Single-Frame Method to Obtain Several Age-Specific Immature Worker or Drone Honey Bee Cohorts

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Short Title: Single-Frame Method for Rearing Several Brood Cohorts

Abstract

Scientists who use honey bees for their experiments sometimes need to obtain known ages of honey bees, mature or immature, for use in their research. We updated a technique commonly-used to obtain known-age brood cohorts (worker or drone) using a frame cage made of queen excluder. We repeatedly confined the queen bee overnight using 4-day intervals to obtain easily-distinguishable brood cohorts on the same brood frame. This helped us to efficiently organize our time and saved brood, equipment and sampling time in the laboratory. It was also less disruptive than placing several cages into the broodnest. We found it imperative to begin feeding colonies 1 week prior to caging the queen, and to maintain the supplemental food throughout the brood sampling. Larger colonies contained in 2 deep Langstroth hive bodies were less hindered by the frame cage and were able to more consistently produce and support large brood cohorts to maturity. But mid-sized colonies with 8 to 10 deep frames of bees produced approximately 50% more eggs than either larger or smaller colonies. This technique was easy to use, and could be used in many kinds of operations or for many aspects of bee research or breeding.

Keywords: brood age, queen excluder cage, honey bee, brood rearing, egg laying

Introduction

Honey bee researchers and queen breeders sometimes need known ages of bees, mature or immature, for various aspects of their work [1, 2, 3]. Typically, large cohorts of brood or bees are required that are the same age. While adult bee cohorts can easily be collected at emergence, the production of known-age immature brood usually requires that the queen be restricted to laying eggs on a specific comb or sampling frame.

There are limits to the size of brood cohorts that one queen can produce. Queens normally lay from 1000 to 1500 eggs per day during the peak brood rearing season [4, 5, 6], although a few higher rates have been recorded [7]. Typically, it is unreasonable to expect a queen to produce an entire deep frame (approximately 4,000 to 6,000 brood cells) of same-age worker brood in less than 24 hours. It is even more unreasonable to expect a queen to lay an entire frame of same-age drone brood. A full frame of drone brood represents about half of the maximum drone brood area that colonies will typically support [8], and this is usually divided among many different ages of drone brood.

Nonetheless, we strive to produce the largest possible cohorts of same-age brood, requiring the queen to lay near her maximum rate. To facilitate this, colonies must have optimum brood rearing conditions, including large populations, surplus honey (nectar) and excess pollen [8]. Empty brood comb must be available for the queen to fill with new eggs. Many young workers (less than 12 days old) are needed to feed the larvae and to care for the queen, as these workers feed the queen every 20-30 minutes during peak periods of egg-laying [9]. If the colony lacks any of these conditions, it may reduce or curtail brood rearing and may abort cohorts at any stage of development [10, 11, 12].

Some additional problems can arise, such as queens laying fewer than the required number of eggs in the cohort, bees removing eggs or larvae, or bees filling the sample frame with nectar instead of eggs. We encountered these problems and others when collecting brood for analysis of volatile compounds produced by immature workers and drones [13, 14]. Our work required sampling known-age cohorts of immature bees from egg to teneral adult. At first, we used the brood-rearing method described by Boot and Calis [15]; but we found that we needed to expand their technique. Since we repeatedly sampled several brood ages from many colonies, it became imperative to rear several different brood cohorts on a single sampling frame to streamline brood collection. There was not enough space to maintain more than 2 caged sample frames in each colony and it was very difficult to maintain a normal broodnest if 2 or more cages were used.

Here we report results from experimentation on rearing brood in frame cages, on repeatedly confining the queen and on developing a method of rearing several ages of brood cohorts on one frame. We monitored egg-laying during one trial to determine whether colony strength or the presence of brood on the sample frame affected the size of brood cohorts produced with these methods.

Figure 1. Sample frame (with brood, honey and pollen) being placed into the frame cage made of queen excluder and galvanized metal. The external cage dimensions were approximately 45 cm wide by 23.5 cm high by 4.5 cm deep, which tightly surrounds 1 brood frame. Cages were closed with a metal strip that slides into the top grooves. A drone brood frame (from drone foundation) used to rear drone cohorts is pictured. (photo by D. Sammataro)

Method and Materials

<u>*Preparing the colony:*</u> We typically applied this method to colonies that contained 8 to 20 deep frames covered by adult bees and 6 or more deep frames of brood. Preparation began approximately one month before brood cohorts were needed. We

reduced larger colonies to two 10-frame deep Langstroth hive bodies. We removed any honey supers to facilitate access to the broodnest. Up to 4 deep honey frames were removed to allow installation of a 1-gallon (3.8L) in-hive syrup feeder near the broodnest, placement of a queen-excluder frame cage (Figure 1) containing the sampling frame into the broodnest, and insertion of 1 or 2 frames of foundation to provide space for new honey stores. We ensured that all colonies contained enough stored pollen and honey to maintain broodrearing at all times.

Choosing queen caging dates: We determined which age(s) of brood were required on a particular sampling day. Next, we subtracted the required brood age from the sampling day to determine the queen caging date (Day 0) needed for each brood cohort (Table I). For example, if we wanted to test both Day 4 (1st instar larvae) and Day 1 (egg) brood on a Friday, we needed to cage the queen on the preceding Monday (Day 4

Day of Week	Queen Caging Night and Resulting Schedule of Brood Ages (in Days)						
	(caged ↓)						
Monday	**MON PM**						
Tuesday	Day 1	**TUES PM**					
Wednesday	2	Day 1	**WED PM**	(caged ↓)			
Thursday	3	2	Day 1	**THUS PM**			
Friday (sample)	4	3	2	Day 1	**FRI PM**		
Saturday	5	4	3	2	Day 1	**SAT PM**	
Sunday	6	5	4	3	2	Day 1	**SUN PM**
Monday	7	6	5	4	3	2	Day 1
Tuesday	8	7	6	5	4	3	2
Wednesday	9	8	7	6	5	4	3
Thursday	10	9	8	7	6	5	4
Friday	11	10	9	8	7	6	5
Saturday	12	11	10	9	8	7	6
Sunday	13	12	11	10	9	8	7
Monday	14	13	12	11	10	9	8
Tuesday	15	14	13	12	11	10	9
Wednesday	16	15	14	13	12	11	10
Thursday	17	16	15	14	13	12	11
Friday	18	17	16	15	14	13	12
Saturday	19	18	17	16	15	14	13
Sunday	20	19	18	17	16	15	14
Monday	21	20	19	18	17	16	15
Tuesday		21	20	19	18	17	16
Wednesday			21	20	19	18	17
Thursday				21	20	19	18
Friday					21	20	19
Saturday						21	20
Sunday							21

Table I. Sample queen caging schedule for worker brood. Numbers indicate the age in days from egg to emergence (e.g. 21 days for European bees). If Day 4 larvae and Day 1 eggs are needed for a Friday sampling, the queen is caged on the previous Monday (Day 4) and Thursday evenings (Day 1).

larvae) and on the preceding Thursday (Day 1 eggs). This chart was helpful in scheduling our time, especially for older cohorts and multiple-age samplings.

Feeding the Colony: Beginning 7 to 10 days prior to the first queen caging, we simulated a honey and pollen flow by feeding the colonies with syrup and pollen. We filled the in-hive feeder with either fructose syrup (4:3 mixture of 65% high fructose corn syrup and water), or sucrose syrup (1:1 mixture of white granulated sugar and water). We placed a 1/2-pound (227 g) pollen patty on the top bars of the broodnest. Pollen patties were made by flattening a pollen paste (a 1:1:1 mixture of fresh bee-collected pollen pellets (stored frozen), white granulated sugar and Drivert sugar (C & H Sugar, Hawaii, USA) with water added) between sheets of waxed paper. The patties were frozen until used. We maintained the supply of supplemental food throughout the brood sampling period, replenishing it every 1 to 3 days, for periods as long as 4 months.

As soon as our original foundation frames were fully-drawn but not yet filled with honey, we removed 1 or 2 fully-capped honey frames from each colony. These were replaced with new foundation frames to prevent colonies from becoming honey-bound.

Preparing the sampling frame: To prepare a sampling frame on which to rear the brood cohorts, we selected a worker brood frame from each colony that contained mostly capped, late-stage pupae. We placed this frame into the frame cage 7 to 10 days prior to the first queen caging (Figure 1). The queen was kept out of the cage to prevent her from adding new brood to the sample frame. Meanwhile, the pupae on the caged frame matured and emerged, leaving large areas of empty cells available for new brood cohorts. Any small amounts of non-cohort brood remaining on the sampling frame were significantly older than subsequently-produced cohorts, and thus easily distinguishable.

If known-age drones were required, we placed a frame of capped drone brood (drawn from drone foundation) into the frame cage and allowed pupae to mature as described above. Just prior to caging the queen, we first released any newly-emerged adult drones from the cage, since drones can not cross through the excluder sides.

Caging the Queen: Our frame cage was constructed of zinc wire-type queen excluder material and galvanized metal (Figure 1). The external cage dimensions were approximately 18 x 9-1/8 x 1-3/4 in. (45 cm wide by 23.5 cm high by 4.5 cm deep), which enclosed one deep Langstroth brood frame and fit inside a deep hive body. We slid a sheet of metal into grooves at the top to close the cage and seal the sampling frame inside. When in place in the broodnest, worker bees (but not queens or drones) passed through the queen excluder sides of the cage to reach the enclosed sampling frame and tend the brood cohorts.

On the first scheduled queen caging date (Day 0), each colony's queen was located and placed onto the prepared sampling frame inside the frame cage (Figure 1). The cage was closed and inserted near the center of the broodnest. Colonies were fed with additional syrup and pollen patty at this time.

Previously, we found that the queens laid approximately twice as many eggs at night as they did during equivalent periods of daylight (data not reported). In this study, we placed queens into the frame cages in the evening; they remained caged overnight (12 to 16 hours), and were released into the colony the next morning. The sampling frame containing the new cohort of eggs was returned to the frame cage which prevented the addition of non-cohort brood to the frame. The caged frame was then returned to the center of the broodnest, so worker bees could care for the eggs.

After 2 to 5 days (48 to 120 hours), the queen was again placed into the frame cage on the same sampling frame, to lay new eggs for a second brood cohort overnight. The following morning, the queen was released into the colony and the sampling frame was returned to the cage and replaced in the broodnest. After another 2 to 5 days, the queen was caged a third time, after which she was released as before. In this way, we obtained 3 or more known-age brood cohorts on a single sampling frame.

Sampling the Brood: On brood sampling days, we removed sampling frames from the colonies and brought them into the lab. We used clean grafting tools to remove eggs and young larvae and used forceps to remove older larvae and pupae. Please see our other publications for the results of these experiments [13, 14].

Egg-laying Trial: To determine if the presence of brood on the sample frame or colony strength affected the size of brood cohorts produced, we monitored egg-laying during an autumn, 2006, trial. We selected 10 colonies with bee populations ranging from 4 to 16 deep frames covered by adult bees and from 3 to 10 deep frames of brood. Colonies were grouped into 3 size/strength categories: *Small* colonies (n= 3) contained 4 to 5.5 deep frames of adult bees; *Mid-sized* colonies (n= 4) contained 8 to 10 deep frames of bees; and *Large* colonies (n= 3) contained 14 to 16 deep frames of bees.

All colonies and sample frames were prepared as described above. Queens were caged twice to produce 2 brood cohorts. For the first caging, we caged queens on an empty frame of worker brood comb on Thursday evening, September 28, 2006. We released queens the next morning after 16 hours. We immediately brought the sample frames into the lab and marked the cells containing eggs on a transparent sheet placed over the frame. We recorded the total number of first cohort eggs laid in each colony. We placed the sample frames back into cages and returned them to their respective colonies. For the second caging, we caged queens again 3 days (72 hours) later, using the sample frames containing the first brood cohort. We released the queens the next morning after 17.5 hours. Eggs from the second caging were counted using the above procedures, except that we waited 24 hours after queens were released to count the eggs. The delay guaranteed that all of the first cohort eggs had hatched to larvae before we counted the second cohort, ensuring that we could not confuse the cohorts.

Since our caging times differed (first = 16 hrs; second = 17.5 hrs), we standardized the cagings by dividing the number of eggs laid in each cohort by the hours that the queen was caged (cage hours). We used these estimates of the number of eggs laid per cage hour (Table II) in our comparative statistics. We used a univariate analysis of variance (ANOVA) to determine whether caging (1st or 2nd) or colony size (S, M, L) affected egg-laying. Individual comparisons were made using Tukey's HSD to determine significant differences within variables.

Finally, we multiplied the eggs laid per cage hour by 24 to estimate the number of eggs each queen may have been able to lay in 24 hours (estimated eggs laid in 24 hours, Table II), assuming that the queen's laying rate was approximately constant. We compared these to published values.

Results

General Caging Results: We successfully used our brood rearing method to rear known-age worker brood cohorts during the spring, summer and late fall, in conditions varying from spring surplus honey flow to fall dearth. We also produced large drone brood cohorts during the hottest part of the desert summer (daily high temperatures of

105 degrees F or 41C) when drone brood production is normally very low. It is important to note that only strong, well-provisioned colonies will rear and maintain substantial drone cohorts. Larger colonies contained in 2 deep Langstroth hive bodies were less hindered by the frame cage and were able to more consistently produce and support large worker or drone brood cohorts to maturity.

We caged queens up to 9 consecutive times on the same sample frame with good results. This was possible because we constantly removed brood individuals for our experiments [13, 14], producing adequate open cells for new cohorts. If we had not removed brood, approximately 4 cagings would fill the sample frame to capacity.

We varied the timing between queen cagings considerably in order to schedule particular brood ages on certain sampling days. We spaced our queen cagings from 2 days (48 hours) to 10 days (240 hours) apart. We recommend a 4-day (96-hour) spacing between cagings to produce easily-identifiable differences in brood cohorts at all times from egg to emergence (capped brood must be uncapped before brood age can be identified). If only pupae of known ages are needed, spacing between cagings can be reduced to 2-day (48-hour) intervals. This produced easily-identifiable compound eye and body color differences between the cohorts at the pupal stage, but it was difficult to visually separate the cohorts during the egg and young larval stages.

Frame Cage Location: We observed that frame cage location within the broodnest played a role in the success of some brood cohorts. Initially, we inserted all frame cages into the center of the bottom deep hive body and obtained good results. Later, we often shifted cages containing worker cohorts to the side of the broodnest, or into the top deep hive body, to minimize the cage's interference with normal broodrearing. Frame cages were placed with at least one frame of brood adjacent to each side of the cage at all times. We found that frame cage location had little effect on the size of worker cohorts or the care that they received.

However, colonies responded differently to drone brood cohorts and frame cage location influenced their success. We reared the largest drone cohorts when the frame cage was located in the center of the bottom deep hive body. If we moved the frame cage near the edge of the broodnest or into the top hive body, queens laid fewer eggs and drone cohorts were smaller. Bees also removed more brood from drone cohorts in these locations. Free [16] reported that drone eggs and larvae were more likely to be destroyed in the peripheral areas of the broodnest, rather than from the central frames.

Feeding: For maximum egg production, it was important to begin feeding 7 to 10 days prior to caging to give colonies time to react to increased food supplies. It was crucial to re-supply colonies before all the supplemental food was consumed. Any perceived reduction in incoming food resources, food availability or colony stores may have caused bees to reduce brood care and/or remove brood from the sampling frame [10, 11, 12], putting cohorts at risk. For worker cohorts, we maintained supplemental feeding from egg at least through capping. Once worker cohorts were capped, colonies reared most individuals to emergence whether feeding continued or not. However, we found drone brood to be much more vulnerable to cannibalization. Bees remove immature drones first if there is a decrease in the incoming food supply [12, 16]. We found that feeding must be maintained through drone emergence to maximize the survival of drone cohorts.

It was also important to continuously provide the colonies with new foundation or empty comb. The presence of new foundation helped to prevent colonies from filling the empty sampling frame and the broodnest with food stores, which could drastically reduce brood production. However, caution must be used when removing capped honey frames to make space for foundation. Again, any reduction in colony stores may cause the bees to remove cohort brood, particularly drones.

Egg-laying Trial: In the egg-laying test of our feeding and caging system, egg production ranged from 311 to 902 eggs per colony in the first caging (16 hrs) with a mean±std. dev. of 513±210 eggs and from 220 to 1019 eggs per colony in the second caging (17.5 hrs) with 584±259 eggs (Table II). When we standardized the 2 cohorts by dividing by the number of cage hours, we observed that egg-laying rates varied from 19 to 56 eggs per cage hour in the first caging (32±13) and from 13 to 58 eggs per cage hour in the second caging (33±15).

		1st Queen Caging (Thursday evening 9/28/06)			**2nd Queen Caging** (Sunday evening 10/1/06)		
Colony Size	**Deep Frames of bees**	Eggs Laid in Cage (16 hrs)	Estim. Eggs per hr	Estim. Eggs in 24 hrs	Eggs Laid in Cage (17.5 hrs)	Estim. Eggs per hr	Estim. Eggs in 24 hrs
Small	4	402	25	603	346	20	475
Small	4	346	22	519	220	13	302
Small	5.5	338	21	507	408	23	560
Mid	10	902	56	1353	649	37	890
Mid	8	713	45	1070	972	56	1333
Mid	8	776	49	1164	1019	58	1397
Mid	9.5	474	30	711	575	33	789
Large	16	366	23	549	702	40	963
Large	14	503	31	755	490	28	672
Large	15	311	19	467	461	26	632
MEANS							
Small	**5**	**362** ± 35	**23** ± 2	**543**	**325** ± 96	**19** ± 6	**445**
Mid	**9**	**716** ± 180	**45** ± 11	**1074**	**804** ± 224	**46** ± 13	**1102**
Large	**15**	**393** ± 99	**25** ± 6	**590**	**551** ± 132	**32** ± 8	**756**
All Colonies	**9.4**	**513** ± 210	**32** ± 13	**770**	**584** ± 259	**33** ± 15	**801**

Table II. Results of Egg-Laying Trial in autumn, 2006. Queens in Large, Mid-Sized, and Small colonies were caged overnight on an empty sample frame and eggs laid were counted. We caged queens a second time 3 days later, using the same sample frames containing brood from the first caging. Means of eggs laid (+/- std. dev.), estimates of eggs laid per hour (during caged period) and estimated number of eggs laid in 24 hours are provided.

As one might expect, total egg production and egg-laying rates (per cage hour) varied considerably with colony strength (Table II). Small colonies (n= 3) produced the fewest eggs and laid an average of 23±2 eggs per cage hour in the first caging. In the second caging, their egg production decreased to an average of 19±6 eggs per cage hour. Mid-Sized colonies (n= 4) produced approximately twice as many eggs as Small colonies, with an average of 45±11 eggs per cage hour in the first caging. This laying rate was maintained in the second caging where they averaged 46 ± 13 eggs per cage hour. Large colonies (n= 3) produced only slightly more eggs than Small colonies and laid an average of 25 ± 6 eggs per cage hour in the first caging. This increased to an average of 32 ± 8 eggs per cage hour in the second caging.

When we analyzed the egg-laying rates (eggs per cage hour) in the two cohorts (univariate ANOVA: p= 0.004; F= 5.922; df = 5, 19), we found that caging (1st or 2nd) did not produce significant differences (p= 0.748) (Figure 2). Therefore, the presence of first cohort brood on the sample frame did not adversely affect the egg production in the second cohort (Table II). We predict that there would be adequate brood space for perhaps 4 brood cohorts before the sample frame would be filled with brood. On the other hand, colony size (Small, Mid-sized, Large) produced highly-significant differences in egg-laying rates (p= 0.000). Mid-sized colonies produced approximately twice as many eggs per cage hour than both Small (Tukey HSD: p= 0.000, α= 0.05) or Large colonies (p= 0.008). Large and Small colonies were similar (p= 0.350, α= 0.05). The interaction between caging and colony size was not significant (p= 0.583).

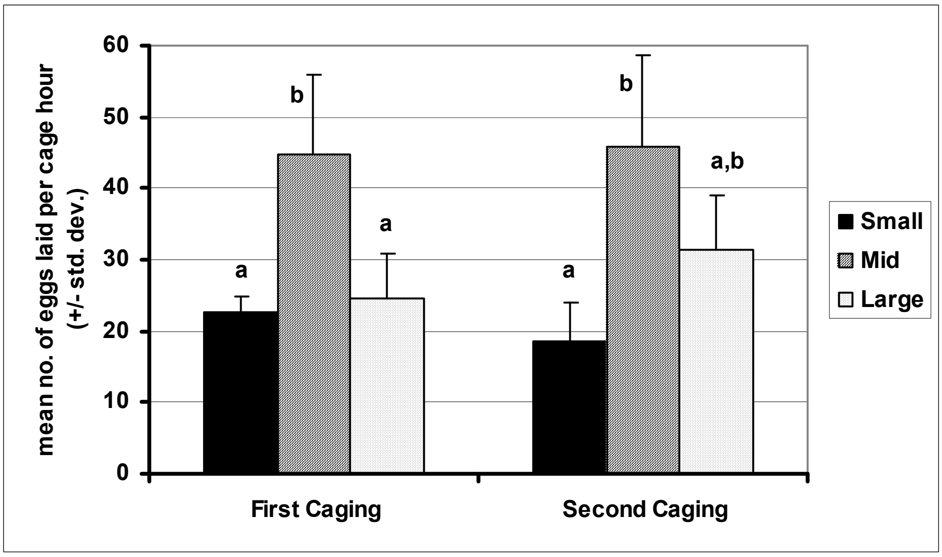

Figure 2. Mean egg-laying per cage hour in Small, Mid-Sized and Large colonies in the First and Second Queen Cagings of our Egg-laying Trial with this feeding and caging system, autumn, 2006. Mid-sized colonies laid significantly more eggs per cage hour than both Small (Tukey HSD: p= 0.000, α= 0.05) or Large colonies (p= 0.008). Columns marked with different letters denote means that are significantly different.

If the egg-laying rates are extrapolated out to 24-hour periods (Table II), our observed egg laying rates compare favorably with daily egg production rates previously reported by others [4, 5, 6, 7, 17]. Assuming that egg-laying rates remained constant (which may not be the case), we estimated that Small, Mid-sized and Large colonies would produce means of 543, 1074 and 590 first cohort eggs, respectively, if queens had continued to lay at the same rates for 24 hours. For the second cohort, we estimated that the same colonies would produce averages of 445, 1102 and 756 eggs per day if they continued for 24 hours. These are all within range of other previously reported daily egg production estimates, which vary from 311±78 eggs per day reported by Harbo [17] to an average of 1,301 eggs per day reported by Moeller [5].

We expected that our Small colonies would have the lowest egg production, since vast quantities of brood cannot be reared with limited bee populations and resources [5, 6]. Our supplemental feeding stimulated the queens of Small colonies to increase egg production at the beginning of the trial, but they started to reduce egg-laying in a subsequent caging, possibly as available nurse bees and brood care became limiting. We expected to see the highest egg production in our Large colonies. With high populations and excess resources, large colonies are typically capable of supporting the largest quantities of brood [6]. But unexpectedly, our Large colonies only produced as many eggs as our Small colonies. Perhaps their best use of resources was not a maximum investment in rearing brood during autumn when we conducted this test [5, 9, 11, 16]. We were surprised that our Mid-sized colonies achieved and sustained the highest egg production. They apparently had adequate populations and resources to support intensive broodrearing and could benefit by investing supplemental feed into brood to increase autumn bee populations. Therefore, we recommend using mid-sized colonies with 8 to 10 frames of adult bees if very large brood cohorts are needed.

Discussion

The presence of the frame cage somewhat hindered the free movement of the uncaged queen in the broodnest. Although worker bees were able to travel around or through the cage to the other side of the broodnest, we found that the queen was sometimes restricted to the brood frames on one side of the cage. This could potentially limit normal brood production to a small portion of the broodnest. Researchers can minimize any adverse impact on broodrearing by routinely switching the queen to frames on the other side of the cage during manipulations.

One limitation of our method was that the queen had access to the entire caged frame, and could choose to lay eggs anywhere on the sample frame. Although queens typically group eggs together, multiple cagings on the same frame resulted in some mixed-cohort brood patches with different ages interspersed together. While the ages of uncapped brood cells were easily distinguished by looking into the cells, we had to remove cappings from older cohorts before we could determine their ages. We wasted some capped brood and time searching for the proper cohort among capped cells. To solve this, we sometimes sketched maps of different cohort locations on transparent sheets placed over the frames. And with practice, the capped cohorts could be reliably distinguished by the appearance and texture of the cappings. We also devised a smaller push-in queen-excluder cage that produces separate patches of single-age brood on the same frame but still allows workers to access developing brood (unpublished data).

Except for these few limitations, our single-frame method was easy to use and is flexible for use in many different types of bee research. We successfully obtained multiple sets of known-age brood cohorts of both workers and drones. We saved time, colony resources, space and equipment by rearing several brood cohorts on the same frame. Our 4-day caging schedule produced sequential brood cohorts that were easily distinguished at all ages of development. In general, large colonies were less hindered by the frame cage and were able to more-consistently support large brood cohorts to maturity. However, mid-sized colonies with 8 to 10 deep frames of bees produced 50% more eggs and we recommend using these to rear the largest brood cohorts.

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