Abstract
Queen honey bees (Apis mellifera) can be reared on demand by the use of various queen-rearing methods. One simple method of rearing queens in queenright honey bee colonies has been used extensively by the authors and is described in detail. The method consists of raising frames of brood above a queen excluder in a strong colony, and grafting 12-18 hr old larvae into queen cell cups next to the brood in the upper chamber. A brood frame rotation schedule maintains the colony as a queen rearer for further batches of queen cells. The overall acceptance rate of 6666 grafts was 81%. In a small study comparing the queens reared in a queenright colony with those reared in a queenless colony, the queen pupae were weighed just before emergence, and the lengths of the queen cells were measured. There was no significant difference in the weights of the queen pupae reared in the queenright or the queenless colony. The cells reared in the queenless colony were significantly longer than those raised in the queenright colony. The relevance of these findings to queen quality is discussed.

Keywords: queen rearing, queen honey bees, queen cells

Introduction
Honey bees (Apis mellifera) have needed to rear new queens for millions of years in order to survive as a species. They have evolved to rear queens in response to various conditions, such as accidental loss of the queen, or congestion of the nest cavity. Ever since Langstroth developed his moveable-frame hive, beekeepers have been devising ingenious methods of inducing their bees to rear queens 'on demand', whether it be a few queens for hobby beekeepers, or thousands of queens for commercial queen breeding and production programs.

Laidlaw (1979) describes in excellent detail many of the practical queen rearing methods in use around the world today, and numerous other books have been written on the subject (Doolittle 1889, Snelgrove 1949, Morse 1979, Ruttner 1983, Cook 1986, Laidlaw and Page 1997, Fert 1997).

Beekeepers commonly transfer (graft) very young worker larvae into artificial queen cell cups, and introduce these into a queenless colony for acceptance and initial feeding. This starter colony is purposefully made queenless to take advantage of the natural response to this. If a queen is lost or killed, a sudden reduction in the level of queen pheromones in the hive usually triggers the worker bees to build emergency queen cells to rear a replacement queen (Huber 1814, Butler 1954, Review by Butler 1959, Ruttner 1983, Cook 1986, Laidlaw and Page 1997, Fert 1997).

Bees also naturally rear queens while in a queenright state. A new queen may be reared in a supersedeure cell to replace a substandard or failing queen, and it is not unusual to later find the two queens, mother and daughter, laying in the same colony. Populous colonies preparing to swarm, will rear numerous swarm cells while the mother queen is still present in the colony. The factors that induce supersedeure or swarming are complex, but queen pheromones again are believed to be a factor – poor distribution and low levels of pheromones per worker probably being important triggers (Butler 1954, 1960, Free 1987, Winston 1991).

Doolittle (1889) successfully reared queens in queenright colonies. Larvae were grafted, and queen cells accepted, fed and finished in one colony with no queenless period required. The general principles of a queenright starter-finisher are described by Laidlaw (1979, p 66-70) and by Laidlaw and Page (1997 p 72-73), and the method is commonly used to produce royal jelly or queens commercially. The queenright starter-finisher has been the main method of rearing queens at the Central Science Laboratory’s National Bee Unit (NBU) for over a decade. However, since so many beekeepers have shown surprise that quality queens can be produced by such a simple method, we felt it worthwhile to describe the method in detail and to publish our comparative study results.

Method
The queen rearing method described below was used by the NBU in 1989 originally to produce royal jelly and is a modification of the royal jelly production method used in commercial apiaries in France. Very little further modification of the method was required to produce queens, and this is the preferred method of queen rearing used by the NBU.

Colonization
Around the beginning of May in the UK, once there are ample quantities of pollen and nectar, colonies in the queen-rearing apiary are assessed for size in terms of equivalent full combs of bees, brood, honey, and pollen, and for docility and freedom from dis-
The completion of the grafting process typically occurs through the following steps:

1. **Preparation of the Graft Frame**
   - The graft frame consists of a normal brood frame without wax, modified to accept two horizontal wooden cell bars. These cell bars are temporarily removed from the frame for ease of grafting. The cell bars have about two inches (5cm) free space beneath them. The cell bars are temporarily removed from the frame for ease of grafting. Large colonies over-wintered on a double brood-box system have often been the most suitable.
   - Two or three colonies are selected as breeder colonies, according to their lineage, past records, how well they over-wintered, and their temperament. Details of the breeder-selection process are not covered here, though its importance can’t be over-emphasized.

2. **Colonies and Set-up**
   - Eight to 24 hours before the first grafting, each rearer is arranged so most of the sealed brood is above a queen excluder, and the queen and most of the unsealed brood are below the queen excluder, as shown in figure 1. If the queen is not found, combs are shaken free of bees before they are placed above the queen excluder. At the same time the graft frame containing 12 - 24 empty plastic cell cups is added to the top brood box to allow the bees to polish the cell cups and add a small rim of beeswax to each. This also ensures that the cups are warmed to brood-nest temperature.
   - It is not known how much each of these factors contribute to good graft acceptance, but this preparatory period for the cups takes virtually no extra effort on the beekeeper’s part. A comb of pollen is put in the top box close to the graft bar, and a comb of young larvae, preferably also with some pollen stores, is also placed adjacent to the graft bar. This young brood attracts nurse bees to the graft area. If there is not a reliable nectar flow occurring naturally, one is simulated by feeding one to two litres of 60% (w/w) sugar syrup (1 kg white granulated sugar per 650 ml water) per week, either in a frame-feeder or a contact feeder. Extra boxes are added on top if required.

3. **Grafting**
   - Eight to 24 hours after set-up, the graft frame is removed, and young larvae are collected from a breeder colony, brushing the bees off the frames rather than shaking to avoid dislodging the larvae. Grafting has been successful whether done inside or outside, as long as the larvae are sheltered from direct sun and wind to prevent them from drying out. The method preferred by the NBU is to transport the combs and graft-bars in a box, and graft in a vehicle or building.
   - Worker larvae aged 12 to 18 hours from hatching are chosen for grafting. We select within this age range to ensure they have the maximum time for the larva to be reared as a queen and yet also be large enough to be grafted with reasonable acceptance rates. It is possible to graft older larvae, but some of the queens may be of inferior quality (Snegrove 1949 p64-68, Dedej 1998). To obtain large numbers of larvae of the right age for grafting, empty brood combs can be added to a breeder colony, or a breeder queen caged overnight on an empty comb (ie using a queen excluder cage), four days prior to grafting.
   - Grafting is either done with a flexible ‘spatula-like’ tool, such as the Chinese grafting tool, or a solid metal tool, such as a dentist’s excavator. The Chinese grafting tool has the advantage of transferring a bed of royal jelly along with the larvae, but good acceptance rates have been obtained from dry grafting with a metal tool or a fine wetted paintbrush. Each larva is picked up by approaching from the outer convex curve of its ‘c’ shape.

   - The completed grafts are returned to the rearer colony as soon as possible.

4. **Checking Acceptance**
   - Between one and three days after grafting, the graft frame is checked to assess cell acceptance. It is always handled gently without shaking or jarring, but can be turned upside down to check the contents of the cells. Normally the bees have further extended the walls of accepted cells with beeswax (see figure 2), and each accepted larva is floating on a deep bed of royal jelly (see figure 3).
   - We have sometimes found that the first one or two batches of grafts of the year placed in a rearer have a poor acceptance, but then the batches to follow have a high acceptance rate. Occasionally, however, a colony keeps giving poor graft acceptance rates, or destroys cells it has started. Possible reasons include the presence of a second queen located in the upper broodbox, or a damaged queen excluder allowing the queen to move through.

5. **Destroying Emergency Cells**
   - Sometimes the bees rear emergency cells on the brood frames in the upper brood box. Six or seven days after grafting, all combs of brood in the top brood box are shaken free of bees and any such queen cells destroyed. The removal of emergency queen cells can be performed at the same time as doing the brood rotation described later.
Removal of graft cells

The graft cells are removed from the rearer before they emerge. Figure 4 shows ripe queen cells ready for transfer. The queen cells are left in the rearer for most of their development, as the mature queen pupae are then more robust and can be moved with less risk of damage. The queen cells are removed 11 days after grafting—about one day before the queens emerge. If consecutive batches are being grafted, the frames are marked to ensure the correct batch is removed. Queen cells are put into queenless nucleus colonies as soon as possible after removal from the rearer, and if there is any delay, they are kept warm either by bees or in an incubator.

Brood Rotation

A queen rearer with sufficient numbers of nurse bees can rear further batches of grafts. Each rearer can normally rear one or two batches of 24 cells each week, continuously for several months. If further grafts are to be given to the rearer, it is re-arranged once a week by ‘rotating the brood’. Combs of recently sealed brood found below the queen excluder are moved to the upper brood box. Combs from which most or all of the brood has emerged are moved down below the queen excluder for the queen to lay eggs again. A frame of young brood is also moved up into position. Usually one or two brood combs are rotated each week. The queen need not be found as the bees are shaken off the brood frames to be moved up. The time spent searching for brood frames to move up or down is minimized by adding them towards one end of the brood-box, and removing them from the other. After two or three weeks a simple rotation pattern is established.

Acceptance rates

The acceptance rates of 6666 grafted larvae given to queenright queen rearers were recorded for 152 batches of queen cells. Between 1989 and 1991 the queenright rearer method was being used for royal jelly production, and between 1999 and 2001 the method was being used for rearing queens.

Comparison with a Queenless Rearer method

To compare the queenright method above with a more traditional method of using a queenless rearer, a small study was set up in Warwickshire in the UK in June 1991 to compare the pupal weights of queens reared by the two methods. Two colonies of equal size and headed by sister queens, were prepared for queen rearing. Colony 53 was set up as a queenless rearer using the above method. Colony 90 was arranged in the same manner except that the brood box with the queen was placed several metres away, and the queenless brood box was left on the original site. Since many of the flying bees returned from the new to the old site, the queenless part was particularly well stocked with bees. A grafting frame with 12 plastic queen cups (two bars of six) was inserted into each colony. About eight hours after set-up, young larvae were grafted into the 12 plastic queen cells and inserted back into each colony.

Seven days after grafting, the colonies were checked and any emergency (non-graft) queen cells were destroyed. Eleven days after grafting, both sets of grafted queen cells were removed and transported to the laboratory where each cell was carefully opened at the joint between the plastic and wax sections of the cell, and each pupa removed and individually weighed. The lengths of the queen cells were also measured from the internal base of the plastic cup, to the tip of the wax section. The data were statistically analyzed using Student t-tests.

Results

Graft Acceptances

Table 1 shows the graft acceptances of 6666 larval grafts in queenright colonies. The overall graft acceptance rate for the presented data is 81%. Although comparable acceptance rates in queenless colonies are not available, the acceptance rates for the queenright rearer have been sufficiently high every year to fully meet the queen cell requirements of the NBU’s queen rearing program. Colony 76 and 94, however, consistently gave lower acceptance rates and their use as queen rearers was discontinued.

Comparison of a Queenright and a Queenless Rearer

Table 2 shows the weights of the queen pupae and lengths of the queen cells reared under the two methods tested. The mean weight (0.2540g) of the queen pupae reared in the queenright colony was not significantly different from the mean weight (0.2488g) of those in the queenless colony (t = 1.17, p > 0.05). There was also no significant difference between the mean weights of pupae reared on the top grafting bars and those on the lower bars (t = 1.04, p > 0.05).

The mean length (30.82mm) of the queen cells reared in the queenless colony was significantly greater than the mean length (26.70mm) in the queenright colony (t = 9.31, p < 0.001). In general observations, it was noticed that all the cells in both groups had surplus royal jelly in the cell base. It was also noticed...
significant difference between the two queen-rearing methods, it does not allow an objective evaluation of this problem.” Weiss (Ruttner 1983 p123) reviewed some of the conflicting reports on this subject, and concluded that “colony-specific differences in nursing capabilities do not allow an objective evaluation of this problem.”

While the results of the study on pupal weights showed no significant difference between the two queen-rearing methods, it does not prove that queenright and queenless methods are equally good. It must be remembered that it was a small study, and only two methods were compared; there are many other queen-rearing methods. Furthermore the evaluation was at a basic level, looking only as far as the pupal weights just before emergence. To fully assess the methods, it would be necessary to do much bigger trials, allowing queens to fully develop, and including full assessments of queen egg laying performance and longevity, over several seasons, and in different countries, regions, and climates. The results of the small trial provides good circumstantial evidence that the queenright rearing method is as good at producing good quality queens as a queenless rearing method. The fact that surplus royal jelly is invariably found at the base of the queen cells after pupation strongly suggests that the queen larvae have ample nutrition during their development.

The maximum number of queen cells a single queenright rearer can produce without any reduction in queen quality is not known. It is likely to depend on factors such as numbers of nurse bees available, quantities of brood food in their hypopharyngeal glands, and the genetic predisposition of the race of bee to rear queen cells. Each rearer is usually provided with 24 graft cells on the average, and it is likely that a commercial queen rearing outfit with more frequent grafting practice, even higher acceptance rates would be achieved. Brother Adam (1975), using the queenright method, reported an average acceptance rate of about 80%. Although Brother Adam (1975), using the queenright method to rear the queens at Buckfast Abbey, reported an average acceptance rate of about 80%. Although Brother Adam was convinced that queens of the highest quality could be raised using the queenright method, he changed over to a more complex queenless system, reporting that an average acceptance rate of about 90% was obtained with the latter, and a larger number of queen cells could be raised more reliably. It is not clear whether Brother Adam compared the two methods in the same year. Certainly a larger trial than the one reported here would be required to determine whether there is any significant difference in the graft acceptance rates between queenright and queenless rearers.

The quality of queens reared in queenright or queenless colonies has long been disputed. Weiss (Ruttner 1983) due to the speed with which they develop laying workers. In particular, queenless capensis colonies develop laying workers which produce worker brood (helytokous reproduction) and the workers fight each other, causing much colony disruption. Various complicated methods have been devised to rear queen honey bees in the Cape bee to avoid laying worker problems. Some of these methods also have the disadvantage that the queen rearing colony can only be used for one batch of queen cells and then another colony has to be chosen (Allsopp - personal communication). This suggests that the method should be investigated further in South Africa, as under proper conditions, the acceptance rates may be as high as those with the European bee in Europe. There are particular difficulties with rearing queens in queenless rearers with the African bees (Taber 1983) due to the speed with which they develop laying workers. In particular, queenless capensis colonies develop laying workers which produce worker brood (helytokous reproduction) and the workers fight each other, causing much colony disruption. Various complicated methods have been devised to rear queen honey bees in the Cape bee to avoid laying worker problems. Some of these methods also have the disadvantage that the queen rearing colony can only be used for one batch of queen cells and then another colony has to be chosen (Allsopp - personal communication). The queenright method has the advantage that the rearers are not separated from their queen at any time, except to the

Table 2. Weights of queen pupae (grams) and lengths of queen cells (mm) reared in a queenright or a queenless colony.

<table>
<thead>
<tr>
<th>Pupal Weight (g)</th>
<th>Queen Cell Length (mm)</th>
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<tbody>
<tr>
<td>Queenright</td>
<td>Queenless</td>
</tr>
<tr>
<td>0.2714</td>
<td>0.2603</td>
</tr>
<tr>
<td>0.2408</td>
<td>0.2530</td>
</tr>
<tr>
<td>0.2381</td>
<td>0.2528</td>
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<tr>
<td>0.2597</td>
<td>0.2591</td>
</tr>
<tr>
<td>0.2535</td>
<td>0.2483</td>
</tr>
<tr>
<td>0.2512</td>
<td>0.2472</td>
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<tr>
<td>0.2608</td>
<td>0.2271</td>
</tr>
<tr>
<td>0.2480</td>
<td>0.2558</td>
</tr>
<tr>
<td>Average:</td>
<td>0.2540</td>
</tr>
<tr>
<td>SD:</td>
<td>0.0109</td>
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<tr>
<td></td>
<td>0.2488</td>
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<tr>
<td></td>
<td>26.70</td>
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<td></td>
<td>30.82</td>
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Discussion

The queenright rearer method is simple and requires a minimum of extra equipment. Most beekeepers can, with some practice, graft successfully, and this is rarely a long-term cause of failure. People who have difficulty seeing the larvae can sometimes graft successfully by using additional magnification and a small flashlight. The key to success with this method is the initial choice of a large-enough colony, and many beekeepers fail at the outset by choosing too small a colony. We have always aimed to select a rearer that has two brood boxes crowded with bees (i.e. 20 brood combs well covered with adult bees). If the rearer is not populous enough, it is possible to make it so by adding young bees from another colony or to unite a small colony on top. We have used the queenright method successfully with different races/types of honey bee including Apis mellifera ligustica, Apis mellifera carnica, and Buckfast bees. The overall acceptance rate of 81% reported here was more than sufficient to provide all the queen cells required by the NBU, and it is likely that in a commercial rearing outfit with more frequent grafting practice, even higher acceptance rates would be achieved. Brother Adam (1975), using the queenright method to rear the queens at Buckfast Abbey, reported an average acceptance rate of about 80%. Although Brother Adam was convinced that queens of the highest quality could be raised using the queenright method, he changed over to a more complex queenless system, reporting that an average acceptance rate of about 90% was obtained with the latter, and a larger number of queen cells could be raised more reliably. It is not clear whether Brother Adam compared the two methods in the same year. Certainly a larger trial than the one reported here would be required to determine whether there is any significant difference in the graft acceptance rates between queenright and queenless rearers.

The queenright rearer method is as good at producing good quality queens as a queenless rearing method. The fact that surplus royal jelly is invariably found at the base of the queen cells after pupation strongly suggests that the queen larvae have ample nutrition during their development.

The finding that the queen cells raised in the queenless colony were longer and more sculptured than those raised in the queenright one is intriguing. The reason for these differences is not known. It may have been the result of small genetic differences in the colonies used, or due to a different distribution of the cell-building bees. Since the trial we have since seen well-sculptured queen cells produced in queenright rearers. Queen cell length is only likely to have an effect on the quality of the queen developing inside if the queen cell is so short that the pupal development is physically restricted. None of the cells in the study showed any sign of the queen pupae being deformed.

Occasionally a queen in the lower box of a queen rearing has swarmed, but this is very much the exception. Many queenless cell-starter methods use queenright cell-finishers without swarm problems. However, we have had swarming problems when the colony chosen as a reared had already been making swarm preparations that year. We therefore now avoid such colonies for rearing queen cells. Ensuring that a rearing colony is headed by a young queen reared the previous season probably also helps reduce the risk of it swarming.

It is of interest to note that in a preliminary test in South Africa in 2001, DW had some success with the queenright rearer method, using a hybrid of Apis mellifera scutellata and Apis mellifera capensis near Heidelberg in the Cape. The graft acceptance, although low (8 out of 24 = 33%), was higher than expected as the colony was small (only 10 combs of bees and 3 combs of brood). Furthermore, the time of year was July (mid-winter in South Africa), which is a poor time of year to rear queens in the Cape (Allsopp - personal communication). This suggests that the method should be investigated further in South Africa, as under proper conditions, the acceptance rates may be as high as those with the European bee in Europe. There are particular difficulties with rearing queens in queenless rearers with the African bees (Taber 1983) due to the speed with which they develop laying workers. In particular, queenless capensis colonies develop laying workers which produce worker brood (helytokous reproduction) and the workers fight each other, causing much colony disruption. Various complicated methods have been devised to rear queen honey bees in the Cape bee to avoid laying worker problems. Some of these methods also have the disadvantage that the queen rearing colony can only be used for one batch of queen cells and then another colony has to be chosen (Allsopp - personal communication). The queenright method has the advantage that the rearers are not separated from their queen at any time, except to the
extent that the queen excluder may act as a barrier to queen pheromone dispersal. It is therefore possible that the queenright method could be re-used for successive batches of queen cells, without causing laying worker problems. The ability to rear queens in South Africa using this queenright method could be a very important tool, particularly as the beekeepers there may have to rely more on queen rearing if the supply of swarms reduces as a result of the varroa mite spreading through the wild bee population. We are therefore encouraging colleagues in South Africa to do some controlled trials to compare the queenright method with the more complicated queen-rearing methods currently used in South Africa.

At the National Bee Unit in the UK we aim to replace queens on a regular basis – at least every second year – to maintain young prolific queens, and so minimizing the risk of swarming, and maximizing productivity. For the past eleven years we have used the queenright method to produce most of the queens required in our queen-replacement program, and the queens so produced have been prolific and headed strong productive colonies.

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