

# Chapter 6

## Wax Secretion, Comb Construction and the Queen

**Abstract** Discovery of queen substance led to the experimental dissection of the importance of these chemical signals in comb construction, especially because more combs are produced in the presence of mated queens than with virgin queens, whose pheromonal bouquets substantially differ. In a series of experiments, Whiffler and Hepburn (1991a) showed that bees secrete the same amount of wax whether queenless or queenright, with either mated or virgin queens, and living or dead. Moreover, removing mandibular glands or restricting workers access to the pheromones of queens has no effect on wax secretion. Similarly, wax secretion does not significantly differ among colonies with caged or division board queens, with intact mandibular and abdominal tergite glands or not. The actual secretion of wax is independent of queen status. However, comb-building differs because colonies headed by mated queens construct significantly more comb than queenless colonies, results consistent with other studies on *A. mellifera* and *A. cerana*. Collectively, these results indicate that the bouquet of the queen's mandibular gland cannot alone fully explain enhanced comb-building by queenright workers. Whatever the source of the comb-building stimulus, its effect requires direct contact with the queen because most comb is always built when workers have full access to a mated free-running, physical and chemical queen; and, little comb is built when the colony has access only to the 'chemical' or 'physical' queen. The independence of wax secretion, as opposed to comb-building, from the pheromonal influence of the queen (Whiffler and Hepburn 1991a) was subsequently confirmed in experiments by Ledoux et al. (2001).

### 6.1 Introduction

That the queen may have a specific relationship to the synthesis and secretion of wax as well as to comb-building has been moot for a few centuries. Indeed, de Réaumur (1740) was the first to note that a caged colony of queenless bees constructed comb after 2 days confinement. However, he had given the bees some

queen cells and, unfortunately, we do not know how long the bees had been queenless prior to their incarceration or whether they had been given any other brood. A far more instructive experiment was performed by Schirach (1770), who observed that on the loss or removal of a queen, a colony would construct emergency queen cells over some of the worker cells containing eggs or larvae, and new queens would be reared from them. This important result was confirmed in numerous experiments by Huber (1814), and is the basis for the queen-rearing industry of today. Huber also knew that queenright colonies normally construct queen cells in the spring, as a prelude to reproductive swarming. So queen cells may be constructed in the presence or absence of a queen. These somewhat ambivalent results led Huber (1814) to another experiment, in which a hive was so divided that about half of the bees were in direct contact with their queen, while the other half had access only to the odour and sounds of the queenright half of the colony. In this situation, the 'queenless' half of the colony began the construction of queen cells, the other half did not.

Turning to comb construction, the first recorded observations relating the queen to comb construction are those of Huber (1814), who noted that queenless bees construct little comb, a point confirmed often since for *A. mellifera* (Gundelach 1842; Dreischer 1956; Goetze and Bessling 1959; Darchen 1968) and *A. cerana* (Rajashekharappa and Channabasavanna 1979). These observations, coupled to the demonstration that pheromones of the queen also suppress ovarian development in workers (Butler 1954; Pain 1955), suggest that the queen and/or her pheromones may well affect the secretions of wax and the construction of combs by worker bees (Darchen 1956a, b, 1957; Hepburn 1986). Suppression of queen cell construction and ovarian development require direct physical contact between workers and queen (Müssbichler 1952; Butler 1954; de Groot and Voogd 1954; Pain 1954; Verheijen-Voogd 1959), effects that have been attributed to her pheromones (cf. Free 1987; Slessor et al. 1988, 1990). Furthermore, the bouquets of mated and virgin queens differ within and between honeybee races of *A. mellifera* (Crewe and Velthuis 1980; Crewe 1982, 1988), and comb construction varies with queen quality in both *A. mellifera* (Darchen 1956a, b, 1957; Verheijen-Voogd 1959) and *A. cerana* (Rajashekharappa and Channabasavanna 1979).

While the production of queen cells is of obvious importance to the honeybee colony, so too is the regulated construction of comb cells in which to rear brood and to hold stores. For example, Gundelach (1842) observed that when queenless bees were caged and given honey, they secreted wax scales within 2 days, but did not construct combs in the absence suitable young larvae from which to rear queens. Against this, Dreischer (1956) found that a queenright and a queenless colony of the same size both produced comb, but the former was fourfold greater than the latter. The same kind of experiment has been performed several times on various races of *A. mellifera* with the same results (Darchen 1956a, 1957; Free 1967; Jay and Jay 1983; Hepburn et al. 1984). This provides the historical background from the 18th to the mid-20th century. It can be noted that virtually all of this research pertains to *A. mellifera*, as does most of the discussion in this chapter, with an occasional reference to *A. cerana*. This is simply the state of play with

respect to queens and comb-building in *Apis*. However, it is worth noting that the mandibular gland pheromones *A. andreniformis*, *A. florea* and *A. dorsata* queens have now been identified (Plettner et al. 1997), so hopefully further progress with field experiments on queens and comb-building with these species will soon be conducted.

## 6.2 The Queen: A Necessary Stimulus for Comb-Building?

Following the discovery of queen substance (Butler 1954; de Groot and Voogd 1954; Pain 1954), the slow experimental dissection of the importance of these chemical signals in comb construction began. For example, because of the ever-present group effects which influence the behaviour of workers, it was desirable to know how the relative size of a colony might relate to comb production by *A. mellifera*. Darchen (1956b, 1957) investigated how comb production was related to different stocking rates under different queening conditions. He formed colonies of 6-day-old bees and to some he gave normal, mated and laying queens, to others virgin or dead queens and, finally, some remained queenless. The results of these experiments are given in Table 7.1. They show that a queenright colony of only 50 bees is just sufficient for comb construction, given a live queen. Even a dead queen could stimulate some construction by 200 bees, but 1000 queenless bees produced no comb at all (the latter point being confirmed by Frichot-Riera 1961) (Table 6.1).

In terms of the expected efficacy of the pheromones, it is not obvious why fewer than 200 bees headed by the corpse of a queen would not construct combs, while more than this number did. In any event, this led Darchen (1956b, 1957) to attempt to separate the signals of the queen from her physical presence. He encased a queen in such a way that the workers of a small colony could smell but not touch her (a technique used to great advantage by Müssbichler 1952). In this experiment, like that of Huber (1814), the bees did not construct combs. In a different experiment, Darchen confined a queen so that her head was accessible to one group of bees and the rest of her body accessible to a different group of bees. Those bees having access to the head of the queen (and to the queen substance of the mandibular gland) began to construct combs, while the bees lacking such access did not.

Comb-building in relation to the queen has also been studied in the Asian honeybee, *A. cerana*, by Rajarhekarappa and Channabasavanna (1979) who established replicate colonies (size not stated) from queenright stock. One pair of these colonies was made queenless, each of a second pair was given a virgin queen, and each of a third pair was given a mated, laying queen. Performance was measured as the area of comb built over 10 days. On final examination, the queenless bees and those headed by virgin queens had both produced the same amount of comb, 89 cm<sup>2</sup>/colony; those headed by mated queens had produced, on average, 341 cm<sup>2</sup>/colony: nearly four times as much. On the 10th day of their experiment, Rajarhekarappa and Channabasavanna gave mated queens to those

**Table 6.1** Comb construction by *A. mellifera* of different colony sizes given mated, virgin, dead queens or under queenless conditions (Darchen 1956b, 1957)

Colony size (no. of bees)	Queenless	Dead queen	Virgin queen	Mated queen
<i>Queen conditions</i> <sup>a</sup>				
0–25	None	None	None	None
26–50	None	None	None	None
51–75	None	None	Construction	Construction
76–100	None	None	Construction	–
101–200	None	None	–	Construction
201–300	None	Construction	Construction	
301–400	None	Construction		Construction
401–500	None	–	–	–
501–600	None	–	–	–
601–700	None	–	–	–
701–800	None	–	–	–
801–900	None	–	–	Construction + egg laying
901–1000	None	–	–	–

<sup>a</sup> From Darchen (1956b, 1957). *Dashes* indicate that no tests were made for conditions stated

colonies which had previously been queenless and to those with virgin queens. The colonies formerly headed by mated queens were now made queenless. After 2 days, the now queenless bees (which had constructed a great deal of comb when queenright), had not constructed comb while the now queenright colonies both produced about the same amount of comb.

In these experiments more combs were always produced in the presence of a mated queen than was obtained under virgin queens, possibly implying pheromonal differences between virgin and mated queens. In a recent study of the pheromones of queens, Crewe (1982) showed that the pheromonal bouquets substantially differ between virgin and mated queens in three races of *A. mellifera*, so there is good reason to believe that large scale comb construction in *A. cerana*, like *A. mellifera*, depends upon the full pheromonal bouquet such as is obtained from mated queens or from egg-laying workers that have become pheromonally false queens.

### 6.3 Comb-Building Experiments by Whiffler and Hepburn (1991a)

#### 6.3.1 Queenright and Queenless Colonies

Whiffler and Hepburn (1991a) reported the results of experiments to further investigate the relationship of queen state and source of pheromones (head or abdomen) in the secretion of wax and the building of combs by worker honeybees. All experiments were performed with queenright honeybee colonies (*A. m. capensis*

**Table 6.2** Festoon bees with wax scales, weights of the scales per bee, comb construction and colony size of *A. m. capensis* and *A. m. scutellata* colonies with differing queenship status (Whiffler and Hepburn 1991a)

Treatment <sup>a</sup>	<i>n</i>	Colony size	% Bees bearing wax	Wax/bee (µg)	Comb weight (µg)
<i>Queenship status in experiment</i> $\bar{X} \pm \text{SD}$					
<i>A. cap/A. cap</i>	4	9355 ± 3622	67 ± 15	473 ± 66	2745 ± 591
<i>A. cap/Q'less</i>	4	3793 ± 1481	55 ± 14	576 ± 250	325 ± 402
<i>A. scut/A.scut</i>	9	9205 ± 3441	75 ± 13	576 ± 128	3494 ± 1562
<i>A. scut/Q'less</i>	4	7399 ± 3842	73 ± 5	622 ± 70	1129 ± 721
<i>A. cap/A. scut</i>	4	6371 ± 3025	61 ± 18	470 ± 68	3240 ± 1061
<i>A. scut/A. cap</i>	4	6992 ± 3058	77 ± 3	639 ± 52	3925 ± 668
<i>A. cap/Virgin Q</i>	4	6464 ± 4664	47 ± 1	358 ± 75	1039 ± 870

<sup>a</sup> *A. cap/A. cap* = *A. capensis* colonies with mated *A. capensis* queen; *A. cap/Q'less* = queenless *A. capensis* colonies; *A. scut/A. scut* = *A. scutellata* colonies with mated *A. scutellata* queen; *A. scut/Q'less* = queenless *A. scutellata* colonies; *A. cap/A. scut* = *A. capensis* colonies with mated *A. scutellata* queen; *A. scut/A. cap* = *A. scutellata* colonies with mated *A. capensis* queen; *A. cap/Virgin Q* = *A. capensis* colonies with virgin *A. capensis* queen

and *A. m. scutellata*) in five-frame nucleus hives containing: 1 frame of brood, 2 of honey and pollen and 2 empty frames for comb construction. Colonies were routinely dequeened early in the morning and requeened in the evening. Each colony had access to feeders. Each treatment ran for 1 week after which colony size was estimated by weighing the bees, newly constructed combs and samples of festoon bees were collected from each colony. The percentages of bees bearing wax scales were recorded and the wax scales of individual bees and constructed combs were weighed. Queens were decapitated and their heads analyzed using standard gas chromatographic techniques (Crewe 1982), and identification of compounds of the secretions was made by comparison of the retention times with those of authentic standards.

The experimental methodology largely consisted of comparing free-running queens with other queens in cages or division boards, a well established technique (Müssbichler 1952; Ribbands 1953; Free 1987; Hepburn 1986). In the first experiment, queenright colonies were dequeened. After a week all samples were collected and reciprocal exchanges of queens were performed for both subspecies. A week later, the queenright *A. m. capensis* colonies were requeened with virgin *A. m. capensis* queens. This experiment showed that the percentage of festoon bees with wax scales and the weights of the scales did not significantly differ among the queenright and queenless colonies or between races (Table 6.2). The amount of raw wax available for comb-building was essentially the same for queenright and queenless bees. In the reciprocal transfer of queens, *A. m. capensis* colonies with *A. m. scutellata* queens, there was significantly less wax/bee than colonies in the reverse arrangement (Table 6.2). The queenless colonies constructed significantly less comb than queenright ones in both *A. m. capensis* and *A. m. scutellata* colonies (Table 6.2). No differences in the amount of comb constructed arose in the

reciprocal transfer of queens between races (Table 6.2). Colonies headed by *A. m. capensis* virgin queens constructed comb equivalent to that of queenless colonies (Table 6.2). The amounts of 9-ODA, 9-HDA and 10-HDA in the mandibular glands did not significantly differ within or among queens for both races. There were no correlations between the amounts or ratios of pheromones and any of the other variables measured.

### 6.3.2 *Free-Running and Confined Queens*

In another experiment, Whiffler and Hepburn (1991a) tested queenright *A. m. capensis* colonies to compare the effects of free-running queens, queens confined in either single or double layered gauze cages (as in Müssbichler 1952; Butler 1954), dead queens and queenless colonies. Here the percentage of festoon bees with wax scales and the weights of wax scales did not differ significantly among the colonies (Table 6.3). Colonies with dead queens, caged queens (single or double-layered cages) and queenless colonies constructed significantly less comb than those headed by free-running, mated queens (Table 6.3). There were no significant differences in mandibular gland acids of the queens (Table 6.4), nor were the pheromones correlated with any of the construction variables measured. Variations in colony size were not significant. The percentage of festoon bees with wax scales and the weight of the scales did not significantly differ among queenright and queenless colonies or between races.

### 6.3.3 *Division Board Experiments*

In an experiment using division boards, Whiffler and Hepburn (1991a) placed queenright colonies of *A. m. capensis* in five-frame nucleus hives (in pairs), but with their entrances in opposite directions. Each colony was transferred to one side of a ten-frame Langstroth hive divided in half using a division board with a hole near its top. Each half of the hive had separate and opposite entrances. Queens were placed in the hole, giving one colony access to the head and thorax, and the other colony only the abdomen. After dequeening, the paired colonies were given (never their own) living or dead queens of all permutations of living queens with/without mandibular glands and with/without occluded abdominal tergite glands. Mandibular glands were surgically excised after anaesthesia on ice (Gary 1961), and abdominal tergites occluded with varnish (Velthuis 1970). Four other completely different colonies were each given a queen in a division board that only extended downwards one-third of the distance between the top and bottom of the Langstroth hive, such that all the worker bees had access to both the head and abdomen of the queen.

**Table 6.3** Percentages of festoon bees with scale wax, weights of scales comb construction and colony size in caged queen experiments with *A. m. capensis* queens (Whiffler and Hepburn 1991b)

Treatment	<i>n</i>	Colony size	% Bees bearing wax	Wax/bee ( $\mu\text{g}$ )	Comb weight ( $\mu\text{g}$ )
1. Double caged queen	3	5255 $\pm$ 2188	78 $\pm$ 18	696 $\pm$ 462	0
Free queen	3	4142 $\pm$ 6165	54 $\pm$ 10	819 $\pm$ 264	312 $\pm$ 448
2. Single caged queen	5	3876 $\pm$ 2307	64 $\pm$ 20	636 $\pm$ 245	0.3 $\pm$ 0.6
Free queen	5	4946 $\pm$ 2992	73 $\pm$ 17	491 $\pm$ 210	189 $\pm$ 336
Dead queen	3	3571 $\pm$ 1803	54 $\pm$ 11	869 $\pm$ 103	125 $\pm$ 109
3. Free queen	3	1761 $\pm$ 1655	58 $\pm$ 10	801 $\pm$ 463	572 $\pm$ 189
Queenless	3	4487 $\pm$ 2757	63 $\pm$ 25	564 $\pm$ 329	0
Total of free queens	19	5127 $\pm$ 3770	65 $\pm$ 15	630 $\pm$ 457	609 $\pm$ 1183

**Table 6.4** Distribution of mandibular gland components of mated *A. m. capensis* and *A. m. scutellata* queens (Whiffler and Hepburn 1991b)

Queen	<i>n</i>	% Composition of the components present <sup>a</sup>			Total ( $\mu\text{g}/\text{head}$ )
		9-ODA	9-HDA	10-HDA	
<i>Experiment 1</i>					
<i>A. capensis</i>	4	73 $\pm$ 26	16 $\pm$ 27	11 $\pm$ 21	70 $\pm$ 12
<i>A. scutellata</i>	9	51 $\pm$ 49	43 $\pm$ 55	15 $\pm$ 11	143 $\pm$ 68
<i>Experiment 2</i>					
<i>A. capensis</i>					
Double caged queens	3	46 $\pm$ 3	54 $\pm$ 3	0.5 $\pm$ 0.1	61 $\pm$ 0
Single caged queens	5	59 $\pm$ 14	29 $\pm$ 18	12 $\pm$ 17	103 $\pm$ 124
Free queens	3	55 $\pm$ 1	44 $\pm$ 14	0.8 $\pm$ 0.1	60 $\pm$ 11

<sup>a</sup> 9-ODA 9-oxo-2-decenoic acid; 10-HDA 10-hydroxy-2-decenoic acid; 9-HDA 9-hydroxy-2-decenoic acid

In this experiment, the percentage of festoon bees with wax scales did not significantly differ among colonies having access to the whole live queen, or only to her head or abdomen (Table 6.5). The amount of wax/bee did not significantly differ among colonies with free-running queens (Table 6.5). Given access only to the head of a queen, colonies with intact queens bore more wax/bee than those with queens that lacked mandibular glands (Table 6.5). Those bees with access only to the abdomen of the queen were equivalent to those with normal, whole queens (Table 6.5). Despite large variations, there were no significant differences in comb constructed in the various permutations of queens (with/without mandibular glands and with/without abdominal tergal glands) when the workers had access to the whole queen (Table 6.5). Among division board colonies, those bees with access to only the abdomen of the queen (intact or not) constructed no comb, while those with access to only the head (mandibular glands present or not) did not

**Table 6.5** Festoon bees with wax scales, scale weights and comb constructed by *A. m. capensis* where mated queens were in division boards (Whiffler and Hepburn 1991b)

Treatment	% Bees bearing wax						Wax/bee ( $\mu\text{g}$ )						Comb weight ( $\mu\text{g}$ )					
	Free moving queen		Queens in division boards		Abdomen of queen		Free moving queen		Queens in division boards		Abdomen of queen		Free moving queen		Queens in division boards		Abdomen of queen	
	n	Whole queen	n	Head of queen	n	Abdomen of queen	n	Whole queen	n	Head of queen	n	Abdomen of queen	n	Whole queen	n	Head of queen	n	Abdomen of queen
+m/+t	9	68 ± 16	3	57 ± 15	3	61 ± 6	9	357 ± 213	3	554 ± 245	3	278 ± 151	9	1304 ± 1241	3	117 ± 202	3	0
-m/+t	7	62 ± 11	3	53 ± 13	3	65 ± 6	7	273 ± 97	3	242 ± 114	3	382 ± 190	7	380 ± 615	3	409 ± 376	3	0
+m/-t	7	78 ± 11	3	68 ± 13	3	65 ± 1	7	376 ± 125	3	478 ± 207	3	344 ± 95	7	1334 ± 1112	3	1023 ± 978	3	0
-m/-t	6	64 ± 11	3	63 ± 1	3	62 ± 21	6	357 ± 71	3	371 ± 161	3	494 ± 443	6	1236 ± 1805	3	582 ± 1008	3	0
+m/+t (dead)	4	63 ± 10	3	64 ± 16	3	80 ± 4	4	322 ± 167	3	236 ± 72	3	420 ± 68	4	264 ± 591	3	0	3	0
+m/+t controls	3	64 ± 6				57 ± 21	3	361 ± 179					3	439 ± 360				

+m/+t queen with mandibular and abdominal tergal glands intact; -m/+t queen without mandibular gland and with tergal gland; +m/-t queen with mandibular but without tergal glands; -m/-t queen without mandibular and tergal glands; +m/+t (dead) dead but intact queen; +m/+t controls intact live queen

**Table 6.6** Distribution of mandibular gland components of *A. m. capensis* queens in division board experiments (Whiffler and Hepburn 1991b)

Treatment <sup>a</sup>	n	Percentage distribution of the components measured				Total ( $\mu\text{g}/\text{head}$ )
		9-ODA	9-HDA	10-HDA	10-HHDA	
<i>Free queens</i>						
+m/+t	9	69 $\pm$ 32	23 $\pm$ 28	6 $\pm$ 10	2 $\pm$ 5	150 $\pm$ 376
-m/+t	7	28 $\pm$ 20	33 $\pm$ 34	14 $\pm$ 16	25 $\pm$ 29	6 $\pm$ 7
+m/-t	7	66 $\pm$ 31	21 $\pm$ 19	10 $\pm$ 17	3 $\pm$ 4	14 $\pm$ 14
-m/-t	6	78 $\pm$ 5	4 $\pm$ 5	11 $\pm$ 8	7 $\pm$ 2	0.1 $\pm$ 0.1
<i>Queens in division boards</i>						
+m/+t	3	72 $\pm$ 48	26 $\pm$ 45	1 $\pm$ 1	1 $\pm$ 2	12 $\pm$ 18
-m/+t	3	32 $\pm$ 29	26 $\pm$ 45	2 $\pm$ 4	40 $\pm$ 33	5 $\pm$ 7
+m/-t	3	83 $\pm$ 12	9 $\pm$ 14	2 $\pm$ 2	5 $\pm$ 4	9 $\pm$ 12
-m/-t	3	71 $\pm$ 14	9 $\pm$ 10	16 $\pm$ 10	5 $\pm$ 5	2 $\pm$ 3

<sup>a</sup> Symbols for the free queen entries as in Table 6.5. Division board queens as follows: +m/+t colonies with access to only head and thorax but head and tergal glands intact; -m/+t colonies with access to head (without mandibular gland) and thorax only but tergal glands intact; +m/-t colonies with access to intact head and thorax only but tergite occluded; -m/-t colonies with access to head (without mandibular gland) and thorax only, and tergite occluded. 9-ODA 9-oxo-2-decenoic acid; 9-HDA 9-hydroxy-2-decenoic acid; 10-HDA 10-hydroxy-2-decenoic acid; 10-HHDA 10-hydroxy-decenoic acid

differ significantly in the amount of comb constructed (Table 6.5). Finally, significantly more comb was constructed by colonies having access to a whole queen (glands present or not) than by any colony having access to only part of the queen (Table 6.6).

Queens with intact mandibular glands tended to have more total queen substance acids than queens whose mandibular glands had been extirpated (Table 6.6). Although the queens lacking both mandibular and tergal glands tended to have relatively higher percentages of 9-ODA than intact queens, the individual titres are actually quite small in terms of total acid recovered (Table 6.6). The amounts of 9-HDA and 10-HDA did not differ significantly among the various queens. However, more 10-HHDA and 10-HDA were associated with the abdomen than with the heads of queens (Table 6.6). In the division board part of the experiment, queens with intact mandibular glands tended to have more 9-ODA than queens without mandibular glands. Queens without mandibular and tergal glands had significantly more 10-HDA than the other division board queens (Table 6.6). There were no significant differences between the colonies for 9-HDA (Table 6.6). Queens with intact mandibular glands (with/without tergal glands) had significantly more 9-ODA than queens without mandibular glands (Table 6.6). The percentage composition of these pheromones in the abdomens of the division board queens was also measured, but only small amounts of queen substance acids were found (Table 6.6). No correlations between any of the pheromones (from the head or abdomen) and the comb construction variables were found.

**Table 6.7** Festoon bees with wax scales, scale weight, comb constructed and colony size in colonies headed by *A. m. capensis* virgin queens (Whiffler and Hepburn 1991b)

Treatment <sup>a</sup>	<i>n</i>	Colony size	% Bees bearing wax	Wax/bee ( $\mu\text{g}$ )	Comb weight ( $\mu\text{g}$ )
+m/+t control	6	10909 $\pm$ 4103	70 $\pm$ 20	426 $\pm$ 130	3947 $\pm$ 2753
+m/+t	6	9221 $\pm$ 5316	80 $\pm$ 10	379 $\pm$ 99	1653 $\pm$ 1660
-m/+t	5	7574 $\pm$ 3619	81 $\pm$ 15	480 $\pm$ 89	3211 $\pm$ 4646
+m/-t	3	5489 $\pm$ 3631	81 $\pm$ 10	392 $\pm$ 136	958 $\pm$ 1659
-m/-t	3	9443 $\pm$ 8100	-	-	0
dq	13	8479 $\pm$ 3902	71 $\pm$ 5	298 $\pm$ 50	291 $\pm$ 665
q-	2	10541 $\pm$ 8512	70	351	33 $\pm$ 47
+m	4	3379 $\pm$ 2823	73 $\pm$ 24	2540 $\pm$ 45	0
+t	4	3777	64	494	0

<sup>a</sup> +m/+t control mated queen, with intact mandibular and tergite glands control; +m/+t mated queen with intact mandibular and tergite glands; -m/+t virgin queen, with mandibular and abdominal tergite glands present; +m/-t mated queen, with mandibular and abdominal tergite glands absent; -m/-t virgin queen, mandibular and abdominal tergite glands absent; dq dead virgin queen, glands intact; q- queenless; +m virgin queen, colonies with access only to intact head and thorax; +t virgin queen, colonies with access only to intact abdomen

The percentages of festoon bees bearing wax and the amounts of wax borne by these bees did not differ significantly among groups with the exception of colonies led by virgin queens without mandibular glands and whose abdominal tergal glands were occluded (Table 6.7). The colonies with mated queens constructed significantly more comb than the other colonies. Although colonies headed by intact virgin queens constructed more comb than was constructed in almost all of the permutations of the virgin queen colonies, there were no significant differences among any of the virgin queen colonies (Table 6.7). No comb was constructed by colonies having virgin queens in division boards (Table 6.7). All of the queens with intact mandibular glands had significantly greater amounts of pheromones than queens without mandibular glands; but there were no significant differences in the percentages of pheromones among the various queens (Table 6.8). There were no colony size effects.

### 6.3.4 General Conclusions from the Experiments of Whiffler and Hepburn (1991a, b)

The results from many field colonies showed that festoon bees bore the same amount of wax scales, whether queenless or queenright, with either mated or virgin queens, living or dead (Whiffler and Hepburn 1991a). Moreover, removing glands or restricting worker access to the pheromonal sources of the queen had no effect on the amount of wax scales on the festoon bees (Figs. 4.5 and 4.7). Similarly, the amount of wax recovered from individual festoon bees was the same in colonies

**Table 6.8** Distribution of mandibular gland components of *A. m. capensis* virgin queens (Whiffler and Hepburn 1991b)

Treatment <sup>a</sup>	Percentage of the components measured			Total ( $\mu\text{g}/\text{head}$ )	
	<i>n</i>	9-ODA	9-HDA		10-HDA
+m/+t control	6	71 $\pm$ 29	28 $\pm$ 28	0.8 $\pm$ 1	34 $\pm$ 33
+m/+t	6	81 $\pm$ 21	0	19 $\pm$ 21	70 $\pm$ 110
–m/+t	5	87 $\pm$ 22	0	13 $\pm$ 22	8 $\pm$ 4
+m/–t	3	65 $\pm$ 5	14 $\pm$ 16	21 $\pm$ 12	116 $\pm$ 153
–m/–t	3	64 $\pm$ 32	0	36 $\pm$ 32	2 $\pm$ 2
dq	13	72 $\pm$ 26	1 $\pm$ 2	27 $\pm$ 24	10 $\pm$ 9
+m	4	43 $\pm$ 51	0	24 $\pm$ 41	4 $\pm$ 4

<sup>a</sup> Symbols under treatment are the same as those in legend for Table 6.7. 9-ODA 9-oxo-2-decenoic acid; 9-HAD hydroxyl-2decenoic acid; 10-HAD 10-hydroxy-2-decenoic acid

preparing to swarm and in moving swarms (Hepburn 1988; Hepburn and Whiffler 1988). That *A. m. scutellata* workers with an *A. m. capensis* queen bore significantly more wax/bee than was recorded in the reverse arrangement is regarded as anomalous. The data overwhelmingly support the conclusion that the actual secretion of wax by workers is not influenced by queen status. The percentage of bees bearing wax scales was the same whether the bees were queenright or queenless, and whether headed by their own queen or one of a different race (Table 6.3). Similarly, the percentage of bees bearing wax did not significantly differ among colonies whose queens were caged or held in division boards, whether the mandibular and abdominal tergal glands were intact or not (Tables 6.2, 6.4, 6.5).

While the percentage of festoon bees bearing wax and the mass of wax actually borne by bees are independent of queen status, comb-building is entirely different. Colonies headed by mated queens constructed significantly more comb than did queenless colonies of both *A. m. capensis* and *A. m. scutellata* (Table 6.3). These results are entirely consistent with those of all other similar studies on *A. mellifera* (Gundelach 1842; Darchen 1957; Goetze and Bessling 1959; Verheijen-Voogd 1959; Jay and Jay 1983), and *A. cerana* (Rajashekharappa and Channabasavanna 1979). Because the mandibular gland bouquets of *A. m. capensis* virgin queens, unlike those of other races, approximate those of mated queens (Crewe 1988; and Tables 6.5 and 6.7), colonies headed by mated and virgin queens may construct similar amounts of comb. However, colonies headed by virgin *A. m. capensis* queens constructed as little comb as did queenless *A. m. capensis* colonies (Tables 6.1, 6.2, 6.6). This result is the same as that obtained for both *A. mellifera* (Verheijen-Voogd 1959) and *A. cerana* (Rajashekharappa and Channabasavanna 1979). Collectively, these results indicate that the bouquet of the mandibular gland of the queen cannot alone fully explain enhanced comb-building by queenright workers.

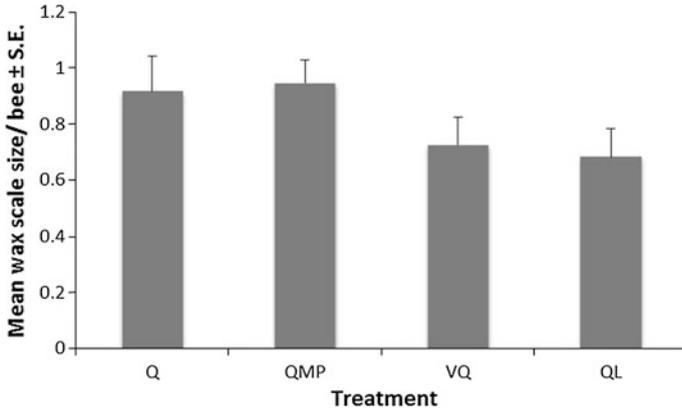
Whatever the source of the comb-building stimulus, its effect requires direct contact with the queen (Table 6.2) because more combs are built when workers have full access to a free-running, mated queen (both a *physical* and *chemical*—pheromonal—queen). Indeed, a queen moving around the nest maybe essential to

its means of chemical communication (Velthuis 1976, 1985, 1990). Little comb is built when the colony has access to only the ‘chemical’ queen (double-layered caged queen) or the ‘physical’ queen (=dead queen). The limitations of the ‘chemical’ queen are further indicated by the fact that there were no significant differences in the percentage composition of mandibular gland secretions of the various queens.

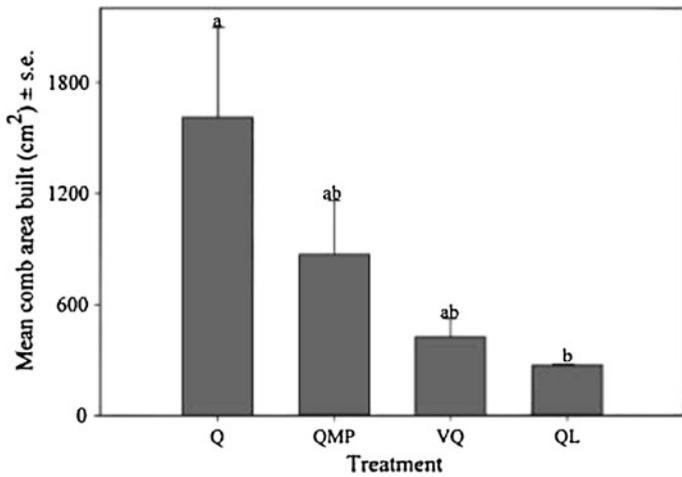
In summary, the results indicate the likelihood that pheromones of the queen, whether from the mandibular glands or elsewhere in the head, acquired through contact chemoreception, stimulate comb construction in honeybees. Chemoreception is as important for comb-building as it is for the inhibition of ovarian development in worker bees (Verheijen-Voogd 1959; Velthuis 1970) and for emergency queen cell construction (Butler 1960). It is also evident that the queen has little effect on wax secretion, a physiological process aptly described in the 19th century as the ‘involuntary’ secretion of wax (Gundelach 1842). The independence of wax secretion, as opposed to comb-building, from the pheromonal influence of the queen (Whiffler and Hepburn 1991a) was also confirmed in a different set of experiments by Ledoux et al. (2001).

#### 6.4 Comb-Building Experiments of Ledoux et al. (2001)

Apparently unaware of the earlier work by Whiffler and Hepburn (1991a, b), Ledoux et al. (2001) reinvestigated the role of the queen in comb-building. They investigated the influence of the queen and her pheromonal signals on comb-building using four groups of *A. mellifera* colonies as follows: (1) 8 colonies with mated queens; (2) 8 others with virgin queens; (3) 8 others queenless but containing a synthetic queen substance pheromone dispenser and finally (4) 8 colonies lacking queens and pheromone dispensers. After 10 days the combs produced and the sizes of the wax scales were measured. Ledoux et al. (2001) estimated mean wax scale size per *A. mellifera* worker bee for colonies with mated queens, others with synthetic queen substance dispensers, others with virgin queens as well as queenless colonies (Fig. 6.1) which again confirm the results of Whiffler and Hepburn (1991a) on the physiological independence of wax secretion from any pheromonal effects of the queen. Their results clearly show that the colonies with mated queens constructed significantly more comb by area (Fig. 6.2) and weight (Fig. 6.3) than the other colonies (Fig. 6.4). Ledoux et al. (2001) also reconfirmed that queenless workers build substantially less comb and usually drone size cells indicating that both cell size and the quantity of comb built are mediated through the queen. These results have been observed many times previously for *A. mellifera* (Dreischer 1956, Goetze and Bessling 1959, Darchen 1968, Whiffler and Hepburn 1991a).



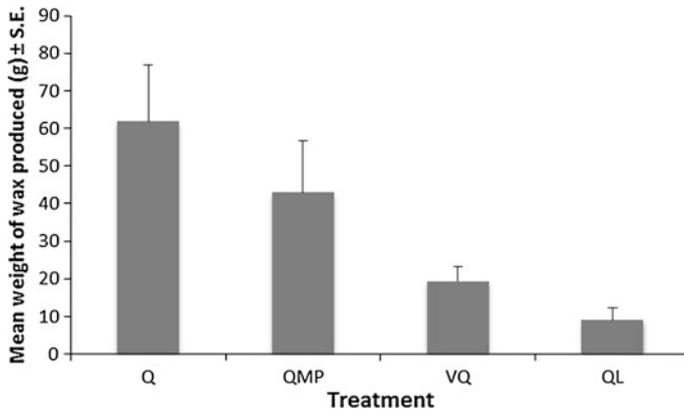
**Fig. 6.1** Mean wax scale size/bee obtained from colonies headed by mated queens (*Q*), virgin queens (*VQ*), queen pheromone dispenser (*QMP*) and queenless (*QL*) *A. mellifera* colonies (Ledoux et al. 2001)



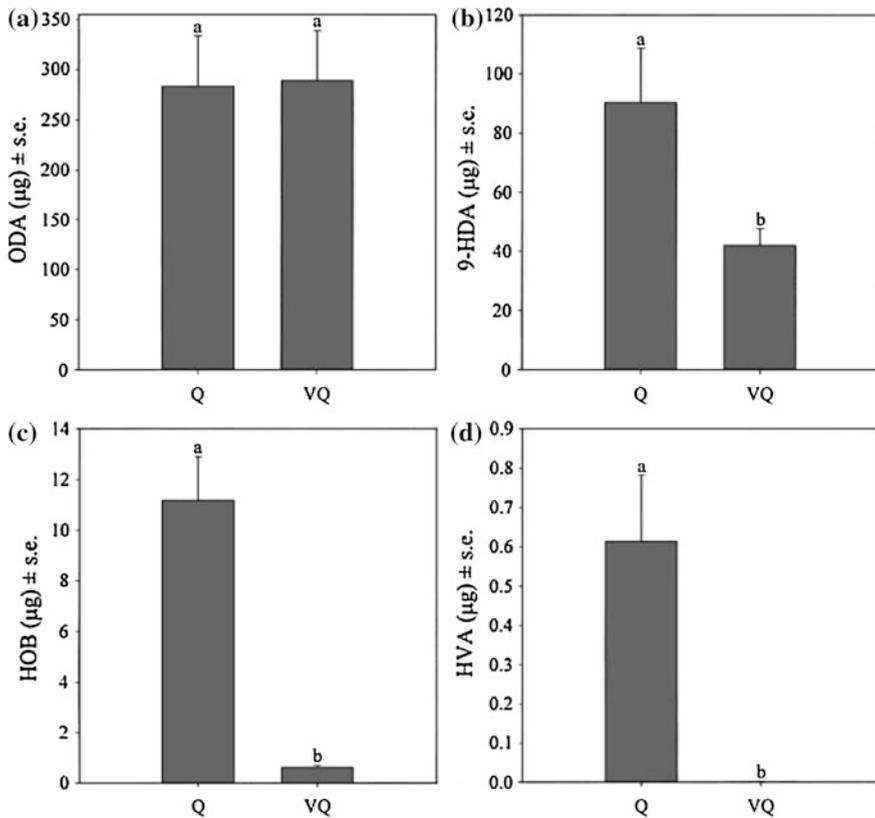
**Fig. 6.2** Mean comb area constructed by colonies headed by mated queens (*Q*), virgin queens (*VQ*), queen pheromone dispenser (*QMP*), and queenless (*QL*) *A. mellifera* colonies (Ledoux et al. 2001)

## 6.5 Perception of Queenrightness

The construction and the repairs of combs is the very last step in the elaboration of wax by bees. Clearly manipulations of wax must be preceded by the entrainment and development of the wax gland system itself, and then by the actual secretion of wax. In the various experiments described above, attempts were made to assay the



**Fig. 6.3** Mean weight of comb constructed by colonies headed by mated queens (*Q*), virgin queens (*VQ*), queen pheromone dispenser (*QMP*), and queenless (*QL*) *A. mellifera* colonies (Ledoux et al. 2001)



**Fig. 6.4** Comparison of queen substance components between mated and virgin *A. mellifera* queens. *Q* mated queen, *VQ* virgin queen, *A* 9-ODA, *B* 9-HDA, *C* HOB, *D* HVA (Ledoux et al. 2001)

role of the queen or of queen-like odours in the separate development of each of the three phases. Knowledge in these areas is fragmentary. Although Dreischer (1956) did not mention the size of her colonies (they must have been smallish to have been kept in observation hives), she found that the histological development of the wax glands (measured as the height of the epidermis) were more or less the same in bees from either queenright or queenless colonies. The more precise experiments and measurements of Goetze and Bessling (1959) also showed that there were no significant differences in the extent of wax gland development in small (100) queenright or queenless colonies of bees. Dreischer, Darchen, Goetze and Bessling and Free all worked in apiaries, so that queenless bees were never physically far removed from normal queenright colonies. Thus the possibility of shared pheromones was not entirely precluded (an effect well known in pallet beekeeping).

How a honeybee queen is perceived by the workers of her colony has long been a question of considerable interest. In one such study on wax glands, Hepburn et al. (1984) compared the development of the wax glands of 12-day-old bees taken from queenright and queenless colonies of 500 bees each. The queenless colonies were of two kinds: some, although kept in their own hives, shared an environmental chamber with queenright colonies; the other queenless bees were kept well isolated from queenright bees in a room in which bees had never been kept. Under these conditions, there was no significant difference in the development of the wax glands between the queenright bees and those queenless bees sharing the compartment; however, the wax glands were significantly less well-developed in those queenless bees which had been kept apart. The matter of the actual secretion of wax, after development of the glands and before comb construction, has always been extremely difficult to assess in a direct experimental way.

If a given bee of suitable age is examined and found to lack wax scales, their absence does not necessarily indicate that the bee is not actively secreting wax—it may have just contributed wax scales to the building effort. Nonetheless, Goetze and Bessling (1959) tried to assess secretory activity by measuring the standing crop of scales in 6-, 12- and 20-day-old bees taken from queenright and queenless colonies. The queenright bees bore a 40 % greater mass of wax than did the queenless ones. More convincingly, on the 20th day of the experiment, the queenright bees had constructed about 20 % more comb (713 mg) than the queenless bees (586 mg). In the absence of any analysis of the pheromones of these bees, one would most likely conclude that the rates of secretion of wax, as well as comb construction, are modulated by queen pheromones. It would appear, then, that young bees are capable of developing their wax glands in the absence of the queen bouquet, but that the extent of this development might be slightly enhanced by her presence. Given developed wax glands, the same would be true for the rate of secretion. Comb construction itself depends greatly upon the quality or 'state' of the queen. All things being equal, some egg-laying-workers and virgin queens stimulate comb construction, but not to the same extent as mated queens, which strongly indicates the importance of the relative composition of the queen-like scent as the driving force in comb construction.

The perception of “queenness” by bees had led Darchen (1956b, 1957) to believe that there is a construction pheromone which lingers on after the death of a queen. He and his colleagues (Chauvin et al. 1961) and another worker (Frichot-Riera 1961) prepared crude ether/acetone extracts of queens and were able to induce comb construction in the absence of a queen by giving bees these compounds on filter paper. That the perception of these compounds is by smell is supported by two observations. When the extracts were combined in a candy or, indeed, if the queens themselves were added to a candy paste and fed to the bees, the bees did not construct combs. Nonetheless, the possible significance of the tactile properties of the queen cannot be ignored, as was shown in the experiments of Müssbichler (1952) and other workers.

Finally, Darchen (1956b, 1957) extended his experiments to comb-building by queenless bees; he used 5,000 and 15,000 workers in two different colonies. His results are quite interesting (Table 6.9). The 5,000 queenless bees began to construct combs after two weeks had passed and the presence of laying-workers had been confirmed. In the case of the 15,000 bees, virtually the obverse result was obtained (Table 6.9). These seemingly anomalous results can now be satisfactorily interpreted with respect to queen substance or pheromones, following a brief digression on laying-workers.

Although Riem (1770) was apparently the first to observe that worker bees sometimes lay eggs, it was the redoubtable Huber (1814) who established time and again that some workers lay eggs in the absence of a queen. Moreover, he had shown that the ovaries of such bees were more developed than those of ordinary workers which did not lay eggs. This has been confirmed many times and it has also been shown that ovarian development, in the absence of a queen, proceeds independently of age (Perepelova 1928), but is certainly subject in some way to group effects (Hess 1942). Nonetheless, laying-workers certainly occur in perfectly normal queenright colonies of the Cape honeybee, *A. m. capensis* (Onions 1912). An historical account of the research related to the origin of laying-workers is beyond our present needs and the subject has been adequately reviewed over the years (Ribbands 1953; Velthuis et al. 1965; Visscher 1989; Hepburn 1994).

Following the discovery that ‘queen substance’ is actually a collection of several different compounds (Boch et al. 1979), Crewe and Velthuis (1980) were able to recover these same chemicals from worker bees. Moreover, they were able to recognise two kinds of laying-workers in pheromonal terms: those that develop all the components of a queen-like bouquet and thus function as false queens, and those that retain the characteristic aroma of worker bees (Table 6.10). These classes cannot yet be readily resolved with the ‘anatomical’ and ‘physiological’ laying worker classes mooted by Perepelova (1926), but actually occur in a graded spectrum of such bees (Hepburn 1994). These important results of Crewe and Velthuis (1980) allow us some latitude in explaining Darchen’s (1956b, 1957) final experiments on comb construction by queenless bees. We note, referring to Table 6.10 that the appearance of laying-workers coincided with comb construction in the one case, which would be consistent with the development, in at least one of those laying-workers, of a queen-like complement of chemical signals.

**Table 6.9** Comb construction by queenless *A. mellifera* colonies (Darchen 1956b, 1957)

<i>A. Colony of 5,000 bees</i>													
Experimental days	1–13	15	16	17	18–19	20–21	22–23	24	25				
Comb (cm <sup>2</sup> )	0	11	46	60	124	226	76	32	25				
<i>B. Colony of 15,000 bees</i>													
Experimental days	1–2	3–4	5	6	7	8	9	10–11	12	13	14	15	16
Comb (cm <sup>2</sup> )	270	89	126	78	44	28	62	96	12	5	13	5	3

**Table 6.10** Mandibular gland substances in *A. mellifera* workers and queens and workers in relation to activation of the ovaries (Crewe and Velthuis 1980)

Group <sup>a</sup>	Total acids (µg/head)	Components present (%)							
		1	2	3	4	5	6	7	
A	4 individuals	1.5	100.0						
	10 individuals	3.0	87.0	13.0					
	1 individuals	4.3	78.4	15.7	5.9				
	2 laying workers	4.5	81.4	13.6	5.1				
B	3 laying workers	5.4	65.2	34.8					
	7 laying workers	6.0	80.3	12.2	7.5				
C	1 laying worker	22.5	52.5	7.8	12.0	18.3	9.4		
	17 <i>A.cap/A.cap</i>	61.5	42.4	3.0	6.8	33.9	14.0		
D	3 <i>A.cap/A.mell</i>	22.4	5.5	0.5	7.7	76.2	10.0		
	7 1-day queens	136.7	61.8	1.8	7.0	26.5	2.3	0.6	
	5 mated, laying queens	197.2	12.1	7.9	32.2	2.4	36.1	6.9	2.3

<sup>a</sup> Groups are as follows: A 2 laying-workers and 15 other workers (chosen at random) from an isolated group of 50 *A. m. mellifera* workers; B 11 laying-workers from a colony of queenless *A. m. mellifera* workers; C individual *A. m. capensis* workers from groups of either 5 *A. m. capensis* workers (*A. cap/A. cap*) or one *A. m. capensis* and 4 *A. m. mellifera* workers (*A. cap/A. mell*); D *A. m. mellifera* queens of different ages. Abbreviations for the compounds present are as follows: 1 = (E)-10-hydroxy-2-decenoic acid; 2 = 10-hydroxydecanoic acid; 3 = (E)-9-hydroxy-2-decenoic; 4 = (E)-9-oxo-2-decenoic acid; 6 = 8-hydroxyoctanoic acid; 7 = methyl *p*-hydroxybenzoate (tentative) (Crewe and Velthuis 1980)

On the other hand, in the queenless colony of 15,000 bees that initially produced combs and then curtailed their operations, we would surmise for the sake of consistency that a pheromonally acceptable false queen was present over the first 10 days, following which its queen-like bouquet waned—just as is thought to be the case in queen cell construction under supersedure conditions.

In yet another study of comb-building, Yang et al. (2010) reported the results of studies on comb-building in mixed-species, *A. cerana* and *A. mellifera*, colonies of honeybees in which three colonies of mixed workers in one group were given *A. cerana* queens, and three others *A. mellifera* queens. Three additional colonies of each species headed by their own queens served as controls. Although they were

interested in the nature of comb-building (numbers of each species of workers in festoons, cell sizes, etc.) under the different treatments (which is discussed elsewhere (cf. Chap. 3). What is germane here is that both species of workers engaged in comb-building whether headed by conspecific or heterospecific queens. This was despite some obvious differences in the relative composition of the mandibular glands of *A. cerana* and *A. mellifera* queens.

## 6.6 Comb-Building Experiments of Maisonnasse et al. (2010)

Maisonnasse et al. (2010) reinvestigated the role of queen substance in comb-building in *A. mellifera*, and noted that although pleiotropic effects on colony regulation are accredited to queen substance, it does not elicit the same range of worker response observed in the presence of a queen, suggesting that yet other compounds may come into play. They tested the hypothesis of a pheromone redundancy in honeybee queens by comparing the effects of queens with and without mandibular glands on a variety of worker behaviours, of which here we only consider the comb-building experiments. The experiments by Maisonnasse et al. (2010) confirmed that 9-ODA is uniquely produced in the queen mandibular glands and suggested the existence of another source of the production of HOB and 9-HAD, as found in *A. m. capensis* and *A. m. scutellata* queens by Whiffler and Hepburn (1991a).

Maisonnasse et al. (2010) asked whether queens lacking mandibular glands were as effective as normal queens in regulating ovary activation, comb construction and retinue behaviour. Maisonnasse et al. (2010) tested the effects of queens lacking mandibular glands and normal queens on comb construction in cage experiments. Three different groups were tested: cages with normal queens (MG+: positive control); queenless cages (QL: negative control); and cages with demandibulated queens (MG-). After 2 weeks, the combs from each cage were collected and the number of cells counted. The mean diameter of 20 cells per treatment cage was determined, and divided into two categories according to their size. In addition, the number of queen cells was counted in the different groups. Three replicates were performed giving a total of 125 data sets. Maisonnasse et al. (2010) found that comb size significantly increased in the presence of queens (MG+, MG-) compared to QL, however no differences were detected between the two types of queens. Workers reared with MG+ and MG- queens built worker-sized cells that did not differ, but QL workers built drone-sized cells. No queen cells were constructed in either MG+ or MG- groups; however, QL workers constructed one to three queen cells per cage.

These results clearly show that demandibulated queens retain their full regulatory functions, which is in agreement with the studies of Velthuis and Van Es (1964) and Velthuis (1970). The data of Maisonnasse et al. (2010) suggest that

queen substance is not solely responsible for the regulation of colony function by the queen. In addition, by testing the effect of mandibular gland removal on the composition of 9-ODA, 9-HDA and HOB, they showed that demandibulated virgin queens were as effective as normal virgin queens in regulating colony function. Interestingly, workers in MG– group produced worker-sized cells, and built a large number of cells, as in the MG+ group, in contrast to the QL group in which workers constructed a small number of drone-sized cells. Thus, their results indicate that comb construction is also regulated by queen chemicals other than ‘classical’ queen substance.

In the absence of queens, *A. m. capensis* workers that reproduce via thelytokous parthenogenesis, and *A. m. scutellata* that reproduce via arrhenotokous parthenogenesis, build only worker or drone cells respectively, but queenless hybrid colonies produce both or only worker cells (Neumann et al. 2000). This would support the idea that comb construction can be regulated by chemicals other than queen-derived substances that are also produced by some workers. However, since *A. m. capensis* workers develop queen-like pheromonal bouquets high in 9-ODA (Simon et al. 2001), the construction of worker cells in those queenless colonies could also be due to this pheromone.

All the experiments on comb construction (excluding queen cells) lead us to believe that the presence of certain chemicals, formerly designated as ‘queen substance’, result in the construction of considerably more comb than is produced in their absence, whether the chemicals come from a queen or a false queen (laying-worker). Nonetheless, one still observes that some bees will secrete wax and construct comb whether these signals are present or not. A pheromonal basis for construction was suggested by Darchen (1956b, 1957). The subsequent identification of these substances by Boch et al. (1979) and their recognition in both queens and workers (Crewe and Velthuis 1980) provide a platform for a further analysis of these compounds in relation to wax synthesis and comb-building. The pheromonal quality of a queen is obviously important and it seems inescapable that some vehicle, in addition to olfactory perception of a ‘queen’, is required. The construction of emergency queen cells appears to work in exactly the same way (Huber 1814; Müssbichler 1952; Verheijen-Voogd 1959).

## 6.7 The Construction of Queen Cells

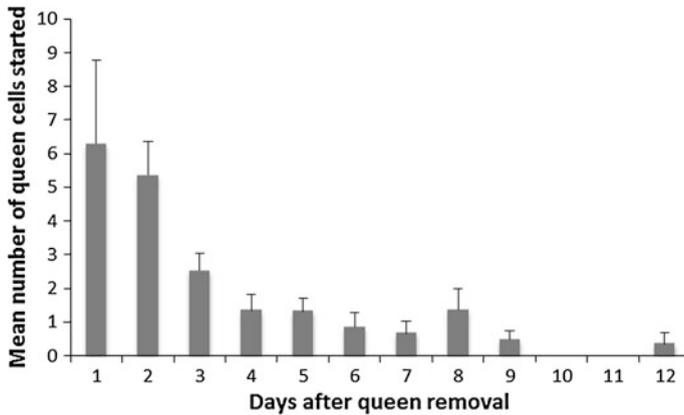
At first sight it may appear that secretions from the mandibular glands in living queens are necessary to stimulate comb-building, because significantly more combs are constructed by bees with access to whole queens having intact mandibular glands than bees whose queens lacked them (Fig. 6.4). The results of the division board experiments do not fully support this idea because workers having access to only the head of a queen constructed similar amounts of comb whether mandibular glands were present or not (Table 6.4). It is possible that some pheromonal secretions of the queen’s head other than those of the mandibular glands, provide a

comb-building stimulus. Any contribution from possible secretions from their abdomens is doubtful because no combs were built when workers only had access to the abdomens of queens (Table 6.4). This interpretation would be consistent with observations that queens without mandibular glands still maintain control of colony behaviour (Gary and Morse 1962; Velthuis and van Es 1964; Velthuis 1970; Butler et al. 1974; Free 1987), and with others that challenge the role of the queen's mandibular gland (Slessor et al. 1990).

That the queen may have a specific relationship to the synthesis and secretion of wax as well as to comb-building has been moot for several centuries. Indeed, de Réaumur (1740) was the first to note that a caged colony of queenless bees had constructed comb after 2 days confinement. However, he had given the bees some queen cells and, unfortunately, we do not know how long the bees had been queenless prior to their incarceration, or whether they had been given any other brood. A far more instructive experiment was performed by Schirach (1770) who observed that, on the loss or removal of a queen, a colony would construct emergency queen cells over some of the worker cells containing eggs or larvae, and new queens would be reared from them. This important result was confirmed in numerous experiments by Huber (1814) and is the basis for the queen-rearing industry of today. Huber also knew that queenright colonies normally construct queen cells in spring in preparation for reproductive swarming. So queen cells may be constructed in the presence or absence of a queen.

These somewhat ambivalent results led Huber (1814) to another experiment in which a hive was so divided that about half the bees were in direct contact with their queen, while the other half had access only to the odour and sounds of the queenright half of the colony. In this situation, the 'queenless' half of the colony began to construct queen cells, the other half did not. In a slightly different experiment, Huber (1814) simply placed a queen in a cage and inserted it in a colony of bees. All the workers were able, in theory, to feel the queen through the cage with their antennae. In this case, no queen cells were constructed. Huber's results have subsequently been confirmed by Lehnart (1935) and their complexity extended by the work of Müssbichler (1952). The latter author added the refinement of dividing the colony with a screen through which the queenless half of the colony could reach the queen with their antennae. Nonetheless, these bees began to construct queen cells. If, as in Huber's experiment, the queen was simply caged among the bees, no queen cells were built. However, if a caged queen was placed on one side of a screen, thus dividing a colony in half, those bees on the opposite side of the screen, away from the queen, began the construction of queen cells.

That the absence of a queen may provoke the building of queen cells (Schirach 1770; Huber 1814), coupled with the results of Müssbichler (1952), suggest a restriction in the flow of some material from the queen. This inevitably leads us to consider: (1) the means by which workers are aware of their queen; (2) how the presence or absence of a queen affects the behaviour of workers; and (3) what precisely do the worker bees do with respect to wax secretion or the building of emergency cells. Within a few hours after the loss of a queen, a general agitation spreads amongst the workers (Huber 1814; Fell and Morse 1984; Skirkevicius



**Fig. 6.5** Mean number of queen cells begun each day after removal of an *A. mellifera* queen, but before the emergence of a new queen.  $N = 13$  for days 1–9,  $n = 12$  on day 10,  $n = 11$  for day 11, and  $n = 9$  for day 14 (Fell and Morse 1984)

2004), the rate at which this happens being apparently related to the size of the colony. When disturbed, such bees make a noise by vibrating their wings and they also release a scent. All this kind of behaviour is however suppressed if such queenless bees are given a dead queen. Fell and Morse (1984) quantified some of the changes associated with the removal of a queen. The rate of queen cell construction is at first high and then rapidly declines (Fig. 6.1). Similar results were obtained with *A. m. capensis* (Hepburn et al. 1988) (Fig. 6.5).

Queen cell construction is inversely related to scenting behaviour (an index of the degree of agitation) of the queenless colony (Skirkevicus 2004). All these observations on the construction of queen cells in the face of apparent emergency (and others on the suppression of ovarian development in workers) finally led to experimental confirmation of the increasingly pervasive idea that a queen secretes substances, the presence or absence of which modifies the behaviour of the worker bees (Butler 1954; Pain 1954; Voogd 1955). The importance of queen substance to building is dramatically illustrated in the experiment by Darchen (1960) in which a small colony of several hundred bees, which had been queenless for 3 months, began the construction of comb only 2 days after the introduction of a dead queen.

In an entirely different set of experiments, Lensky and Darchen (1962) introduced two caged queens (presumably having twice the amount of queen substance) into a small colony, but the workers soon began the construction of queen cells. In a second experiment they placed three queens (2 old and 1 young one) into such a colony and again queen cells were constructed. Finally, three young caged queens did not inhibit the construction of queen cells. Earlier experiments with *A. mellifera* similar to these were performed by Melnik (1951) and another by Kovtyn (1949) with the opposite result. Finally, it has been shown that certain anxiolytic drugs enhance queen cell construction in queenless bees (Leonard and Darchen 1978).

The interpretation of all these results is difficult in the absence of any measurements of queen substance. Such signals might have come from laying workers (discussed below), and then there are the possible synergistic effects of workers in the even distribution of queen substance. Even though it has been shown that the presence of a queen sometimes inhibits queen cell construction, an effect specifically attributed to 9-ODA secreted by the queen (Butler and Callow 1968; Boch and Lensky 1976; Lensky and Slabezki 1981), an explanation for switching on or off queen cell production under emergency conditions or otherwise, based solely on the constituents of queen substance, is inadequate (Winston et al. 1990, 1991). Nonetheless, Grozinger et al. (2003) demonstrated that queen mandibular pheromone caused changes in gene expression in the brain of adult worker honeybees, and that these changes can be correlated to downstream behavioural responses induced by queen mandibular pheromone. Their data demonstrate that queen substance regulates expression of several hundred genes either transiently or chronically. Clearly, pheromone-mediated gene expression could be expected to modulate worker behaviour in the inhibition of queen cell construction.

Other queen cell constructions, probably more common in nature, have received less attention than has the emergency queen cell. The little concrete information available on the construction of queen cells for the purposes of reproductive swarming or supersedure has been summarised by Ribbands (1953) and Butler (1957, 1974). Of the former, we know that comb construction abates and that queen cell construction begins despite the fact that one of the queen substances, 9-ODA, appears in quantities that are indistinguishable in swarming and non-swarming queens (Seeley and Fell 1981). Obviously the presence of a single component of the queen signal is neither necessary nor sufficient to explain the commencement or cessation of either comb construction or the building of queen cells. As to queen cell construction for supersedure, it is commonly believed to be related to a decline in the production of essential queen substances, but there is no experimental evidence to support this idea. Moreover, were this true, it is likely that supersedure is only quantitatively different from queen cell construction under emergency conditions (Ruttner 1983).

It has long been supposed that new queens are not produced by workers if adequate amounts of queen mandibular gland pheromone are present and circulating among the bees (Butler 1954; Butler and Simpson 1958; Winston et al. 1989, 1990, 1991). However, only relatively recently, Naumann et al. (1993) explored the possible transfer and dissemination of queen pheromone by comparing populous colonies with other less populous ones. They used synthetic queen substance containing tritiated 9-keto-2(E)-decenoic acid, as a marker component of queen substance. Considering their results, we think that colony crowding does not significantly affect the dissemination and transportation of queen substance and that their data better support their alternate sub-hypothesis: that queen-rearing could be associated with changes in the thresholds of the worker bees at swarming time.

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