudden loss of the queen, all colonies policed eggs however, this is not restricted to eggs laid by workers but, in colony D, the eating of eggs produced by the queen was also observed. It was thought that such policing was due to the presence of a foreign queen shortly after the initial dequeening.

4.3 Behaviour in queenless colonies

Unconventional behaviour was recorded in all four colonies following queen loss. The reduction in basic housekeeping was noted, where chalk brood was not immediately removed but rather left in a cell for days. This would suggest that, with colonies steadily dwindling numbers, the emphasis placed on housekeeping would also be reduced.

The increased pollen and nectar found could possibly suggest an increase in the number of forager bees in the hive due to the need for less nurse bees with the lack of brood. It could also be that, with the lack of young and developing brood, there is a reduced need for the utilization of stores in the feeding of larvae, hence an increase in stores available. Ribbands (1952) suggested that it was possible that in a honeybee colony the division of labour was not solely determined by the age of the workers but rather on the requirements of the colony at any given time.

The uncapping of capped brood was noted on several occasions, exposing either a larva or pupa. The recapping of said cell in subsequent photographic intervals was also recorded. However, on occasion the cell remained uncapped for more than one photographic interval and the presumed dead larva or pupa removed. Again, this adds to the idea of reduced emphasis of particular duties within the colony.

To summarize, colonies all utilised the pathways to one degree or another observed by Hepburn (1994). Foreign queens invaded two colonies which was thought to be a new pathway available to queenless colonies of *A. m. capensis*. The number of queen cells constructed varied between colonies yet in all colonies significantly more were built in the swarm position. Worker policing and cannibalism of larvae was recorded in all colonies; however accurate numbers for such cannibalism could not be determined as, in addition to some being eaten, larvae and eggs were moved from their original cells to a new ones, presumably by worker bees. The behaviour of the colonies following queen loss varied, yet in all colonies, a reduction in housekeeping was observed where chalk brood, dead larvae and pupae were left in cells for days without being removed.

V

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Empty	Empty	Empty	Empty	Empty	Empty	Empty
Stores	Stores	Stores	Stores	Stores	Stores	Stores
Empty	Empty	Empty	Empty	Empty	Empty	Empty
Empty	Empty	Empty	Empty	Empty	Empty	Empty
Stores	Stores	Stores	Stores	Stores	Stores	Stores
Stores	Stores	Stores	Stores	Stores	Stores	Stores
Empty	Empty	Empty	Empty	Empty	Empty	Empty
Empty	Empty	Empty	Empty	Empty	Empty	Empty
Empty	Empty	Empty	Empty	Empty	Empty	Empty
Empty	Empty	Empty	Empty	Empty	Empty	Empty
Stores	Stores	Stores	Stores	Stores	Stores	Stores
Empty	Empty	Empty	Empty	Empty	Empty	Empty
Empty	Empty	Empty	Empty	Empty	Empty	Empty
Empty	Empty	Empty	Empty	Empty	Empty	Empty
Empty	Empty	Empty	Empty	Empty	Empty	Empty
Empty	Empty	Empty	Empty	Empty	Empty	Empty

APPENDIX B

Appendix B Colony B: Contents of the cells for each square sampled for the duration of the experiment

FRAME	CUBE	# of CELLS	D1	D2	D3	D4	D5	D6	D7	D8
2Ba	D4	32	Empty	Empty	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	D4		Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
2Ba	D4		Empty	Empty	Empty	Empty	Stores	Stores	Stores	Empty
2Ba	D4		Empty	Empty	Empty	Empty	Stores	Stores	Stores	Empty
2Ba	D4		Empty	Empty	Empty	Empty	Empty	Stores	Stores	Empty
2Ba	D4		Brood	Brood	Brood	Empty	Empty	Stores	Empty	Empty
2Ba	D4		Brood	Brood	Brood	Empty	Empty	Empty	Stores	Empty
2Ba	D4		Brood	Brood	Brood	Empty	Empty	Empty	Empty	Empty
2Ba	D4		Brood	Brood	Brood	Empty	Stores	Stores	Stores	Empty
2Ba	D4		Empty	Empty	Empty	Stores	Empty	Stores	Stores	Empty
2Ba	D4		Brood	Brood	Brood	Empty	Empty	Stores	Stores	Empty
2Ba	D4		Brood	Brood	Brood	Brood	Empty	Empty	Empty	Empty
2Ba	D4		Brood	Brood	Brood	Empty	Empty	Empty	Stores	Empty
2Ba	D4		Brood	Brood	Brood	Empty	Empty	Empty	Empty	Empty
2Ba	D4		Brood	Brood	Brood	Brood	Empty	Empty	Stores	Empty
2Ba	D4		Brood	Brood	Brood	Empty	Empty	Empty	Stores	Empty
2Ba	D4		Brood	Brood	Brood	Empty	Empty	Empty	Empty	Empty
2Ba	D4		Brood	Brood	Brood	Brood	Empty	Empty	Stores	Empty
2Ba	D4		Brood	Brood	Brood	Empty	Empty	Empty	Stores	Empty
2Ba	D4		Brood	Brood	Brood	Empty	Empty	Empty	Empty	Empty
2Ba	D4		Brood	Brood	Brood	Empty	Empty	Empty	Stores	Empty
2Ba	D4		Brood	Brood	Brood	Empty	Empty	Stores	Stores	Empty
2Ba	D4		Brood	Brood	Brood	Empty	Empty	Stores	Stores	Empty
2Ba	D4		Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
2Ba	D4		Brood	Brood	Brood	Stores	Empty	Stores	Stores	Empty
2Ba	D4		Brood	Brood	Brood	Empty	Empty	Stores	Empty	Empty
2Ba	D4		Brood	Brood	Empty	Empty	Empty	Empty	Empty	Empty
2Ba	D4		Brood	Brood	Empty	Empty	Empty	Stores	Stores	Empty
2Ba	D4		Brood	Brood	Empty	Empty	Empty	Empty	Empty	Empty
2Ba	D4		Brood	Brood	Brood	Brood	Empty	Stores	Stores	Empty
2Ba	E6	32	Brood	Brood	Brood	Brood	Brood	Empty	Empty	Empty
2Ba	E6		Brood	Brood	Empty	Stores	Stores	Empty	Empty	Empty
2Ba	E6		Brood	Brood	Brood	Empty	Empty	Empty	Empty	Empty
2Ba	E6		Empty	Empty	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	E6		Brood	Brood	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	E6		Brood	Brood	Brood	Empty	Empty	Empty	Empty	Empty
2Ba	E6		Empty	Empty	Empty	Empty	Empty	Empty	Stores	Empty

2Ba	F3		Egg	Larva	Larva	Larva	Brood	Brood	Brood	Empty
2Ba	F3		Empty	Empty	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	F3		Empty	Empty	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	F3		Empty	Empty	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	F3		Brood	Empty	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	F3		Empty	Empty	Empty	Larva	Brood	Empty	Stores	Empty
2Ba	F3		Egg	Larva	Larva	Larva	Brood	Brood	Brood	Empty
2Ba	G6	25	Brood	Brood	Empty	Empty	Stores	Empty	Stores	Empty
2Ba	G6		Brood	Empty	Empty	Empty	Stores	Empty	Stores	Empty
2Ba	G6		Brood	Brood	Empty	Empty	Stores	Stores	Empty	Empty
2Ba	G6		Brood	Brood	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	G6		Brood	Empty	Empty	Empty	Empty	Empty	Empty	Empty
2Ba	G6		Brood	Empty	Larva	Empty	Empty	Empty	Empty	Empty
2Ba	G6		Brood	Empty	Larva	Stores	Stores	Stores	Stores	Egg
2Ba	G6		Brood	Empty	Larva	Stores	Stores	Stores	Stores	Empty
2Ba	G6		Brood	Empty	Empty	Stores	Stores	Stores	Empty	Empty
2Ba	G6		Brood	Brood	Brood	Empty	Empty	Empty	Empty	Empty
2Ba	G6		Empty	Empty	Empty	Empty	Empty	Empty	Egg	Empty
2Ba	G6		Brood	Empty	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	G6		Brood	Empty	Empty	Stores	Stores	Empty	Empty	Empty
2Ba	G6		Brood	Brood	Brood	Empty	Empty	Empty	Stores	Empty
2Ba	G6		Empty	Empty	Larva	Empty	Empty	Empty	Empty	Empty
2Ba	G6		Brood	Brood	Brood	Empty	Empty	Empty	Empty	Empty
2Ba	G6		Brood	Empty	Larva	Empty	Empty	Empty	Stores	Empty
2Ba	G6		Brood	Brood	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	G6		Brood	Brood	Brood	Empty	Empty	Empty	Stores	Empty
2Ba	G6		Brood	Empty	Empty	Stores	Stores	Empty	Stores	Empty
2Ba	G6		Empty	Empty	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	G6		Brood	Brood	Empty	Empty	Empty	Empty	Empty	Empty
2Ba	G6		Brood	Brood	Brood	Empty	Empty	Empty	Empty	Empty
2Ba	G6		Brood	Brood	Brood	Empty	Empty	Empty	Empty	Empty
2Ba	G6		Brood	Empty	Empty	Empty	Empty	Empty	Empty	Empty
2Ba	K5	33	Larva	Larva	Brood	Brood	Brood	Brood	Brood	Empty
2Ba	K5		Empty	Larva	Larva	Brood	Brood	Brood	Brood	Empty
2Ba	K5		Larva	Larva	Larva	Brood	Brood	Brood	Brood	Empty
2Ba	K5		Brood	Empty	Empty	Empty	Egg	Empty	Egg	Empty
2Ba	K5		Larva	Larva	Larva	Brood	Brood	Brood	Brood	Empty
2Ba	K5		Larva	Empty	Empty	Empty	Empty	Empty	Empty	Empty
2Ba	K5		Empty	Empty	Larva	Empty	Empty	Empty	Empty	Empty
2Ba	K5		Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
2Ba	K5		Empty	Empty	Larva	Empty	Empty	Empty	Stores	Empty
2Ba	K5		Empty	Empty	Empty	Empty	Empty	Empty	Egg	Empty
2Ba	K5		Larva	Empty	Empty	Empty	Empty	Empty	Empty	Empty
2Ba	K5		Larva	Larva	Empty	Empty	Empty	Empty	Empty	Empty

2Ba	K5		Larva	Larva	Brood	Brood	Brood	Brood	Brood	Empty
2Ba	K5		Larva	Empty	Empty	Empty	Empty	Stores	Stores	Empty
2Ba	K5		Larva	Larva	Brood	Stores	Stores	Stores	Stores	Empty
2Ba	K5		Larva	Empty	Larva	Brood	Brood	Brood	Brood	Empty
2Ba	K5		Brood	Brood	Empty	Empty	Empty	Empty	Empty	Empty
2Ba	K5		Empty	Larva	Empty	Empty	Empty	Empty	Empty	Empty
2Ba	K5		Empty	Empty	Empty	Empty	Stores	Stores	Stores	Empty
2Ba	K5		Brood	Brood	Empty	Empty	Stores	Stores	Stores	Empty
2Ba	K5		Larva	Larva	Larva	Brood	Brood	Brood	Brood	Empty
2Ba	K5		Empty	Empty	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	K5		Egg	Larva	Larva	Empty	Larva	Empty	Chalk	Empty
2Ba	K5		Larva	Larva	Larva	Empty	Empty	Empty	Empty	Empty
2Ba	K5		Egg	Larva	Larva	Empty	Empty	Empty	Stores	Empty
2Ba	K5		Brood	Empty	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	K5		Brood	Brood	Empty	Empty	Empty	Empty	Empty	Empty
2Ba	K5		Brood	Brood	Empty	Empty	Empty	Empty	Empty	Empty
2Ba	K5		Brood	Empty	Empty	Empty	Empty	Empty	Empty	Empty
2Ba	K5		Empty	Empty	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	K5		Brood	Brood	Empty	Empty	Larva	Empty	Empty	Empty
2Ba	K5		Brood	Brood	Empty	Empty	Empty	Empty	Empty	Empty
2Ba	K5		Empty	Empty	Empty	Empty	Empty	Stores	Stores	Empty
2Ba	H4	32	Empty	Empty	Larva	Larva	Brood	Brood	Empty	Empty
2Ba	H4		Larva	Larva	Larva	Brood	Brood	Brood	Brood	Empty
2Ba	H4		Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
2Ba	H4		Larva	Larva	Larva	Brood	Brood	Brood	Brood	Empty
2Ba	H4		Larva	Larva	Brood	Brood	Brood	Brood	Brood	Empty
2Ba	H4		Larva	Larva	Brood	Brood	Brood	Brood	Brood	Empty
2Ba	H4		Larva	Larva	Larva	Brood	Brood	Brood	Brood	Empty
2Ba	H4		Larva	Larva	Brood	Brood	Brood	Brood	Brood	Empty
2Ba	H4		Empty	Larva	Larva	Brood	Brood	Brood	Brood	Empty
2Ba	H4		Empty	Larva	Larva	Brood	Brood	Brood	Empty	Empty
2Ba	H4		Larva	Larva	Brood	Empty	Empty	Empty	Egg	Empty
2Ba	H4		Empty	Larva	Empty	Stores	Empty	Empty	Stores	Empty
2Ba	H4		Empty	Empty	Empty	Brood	Brood	Brood	Brood	Empty
2Ba	H4		Larva	Larva	Brood	Empty	Empty	Empty	Stores	Empty
2Ba	H4		Empty	Larva	Larva	Brood	Brood	Brood	Empty	Empty
2Ba	H4		Empty	Larva	Larva	Brood	Brood	Brood	Brood	Empty
2Ba	H4		Larva	Empty	Empty	Brood	Brood	Brood	Brood	Empty
2Ba	H4		Larva	Larva	Brood	Brood	Brood	Brood	Brood	Empty
2Ba	H4		Larva	Larva	Larva	Empty	Empty	Empty	Empty	Empty
2Ba	H4		Larva	Larva	Brood	Brood	Brood	Brood	Brood	Empty
2Ba	H4		Larva	Larva	Brood	Brood	Brood	Brood	Brood	Empty
2Ba	H4		Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty

2Ba	H4		Larva	Larva	Larva	Brood	Brood	Brood	Brood	Empty
2Ba	H4		Larva	Larva	Brood	Brood	Brood	Brood	Brood	Empty
2Ba	H4		Larva	Larva	Brood	Brood	Brood	Brood	Brood	Empty
2Ba	H4		Empty	Larva	Larva	Brood	Brood	Brood	Brood	Empty
2Ba	H4		Larva	Larva	Larva	Brood	Brood	Brood	Empty	Empty
2Ba	H4		Empty	Empty	Larva	Brood	Brood	Brood	Brood	Empty
2Ba	H4		Larva	Larva	Brood	Brood	Brood	Brood	Brood	Empty
2Ba	H4		Larva	Empty	Empty	Brood	Brood	Brood	Brood	Empty
2Ba	H4		Larva	Larva	Larva	Brood	Brood	Brood	Brood	Empty
2Ba	L4	31	Empty	Empty	Empty	Empty	Empty	Empty	Egg	Empty
2Ba	L4		Egg	Larva	Larva	Brood	Brood	Brood	Brood	Empty
2Ba	L4		Empty	Empty	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	L4		Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
2Ba	L4		Egg	Larva	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	L4		Egg	Larva	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	L4		Egg	Larva	Larva	Brood	Brood	Brood	Brood	Empty
2Ba	L4		Egg	Larva	Empty	Empty	Empty	Empty	Empty	Empty
2Ba	L4		Empty	Empty	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	L4		Brood	Brood	Brood	Empty	Empty	Empty	Stores	Empty
2Ba	L4		Stores	Larva	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	L4		Brood	Larva	Empty	Empty	Empty	Empty	Empty	Empty
2Ba	L4		Brood	Empty	Empty	Empty	Empty	Empty	Empty	Empty
2Ba	L4		Empty	Empty	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	L4		Brood	Larva	Larva	Empty	Empty	Empty	Empty	Empty
2Ba	L4		Brood	Brood	Brood	Brood	Brood	Brood	Stores	Empty
2Ba	L4		Brood	Empty	Empty	Empty	Empty	Empty	Empty	Empty
2Ba	L4		Empty	Empty	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	L4		Brood	Brood	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	L4		Empty	Larva	Larva	Empty	Empty	Empty	Stores	Empty
2Ba	L4		Empty	Larva	Larva	Empty	Empty	Empty	Stores	Empty
2Ba	L4		Brood	Brood	Empty	Empty	Empty	Empty	Empty	Empty
2Ba	L4		Brood	Brood	Empty	Empty	Empty	Empty	Egg	Empty
2Ba	L4		Brood	Brood	Empty	Empty	Empty	Empty	Empty	Empty
2Ba	L4		Brood	Brood	Empty	Empty	Empty	Stores	Stores	Empty
2Ba	L4		Empty	Larva	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	L4		Empty	Larva	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	L4		Empty	Larva	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	L4		Brood	Empty	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	L4		Brood	Empty	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	L4		Brood	Brood	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	M5	30	Brood	Empty	Empty	Empty	Empty	Empty	Empty	Empty
2Ba	M5		Empty	Empty	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	M5		Brood	Empty	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	M5		Empty	Larva	Larva	Empty	Empty	Empty	Stores	Empty

2Ba	M5		Brood	Brood	Brood	Empty	Empty	Empty	Stores	Empty
2Ba	M5		Brood	Brood	Empty	Empty	Empty	Empty	Empty	Empty
2Ba	M5		Empty	Empty	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	M5		Brood	Empty	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	M5		Empty	Empty	Empty	Empty	Empty	Empty	Larva	Empty
2Ba	M5		Brood	Empty	Empty	Stores	Empty	Empty	Empty	Empty
2Ba	M5		Empty	Larva	Larva	Empty	Empty	Empty	Stores	Empty
2Ba	M5		Brood	Brood	Brood	Empty	Empty	Empty	Empty	Empty
2Ba	M5		Brood	Empty	Empty	Empty	Empty	Empty	Empty	Empty
2Ba	M5		Brood	Brood	Empty	Empty	Empty	Empty	Empty	Empty
2Ba	M5		Brood	Brood	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	M5		Brood	Brood	Empty	Empty	Empty	Empty	Empty	Empty
2Ba	M5		Brood	Brood	Empty	Stores	Empty	Empty	Stores	Empty
2Ba	M5		Empty	Empty	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	M5		Empty	Empty	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	M5		Empty	Empty	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	M5		Empty	Larva	Larva	Empty	Empty	Empty	Empty	Empty
2Ba	M5		Brood	Empty	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	M5		Brood	Empty	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	M5		Brood	Brood	Brood	Empty	Empty	Empty	Stores	Empty
2Ba	M5		Empty	Empty	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	M5		Brood	Empty	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	M5		Larva	Larva	Brood	Empty	Empty	Empty	Stores	Empty
2Ba	M5		Brood	Brood	Empty	Stores	Empty	Empty	Empty	Empty
2Ba	M5		Brood	Brood	Empty	Empty	Empty	Empty	Stores	Empty
2Ba	M5		Brood	Brood	Empty	Empty	Empty	Empty	Stores	Empty

APPENDIX C

Appendix C Colony C: Contents of the cells for each square sampled for the duration of the experiment

FRAME	CUBE	# of CELLS	1	2	3	4	5	6	7
2Cb	G6	25	Brood	Brood	Empty	Stores	Stores	Stores	Empty
2Cb	G6		Larva	Stores	Stores	Stores	Stores	Stores	Empty
2Cb	G6		Larva	Stores	Stores	Stores	Stores	Stores	Empty
2Cb	G6		Brood	Empty	Stores	Stores	Empty	Empty	Empty
2Cb	G6		Brood	Brood	Brood	Stores	Empty	Empty	Empty
2Cb	G6		Larva	Stores	Empty	Stores	Stores	Stores	Empty
2Cb	G6		Larva	Stores	Stores	Stores	Stores	Stores	Empty
2Cb	G6		Larva	Empty	Stores	Stores	Stores	Stores	Empty
2Cb	G6		Larva	Empty	Empty	Empty	Empty	Empty	Empty
2Cb	G6		Larva	Brood	Empty	Empty	Empty	Empty	Empty
2Cb	G6		Larva	Stores	Stores	Stores	Empty	Stores	Empty
2Cb	G6		Larva	Brood	Brood	Brood	Brood	Brood	Empty
2Cb	G6		Empty	Empty	Stores	Stores	Stores	Stores	Empty
2Cb	G6		Brood	Brood	Brood	Empty	Stores	Stores	Empty
2Cb	G6		Empty	Empty	Empty	Empty	Stores	Stores	Empty
2Cb	G6		Larva	Empty	Empty	Empty	Empty	Empty	Empty
2Cb	G6		Larva	Brood	Brood	Brood	Brood	Empty	Empty
2Cb	G6		Larva	Stores	Empty	Stores	Stores	Stores	Empty
2Cb	G6		Brood	Brood	Brood	Stores	Stores	Stores	Empty
2Cb	G6		Larva	Empty	Stores	Stores	Stores	Stores	Empty
2Cb	G6		Brood	Brood	Brood	Brood	Brood	Empty	Empty
2Cb	G6		Stores	Stores	Stores	Empty	Stores	Stores	Empty
2Cb	G6		Brood	Brood	Brood	Empty	Stores	Stores	Empty
2Cb	G6		Brood	Brood	Brood	Empty	Stores	Empty	Empty
2Cb	G6		Stores	Empty	Empty	Empty	Empty	Stores	Empty
2Cb	F5	30	Larva	Empty	Stores	Stores	Stores	Stores	Empty
2Cb	F5		Larva	Empty	Empty	Empty	Empty	Empty	Empty
2Cb	F5		Larva	Brood	Brood	Brood	Brood	Brood	Empty
2Cb	F5		Larva	Empty	Empty	Empty	Stores	Stores	Empty
2Cb	F5		Brood	Brood	Empty	Empty	Stores	Stores	Empty
2Cb	F5		Egg	Empty	Empty	Empty	Empty	Stores	Empty
2Cb	F5		Egg	Empty	Stores	Stores	Empty	Stores	Empty
2Cb	F5		Larva	Empty	Empty	Empty	Empty	Stores	Empty
2Cb	F5		Larva	Empty	Empty	Empty	Empty	Empty	Empty
2Cb	F5		Larva	Stores	Empty	Empty	Empty	Empty	Empty
2Cb	F5		Brood	Brood	Brood	Brood	Brood	Empty	Empty
2Cb	F5		Larva	Empty	Empty	Stores	Stores	Empty	Empty

2Cb	F5		Larva	Empty	Empty	Stores	Stores	Stores	Empty
2Cb	F5		Brood	Brood	Brood	Stores	Empty	Empty	Empty
2Cb	F5		Brood	Brood	Brood	Empty	Stores	Stores	Empty
2Cb	F5		Larva	Empty	Stores	Stores	Stores	Empty	Empty
2Cb	F5		Larva	Empty	Stores	Stores	Stores	Empty	Empty
2Cb	F5		Empty	Empty	Stores	Stores	Empty	Empty	Empty
2Cb	F5		Brood	Brood	Brood	Empty	Empty	Stores	Empty
2Cb	F5		Empty	Empty	Empty	Empty	Stores	Stores	Empty
2Cb	F5		Empty	Empty	Stores	Stores	Stores	Stores	Empty
2Cb	F5		Larva	Stores	Stores	Stores	Stores	Stores	Empty
2Cb	F5		Brood	Brood	Brood	Brood	Brood	Stores	Empty
2Cb	F5		Larva	Empty	Empty	Stores	Empty	Stores	Empty
2Cb	F5		Brood	Brood	Brood	Stores	Stores	Empty	Empty
2Cb	F5		Larva	Stores	Stores	Stores	Stores	Stores	Empty
2Cb	F5		Larva	Empty	Stores	Stores	Stores	Stores	Empty
2Cb	F5		Larva	Brood	Stores	Stores	Stores	Stores	Empty
2Cb	F5		Brood	Brood	Brood	Empty	Empty	Empty	Empty
2Cb	F5		Brood	Brood	Brood	Empty	Empty	Empty	Empty
2Cb	L5	25	Larva	Stores	Stores	Empty	Empty	Empty	Empty
2Cb	L5		Empty	Empty	Empty	Empty	Stores	Stores	Empty
2Cb	L5		Larva	Brood	Brood	Brood	Brood	Empty	Empty
2Cb	L5		Larva	Empty	Empty	Empty	Empty	Empty	Empty
2Cb	L5		Empty	Stores	Empty	Empty	Empty	Empty	Empty
2Cb	L5		Larva	Empty	Empty	Empty	Stores	Empty	Empty
2Cb	L5		Brood	Brood	Brood	Brood	Brood	Stores	Empty
2Cb	L5		Empty	Empty	Stores	Stores	Stores	Stores	Empty
2Cb	L5		Larva	Empty	Empty	Stores	Stores	Stores	Empty
2Cb	L5		Empty	Stores	Empty	Empty	Empty	Empty	Empty
2Cb	L5		Larva	Brood	Brood	Brood	Brood	Empty	Empty
2Cb	L5		Brood	Brood	Brood	Brood	Brood	Empty	Empty
2Cb	L5		Larva	Stores	Stores	Empty	Stores	Stores	Empty
2Cb	L5		Empty	Stores	Empty	Empty	Empty	Stores	Empty
2Cb	L5		Empty	Stores	Stores	Empty	Empty	Empty	Empty
2Cb	L5		Empty	Empty	Empty	Empty	Stores	Empty	Empty
2Cb	L5		Empty	Empty	Stores	Stores	Stores	Stores	Empty
2Cb	L5		Egg	Empty	Stores	Stores	Stores	Stores	Empty
2Cb	L5		Egg	Empty	Stores	Stores	Stores	Stores	Empty
2Cb	L5		Brood	Empty	Empty	Stores	Stores	Stores	Empty
2Cb	L5		Egg	Empty	Empty	Empty	Empty	Stores	Empty
2Cb	L5		Egg	Stores	Stores	Empty	Empty	Empty	Empty
2Cb	L5		Egg	Stores	Empty	Empty	Empty	Empty	Empty
2Cb	L5		Egg	Empty	Empty	Empty	Empty	Stores	Empty
2Cb	L5		Larva	Empty	Empty	Empty	Empty	Empty	Empty
2Cb	H4	30	Egg	Larva	Empty	Stores	Stores	Stores	Empty

2Cb	H4		Larva	Larva	Empty	Stores	Empty	Stores	Empty
2Cb	H4		Larva	Empty	Empty	Empty	Empty	Stores	Empty
2Cb	H4		Larva	Empty	Empty	Empty	Empty	Empty	Empty
2Cb	H4		Larva	Empty	Empty	Empty	Empty	Stores	Empty
2Cb	H4		Brood	Brood	Brood	Brood	Brood	Stores	Empty
2Cb	H4		Larva	Stores	Empty	Stores	Empty	Empty	Empty
2Cb	H4		Larva	Empty	Empty	Empty	Empty	Empty	Empty
2Cb	H4		Larva	Empty	Stores	Stores	Stores	Empty	Empty
2Cb	H4		Larva	Empty	Stores	Stores	Stores	Stores	Empty
2Cb	H4		Larva	Empty	Stores	Stores	Stores	Stores	Empty
2Cb	H4		Larva	Empty	Empty	Stores	Stores	Stores	Empty
2Cb	H4		Larva	Empty	Empty	Empty	Empty	Stores	Empty
2Cb	H4		Larva	Empty	Empty	Empty	Empty	Stores	Empty
2Cb	H4		Stores	Empty	Stores	Empty	Stores	Stores	Empty
2Cb	H4		Larva	Empty	Stores	Stores	Stores	Stores	Empty
2Cb	H4		Larva	Brood	Brood	Empty	Stores	Stores	Empty
2Cb	H4		Larva	Brood	Brood	Brood	Brood	Brood	Empty
2Cb	H4		Larva	Brood	Brood	Brood	Brood	Stores	Empty
2Cb	H4		Larva	Empty	Stores	Stores	Stores	Stores	Empty
2Cb	H4		Larva	Empty	Stores	Stores	Stores	Empty	Empty
2Cb	H4		Larva	Stores	Stores	Stores	Stores	Stores	Empty
2Cb	H4		Larva	Empty	Empty	Empty	Empty	Stores	Empty
2Cb	H4		Larva	Empty	Empty	Empty	Empty	Stores	Empty
2Cb	H4		Larva	Stores	Stores	Empty	Stores	Stores	Empty
2Cb	H4		Larva	Stores	Stores	Stores	Stores	Stores	Empty
2Cb	H4		Larva	Brood	Brood	Brood	Brood	Brood	Empty
2Cb	H4		Larva	Brood	Brood	Brood	Brood	Brood	Empty
2Cb	G3	25	Brood	Brood	Brood	Brood	Brood	Empty	Empty
2Cb	G3		Larva	Empty	Empty	Empty	Empty	Stores	Empty
2Cb	G3		Larva	Empty	Empty	Empty	Empty	Empty	Empty
2Cb	G3		Larva	Stores	Empty	Empty	Empty	Empty	Empty
2Cb	G3		Empty	Empty	Empty	Stores	Stores	Stores	Empty
2Cb	G3		Brood	Brood	Empty	Empty	Empty	Empty	Empty
2Cb	G3		Larva	Empty	Empty	Empty	Empty	Empty	Empty
2Cb	G3		Larva	Empty	Empty	Empty	Empty	Empty	Empty
2Cb	G3		Brood	Brood	Empty	Empty	Empty	Empty	Empty
2Cb	G3		Empty	Empty	Empty	Empty	Empty	Empty	Empty
2Cb	G3		Larva	Brood	Brood	Brood	Brood	Empty	Empty
2Cb	G3		Brood	Brood	Empty	Empty	Empty	Empty	Empty
2Cb	G3		Larva	Empty	Empty	Empty	Empty	Empty	Empty
2Cb	G3		Brood	Brood	Brood	Brood	Brood	Empty	Empty
2Cb	G3		Brood	Brood	Brood	Brood	Brood	Empty	Empty

2Cb	G3		Larva	Empty	Empty	Empty	Empty	Empty	Empty
2Cb	G3		Stores	Empty	Empty	Empty	Empty	Empty	Empty
2Cb	G3		Brood	Brood	Brood	Brood	Brood	Empty	Empty
2Cb	G3		Stores	Empty	Empty	Empty	Empty	Empty	Empty
2Cb	G3		Brood	Empty	Empty	Empty	Empty	Empty	Empty
2Cb	G3		Brood	Brood	Empty	Empty	Empty	Stores	Empty
2Cb	G3		Empty	Empty	Empty	Empty	Stores	Empty	Empty
2Cb	G3		Egg	Larva	Larva	Stores	Stores	Empty	Empty
2Cb	G3		Brood	Empty	Empty	Empty	Stores	Empty	Empty
2Cb	G3		Empty	Empty	Stores	Stores	Stores	Stores	Empty
2Cb	K5	26	Brood	Brood	Brood	Brood	Brood	Stores	Empty
2Cb	K5		Larva	Brood	Brood	Brood	Brood	Stores	Empty
2Cb	K5		Larva	Empty	Stores	Stores	Stores	Stores	Empty
2Cb	K5		Larva	Empty	Stores	Stores	Stores	Stores	Empty
2Cb	K5		Larva	Empty	Stores	Stores	Stores	Stores	Empty
2Cb	K5		Larva	Brood	Brood	Brood	Brood	Empty	Empty
2Cb	K5		Larva	Stores	Empty	Empty	Empty	Stores	Empty
2Cb	K5		Larva	Empty	Empty	Stores	Stores	Empty	Empty
2Cb	K5		Larva	Brood	Brood	Brood	Brood	Stores	Empty
2Cb	K5		Larva	Brood	Brood	Brood	Brood	Empty	Empty
2Cb	K5		Larva	Brood	Brood	Brood	Brood	Stores	Empty
2Cb	K5		Larva	Stores	Stores	Stores	Stores	Stores	Empty
2Cb	K5		Larva	Stores	Stores	Stores	Stores	Empty	Empty
2Cb	K5		Larva	Empty	Empty	Empty	Empty	Stores	Empty
2Cb	K5		Empty	Stores	Empty	Empty	Empty	Empty	Empty
2Cb	K5		Empty	Empty	Empty	Stores	Stores	Stores	Empty
2Cb	K5		Brood	Brood	Stores	Stores	Empty	Stores	Empty
2Cb	K5		Empty	Stores	Stores	Stores	Stores	Empty	Empty
2Cb	K5		Larva	Empty	Empty	Stores	Stores	Stores	Empty
2Cb	K5		Brood	Brood	Brood	Brood	Brood	Stores	Empty
2Cb	K5		Empty	Empty	Empty	Empty	Stores	Stores	Empty
2Cb	K5		Empty	Empty	Empty	Stores	Stores	Stores	Empty
2Cb	K5		Empty	Empty	Empty	Stores	Stores	Empty	Empty
2Cb	K5		Larva	Empty	Empty	Stores	Empty	Stores	Empty
2Cb	K5		Empty	Empty	Empty	Empty	Empty	Stores	Empty
2Cb	K5		Empty	Empty	Empty	Stores	Stores	Empty	Empty
2Cb	J4	30	Larva	Larva	Brood	Brood	Brood	Brood	Empty
2Cb	J4		Larva	Stores	Empty	Empty	Empty	Empty	Empty
2Cb	J4		Larva	Empty	Empty	Empty	Empty	Empty	Empty
2Cb	J4		Larva	Brood	Brood	Brood	Brood	Brood	Empty
2Cb	J4		Larva	Empty	Stores	Stores	Stores	Stores	Empty
2Cb	J4		Larva	Brood	Brood	Brood	Brood	Stores	Empty
2Cb	J4		Larva	Empty	Empty	Empty	Empty	Stores	Empty
2Cb	J4		Larva	Empty	Empty	Empty	Empty	Empty	Empty

2Cb	J4		Brood	Brood	Brood	Brood	Brood	Stores	Empty
2Cb	J4		Brood	Brood	Brood	Uncap	Empty	Empty	Empty
2Cb	J4		Larva	Brood	Brood	Brood	Brood	Stores	Empty
2Cb	J4		Larva	Empty	Stores	Empty	Empty	Stores	Empty
2Cb	J4		Larva	Brood	Brood	Brood	Brood	Brood	Empty
2Cb	J4		Larva	Brood	Brood	Brood	Brood	Empty	Empty
2Cb	J4		Larva	Empty	Empty	Empty	Empty	Empty	Empty
2Cb	J4		Larva	Brood	Brood	Brood	Brood	Empty	Empty
2Cb	J4		Larva	Brood	Brood	Brood	Brood	Empty	Empty
2Cb	J4		Larva	Brood	Brood	Brood	Brood	Stores	Empty
2Cb	J4		Brood	Brood	Brood	Brood	Brood	Empty	Empty
2Cb	J4		Larva	Brood	Stores	Empty	Empty	Stores	Empty
2Cb	J4		Brood	Brood	Brood	Brood	Brood	Empty	Empty
2Cb	J4		Larva	Brood	Brood	Brood	Brood	Stores	Empty
2Cb	J4		Larva	Brood	Brood	Brood	Brood	Empty	Empty
2Cb	J4		Larva	Empty	Stores	Empty	Stores	Stores	Empty
2Cb	J4		Empty	Empty	Empty	Empty	Empty	Empty	Empty
2Cb	J4		Larva	Empty	Empty	Empty	Empty	Empty	Empty
2Cb	J4		Larva	Brood	Brood	Brood	Brood	Empty	Empty
2Cb	J4		Larva	Brood	Brood	Brood	Brood	Empty	Empty
2Cb	J4		Larva	Brood	Brood	Brood	Brood	Empty	Empty
2Cb	I3	24	Egg	Larva	Stores	Stores	Stores	Stores	Empty
2Cb	I3		Egg	Larva	Empty	Empty	Empty	Stores	Empty
2Cb	I3		Egg	Larva	Empty	Empty	Stores	Stores	Empty
2Cb	I3		Egg	Empty	Stores	Stores	Stores	Stores	Empty
2Cb	I3		Egg	Empty	Stores	Stores	Stores	Stores	Empty
2Cb	I3		Egg	Larva	Empty	Stores	Stores	Stores	Empty
2Cb	I3		Egg	Larva	Stores	Stores	Stores	Empty	Empty
2Cb	I3		Egg	Larva	Empty	Empty	Stores	Empty	Empty
2Cb	I3		Egg	Larva	Empty	Empty	Stores	Empty	Empty
2Cb	I3		Larva	Brood	Brood	Brood	Brood	Stores	Empty
2Cb	I3		Egg	Larva	Stores	Stores	Stores	Empty	Empty
2Cb	I3		Larva	Larva	Stores	Stores	Stores	Stores	Empty
2Cb	I3		Larva	Larva	Empty	Empty	Empty	Empty	Empty
2Cb	I3		Larva	Larva	Brood	Empty	Empty	Empty	Empty
2Cb	I3		Empty	Empty	Empty	Empty	Empty	Empty	Empty
2Cb	I3		Larva	Brood	Brood	Brood	Brood	Empty	Empty
2Cb	I3		Larva	Larva	Brood	Brood	Brood	Brood	Empty
2Cb	I3		Larva	Empty	Empty	Empty	Empty	Empty	Empty
2Cb	I3		Larva	Larva	Brood	Empty	Stores	Stores	Empty
2Cb	I3		Larva	Empty	Stores	Stores	Stores	Stores	Empty
2Cb	I3		Larva	Larva	Brood	Brood	Brood	Stores	Empty
2Cb	I3		Larva	Larva	Brood	Brood	Brood	Uncap	Empty

2Cb	I3		Larva	Larva	Brood	Brood	Brood	Stores	Empty
2Cb	I3		Larva	Brood	Brood	Brood	Brood	Stores	Empty

APPENDIX D

Appendix D Colony D: Contents of the cells for each square sampled for the duration of the experiment

FRAME	CUBE	# of CELLS	1	2	3	4	5	6	7
3Da	K3	26	Larva	Larva	Brood	Brood	Brood	Brood	Empty
3Da	K3		Larva	Larva	Brood	Brood	Brood	Brood	Empty
3Da	K3		Larva	Brood	Brood	Brood	Brood	Brood	Stores
3Da	K3		Larva	Brood	Brood	Brood	Brood	Brood	Empty
3Da	K3		Empty	Larva	Brood	Brood	Brood	Brood	Empty
3Da	K3		Larva	Larva	Empty	Empty	Empty	Empty	Stores
3Da	K3		Larva	Empty	Stores	Stores	Stores	Stores	Stores
3Da	K3		Larva	Brood	Brood	Brood	Brood	Brood	Empty
3Da	K3		Empty	Empty	Empty	Empty	Empty	Empty	Empty
3Da	K3		Larva	Brood	Brood	Brood	Brood	Empty	Empty
3Da	K3		Larva	Larva	Brood	Brood	Brood	Empty	Stores
3Da	K3		Larva	Larva	Empty	Empty	Empty	Empty	Empty
3Da	K3		Larva	Larva	Brood	Brood	Brood	Brood	Empty
3Da	K3		Larva	Brood	Brood	Brood	Brood	Empty	Stores
3Da	K3		Larva	Brood	Chalk	Chalk	Empty	Stores	Empty
3Da	K3		Empty	Brood	Brood	Brood	Empty	Empty	Empty
3Da	K3		Larva	Larva	Brood	Brood	Brood	Brood	Stores
3Da	K3		Empty	Empty	Empty	Empty	Empty	Empty	Egg
3Da	K3		Larva	Brood	Brood	Brood	Brood	Brood	Empty
3Da	K3		Larva	Brood	Brood	Brood	Brood	Empty	Empty
3Da	K3		Brood	Brood	Brood	Brood	Brood	Empty	Empty
3Da	K3		Larva	Larva	Brood	Brood	Brood	Brood	Empty
3Da	K3		Empty	Empty	Empty	Stores	Empty	Stores	Stores
3Da	K3		Empty	Empty	Empty	Stores	Stores	Stores	Stores
3Da	K3		Larva	Brood	Brood	Brood	Brood	Brood	Empty
3Da	K3		Larva	Brood	Brood	Brood	Brood	Brood	Empty
3Da	D3	24	Larva	Brood	Brood	Brood	Brood	Brood	Empty
3Da	D3		Larva	Brood	Brood	Brood	Brood	Brood	Empty
3Da	D3		Larva	Brood	Larva	Larva	Chalk	Chalk	Empty
3Da	D3		Larva	Brood	Brood	Brood	Brood	Brood	Empty
3Da	D3		Larva	Brood	Brood	Brood	Brood	Brood	Stores
3Da	D3		Larva	Brood	Brood	Brood	Brood	Empty	Empty
3Da	D3		Larva	Brood	Brood	Brood	Brood	Brood	Empty
3Da	D3		Larva	Brood	Brood	Brood	Brood	Brood	Empty
3Da	D3		Larva	Brood	Larva	Larva	Larva	Chalk	Empty
3Da	D3		Larva	Brood	Brood	Brood	Brood	Brood	Empty
3Da	D3		Larva	Brood	Brood	Brood	Brood	Empty	Stores

3Da	D3		Larva	Brood	Brood	Brood	Brood	Empty	Egg
3Da	D3		Larva	Brood	Brood	Brood	Brood	Empty	Empty
3Da	D3		Larva	Brood	Brood	Brood	Brood	Empty	Empty
3Da	D3		Empty	Empty	Empty	Empty	Empty	Empty	Empty
3Da	D3		Larva	Brood	Empty	Empty	Empty	Stores	Stores
3Da	D3		Larva	Larva	Brood	Brood	Brood	Brood	Empty
3Da	D3		Larva	Brood	Brood	Brood	Brood	Empty	Stores
3Da	D3		Larva	Brood	Brood	Brood	Brood	Empty	Empty
3Da	D3		Larva	Brood	Brood	Brood	Brood	Brood	Empty
3Da	D3		Larva	Brood	Brood	Brood	Brood	Brood	Stores
3Da	D3		Larva	Brood	Brood	Brood	Brood	Brood	Empty
3Da	D3		Larva	Brood	Brood	Brood	Brood	Brood	Empty
3Da	D3		Larva	Brood	Brood	Brood	Brood	Brood	Empty
3Da	F5	25	Larva	Larva	Brood	Brood	Brood	Brood	Empty
3Da	F5		Larva	Brood	Brood	Brood	Brood	Brood	Empty
3Da	F5		Larva	Brood	Brood	Brood	Brood	Brood	Empty
3Da	F5		Empty	Empty	Empty	Empty	Empty	Empty	Stores
3Da	F5		Larva	Brood	Brood	Brood	Brood	Brood	Stores
3Da	F5		Larva	Brood	Brood	Brood	Brood	Brood	Empty
3Da	F5		Larva	Brood	Brood	Brood	Brood	Brood	Stores
3Da	F5		Larva	Larva	Brood	Brood	Brood	Brood	Stores
3Da	F5		Empty	Empty	Empty	Empty	Empty	Empty	Empty
3Da	F5		Empty	Empty	Empty	Empty	Empty	Empty	Stores
3Da	F5		Larva	Larva	Brood	Brood	Brood	Brood	Stores
3Da	F5		Empty	Larva	Empty	Empty	Empty	Empty	Empty
3Da	F5		Larva	Empty	Empty	Empty	Empty	Empty	Stores
3Da	F5		Larva	Larva	Brood	Brood	Brood	Empty	Stores
3Da	F5		Larva	Larva	Brood	Brood	Brood	Brood	Stores
3Da	F5		Empty	Empty	Empty	Stores	Empty	Empty	Empty
3Da	F5		Empty	Larva	Empty	Empty	Empty	Empty	Stores
3Da	F5		Empty	Larva	Brood	Brood	Brood	Brood	Stores
3Da	F5		Empty	Empty	Empty	Empty	Empty	Empty	Empty
3Da	F5		Empty	Larva	Brood	Brood	Brood	Brood	Empty
3Da	F5		Larva	Larva	Brood	Brood	Brood	Brood	Empty
3Da	F5		Larva	Brood	Brood	Brood	Brood	Brood	Stores
3Da	F5		Larva	Larva	Brood	Brood	Brood	Brood	Stores
3Da	F5		Larva	Larva	Brood	Brood	Brood	Brood	Stores
3Da	F5		Larva	Larva	Brood	Brood	Brood	Brood	Empty
3Da	N4	30	Brood	Brood	Brood	Empty	Empty	Empty	Stores
3Da	N4		Larva	Brood	Brood	Brood	Brood	Empty	Empty
3Da	N4		Larva	Brood	Brood	Brood	Brood	Empty	Empty
3Da	N4		Larva	Brood	Brood	Brood	Brood	Brood	Empty

3Da	N4		Brood	Brood	Brood	Brood	Brood	Brood	Empty
3Da	N4		Larva	Brood	Brood	Brood	Brood	Empty	Egg
3Da	N4		Larva	Brood	Brood	Brood	Brood	Empty	Empty
3Da	N4		Larva	Brood	Brood	Brood	Brood	Empty	Empty
3Da	N4		Larva	Brood	Brood	Brood	Brood	Empty	Empty
3Da	N4		Larva	Brood	Brood	Brood	Brood	Empty	Empty
3Da	N4		Larva	Brood	Brood	Brood	Brood	Empty	Egg
3Da	N4		Larva	Brood	Brood	Brood	Brood	Empty	Egg
3Da	N4		Empty	Empty	Empty	Empty	Empty	Empty	Stores
3Da	N4		Larva	Brood	Brood	Brood	Brood	Brood	Stores
3Da	N4		Larva	Brood	Brood	Brood	Brood	Empty	Empty
3Da	N4		Larva	Brood	Brood	Brood	Brood	Empty	Egg
3Da	N4		Larva	Brood	Brood	Brood	Brood	Empty	Egg
3Da	N4		Larva	Brood	Brood	Brood	Brood	Empty	Egg
3Da	N4		Brood	Brood	Brood	Brood	Brood	Brood	Empty
3Da	N4		Larva	Brood	Brood	Brood	Brood	Empty	Egg
3Da	N4		Larva	Brood	Brood	Brood	Brood	Empty	Egg
3Da	N4		Larva	Brood	Brood	Brood	Brood	Empty	Egg
3Da	N4		Larva	Larva	Brood	Brood	Brood	Empty	Stores
3Da	N4		Larva	Brood	Brood	Brood	Brood	Empty	Stores
3Da	N4		Brood	Brood	Brood	Brood	Brood	Empty	Egg
3Da	N4		Larva	Brood	Brood	Brood	Brood	Brood	Egg
3Da	N4		Larva	Brood	Brood	Brood	Brood	Empty	Empty
3Da	N4		Larva	Brood	Brood	Brood	Brood	Brood	Egg
3Da	N4		Larva	Brood	Brood	Brood	Brood	Empty	Empty
3Da	N4		Larva	Brood	Brood	Brood	Brood	Empty	Empty
3Da	G3	25	Larva	Brood	Brood	Brood	Brood	Brood	Empty
3Da	G3		Larva	Brood	Brood	Brood	Brood	Brood	Empty
3Da	G3		Empty	Empty	Stores	Stores	Stores	Stores	Empty
3Da	G3		Larva	Brood	Brood	Brood	Brood	Empty	Empty
3Da	G3		Larva	Brood	Brood	Brood	Brood	Brood	Stores
3Da	G3		Larva	Brood	Brood	Brood	Brood	Empty	Stores
3Da	G3		Larva	Brood	Brood	Brood	Brood	Brood	Stores
3Da	G3		Empty	Empty	Empty	Empty	Empty	Empty	Empty
3Da	G3		Larva	Brood	Brood	Brood	Brood	Brood	Empty
3Da	G3		Larva	Brood	Brood	Brood	Brood	Empty	Stores
3Da	G3		Larva	Brood	Brood	Brood	Brood	Empty	Empty
3Da	G3		Larva	Brood	Brood	Brood	Brood	Empty	Empty
3Da	G3		Larva	Brood	Brood	Brood	Brood	Empty	Empty
3Da	G3		Larva	Brood	Brood	Brood	Brood	Empty	Empty
3Da	G3		Larva	Brood	Brood	Brood	Brood	Brood	Stores
3Da	G3		Larva	Brood	Brood	Brood	Brood	Empty	Stores

3Da	G3		Larva	Brood	Brood	Brood	Brood	Brood	Stores
3Da	G3		Larva	Brood	Brood	Brood	Brood	Brood	Empty
3Da	G3		Larva	Brood	Brood	Brood	Brood	Brood	Stores
3Da	G3		Larva	Brood	Brood	Brood	Brood	Empty	Stores
3Da	G3		Larva	Brood	Brood	Brood	Brood	Empty	Empty
3Da	G3		Larva	Brood	Brood	Brood	Brood	Brood	Stores
3Da	G3		Larva	Larva	Larva	Stores	Stores	Stores	Stores
3Da	G3		Larva	Empty	Empty	Empty	Stores	Stores	Empty
3Da	G3		Larva	Larva	Brood	Brood	Brood	Brood	Empty
3Da	L5	30	Egg	Larva	Brood	Brood	Brood	Brood	Empty
3Da	L5		Larva	Brood	Brood	Brood	Brood	Egg	Larva
3Da	L5		Larva	Brood	Brood	Brood	Brood	Egg	Larva
3Da	L5		Larva	Brood	Brood	Brood	Brood	Egg	Larva
3Da	L5		Larva	Brood	Brood	Brood	Brood	Empty	Larva
3Da	L5		Larva	Brood	Brood	Brood	Brood	Egg	Larva
3Da	L5		Larva	Empty	Empty	Empty	Empty	Egg	Larva
3Da	L5		Brood	Brood	Brood	Brood	Brood	Empty	Empty
3Da	L5		Larva	Brood	Brood	Brood	Brood	Egg	Larva
3Da	L5		Larva	Brood	Brood	Brood	Brood	Egg	Larva
3Da	L5		Larva	Larva	Brood	Brood	Brood	Brood	Empty
3Da	L5		Larva	Larva	Empty	Empty	Empty	Egg	Larva
3Da	L5		Larva	Brood	Brood	Brood	Brood	Brood	Egg
3Da	L5		Brood	Brood	Brood	Brood	Brood	Egg	Larva
3Da	L5		Brood	Brood	Brood	Brood	Brood	Larva	Larva
3Da	L5		Larva	Brood	Brood	Brood	Brood	Egg	Larva
3Da	L5		Larva	Brood	Brood	Brood	Brood	Egg	Larva
3Da	L5		Larva	Brood	Brood	Brood	Brood	Egg	Larva
3Da	L5		Larva	Brood	Brood	Brood	Brood	Egg	Larva
3Da	L5		Brood	Brood	Brood	Brood	Brood	Brood	Empty
3Da	L5		Larva	Brood	Brood	Brood	Brood	Egg	Larva
3Da	L5		Empty	Empty	Stores	Empty	Empty	Empty	Empty
3Da	L5		Larva	Brood	Brood	Brood	Brood	Egg	Larva
3Da	L5		Larva	Brood	Brood	Brood	Brood	Egg	Larva
3Da	L5		Larva	Brood	Brood	Chalk	Chalk	Chalk	Chalk
3Da	L5		Larva	Brood	Brood	Brood	Brood	Empty	Empty
3Da	L5		Empty	Empty	Empty	Empty	Empty	Empty	Empty
3Da	L5		Larva	Empty	Empty	Empty	Empty	Egg	Larva
3Da	L5		Larva	Brood	Brood	Brood	Brood	Egg	Egg
3Da	L5		Brood	Brood	Brood	Brood	Brood	Egg	Larva
3Da	M4	26	Larva	Brood	Brood	Brood	Brood	Empty	Empty
3Da	M4		Larva	Brood	Brood	Brood	Brood	Empty	Empty
3Da	M4		Larva	Brood	Brood	Larva	Chalk	Chalk	Chalk

3Da	M4		Brood	Brood	Brood	Brood	Brood	Empty	Empty
3Da	M4		Brood	Brood	Brood	Brood	Brood	Empty	Egg
3Da	M4		Larva	Brood	Brood	Brood	Brood	Empty	Empty
3Da	M4		Larva	Brood	Brood	Brood	Brood	Empty	Egg
3Da	M4		Brood	Brood	Brood	Brood	Brood	Empty	Egg
3Da	M4		Brood	Brood	Brood	Brood	Brood	Egg	Empty
3Da	M4		Larva	Brood	Brood	Brood	Brood	Empty	Egg
3Da	M4		Larva	Brood	Brood	Brood	Brood	Egg	Egg
3Da	M4		Larva	Brood	Brood	Brood	Brood	Empty	Egg
3Da	M4		Larva	Brood	Brood	Brood	Brood	Empty	Egg
3Da	M4		Brood	Brood	Brood	Brood	Brood	Egg	Egg
3Da	M4		Brood	Brood	Brood	Brood	Brood	Empty	Egg
3Da	M4		Brood	Brood	Brood	Brood	Brood	Egg	Larva
3Da	M4		Larva	Brood	Brood	Brood	Brood	Egg	Larva
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3Da	M4		Larva	Brood	Brood	Brood	Brood	Egg	Larva
3Da	M4		Brood	Brood	Brood	Brood	Brood	Egg	Larva
3Da	I3	30	Egg	Larva	Empty	Stores	Stores	Stores	Stores
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Brood	Brood	Empty	Empty	Empty	Empty
Brood	Empty	Empty	Empty	Empty	Empty
Brood	Brood	Empty	Empty	Empty	Empty
Stores	Stores	Stores	Empty	Empty	Empty
Brood	Brood	Brood	Brood	Empty	Empty
Stores	Stores	Stores	Empty	Empty	Empty
Brood	Brood	Brood	Brood	Empty	Empty
Empty	Empty	Stores	Empty	Empty	Empty
Stores	Stores	Stores	Empty	Empty	Empty
Empty	Stores	Empty	Empty	Empty	Empty
Brood	Brood	Brood	Brood	Empty	Empty
Brood	Brood	Brood	Brood	Stores	Stores
Empty	Empty	Empty	Stores	Empty	Empty
Empty	Stores	Empty	Empty	Empty	Empty
Brood	Brood	Brood	Brood	Stores	Empty
Empty	Empty	Empty	Empty	Empty	Empty
Brood	Brood	Brood	Brood	Empty	Empty
Brood	Brood	Brood	Brood	Empty	Empty
Brood	Brood	Brood	Brood	Empty	Stores
Stores	Empty	Empty	Empty	Empty	Empty
Stores	Empty	Empty	Empty	Empty	Empty
Stores	Stores	Empty	Empty	Empty	Empty

Brood	Brood	Brood	Brood	Empty	Empty
Brood	Brood	Brood	Brood	Empty	Empty
Brood	Brood	Brood	Brood	Empty	Empty
Brood	Brood	Brood	Brood	Empty	Empty
Stores	Stores	Empty	Empty	Empty	Empty
Brood	Brood	Brood	Brood	Empty	Empty
Brood	Brood	Brood	Brood	Empty	Empty
Brood	Brood	Brood	Brood	Empty	Empty
Empty	Stores	Empty	Empty	Empty	Empty
Empty	Empty	Stores	Empty	Empty	Empty
Brood	Brood	Brood	Brood	Empty	Empty