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ORIGINAL RESEARCH ARTICLE

Direct introduction of mated and virgin queens using smoke: a method that gives almost 100% acceptance when hives have been queenless for 2 days or more.

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Summary

We compared the acceptance of virgin and mated queens introduced into queenless hives using either artificial queen cells or direct introduction accompanied by smoke. In Experiment 1, virgin queens aged 3-4 days were introduced into 5-frame hives than had been dequeened 1, 2, 3, 4, 5, or 6 days previously. Acceptance increased significantly with the length of time a colony had been queenless, and direct introduction gave significantly greater success than artificial queen cells (between 31% and 100% acceptance vs. 8% to 92% for direct and cell introduction respectively, depending on the period of queenlessness). In Experiment 2, virgin and mated queens were introduced into 2-frame observation hives that had been dequeened 1, 2, 3 and 4 days previously. The probability of acceptance was significantly higher for mated queens than virgins, for direct introduction versus artificial queen cells, and for longer queenless periods. Accordingly, the probability of a queen being balled by the workers declined significantly with the duration of the queenless period, and was significantly less for mated versus virgin queens. Finally, in Experiment 3, we introduced mated queens into medium-sized hives (10 medium Langstroth frames) that had been queenless for 2 days using both the direct introduction and artificial cells. All queens were accepted.

Introducción directa de reinas apareadas y vírgenes utilizando humo: un método con 100% de aceptación cuando las colmenas tienen dos días ó más sin reina.

Resumen
Comparamos la aceptación de reinas vírgenes y apareadas, introducidas en colonias sin reina (huérfanas) por medio de celdas reales artificiales y por introducción directa utilizando humo. En el experimento 1, fueron introducidas reinas vírgenes con edad de 3-4 días en colonias con 5 panales que tenían 1, 2, 3, 4, 5 ó 6 días de huérfanas. La aceptación de las reinas incrementó significativamente en las colonias que tenían más tiempo de huérfanas, y las introducciones directas tuvieron significativamente mayor éxito que las realizadas con celdas reales artificiales (entre un 31 a 100% de aceptación vs. un 8 a 92% respectivamente) dependiendo del periodo de orfandad. En el experimento 2, reinas vírgenes y apareadas fueron introducidas en colonias con 2 panales que tenían 1, 2, 3 y 4 días de huérfanas. La probabilidad de aceptación fue significativamente mayor para las reinas apareadas que para las vírgenes, se obtuvieron resultados similares para la introducción directa versus la introducción con celdas reales artificiales y para colonias con

el mayor tiempo sin reina. Por lo tanto, la probabilidad de que una reina llegue a ser rechazada por las obreras disminuye significativamente con la duración de la orfandad, y fue significativamente menor para reinas apareadas que para reinas vírgenes. Por último, en el experimento 3, introdujimos reinas apareadas en colonias con dos días de huérfanas y 10 panales de tamaño mediano tipo Langstroth usando ambas técnicas de introducción. Todas las reinas fueron aceptadas.

Keywords: *Apis mellifera mellifera*, virgin queen, mated queen, queen introduction, queen rearing, queen balling, observation hive, artificial queen cell

Introduction

Queen introduction is a common procedure used by beekeepers to introduce mated or virgin queens into queenless honey bee colonies (Laidlaw and Page, 1997; Pérez-Sato and Ratnieks, 2006; Pérez-Sato *et al*., 2007). In commercial beekeeping, mated queens are normally introduced to replace failing queens or when dividing colonies. Virgin queens are less often introduced, but the introduction of virgins can be especially useful in commercial queen rearing in order to requeen mating nucleus hives after harvesting the previous mated queen (Pérez-Sato and Ratnieks, 2006; Pérez-Sato *et al*., 2007).

Many methods have been used to introduce mated queens and these can also be used to introduce virgin queens. Queens are often introduced using a cage, such as a 3-hole wooden mailing cage, which is introduced into the queenless hive with the queen being released from the cage several days later (McCutcheon, 2001). The success rate of this method is highly variable, ranging from 33% to 80% (Mantilla and Goncalves, 1987; Medina and Goncalves, 2001; Moretto *et al*., 2004; Pérez-Sato and Ratnieks, 2006). Although the use of cages is probably the most common introduction method used by beekeepers, and is the most frequently recommended method (Table 1), it may well not be the best. An alternative method, in which virgin queens were introduced using either a reused natural queen cell or an artificial plastic queen cell gave greater acceptance, 93–95%, than 3-hole mailing cages (Pérez-Sato and Ratnieks, 2006). Accepted queens left their introduction cells very quickly, within 10 minutes of the cell being placed in a hive (Pérez-Sato *et al*., 2007), which suggests that it is unnecessary for the introduced queen to be left for several days to acquire odours of her new hive and that the direct introduction of queens without an introduction container may be feasible.

Several direct methods of introducing queens have been used by beekeepers (Snelgrove, 1940; McCutcheon, 2001). For example, the queen may be placed at the hive entrance and simply allowed to walk in, after several puffs of cool, dense smoke have been blown into the hive entrance (Snelgrove, 1940; Laidlaw and Page, 1997). Another method is to spray a fine mist of light sugar syrup onto both the queen and frames of brood and worker bees taken from the brood chamber of the recipient hive (Laidlaw and Page, 1997; McCutcheon, 2001). The queen is then released directly on top of the frames and is sprayed again as she walks down the gap between two neighbouring frames (Laidlaw and Page, 1997). In this study, we compare the success of direct introduction using smoke with a highly successful indirect method of introducing queens using artificial queen cells (Pérez-Sato *et al*., 2007), and examine both in relation to the length of time that colonies had been queenless.

Materials and Methods

The study was carried out in a queen mating apiary at Losehill Hall, Derbyshire, UK (Ordnance Survey Grid Reference 154835), during the summer (June to September) of 2006. The honey bees used were a mixture of European subspecies, but predominantly *A. mellifera mellifera*. We carried out three experiments in which we compared the acceptance of queens introduced either in artificial queen cells or directly:

Artificial queen cells

The artificial queen cells used were constructed by cutting the tip from a 1.5 ml plastic Eppendorf® tube to leave a circular opening approximately 7mm in diameter (Fig. 4a). This resulted in a tube very similar in overall shape to the artificial queen cell used in an earlier study (Pérez-Sato *et al*., 2007), and to the queen reintroduction cages used in instrumental insemination. The virgin

Table 1. Queen introduction methods recommended or described in 10 beekeeping books (n=10), and 20 web pages found by Google searching the internet with keywords "Introducing Queen Bees".

	A. Only recommended cage methods (mailing cages, push in cages etc.)	B. Described both cage and direct methods	C. Described smoke method
Books (10)	5 Hooper, 1976 Croft, 1986 Ruttner ,1988 Graham, 1992 Morse, 1994	5 Snelgrove, 1940 Ribbands, 1953 Laidlaw, 1979 Ruttner, 1983 Laidlaw & Page, 1997	2 Snelgrove, 1940 Laidlaw & Page, 1997
Internet (20)	20	0	0

queen was held gently in the fingers and introduced through the open lid of the Eppendorf® tube, which was then closed. The tip of the artificial cell was closed with a small plug of beeswax and honey taken from the hive into which she was being introduced (Pérez-Sato *et al*., 2007). The queen cell was then gently pushed into the wax comb in the brood area of the receiving hive, and the hive was then closed. The hive was lightly smoked on removing the lid. The amount of smoke given was the amount needed to make a normal hive inspection, and far less than used in the direct method below.

Direct introduction with smoke

In this method considerably more smoke was given than is needed to make a hive inspection. First, three or four puffs of smoke were blown into the entrance. The hive was then opened and 6–7 more puffs were blown onto the tops of the frames. This caused many of the workers to leave the frames and congregate on the bottom board and even to start walking out of the entrance. The virgin queen was then introduced. She was first held gently in the fingers and then placed in the gap between the top bars of two adjacent frames. A few seconds after being released, 4–6 more puffs of smoke were blown into the open hive. After the queen had disappeared between the combs, the hive was closed. Finally, 5–6 more puffs of smoke were blown into the entrance. In Experiment 2 (see below), queens were instead introduced into observation hives through a hole on the top of each observation hive, but the protocol was otherwise identical.

Experiment 1: virgin queens introduced into queenless mating hives

The mating hives used in this experiment were in pairs, obtained by using a sheet of thin plywood to divide a Langstroth hive with 10 medium-depth frames into two 5-frame hives with entrances in opposite directions. Each hive had sufficient bees to cover 2–3 frames and had its own feeder in the lid. Hives were fed sucrose syrup as needed during the experiment, but no syrup was fed within one week of queen introduction, either before or after. Virgin queens were obtained following standard queen rearing procedures (Laidlaw and Page, 1997). Ten or eleven days after grafting one-day old larvae from worker cells into queen cells, the ripe queen cells were removed from the starter-finisher colony and placed individually in small glass vials (13 ml) in an incubator at 34°C and 70% relative humidity. Each newly-emerged queen was marked with a numbered disc (Opalithplättchen). The queens were then held individually in new wooden 3-hole mailing cages (Walter T. Kelly Co.) without attendant workers or candy for 3 days at room temperature, c. 20°C. During this period they were fed daily by placing a small drop of honey inside one of the end holes of the cage after briefly removing the cork. After three days in cages, the virgin queens were transported to the mating apiary to be introduced into the mating hives.

Virgin queens were introduced in two trials during the summer (July–August 2006) either directly with smoke or using artificial queen cells. The queens were introduced 1, 2, 3, 4, 5 and 6 days after the hive had been made queenless by removing the egg-laying queen. Any queen cells that the colony had built during the queenless period were removed. Thirteen virgin queens were introduced by each method and for each duration of queenlessness to give a total sample size of 156 queens (= 13 x 2 x 6). We inspected the hives to determine acceptance one day after the queen had been introduced. Rejection of queens typically occurs within a matter of hours of introduction (Robinson, 1984; Pérez-Sato and Ratnieks, 2006; Pérez-Sato *et al*., 2007), and survival of queens one day after introduction is generally considered to indicate acceptance and long-term survival (Mantilla and Goncalves, 1987; Medina and Goncalves, 2001; Moretto *et al*., 2004). Queens that were found alive and uninjured on the combs one day after introduction were thus considered to have been accepted. Queens found being "balled", with injured legs, or dead outside the hive on the next day were considered to have been rejected.

Experiment 2: mated and virgin queens introduced into queenless observation hives

In order to compare the response of workers to queens introduced directly or in artificial queen cells, eight colonies were set up in observation hives, each consisting of 2 deep Langstroth frames, at the Laboratory of Apiculture and Social Insects, University of Sheffield. The number of bees in these colonies was similar to the mating hives studied in Experiment 1. Virgin and mated queens were introduced 1, 2, 3 and 4 days after the observation hives had been dequeened. Four of the eight observation hives were used to introduce virgin queens and the other four were used to introduce mated queens. Six mated and 6 virgin queens were introduced for each period of queenlessness. The mother queens that had been removed from the observation hives were reintroduced after 5–6 days, after which the colonies were undisturbed for 7 days. During this period the queen resumed normal egg-laying activities. The queen was then removed again, to allow more queen introductions. The behaviour of each introduced queen and the workers contacting her were observed for two hours after introduction. During this period queens are often balled by workers (Robinson, 1984; Pérez-Sato *et al*., 2007). A queen was considered balled if workers started attacking her, which led to more workers joining in and pressing tightly against the queen to form a characteristic ball of workers (Robinson, 1984). Other behaviours of the queen and workers were recorded including grooming, forming a court, queen running, queen piping, queen self grooming and trophallaxis. Some of these behaviours were classified according to the categories used by (Gilley, 2001): chase ("a worker pursued a queen closely as she moved rapidly away"), groom ("a worker antennated and licked a queen's abdomen or mandibulated her wings and thorax"), self-groom ("while stationary, the queen cleaned her tongue, wings and legs") and trophallaxis ("food was transferred from worker to queen"). Seven hours after introduction, a second observation lasting two minutes was made to check if queens were being balled. One day after introduction each queen was classified as accepted or rejected, as in Experiment 1.

Experiment 3: mated queens introduced into queenless hives

Both the previous experiments used small hives with five or two frames. We therefore carried out a third experiment in September to confirm that mated queens could be introduced into medium-sized queenless hives either directly or using artificial queen cells. The hives used were in a 10-frame Langstroth

medium box with 6–7 frames covered with bees. The queens were removed from these colonies and the colonies left queenless for two days. The experimental queens were obtained from other hives in the apiary and were held for three days prior to the experiment in new three-hole mailing cages with candy and 4–5 attendant workers. A total of 30 queens were introduced in a single trial, 15 using an artificial plastic cell and 15 using the direct introduction method with smoke. Acceptance was determined as in Experiment 1.

Statistical analyses

For Experiments 1 and 2, generalized linear models with binomial errors were used to compare the number of queens accepted in relation to the introduction method and the length of time that the receiving colony had been queenless. In Experiment 2, mating status of the queen was also included as a factor and the number of queens balled was analysed as well as acceptance. In Experiment 2, we used 2 x 2 chi-square tests to compare the behaviours (grooming of queen, court formation, queen-worker trophallaxis, queens running, being chased, piping, and self-grooming) associated with the virgin and mated queens.

Results

Experiment 1

There was no interaction between introduction method and the length of time the receiving colony had been queenless ($P = 0.166$, Table 2). For both introduction methods, the proportions of queens accepted was positively correlated with the length of time the receiving colony had been queenless ($P < 0.0001$; Fig. 1). More queens were always accepted when introduced directly rather than in artificial queen cells ($P < 0.0001$). Direct introduction gave high acceptance (> 90%) when colonies had been queenless for 3–6 days, whereas similar high levels of acceptance were only achieved using artificial queen cells when colonies had been queenless for 5–6 days (Fig. 1).

Experiment 2

There were no significant interactions between introduction method, the duration colonies had been queenless and whether a queen was virgin or mated ($P > 0.05$ for all two or three-way interactions; Table 2). Experiment 2 produced similar results to Experiment 1 in that a longer queenless period gave greater acceptance ($P < 0.0001$; Fig. 2). Acceptance was always equal or higher for mated queens than for virgin queens ($P < 0.0001$), and for direct introduction than for introduction in artificial cells ($P = 0.042$; Fig. 2). Mated queens had high acceptance (83% or 100% for introduction in cells or directly) after just one day of queenlessness, rising to 100% after 2 days even when introduced in cells. The acceptance of virgin queens was markedly lower with

Table 2. Results of the generalized linear models used for the analysis of the data in Experiments 1 and 2.

Experiment & Treatment	Deviance	Df	Resid. Dev.	*P*
Experiment 1. Acceptance				
Introduction method [a]	21.4	309	231	< 0.0001
Queenless period [b]	105	310	252	< 0.0001
Introduction method x queenless period	1.92	308	229	0.166
Experiment 2. Acceptance				
Introduction method [a]	4.14	94	94.1	0.042
Type of queen [c]	25.2	93	68.9	< 0.0001
Queenless period [d]	21.2	92	47.7	< 0.0001
Introduction method x type of queen	0.249	91	47.5	0.618
Introduction method x queenless period	0.052	90	47.4	0.819
Type of queen x queenless period	0.497	89	46.9	0.481
Three-way interaction	0.002	88	46.9	0.961
Experiment 2. Balling of queen				
Introduction method [a]	4.84	93	79.4	0.028
Type of queen [c]	31.7	92	47.7	< 0.000
Queenless period [d]	14	94	84.3	0.0002
Introduction method x type of queen	0.234	89	46.9	0.629
Introduction method x queenless period	0.095	91	47.6	0.758
Type of queen x queenless period	0.47	90	47.2	0.493
Three-way interaction	0.002	88	46.9	0.961

[a] Queens introduced directly with smoke or in an artificial queen cell. [b] Colonies queenless for 1, 2, 3, 4, 5, 6 days prior to queen introduction. [c] Virgin or mated queens. [d] Colonies queenless for 1, 2, 3 or 4 days prior to queen introduction.

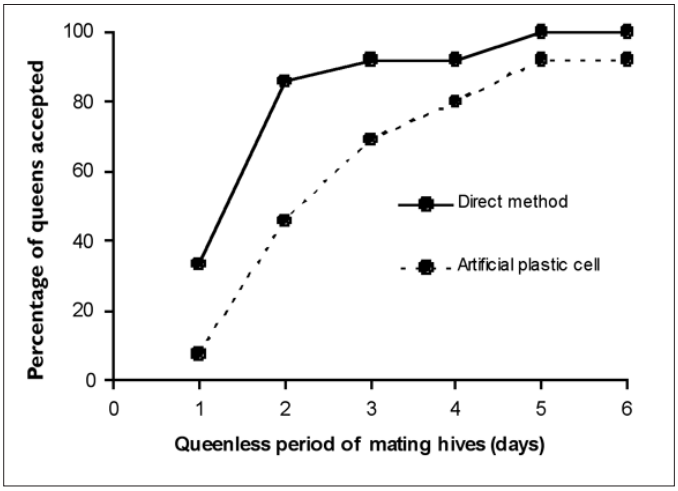

Fig. 1. Percentage of virgin queens accepted into mating nucleus hives that had been dequeened 1, 2, 3, 4, 5, or 6 days previously when introduced using either an artificial plastic queen cell or directly with smoke. N=13 queens per data point.

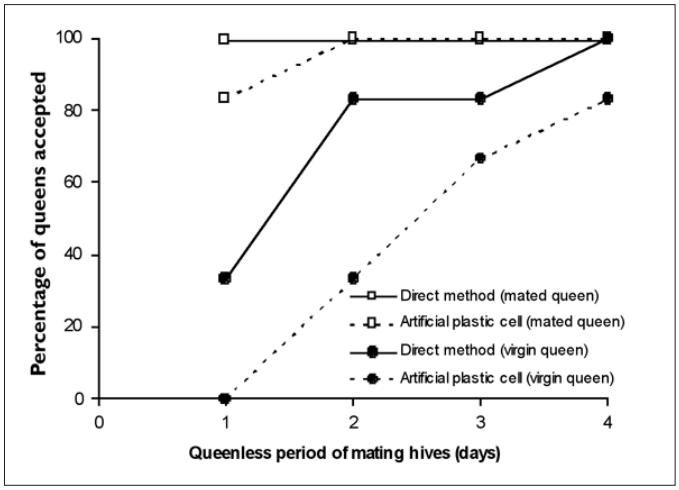

Fig. 2. Percentage of virgin or mated queens accepted into observation hives that had been dequeened 1, 2, 3, 4 days previously when introduced using either an artificial plastic queen cell or directly with smoke. N=6 queens per data point.

both introduction methods, increasing gradually with the duration colonies had been queenless (Fig. 2). Acceptance of virgin queens was very low after a single day of queenlessness and only reached a high (> 90%) level when queens were introduced directly into colonies that had been queenless for 4 days.

All queens that were rejected (Fig. 2) were balled by workers within two hours of introduction, i.e. 19/48 virgin queens and 1/48 mated queens. None of the queens that were accepted were observed being balled, either within the first two hours of being introduced or at seven hours. Accordingly, balling was significantly more common for virgin rather than mated queens ($P < 0.0001$), for queens introduced in cells rather than directly ($P = 0.028$), and for queens introduced after a shorter period of queenlessness ($P < 0.0001$). There were no significant interactions between these factors ($P > 0.05$ in all cases). Balling began on average 12.5 ± 10.5 minutes after a queen was introduced, with balling of the single mated queen to be balled beginning seven minutes after introduction. Once a ball formed it persisted throughout the entire two hour period and was also present at the seven hour observation. On the following day, all the queens that had been balled were found dead outside their hives.

During the first two hours after introduction, all of the queens that went on to be accepted were groomed by workers (Table 3; Fig. 3). However, when a mated queen was groomed, a court of workers was observed around her (Fig. 3f), but workers never formed a court when they were grooming a virgin queen (Fig. 3c) (χ^2 $P < 0.0001$). None of the mated queens was seen running on the combs, piping, or being chased by workers, whereas these behaviours were observed for all accepted virgin queens (χ^2 $P < 0.0001$ for each behaviour). About two thirds (61%) of the mated queens were seen performing trophallaxis with a worker compared with 14% of virgin queens (χ^2 $P < 0.0001$). Self grooming was performed by 100% of the mated queens but by only 7% of the virgin queens (χ^2 $P < 0.0001$). None of the mated queens was seen being clamped by the workers whereas this was seen for 100% of the virgin queens (χ^2 $P < 0.0001$).

Experiment 3

All of the queens introduced either directly or in artificial cells were accepted.

Table 3. Queen-worker interactions observed during the first two hours of a queen being introduced into an observation hive. Data are from the queens that were not balled (i.e., queens that were accepted). All comparisons between mated and virgin queens are highly significant (chi square < 0.001)

Behaviour	Mated queens, n=47	Virgin queens, n=28
Workers seen grooming the queen for at least 10 minutes	100%	100%
Workers formed a court for at least 1 minute when grooming the queen	100%	0%
Queen seen running on combs at least once after being chased by workers	0%	100%
Queen piping at least once	0%	100%
Queen seen performing trophallaxis with a worker at least once	61%	14%
Queen seen self grooming at least once	100%	7%
Workers seen chasing the queen at least once	0%	100%

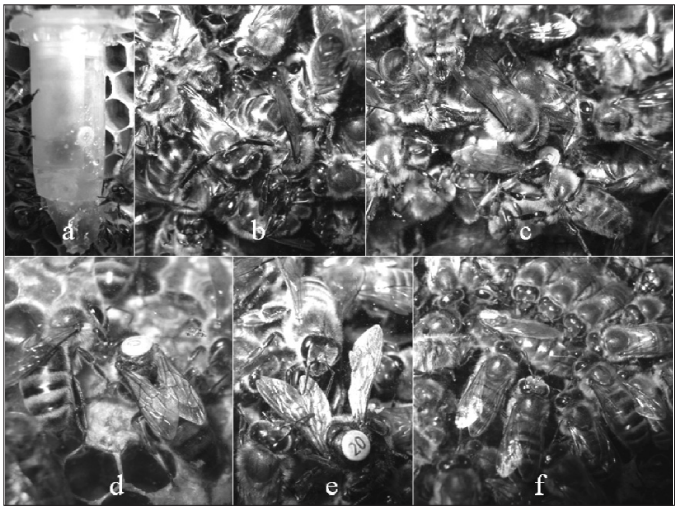

Fig. 3. Introduction methods and worker-queen interactions after queen introduction. a.) Virgin introduced using an artificial plastic queen cell made from an Eppendorf® tube. b.) Virgin queen being attacked after leaving her cell. c.) Workers grooming a virgin after leaving her cell. d.) Workers grooming and mounting a virgin (direct method). e.) Queen opening her wings to allow workers to groom her abdomen (direct method). f.) Workers forming a court around a mated queen a few minutes after introduction (direct method).

Discussion

Experiment 1

The results obtained in this experiment confirm the suggestion of Pérez-Sato *et al*. (2007) that a virgin queen can be introduced directly into queenless hives and that the use of a cage is unnecessary (Pérez-Sato *et al*., 2007). Direct introduction with smoke gave higher acceptance of virgin queens than introduction in artificial queen cells. Direct introduction gave 100% acceptance after 5 or 6 days of queenlessness, and at least 85% for 2, 3 or 4 days of queenlessness. Although the artificial cells also gave high acceptance (92% on average) after 5 and 6 days of queenlessness, acceptance averaged only 65% after 2, 3, and 4 days of queenlessness. The acceptance rates with either method after 5 and 6 days of queenlessness were thus similar to those obtained by Pérez-Sato *et al*. (2007) using either natural queen cells (95%) or artificial cells made from a JZ/BZ queen cell protector (93%) after 2 days of queenlessness. The lower acceptance rate for artificial cells after 2 days queenlessness in the current study compared to that obtained by Pérez-Sato *et al*. (2007) may suggest that plastic cells made from Eppendorf® tubes are less successful than cells made from JZ/BZ queen cell protectors. The difference may, however, also be due to some other uncontrolled factor which varied between the experiments, such as the weather or nectar conditions affecting the behaviour of the workers in the receiving hives. Overall, acceptance rates using both the direct and artificial cell methods were higher than the rates of 65%–75% obtained using new 3-hole mailing cages (Pérez-Sato and Ratnieks, 2006). By comparison, although the acceptance of queens emerging naturally from cells is 100%, the overall success rate is only approximately 70% because some queens fail to emerge (Pérez-Sato and Ratnieks, 2006). Both direct introduction and artificial queen cells gave low acceptance after 1 day of queenlessness (31% and 8% respectively), with acceptance increasing as the period of queenlessness increased. This is in agreement with previous research (Szabo, 1977) and the results of Experiment 2, which showed that workers are less aggressive to queens when the period of queenlessness is greater.

Experiment 2

Experiment 2 produced similar results to Experiment 1 in terms of the acceptance of virgin queens. It confirms that virgin queens are more likely to be accepted using direct introduction rather than an artificial cell, and when the receiving hive has been queenless for longer. After two days of queenlessness, the direct method gave high acceptance (83%) and the cell method low acceptance (33%). As for Experiment 1,the lower acceptance rate for cells than that reported under similar conditions elsewhere (Pérez-Sato *et al*., 2007), may be due to differences in environmental factors or to the use here of Eppendorf® tubes as cells. Queens in this study left their cells after 22 minutes on average, whereas they left their cells within 10 minutes in the study of Pérez-Sato *et al*. (2007). We observed here that colonies took about 10 minutes to settle down when smoke was used to introduce the queen. Whereas queens in the Pérez-Sato *et al*. (2007) had therefore left their cells while colonies were still disturbed by smoke, queens in the current study left after the effects of smoke had worn off. The pacifying effect of smoke on workers is well known, and, as the results with the direct introduction method show, workers are less likely to detect and attack an introduced queen whilst under the influence of smoke. Although we did not gather data on it, our impression was that fewer workers normally contacted a queen in the first minutes after direct introduction than when the use of an artificial cell delayed release by around 20 minutes (c. 2-3 versus c.7 workers respectively). Balling is triggered by the attack of 1–2 workers, after which more workers, including non-aggressive workers, join in (Robinson, 1984). It appeared to be easier for a queen to escape, and so not be balled, if the number of workers that initially attacked her was small.

Mated and virgin queens behaved and were treated differently. All rejected queens were balled in the first few minutes following introduction or release and continued being balled for seven hours or more, whereas accepted queens were never seen being balled. Mated queens were never seen running. It has been observed previously that nervous queens (which run) are more likely to be balled (Szabo, 1977). Accepted virgin queens were seen running on the combs with workers chasing them. After running a short distance they stopped and began to pipe, which caused the nearby workers to also stop moving until the piping was completed. Piping is known to inhibit the emergence of rival queens from cells and also occurs during the elimination of rival queens (Bruinsma *et al*., 1981; Grooters, 1987; Winston, 1987; Schneider *et al*., 2003). Our observations suggest piping also serves to reduce worker aggression (e.g. biting, clamping, balling) towards a queen.

Another observed difference between the mated and virgin queens that were accepted, was how they were groomed by workers. Workers formed a circular court around a mated queen when they groomed her (Fig. 4f), but this was never observed with virgin queens (Fig. 4c). All mated queens walked slowly in the

hive during the first two hours after introduction, and a court of workers formed around a queen when she stood still. In addition, trophallaxis and self grooming were more common with mated queens than virgins. The difference in treatment is probably due to chemical differences between mated and virgin queens (Gilley *et al*., 2006). Pheromones produced by a mated queen honey bee have many effects within the colony (Free, 1987; Winston, 1987). Most evident is the retinue attractant, which encourages workers to feed and groom the queen and to acquire and distribute her pheromone messages to other workers thought the colony (Keeling *et al*., 2003). Virgin queens are not attractive to the workers and do not have a court or retinue of workers.

Experiment 3

Experiment 3 showed that both the artificial cell and direct introduction methods can be used to introduce mated queens into medium strength hives that have been queenless for 2 days. Fifteen queens were introduced using each method and all were accepted. Although these hives were not as populous as full strength honey-producing hives, they were of similar strength to hives in early spring and the results therefore suggest that these methods are suitable for regular beekeeping, not just queen rearing. These results also confirm those of Experiment 2, showing that mated queens have high acceptance after even only 2 days of queenlessness.

Overall conclusions and beekeeping recommendations

Overall, our results clearly show three things about queen acceptance. Firstly, direct queen introduction with smoke gives very high acceptance of both mated and virgin queens, and appears to be a better method than the use of new mailing cages, reused natural queen cells, or artificial queen cells. Secondly, mated queens have higher acceptance rates than virgin queens. Thirdly, the length of time a colony has been queenless greatly affects its likelihood of accepting a queen, with colonies needing to be queenless for a longer period to accept virgin queens than mated queens. Our experiments were carried out between June and September so seem relatively robust to time of year. Whilst Experiments 1 and 2 used small colonies of the size used in queen-rearing operations, Experiment 3 showed that the direct introduction method was also successful in medium-sized colonies and it seems highly likely that similar success would be obtained in larger colonies.

Some 65 different methods have been described by which beekeepers can introduce a queen (Snelgrove, 1940; Johansson and Johansson, 1971; Ruttner, 1983). Despite these many options, the most commonly given advice on queen introduction both in beekeeping books and on the internet is, however, to use cages. For example, a Google search with the key words "Introducing Queen Bees" brought up 20 web pages, in all of which the use of cages was the sole, or most highly, recommended method (Table 1). Similarly, of ten beekeeping books dealing with queen introduction, five recommended only the use of cages. The other five described other methods, but gave most emphasis to the use of cages, or recommended cages over other methods. The method of direct introduction with smoke is only mentioned in two of the books consulted (Snelgrove, 1940; Laidlaw and Page, 1997), but on none of the web pages. Our results show, however, that direct introduction with smoke can give 100% acceptance, which is greater than is ever found in scientific studies of queen introduction using mailing cages or other mesh cages (Mantilla and Goncalves, 1987; Medina and Goncalves, 2001; Moretto *et al*., 2004; Pérez-Sato and Ratnieks, 2006; Pérez-Sato *et al*., 2007).

One possible reason why beekeepers normally use a method of queen introduction that is not the best method is that, for the most part, beekeeping is not based on using methods that have been carefully compared with alternatives in scientific experiments. Rather, beekeeping is largely based on doing what experience has shown to work. Queen introduction with cages does work well if carried out carefully, for example if queens are released from cages at least several days after the cage has been placed in the receiving hive and after checking that workers are not clustering aggressively around the cage. Recommendations often suggest that acceptance of queens can be increased by introducing the queens into small hives, or into hives with young bees, or during a nectar flow. Another reason why direct introduction may not be used is that, on the surface, it seems to be a recipe for disaster because an introduced queen is thought to need to acquire the odour of the new colony before being released. In short, using a cage seems like a more careful method.

We suggest that direct introduction should be more widely used by beekeepers. The method is cheap, requiring no additional equipment beyond a smoker, is simple to carry out and involves little work. Most importantly, it is highly successful and, by allowing a queen to be accepted almost immediately, it probably brings forward the start of egg laying by the new queen by several days.

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