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The effect of social interactions on the production of mandubular gland signals in female african honeybees (apis mellifera adansoni)

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various conditions during the course of the study.

At the end of November 1978 two groups of newly-emerged worker bees were marked on the thorax with paint spots and released into an established queenright observation hive headed by Queen 13, who at the time was only four weeks old. The groups, each numbering two hundred and fifty individuals, were allowed to live normally, and at intervals of roughly every three days, nine bees from each group were removed and analysed in samples of three heads in order to follow changes which occurred in queenright worker mandibular gland secretions with age. Sampling continued until mid-December covering a period of four weeks, and a total of one hundred and forty seven worker heads was analysed in forty nine samples. The ovaries of the bees sampled were not examined as it was assumed that they were undeveloped.

A second study was made on the same observation colony in February 1979. In this study the colony was made queenless and it was hoped that a direct comparison would be possible with the results obtained in the preceding study of queenright bees. This was done in order to determine the degree to which mandibular gland signals of A.m. adansonii workers change during queenless periods, and how significantly they change toward queenlike signals. Other aims of the study were to identify the age group of bees most capable of experiencing changes in their mandibular gland secretions, how soon after dequeening changes occur, and to what extent these changes are related to ovary development.

The study necessitated the identification of the age (in days) of every worker in the colony, and in order to do this, brood was incubated at about 33°C and each day between three and four hundred newly-emerged bees were marked. The marking system required two thoracic paint spots on each bee, one placed centrally on the scutum (indicating the specific day of the week in which the bee emerged) the other placed on the scutellum and into the trans scutal ridge (indicating the week of emergence). As bees are efficient at removing paint spots from each other it was essential to paint the week spot well into the trans scutal ridge, thereby making it more difficult to remove. The system was largely successful for although many spots had been gnawed to some extent, less than 1 per cent had lost their week spot rendering them ageless. As many as 9 per cent had had their day spots removed, while the remainder retained their age identification until the study was terminated.

In toto, approximately fifteen thousand workers were marked during

a seven week period, ten thousand five hundred during the first five weeks prior to dequeening and the remainder afterwards. In order to prevent unmarked bees emerging in the hive it was necessary to debrood the colony on three occasions : at the start of the exercise on the 5th January, sixteen days later and finally the day before the colony was made queenless on the 8th February. The first operations were performed at night - the queen was captured and the combs in the hive replaced with empty drawn comb and comb containing honey and pollen before the queen was released. This method was extremely disruptive since the design of the hive required that it be almost entirely dismantled. Despite the fact that the colony appeared to have settled down by morning it was decided that a more gentle procedure was necessary for the final operation. A second observation hive was assembled containing empty comb and food comb and the queen was removed and caged in this hive. The exit from the occupied hive was sealed and the hives switched in position, the new hive being connected to the exit. The hives were then linked by their perspex windows and the workers allowed to leave the old hive and join the queen. The procedure lasted about three hours and was hastened by the use of smoke. The process appeared to be less drastic than the earlier one used, and many bees had begun inspecting cells long before the operation ended.

After twenty four hours the queen, who had remained caged in the new hive throughout this period, was removed and analysed. The queen (Queen 13) was eight weeks old when the first marked bees were released into the colony and was fifteen weeks old when she was removed. It was decided to continue to release marked bees into the colony for a period of two weeks after dequeening as under normal conditions new bees might emerge for up to three weeks following the loss of the queen.

Sampling of the colony took place just prior to dequeening, and then again three, five, seven, fourteen and nineteen days after dequeening, and finally at the termination of the experiment nearly six weeks after the queen was removed. The bees of each sample were dissected and their ovaries examined to determine the degree of development. Heads were grouped according to ovary development and age, up to seven heads being sampled together. During dissection, severed heads were stored in clean, dry testtubes in a container of liquid nitrogen to prevent loss of volatile head compounds . In addition, the colony was observed repeatedly and a number of individuals

showing laying behaviour were captured. These bees were sampled individually and their ovary development determined.

After sampling on the nineteenth day it was decided to leave the colony for three weeks to enable laying workers to establish their behavioural position ; however the colony began to be seriously disturbed by Iridomyrmex humilis foragers, and after repeated efforts to discourage the ants had failed, the study was prematurely terminated when the bees were captured outside the hive in the process of absconding. The absconding swarm numbered several thousands and as it was practically impossible to analyse each bee, the heads of only one hundred and fifty two randomly selected individuals were extracted for analysis and only forty seven ovary dissections completed. Altogether three hundred and sixty bees were analysed in the study of which two hundred and fifty six had the degree of development in their ovaries determined.

In November 1979 a small colony of queenless workers was transferred to an observation hive. Over a period of four weeks, eighty four bees were removed, their head extracts analysed separately, the degree of development in their ovaries estimated and the number of ovarioles in their ovaries counted. The remaining bees, numbering only eighty six, were then simultaneously killed and similarly analysed except that heads were extracted in groups according to ovary development, the samples containing between three and eight heads.

The ages of the bees analysed in this colony were unknown ; however the fact that it consisted of fewer than two hundred workers suggests that the bees were of a considerable age and had probably been queenless for at least two months. The colony had earlier had laying workers as there were at least ten stunted drones present. At the time of the study, however, there was no live brood although dried up groups of eggs in some cells suggested that certain individuals were still laying.

In March 1980 it was decided to establish small groups of queenless workers in cages in an attempt to study the social interactions occurring between the individuals of each defined group and to compare the social position of each bee with its mandibular gland signal and the condition of its ovaries. Five newly-emerged workers were distinctively marked by thoracic paint spots and released into each of ten small wooden and perspex cages (Liebefeld cages) containing a small (four by four centimetre)

square of undrawn wax plate, and water and food containers (Verheijen-Voogd, 1959). The bees were incubated at about 33°C and were only removed from the incubator for periods of observation. Six such periods were held daily, each lasting for ten minutes. During these periods two major social interactions were recorded, namely the trophollactic actions of 'begging' and 'offering'. Free (1957, 1959) has described these interactions as well as their frequency of occurrence. Begging is described as the thrusting of the proboscis between the mouthparts of a nestmate, while offering describes the movement of a st.'l-folded proboscis downward and forward between open mandibles and is often accompanied by the extrusion of a droplet of regurgitated liquid. Both activities are accompanied by antennial stroking and foreleg tapping. The role a worker will assume appears to depend largely on the state of her crop content at the moment of activity, and often both bees will beg or offer simultaneously before one may give way and commence the opposite action.

As it is thought that the act of receiving food by trophollaxis may be an indication of the dominance of the recipient over the donor, a simple formula was derived to equate the social position of each individual within a group ; this was termed the social index.

$$\text{Social index} = \frac{\frac{(D+F)}{(C+E)}}{\frac{(B+H)}{(A+G)}} \quad \text{or} \quad \frac{(D+F)(A+G)}{(C+E)(B+H)}$$

C was the number of times other bees 'offered' to a specific worker, while E was the number of occasions that the worker 'begged' from others. C+E therefore represented the number of occasions that an individual had the opportunity to feed by trophollaxis. D was the number of times a bee accepted food 'offered', while F was the number of times other workers gave in response to soliciting. D+F then represented the number of occasions that an individual actually received food by trophollaxis, and the ratio of D+F : C+E provided an indication of the degree of dominance of each individual in the group. A was the number of occasions that a bee 'offered' to other workers, and G the number of times other 'begged', A+G representing the opportunities others had of feeding from that individual. B was the number of times bees accepted offerings and H the number of occasions that a bee gave in response to being solicited by others, B+H representing the number of occasions an individual fed others trophollactically. The ratio of B+H : A+G therefore provided an indication of the degree of subordination of an

individual. The social index was in essence a comparison between the degree of dominance and the degree of subordination, the higher the index being, the more dominant the individual.

The experiment was terminated on the tenth day as twenty three bees had died and a number of others appeared to be dying. It was found, upon dissection, that the dead and dying individuals had greatly distended hindguts and had obviously developed an alimentary disorder. The majority of these bees had ceased to interact socially prior to death, and the number of interactions observed in each group was therefore affected.

On the termination of the study (or upon the premature death of any individual) each bee was dissected for ovary assessment and its head extracted for chemical analysis.

In addition to the ovariole counts made on the ovaries of bees from the small queenless colony and the small cages, two further counts were made on queenright workers sampled from field hives in February 1979. In all, the ovarioles of four hundred and nineteen ovaries were counted from over two hundred bees. In the case of the small queenless colony where ovary development was also assessed the relationship between the number of ovarioles and degree of development was tested.

During the preparation of the observation hive for the study of the effect of queenlessness on workers (and whilst the queen was present and marked workers were being added daily to the colony) a survey was conducted of the attendant court constantly present around the queen in order to determine which age groups of workers were responsible for attending the queen.

Analytical Techniques

Ovary dissection and assessment

Dissections were performed on newly-killed bees as it was found that refrigerated ovaries and preserved ovaries tended to undergo changes in texture which often caused them to disintegrate or become detached from the lateral oviducts making assessment extremely difficult owing to the network of tracheal tubes.

Decapitated, chilled bees were pinned dorsally through the thorax onto a perspex dissecting dish containing transparent plastic mounting

material, and covered with a weak ethanol solution. Under a stereomicroscope at a magnification of about ten times, the first three abdominal tergites (tergites II - IV) were removed using jeweller's forceps (Size No. 5), the oesophagus pulled from the thorax, and the entire gut lifted and inverted posteriorly thus revealing the ventral aspect of the hind gut and the ventral, internal floor of the abdomen on which the ovaries lie. The ovaries, whose terminal filaments meet over the dorsal aspect of the hindgut, are loosely attached to the gut by the tracheal tubes, and the critical part of the dissection was the separation of the two. Once this was accomplished, the free ovaries and lateral oviducts were detached from the common oviduct and placed into the dissecting fluid for inspection.

Under a magnification of between thirty and forty times and with the light source directed from beneath the stage, the degree of ovary development could be determined and the number of ovaries counted. The classification of ovary development developed for this study was more elaborate than that of Velthuis (1970b) and followed closely that of Hastings (pers. comm.). The following stages of development were definable (see Figure 2.1) :-

'0' ovaries undeveloped : resting and almost inactive, no compartmentation of vitellarium.

'1' ovaries slightly developed : swelling and compartmentation of vitellarium visible : compartments, or follicles, may be slightly elongated but are generally round, about as wide as long. Follicular contents granular but otherwise vitreous.

'2' Ovarioles having marked increase in size : follicles bean-shaped but may be round, at most twice as long as wide. Follicular contents opaque.

'3' intermediate between '2' and '4'. Eggs up to three times longer than wide : nurse cells clearly visible.

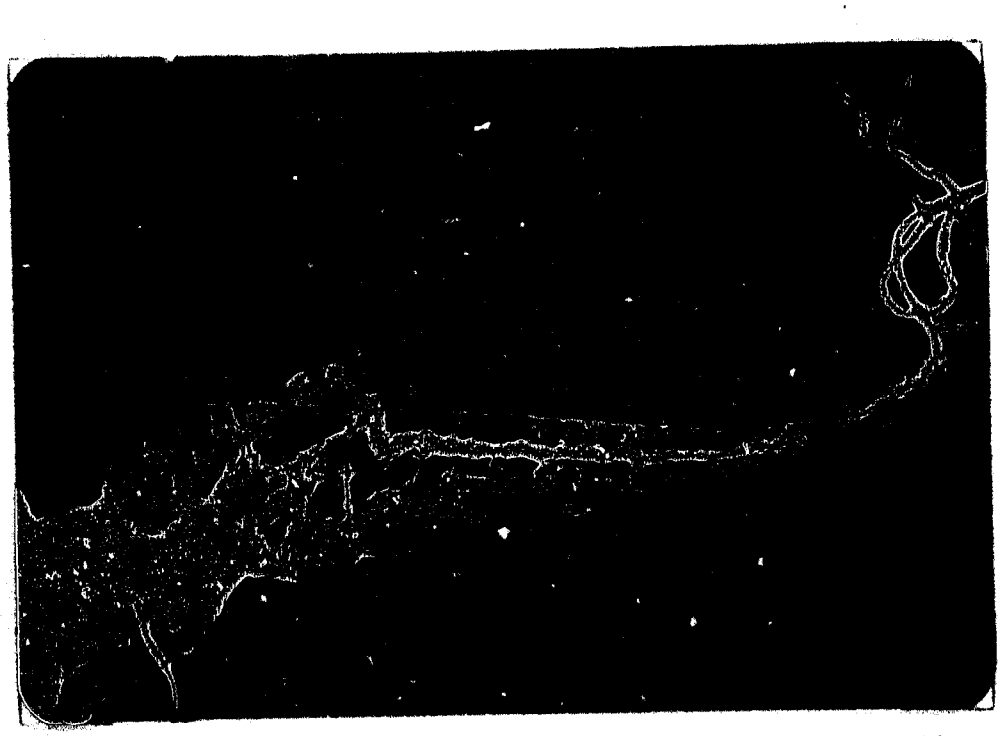
'4' eggs having sausage form.

Solvents and sampling procedure

Throughout the study queens and workers captured for sampling were analysed identically. Captured individuals were placed in darkness for about half an hour in the hope that they would largely regenerate any part of the mandibular gland secretion they may have released during capture. Thereafter they were transferred to a cold room at 4°C where they were immobilised by the low temperature and their heads were cut off and immediately dropped in fifty microlitres dichloromethane (Merck art 6048). Where samples contained more than one head (these were

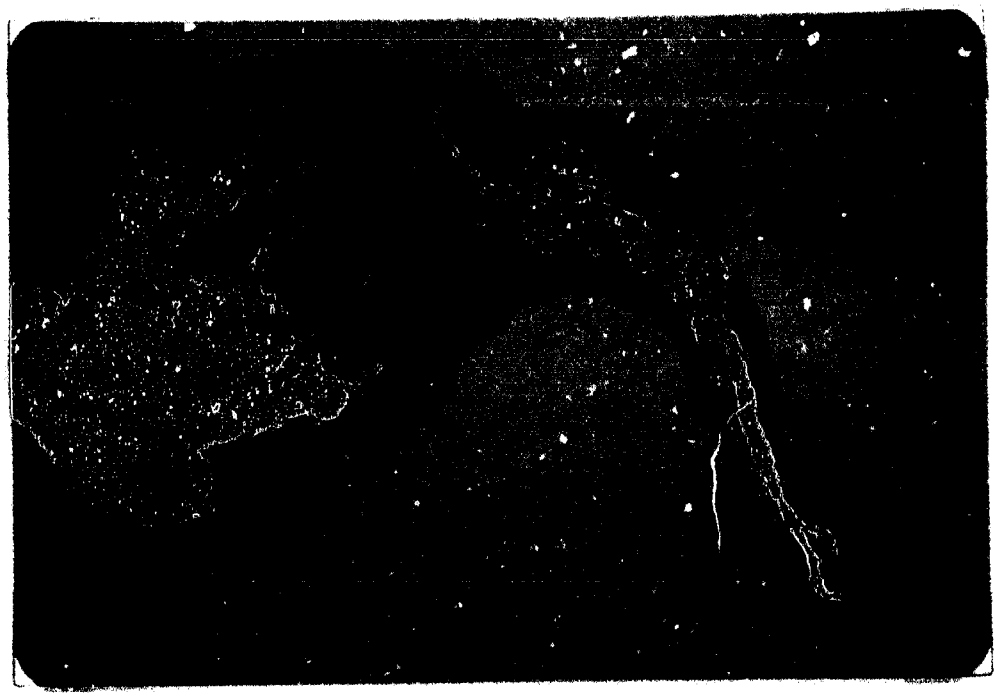


Ovarial development stage '0'. Ovaries undeveloped : resting and almost inactive, no compartmentation of vitellarium



Ovarial development stage '1'. Ovaries slightly developed : swelling and compartmentation of vitellarium visible : compartments, or follicles, may be slightly elongated but are generally round, about as wide as long. Follicular contents granular but otherwise vitreous

Figure 2.1. Stages of ovarial development occurring in the ovaries of queenless A.m.adansonii workers



Ovarial development stage '2'. Ovarioles having marked increase in size ; follicles bean-shaped but may be round, at most twice as long as wide. Follicular contents opaque



Ovarial development stage '3'. Intermediate between '2' and '4'. Eggs up to three times longer than wide ; nurse cells clearly visible

grouped according to the degree of ovary development) heads were frozen temporarily in test tubes in liquid nitrogen until the ovaries had been assessed after which they were transferred to the appropriate sample vial.

Heads were left in solvent for two to three days under standard refrigeration after which they were removed to avoid contamination by larger, less soluble cuticular and glandular compounds which were found to interfere with the resolution of key components of the mandibular gland secretion during the chromatographic process.

Analysis of mandibular gland secretion

It was decided that only eight compounds, common to the secretions of both castes, would be analysed during the study. These were decanoic acid, p-hydroxy benzoic acid, 8-hydroxy octanoic acid, 9-keto decenoic acid, 2-(4-hydroxy-3-methoxyphenyl) ethanol, 9-hydroxy decenoic acid, 10-hydroxy decanoic acid and 10-hydroxy-decenoic acid. In addition, the amount of 2-heptanone in the secretions of some bees was determined.

Owing to the chemical properties of ketones, 2-heptanone was analysed separately using a glass column (2m x 3mm I.D.) packed with 10% SP-1000 on Supelcoport (80/100 mesh). The chromatograph was operated isothermally at 80°C, and after each run the column was flushed at 150°C before reuse. A known amount of 4-methyl-3-heptanone in solution in dichloromethane was added to each sample and was used as an internal standard to quantify amounts of 2-heptanone. The ketone was chosen as the internal standard because it was similar to 2-heptanone in both its detector response and its retention time enabling accurate quantitative analysis. (See Appendix Figure 5).

Immediately following 2-heptanone analysis (where this was performed) the sample solutions were transferred to small five millilitre pyrex reaction vials and evaporated to dryness under a slow stream of gaseous nitrogen in preparation for fatty acid analysis.

The method adopted during this study, derivatized the fatty acids to their N-trimethylsilyl esters using the silylating reagent, bis (trimethylsilyl) trifluoroacetamide (BSTFA). The technique was a modification of one developed for preparing amino acids for gas chromatography (Gehrke and Leimer, 1971).

Dried sample residues were redissolved in fifty microlitres dichloromethane (containing the internal standard n-tetradecane) and

fifty microlitres BSTFA, and the reaction vial was sealed before being heated in an oil bath at 150°C for forty five minutes. After the solution had cooled the derivatives were analysed. A glass column (2m x 3mm I.D.) packed with 5% OV101 on Supelcoport (80/100 mesh) was used, the column oven being operated under a temperature programme initiated at 150°C and rising immediately at three degrees per minute to 250°C. The 5% OV101 liquid phase was selected for use in the fatty acid analysis as it was found to give a better separation of the relevant component chemicals than did 10% OV101 and both the 5% and 10% OV11 liquid phases. n-Tetradecane was chosen as the internal standard as it eluted from the column before the compounds of interest and consequently did not interfere with the resolution of any components of the mandibular gland secretion, as did n-pentadecane and n-hexadecane. In addition, since it was a straight chain hydrocarbon, it was not subject to silylation. Detector responses to n-tetradecane were therefore uniform and reliable, allowing accurate quantitative analysis of the fatty acids.

Because the derivatization of fatty acids to their silylated esters was never complete, detector responses to these esters were relative and the amounts of the fatty acids present in the extracts had to be quantified by means of the relative weight response (RWR). The actual amount of acid was then determined by Formula 1, a simple weight/response ratio corrected by the RWR.

$$\text{Weight(acid)} = \frac{\text{Weight(I.S.)}}{\text{Response(I.S.)}} \times \frac{\text{Response(acid)}}{\text{RWR(acid)}} \qquad \text{(Formula 1)}$$

The RWR for each acid was determined by derivatizing known amounts of authentic acids and using Formula 2, I.S. being the internal standard and response being the area of the peak produced by the acid, as measured by an electronic integrator.

$$\text{RWR(acid)} = \frac{\text{Weight(I.S.)}}{\text{Response(I.S.)}} \times \frac{\text{Response(acid)}}{\text{Weight(acid)}} \qquad \text{(Formula 2)}$$

Silylation of the authentic fatty acid standards was found to occur more rapidly than did amino acid silylation at 150°C, and equilibrium was reached within forty five minutes for all acids. It was found that the use of acetonitrile in place of dichloromethane in the silylation of fatty acids was not necessary, and that the importance of drying the sample residues was not as critical as for amino acid residues, possibly

Table 2.1. Relative weight responses and relative retention times of the silylated ester derivatives of some fatty acids occurring in the mandibular gland secretion

Fatty acid	RWR	RRT
decanoic acid	0,92	1,14
p-hydroxy benzoic acid	0,75	1,24
8-hydroxy octanoic acid	0,75	1,69
9-keto decenoic acid	0,70	1,93
2-(4-hydroxy methoxy phenyl) ethanol	0,75	2,02
9-hydroxy decenoic acid	0,65	2,34
10-hydroxy decanoic acid	0,65	2,37
10-hydroxy decenoic acid	0,60	2,46

because less water was present in the head extracts. The silylated fatty acid derivatives were found to be stable for over two weeks, although this information was not necessary as all samples were analysed earlier.

The RWR determinations used in the study are shown in Table 2.1. ..ther with the relative retention times for each acid, the latter being the ratio of the retention times between the relevant acid and n-tetradecane ($\frac{RT_{acid}}{RT_{I.S.}}$).

All standard solutions and bee extracts were analysed on a Packard 417 gas chromatograph equipped with flame ionisation detectors. Quantification of the responses from the detectors was performed by a Spectra Physics minigrator and chromatograms of these responses were traced by a Nippon U-125M recorder. Identification of the peaks in the extracts was by comparison of relative retention times.

RESULTS

The mandibular gland secretions of A.m. adansonii queens and workers were analysed for only eight compounds, namely decanoic acid (DAA), p-hydroxy benzoic acid (pHBA), 8-hydroxy octanoic acid (8HOAA), 9-keto-2-decenoic acid (9KDA), 2-(4-hydroxy-3-methoxyphenyl) ethanol (43HPE), 9-hydroxy-2-decenoic acid (9HDA), 10-hydroxy decanoic acid (10HDAA) and 10-hydroxy-2-decenoic acid (10HDA). In addition, the amount of 2-heptanone in the secretions of some workers was determined. The decision to analyse the above chemicals only was taken in view of the fact that they constitute the major part of both the queen and worker mandibular gland secretion, other components seldom being found in more than trace amounts in queens, while in workers they were normally absent. Percentages of each component were determined with respect to these compounds alone rather than with respect to the total head extraction. This was necessary as head extracts occasionally contained unidentified, additional compounds eluted between 225-250°C which were probably cuticular in origin. These were therefore ignored in order to obtain a direct comparison between the eight compounds just mentioned.

Mandibular gland secretions of A.m. adansonii queens

The amounts of the eight components of the mandibular gland secretions of A.m. adansonii queens were highly varied (Table 3.1.). The total amount of secretion produced varied from between 230.3 micrograms to 798.9 micrograms in mated queens, but was considerably lower in virgin queens apart from a forty day old virgin in which the secretion was found to be 926.8 micrograms. The most abundant component, 9KDA, varied from 116.6 micrograms to 390.3 micrograms in mated queens, the average being 250.5 micrograms, while 9HDA varied from 38.4 micrograms to 166.8 micrograms and 10HDA from 19.2 micrograms to 81.2 micrograms. In the forty day old virgin queen there was 456.5 micrograms 9KDA, 211.3 micrograms 9HDA and 115.5 micrograms 10HDA, roughly double the quantities found in the average mated queen signal (see Appendix Figure 1).

Although the age, weight, development period, mated state and

Table 3.1. Amounts of eight chemicals in head extracts of A.m.adansonii queens, together with some biological data (a and b indicate individuals from the same colony-sex test)

Biological data regarding queens							Total chemical (µg / head)								
Queen No.	Locality	Date of extraction	Weight (mg)	Age of extraction	Stage of development	Mated state	DAA	pHBA	8HDAA	9KDA	4SHPE	9NDA	10HDAA	10NDA	Total secretion
1	Zimbabwe	–	–	1 hour	–	Virgin			5.0					1.2	6.2
2	Zimbabwe	–	–	24-36 hr	–	Virgin		1.6	2.5	36.8	2.5	19.7	7.7	13.4	84.2
3	Zimbabwe	–	–	36-48 hr	–	Virgin	1.8	1.6	6.7	111.7	6.4	26.2	20.4	30.8	205.6
4	Zimbabwe	–	–	48-60 hr	–	Virgin	7.9	3.0	8.5	86.6	10.0	33.1	5.2	18.1	172.4
5	Transvaal	6/1979	220	24-36 hr	larva	Virgin			3.2	6.3	1.2	3.6	3.6	31.7	49.6
6	Transvaal	8/1979	212	36-48 hr	–	Virgin			1.0	6.9		0.6	0.9	11.2	20.6
7	Transvaal	12/1978	–	40 days	egg	Virgin	5.4	5.1	104.4	456.5	13.9	211.3	15.1	115.1	926.8
8	Zimbabwe	–	–	–	–	Mated	5.6	2.1	6.6	225.5	13.5	44.8	21.2	44.4	363.7
9	Zimbabwe	–	–	–	–	Mated			12.9	257.2	6.3	46.2	19.9	47.8	390.3
10	Transvaal	12/1979	260	14 days	egg	Mated[a]	3.7	13.0	19.0	116.6	8.8	38.4	11.8	15.2	230.5
11	Transvaal	11/1979	255	21 days	–	Mated[a]	1.8	12.2	11.2	118.1	14.0	47.8	13.1	23.1	241.3
12	Transvaal	6/1979	139	–	–	Mated	1.7	2.7	42.4	346.6	4.9	53.7	10.1	80.2	542.3
13	Transvaal	2/1979	–	15 weeks	egg	Mated	2.8	51.2	25.4	270.0	57.3	127.3	38.9	73.3	646.2
14	Transvaal	5/1979	205	–	–	Mated[b]	2.1	56.4	68.9	279.4	37.3	122.4	73.2	81.2	720.9
15	Transvaal	5/1979	205	–	–	Mated[b]	1.5	46.2	56.3	390.3	40.5	166.8	37.8	51.4	798.8
Average mated queen secretion							2.4	23.0	30.2	250.5	22.8	80.9	28.2	53.7	491.8

date of extraction were known for most queens, the sample was too small to indicate why substantial variation exists between queens. Of interest was the fact that queens reared from the same colony at about the same time of year appeared to secrete reasonably similar signals, these queens being the two mated individuals from Zimbabwe and the two pairs of Transvaal queens indicated in Tables 3.1 and 3.2. The weights of these queens were also found to be similar.

The determination of the amount of each component as a percentage of the whole secretion (Table 3.2.) revealed that while absolute amounts vary greatly the proportions of each compound in the signal are relatively constant. Percentage estimates were substantially less varied in mated queen secretions than in virgin queen secretions, the C.V. being only 15 per cent compared with 46 per cent. The high C.V.s obtained for virgin queen data are indicative of the fact that the mandibular glands of these individuals were not as yet secreting a mature queen signal, but are in a transitory phase of activity. The mandibular glands of a pupal queen (Stage 6 according to Thompson 1978) analysed during this study did not have detectable quantities of the eight compounds. An hour-old virgin queen (Queen 1) was found to be secreting small amounts of 8HOAA and 10HDA. The secretion of the remaining compounds began within twenty four hours, and within two to three days virgin queens were found to be secreting all eight components.

There was some evidence that the mandibular glands of Zimbabwe virgin queens matured more rapidly than did those of Transvaal queens. The secretions of one to three day olds had already reached a level where the components were in similar proportions to those in mated queen secretions although the quantity of secretion was somewhat smaller. Secretions of Transvaal virgins of the same age were totally different from those of mated queens. In these queens 10HDA was the most abundant constituent, the levels of 9KDA and 9HDA being significantly lower.

From the data available it would appear that 8HOAA is possibly the first compound secreted by the queen mandibular gland, the secretion of 10HDA, 9KDA, 9HDA and 10HDAA following within hours, and lastly the secretion of DAA, pHBA and 43HPE.

Of particular significance to this study was the possibility of the existence of a mature queen signal. On average about 80 per cent of the mated queen secretion consisted of 9KDA, 9HDA and 10HDA, these components being found in a ratio approximately 10:3:2 (Table 3.2.)

Table 3.2. Percentages of eight chemicals in head extracts of A.m.adansonii queens (a and b indicate individuals from same colony)

Queen No.	Percentage chemical							
	DAA	pHBA	8HDAA	9KDA	43HPE	9HDA	10HDAA	10HDA
1			81.1					18.9
2		1.9	3.0	43.7	3.0	23.4	9.1	15.9
3	0.9	0.8	3.3	54.4	3.1	12.8	9.9	15.0
4	4.6	1.7	4.9	50.3	5.8	19.2	3.0	10.5
5			6.4	12.7	2.3	7.3	7.2	64.0
6			5.1	33.3		3.0	4.3	54.3
7	0.6	0.5	11.3	49.3	1.5	22.8	1.6	12.4
8	1.5	0.6	1.8	62.0	3.7	12.3	5.8	12.2
9			3.3	65.9	1.6	1 .8	5.1	12.2
10[a]	1.6	5.6	8.3	50.6	3.8	16.7	5.1	8.3
11[a]	0.8	5.1	4.7	49.0	5.7	19.9	5.5	9.6
12	0.3	0.5	7.8	63.9	0.9	9.9	1.9	14.8
13	0.4	7.9	3.9	41.8	8.9	19.7	6.0	11.3
14[b]	0.3	7.8	9.6	38.8	5.2	17.0	10.2	11.3
15[b]	0.2	5.8	6.9	48.9	5.1	20.9	4.7	7.6
Average mated queen secretion	0.6	4.2	5.8	52.6	4.4	16.0	5.5	10.9

Table 3.3. X^2 analysis to test goodness of fit for a 10:3:2 ratio of 9KDA, 9HDA and 10HDA in queen secretions (percentage secretion)

Queen No.	9KDA O.	9KDA E.	9HDA O.	9HDA E.	10HDA O.	10HDA E.	X^2value	Probability 2DF	Precise ratio
1	–	12.6	–	3.8	18.9	2.5	122.8	.001 ⟩ P**	0:0:10
2	43.7	55.3	23.4	16.6	15.9	11.1	7.294	.05 ⟩ P *	10:5: 4
3	54.4	54.8	12.8	16.4	15.0	11.0	2.248	.5 ⟩ P. ⟩ .3	10:2; 3
4	50.3	53.3	19.2	16.0	10.5	10.7	0.813	.7 ⟩ P ⟩ .5	10:4: 2
5	42.7	56.0	7.3	16.8	64.0	11.2	287.8	.001 ⟩ P**	6:1:10
6	33.3	60.4	3.6	18.1	54.3	12.1	171.9	.001 ⟩ P**	2:1:10
7	49.3	56.3	22.8	16.9	12.4	11.3	3.037	.3 ⟩ PP⟩ .2	10:5: 3
8	62.0	57.7	12.3	17.3	12.2	11.5	1.808	.5 ⟩ P ⟩ .3	10:2: 2
9	65.9	59.9	11.8	18.0	12.2	12.0	2.740	.3 ⟩ P ⟩ .2	10:2: 2
10	50.6	50.4	16.7	15.1	8.3	10.1	0.491	.8 ⟩ P ⟩ .7	10:3: 2
11	49.0	52.3	19.9	15.7	7.6	10.4	1.393	.5 ⟩ P ⟩ .3	10:4: 2
12	63.9	59.1	9.9	17.7	14.8	11.8	4.590	.2 ⟩ P ⟩ .1	10:2: 3
13	41.8	48.5	19.7	14.6	11.3	9.7	2.971	.3 ⟩ P ⟩ .2	10:5: 3
14	36.8	44.7	17.0	13.4	11.3	8.9	2.393	.5 ⟩ P ⟩ .3	10:5: 3
15	48.9	51.6	20.9	15.5	7.5	10.3	2.730	.3 ⟩ P ⟩ .2	10:4: 2

Table 3.4. X^2 analysis to test goodness of fit for a 10:4:2 ratio of 9KDA, 9HDA and 10HDA in mated queen secretions

Queen No.	9KDA O.	9KDA E.	9HDA O.	9HDA E.	10HDA O.	10HDA E.	X^2value	Probability 2DF
8	62.0	54.1	12.3	21.6	12.2	10.8	5.339	.1 > P > .05
9	65.9	56.2	11.8	22.5	12.2	11.2	6.852	.05 > P*
10	50.6	47.2	16.7	18.9	8.3	9.7	0.630	.8 > P > .7
11	49.0	49.1	19.9	19.6	9.6	8.4	0.009	P < .99
12	63.9	55.3	9.9	22.2	14.8	11.1	9.386	.01 > P**
13	41.8	45.5	19.7	18.2	11.3	9.1	0.956	.7 > P > .5
14	38.8	41.9	17.0	16.8	11.3	9.8	1.233	.7 > P > .5
15	48.9	48.4	20.9	19.4	7.6	9.4	0.576	.8 > P > .7

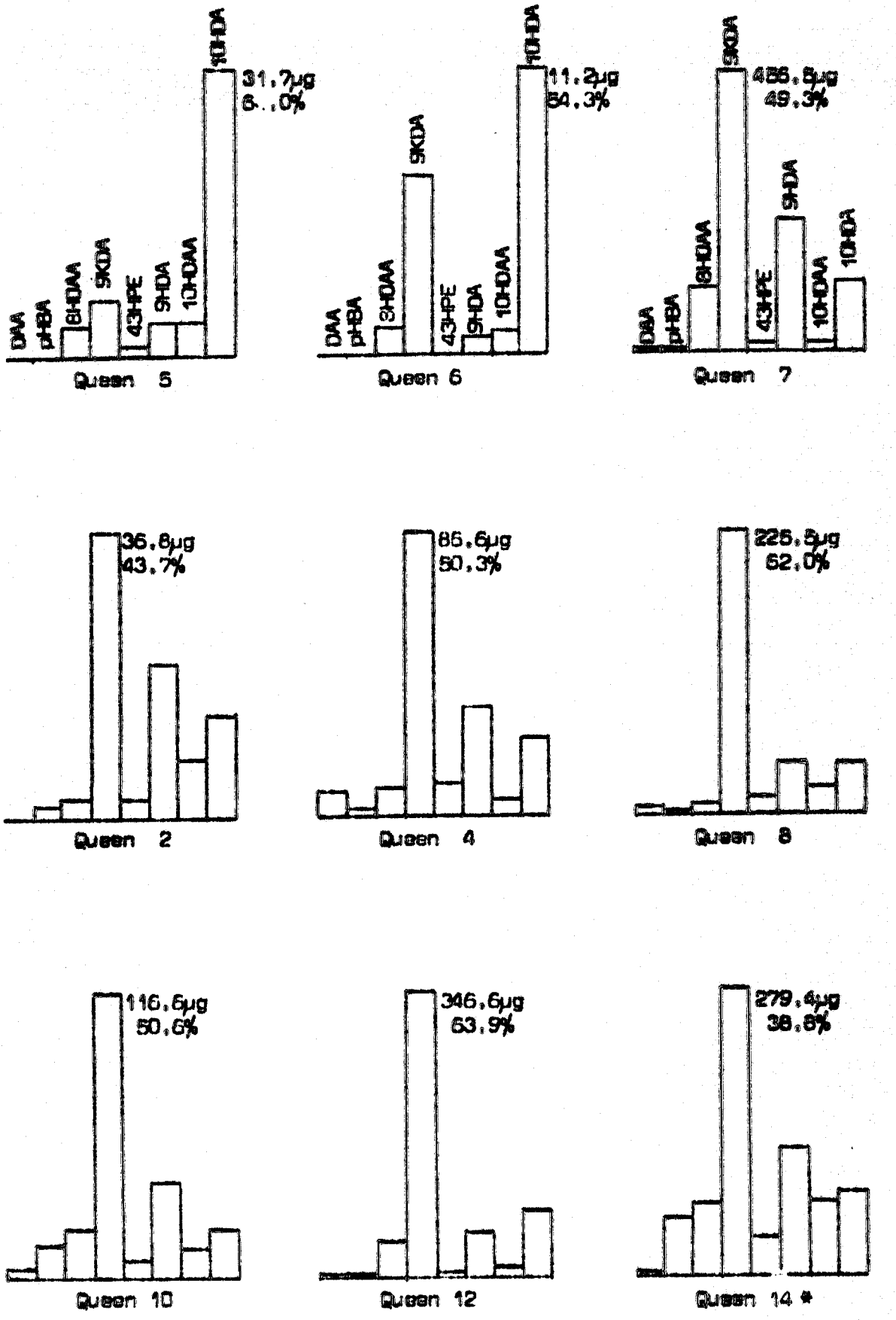


Figure 3.1. Head extracts of mated and virgin A.m.adansonii queens (*see Appendix Figure 1)

In contrast the three components were found in a significantly different ratio in four of the seven virgin queens analysed (Table 3.3) Heterogeneity in the data for mated queens (Table 3.3) was due largely to a somewhat different ratio of 9KDA : 9HDA : 10HDA in queens 8, 9 and 12, the proportion of 9HDA being low, and if these individuals were excluded, the remaining five mated queens (Queens 10, 11, 13, 14 and 15) were found to have remarkably homogenous secretions with regard to 9KDA, 9HDA and 10HDA, these components being found in a ratio of 10;4;2. (Table 3.4).

The other compounds found in the mandibular glands (DAA, pHBA, 8HOAA, 43HPE and 10HDAA) which constituted only 20 per cent of the total secretion, were found to vary considerably and a common ratio was not evident (Table 3.5). Compared with worker data, DAA was noticeably the least abundant component, constituting less than one percent of the total secretion in twelve of the fifteen queens, and never exceeding more than eight micrograms (Figure 3.1).

Table 3.5. Ratios of DAA, pHBA, 8HOAA, 43HPE and 10HDAA in queen mandibular gland secretions (* indicates compound present but less than 1).

Queen N°.	DAA	pHOB	8HOAA	43HPE	10HDAA
2	0	2	3	3	10
3	1	1	3	3	10
4	8	3	8	10	5
7	1	*	10	1	1
8	3	1	3	6	10
9	0	0	6	3	10
10	2	7	10	5	6
11	1	9	8	10	10
12	*	1	10	1	2
13	*	9	4	10	7
14	*	8	9	5	10
15	*	8	10	7	7

<u>The mandibular gland secretions of queenright A.m. adansonii workers of different ages</u>

The amounts of the eight compounds found in the mandibular gland secretions of queenright workers of known ages were highly variable

(Appendix Tables 1 and 2). As expected, 9KDA was not found in any worker regardless of its age, but in addition pHBA was absent from most secretions and where it was present amounts were minute. As in queens, synthetic activity in the mandibular glands probably begins about the time of eclosion, since six to eighteen hour old bees had up to twenty five micrograms of 10HDA (Table 3.6). The highest level of secretion was attained within two to five days where workers had on average over forty micrograms of 10HDA, constituting 55 per cent of the total secretion (Table 3.7). However immediately following this initial period; and co-inciding with the age when workers most often nurse larvae, the amount of 10HDA dropped markedly to an average of only twenty micrograms. Although the level of 10HDA was found to increase again as workers grew older, on average it never constituted more than 40 per cent of the secretion, or about thirty micrograms per head (Figure 3.2).

The ratio of 9HDA to 10HDA was found to vary from 1:20 to 1:1 although 75 per cent of the workers had ratios between 1:10 and 4:10. In all only seven individuals (representing 14 per cent of the sample) had a 9HDA:10HDA ratio similar to that found in the secretions of Queens 8, 9 and 12. None had a ratio approaching that found in the other mated queens (Queens 10, 11, 13, 14 and 15).

An unexpected feature of the mandibular gland secretions of queenright workers was the production of DAA which was found to increase markedly with age (Figure 3.3). In ten older workers DAA constituted over 20 per cent of the whole secretion, and in six individuals was the most abundant component. (see Appendix Figures 3 and 4).

The mandibular gland secretions of queenless A.m. adansonii workers

The mandibular gland secretions of newly queenless A.m. adansonii workers of different ages and different degrees of ovarial development

Samples of queenright bees of various ages captured just prior to dequeening and debrooding the colony in February 1979, were found to have a markedly different mandibular gland secretion from the queenright bees sampled from the same colony in November 1978 (Figure 3.2 and Table 3.6). Half of the workers sampled were secreting much lower amounts of 10HDA causing the 9HDA:10HDA ratio to be inverted with 9HDA being the major component. The levels of DAA were also considerably lower than in the November sample, workers being found to have from 1.5 to 6.1 micrograms,

Table 3.6. Amounts of eight chemicals in head extracts of groups of queenright A.m.adansonii workers of different ages (November 1978) (mean ± S.E.)
Raw data Appendix Table 1

Age of bees	Sample size	Total chemical (µg/head)								
		DAA	pHBA	8HDAA	9KDA	4ΔHPE	9HDA	10HDAA	10HDA	Total
6–18 hr	6	1.4 ± 0.25		3.9 ± 0.86		3.1 ± 0.89	4.7 ± 0.16	4.7 ± 0.47	13.9 ± 2.73	33.8 ± 3.71
2–5 days	8	1.3 ± 0.23	0.2 ± 0.19	4.6 ± 0.83		3.1 ± 0.32	7.8 ± 0.82	16.3 ± 2.91	43.1 ± 7.01	76.4 ± 8.56
7–10 days	9	2.5 ± 0.76	tr ± 0.04	4.8 ± 0.86		4.3 ± 0.74	6.9 ± 0.91	17.9 ± 1.63	20.7 ± 2.11	56.9 ± 3.76
12 days	6	7.1 ± 1.11	0.2 ± 0.15	6.7 ± 1.02		2.8 ± 0.58	8.2 ± 1.56	24.1 ± 1.89	33.8 ± 6.53	82.9 ± 5.53
15–21 days	12	15.9 ± 1.88	0.1 ± 0.10	5.9 ± 0.34		4.3 ± 0.58	8.0 ± 1.24	17.3 ± 2.22	32.3 ± 4.18	83.8 ± 7.62
24–27 days	8	16.0 ± 1.32	0.6 ± 0.39	6.3 ± 1.22		4.4 ± 0.90	7.5 ± 1.55	19.8 ± 1.76	23.3 ± 5.34	77.9 ± 8.16
Average secretion	49	8.1 ± 1.01	0.2 ± 0.08	5.4 ± 0.40		3.8 ± 0.29	7.3 ± 0.49	17.2 ± 1.05	28.4 ± 2.31	70.4 ± 3.64

Table 3.7. Percentages of eight chemicals in head extracts of groups of queenright A.m.adansonii workers of different ages (November 1978) (mean ± S.E.)
Raw data Appendix Table 2

Age of bees	Sample size	Percentage chemical							
		DAA	pHBA	8HDAA	9KDA	4ΔHPE	9HDA	10HDAA	10HDA
6–18 hr	6	4.4 ± 0.59		11.2 ± 1.67		9.0 ± 1.84	14.7 ± 1.64	21.1 ± 1.90	39.7 ± 4.43
2–5 days	8	1.7 ± 0.21	0.2 ± 0.25	6.1 ± 1.1		4.0 ± 0.39	10.5 ± 0.67	21.9 ± 3.37	55.5 ± 4.88
7–10 days	9	4.2 ± 1.28	tr ± –	7.7 ± 1.31		7.4 ± 0.95	11.9 ± 1.11	31.7 ± 2.52	37.1 ± 4.37
12 days	6	9.0 ± 1.75	0.2 ± 0.23	8.2 ± 1.24		3.7 ± 0.86	10.2 ± 2.10	29.2 ± 1.55	39.4 ± 5.34
15–21 days	12	18.9 ± 1.52	0.1 ± 0.08	7.2 ± 0.79		5.5 ± 0.78	10.2 ± 1.55	19.8 ± 1.63	38.0 ± 3.39
24–27 days	8	21.4 ± 1.85	0.7 ± 0.49	7.9 ± 1.34		5.6 ± 1.01	9.2 ± 1.98	26.0 ± 1.56	29.1 ± 3.94
Average secretion	49	10.8 ± 1.25	0.2 ± 0.10	7.8 ± 0.51		5.8 ± 0.46	10.9 ± 0.63	24.6 ± 1.09	39.5 ± 2.05

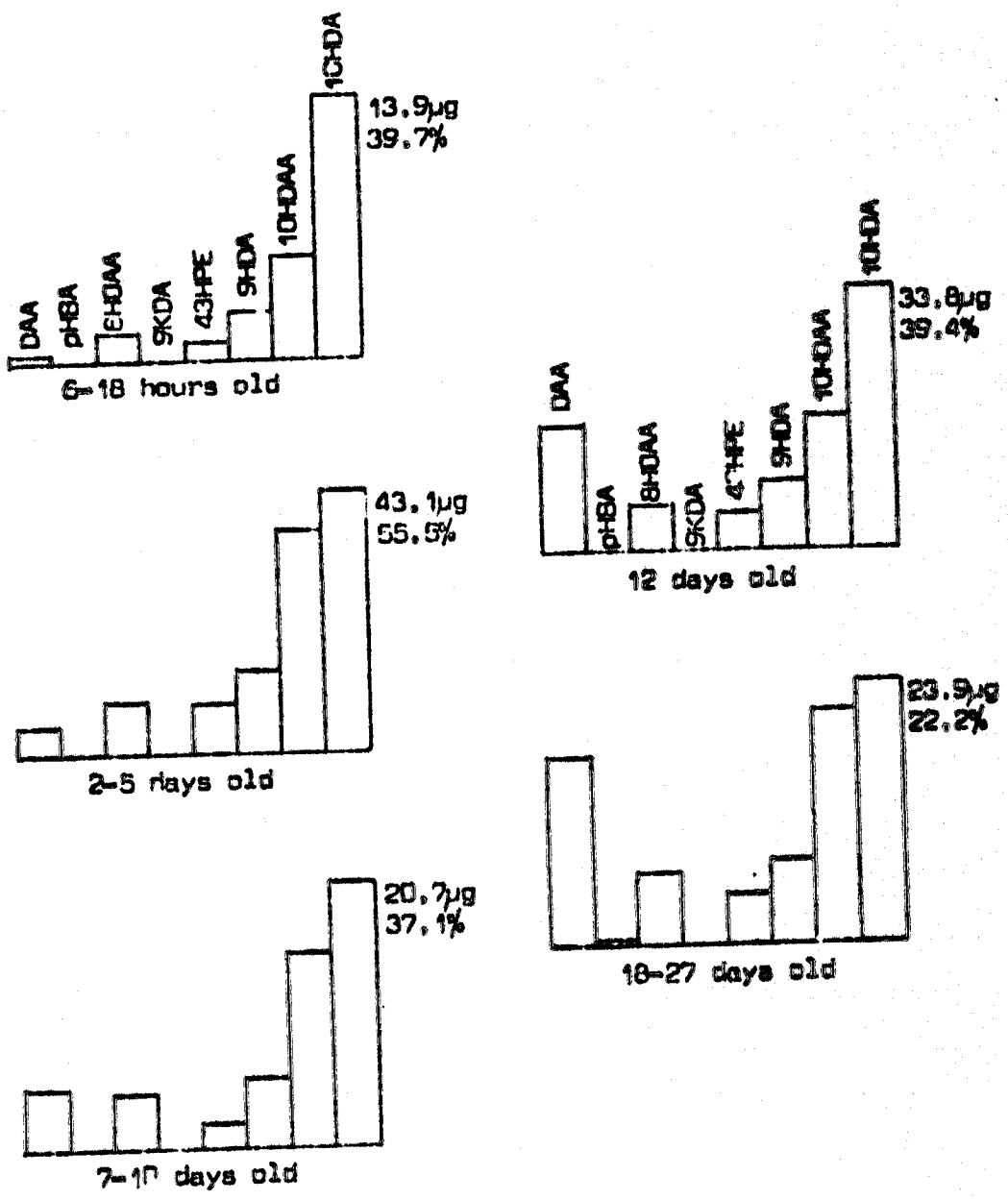


Figure 3.2. Head extracts of queenright A.m.adansonii workers of different ages (November 1978)

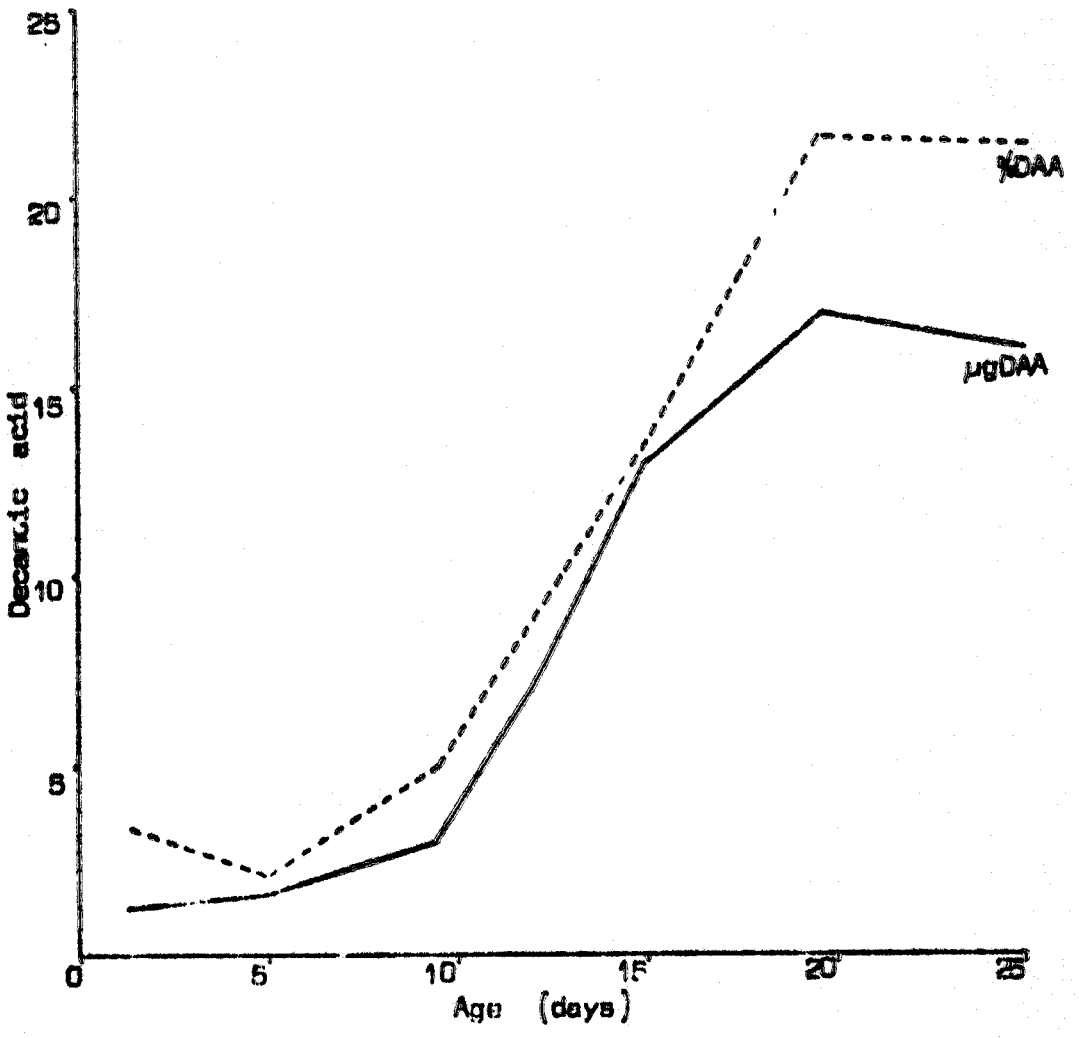


Figure 3.3. Amounts of decanoic acid in head extracts of groups of queenright A.m.adansonii workers of different ages (November 1978)

the latter constituting little over 10 per cent of the whole secretion (Table 3.8).

The average coefficient of variation of the means of the components of the February secretion was 17 per cent, indicating that variation was slightly more pronounced than in the November secretion where the coefficient of variation was only 12 per cent. The secretion of 10HDA was particularly variable, the coefficient of variation being 33 per cent compared to only 8 per cent in November workers. While the workers of both samples were of similar age, and both had equal exposure to immature brood, two factors could have influenced the mandibular gland secretion. The first factor was the difference in season, the affect of which could have been magnified in an observation hive, the second that although both groups had the same queen, she was only a month old in November while by February she was three months old and had probably experienced significant changes in her own mandibular gland secretion which would have had an influence upon the workers.

During the first two weeks of queenlessness, workers experienced substantial ovarial development (complete data relating to ovarial development is presented in Tables 3.25 and 3.26) and highly significant changes in their mandibular gland secretions. The most significant of these was the decrease in the production of 10HDA. Amounts of the chemical dropped from an average of 23.3 micrograms just before queen loss to only 7.9 micrograms, a decrease of about 66 per cent, so that it was only the third most prevalent component of the secretion after 9HDA and 10HDAA (Table 3.9 and Figure 3.4).

Although the production of all compounds other than 10HDAA decreased, the substantial decrease in 10HDA resulted in a 13 per cent increase in the levels of the other components in the secretion, the proportion of 10HDAA largely accounting for this by increasing by 11 per cent.

The average ratio of 9HDA : 10HDA had altered from 7 : 10 prior to queen loss, to approximately 10:9, 75 per cent of workers having more 9HDA. Bees sampled two weeks or more after loss of the queen, however, had an average ratio of 8 : 10 indicating a return toward the original queenright secretion, although nearly half still had more 9HDA than 10HDA.

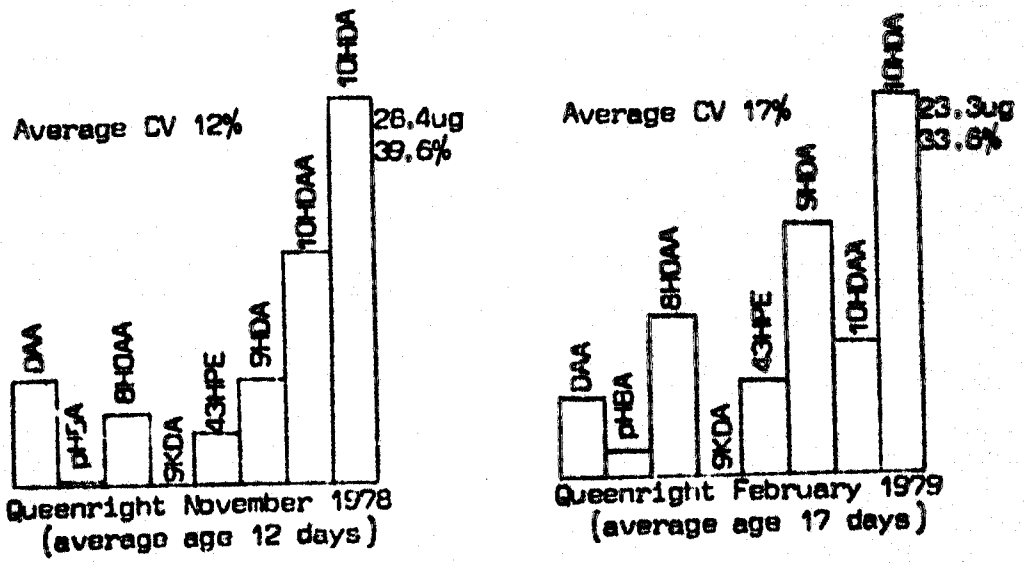
The increase in the level of 9HDA was not closely related to ovary activity and laying behaviour, as four of the seven bees with stage '4' development had more 10HDA than 9HDA, while only five of

Table 3.8. Amounts and percentages of eight chemicals in head extracts of groups of queenright A. a. adamsonii workers of various ages (February 1979) (mean ± S.E.)
Raw data Appendix Tables 3 and 4

Age (days) Range	Mean	Total chemical (µg/head)								
		DAA	pHBA	8HDAA	9KDA	4OHPE	9HDA	10HDAA	10HDA	Total
5-10	8	2.3 ± 0.37	1.9 ± 0.49	7.2 ± 0.97		4.7 ± 0.78	11.3 ± 1.59	6.2 ± 1.54	36.1 ± 13.13	69.7 ± 16.79
20-30	26	5.1 ± 0.45	0.4 ± 0.42	8.3 ± 1.26		4.4 ± 0.28	12.9 ± 0.23	7.3 ± 0.21	10.6 ± 3.4	49.0 ± 3.37
		Percentage chemical								
5-10	8	3.6 ± 0.47	3.6 ± 1.18	11.7 ± 1.92		7.7 ± 1.88	17.8 ± 1.87	8.8 ± 2.11	46.7 ± 7.89	
20-30	26	10.5 ± 1.19	1.0 ± 0.96	17.1 ± 2.76		9.1 ± 0.51	26.9 ± 1.76	15.0 ± 0.93	20.4 ± 4.85	

Table 3. 9. Amounts and percentages of eight chemicals in head extracts of groups of A.m.adansonii workers during the first month of queenlessness (February 1979) (mean ± S.E.) Raw data Appendix Tables 3 and 4

Period queenless	Age (days) Range	Mean	Sample size	Total chemical (µg/head) DAA	pHBA	8HDAA	9KDA	43HPE	9HDA	10HDAA	10HDA	Total
Before dequeening	5-30	17	10	3.7 ± 0.54	1.1 ± 0.39	7.8 ± 0.77		4.6 ± 0.40	12.1 ± 0.80	6.7 ± 0.76	23.3 ± 7.69	59.3 ± 8.78
less than 2 weeks	5-38	17	36	3.7 ± 0.85	0.4 ± 0.13	4.6 ± 0.57	1.8 ± 1.35	4.1 ± 0.41	8.0 ± 0.59	8.9 ± 0.89	7.9 ± 0.77	39.4 ± 2.97
2-6 weeks	5-57	34	40	8.7 ± 1.02	0.7 ± 0.11	5.6 ± 0.40	0.8 ± 0.23	5.2 ± 0.41	10.1 ± 0.72	11.8 ± 1.08	13.4 ± 1.59	56.3 ± 4.05
				Percentage chemical								
Before dequeening	5-30	17	10	7.1 ± 1.30	2.3 ± 0.84	14.4 ± 1.82		8.4 ± 0.95	22.3 ± 1.95	11.9 ± 1.50	33.6 ± 6.17	
less than 2 weeks	5-38	17	36	8.9 ± 1.87	1.6 ± 0.46	10.9 ± 0.90	3.2 ± 2.22	10.0 ± 0.80	22.0 ± 1.36	22.9 ± 1.62	23.8 ± 1.36	
2-6 weeks	5-57	34	40	14.7 ± 1.39	1.3 ± 0.18	10.3 ± 0.48	1.6 ± 0.47	9.6 ± 0.55	18.6 ± 0.85	20.3 ± 1.09	23.2 ± 1.74	



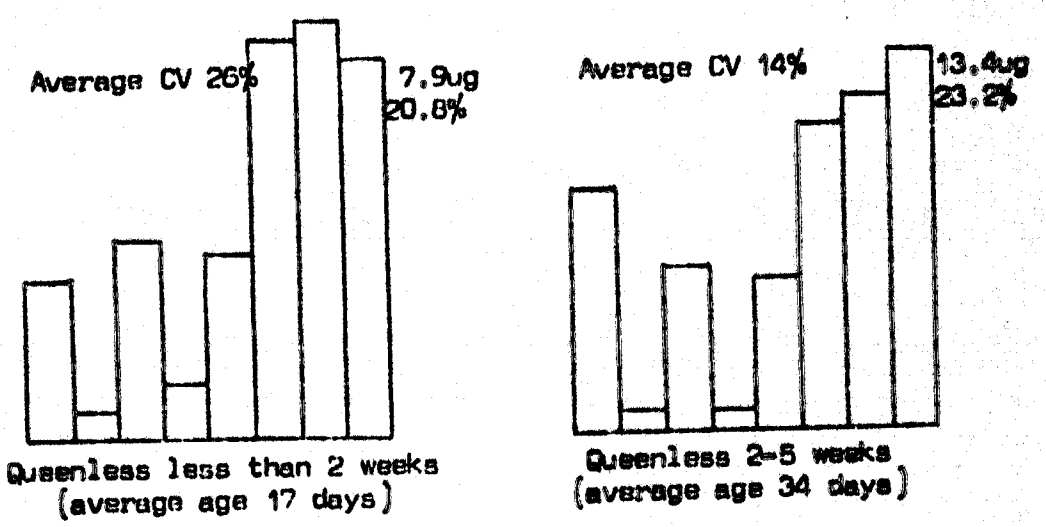


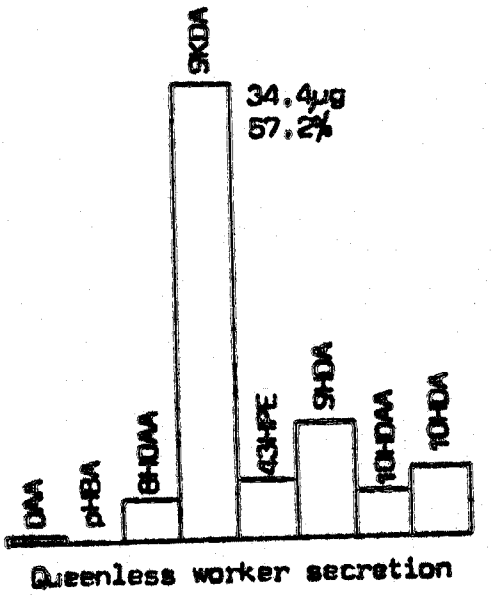
Figure 3.4. Head extracts of groups of queenright A.m.adamsonii workers sampled in November 1978 and February 1979 compared with groups of queenless workers sampled less than two weeks and two to five weeks after loss of the queen

eight laying workers had more 9HDA (Appendix Table 3).

Of one hundred and nine bees analysed in thirty six samples, only two individuals were found to be secreting 9KDA. Although both workers were captured in the brood comb section of the hive, and one was showing egg-laying behaviour, neither was found to have mature eggs. Their mandibular gland secretions had, however, undergone massive changes. The production of 10HDA and 10HDAA was significantly lower than the average production of these compounds by workers of similar age and with the same degree of ovarial development. Of great interest was that the ratio of 9KDA : 9HDA : 10HDA in these bees was not significantly different from a queen signal. 9KDA constituting over 50 per cent of the secretion and 9HDA being more abundant than 10HDA (Figure 3.5).(see also Appendix Table 2).

The only other bees found to be secreting 9KDA were individuals sampled when the colony was killed thirty eight days after the queen had been removed. Of necessity these bees were grouped with others in samples of up to eleven heads, and consequently their individual secretions were lost (Appendix Table 3). 9KDA was identified from ten such samples indicating that at least ten other bees were secreting the compound. The bees of only one sample were dissected and it was found that none of the ovaries were developed beyond the second stage. The amounts of 9KDA varied between 17.0 and 41.8 micrograms per sample, the average being 30.5 micrograms, and when compared with the amounts found in the two 9KDA secreting individuals who had 43.2 and 25.5 micrograms respectively, suggest that these samples probably contained only one or two individuals that were secreting 9KDA. On this assumption, it was estimated that only twelve to twenty bees out of three hundred and sixty (roughly 3 to 6 per cent) analysed were secreting 9KDA.

An alternative method of analysing the data, and one more comprehensive than simply assessing the gross influences of queenlessness on the mandibular gland secretion of workers, was made by relating the secretion to age, the degree of ovarial development and the age of each bee when the queen was removed. The data indicate that changes in ovarial activity cannot be closely correlated with changes in the secretion of the mandibular gland (Tables 3.10, 3.11 and Figure 3.8). Relationship is probably most obvious in the secretion of DAA which was found to decline markedly as workers experienced activity in their ovaries. While no biological function for DAA has been determined, its



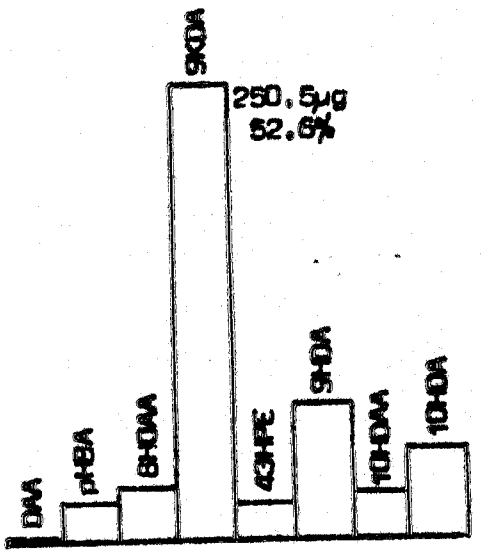


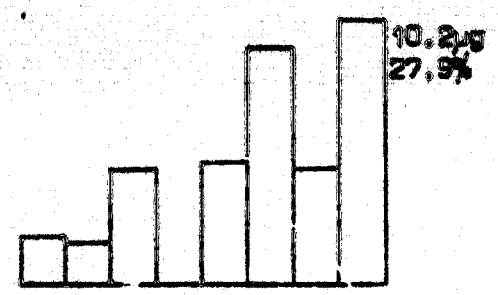
Figure 3.5. Head extracts of mated A.m. adansonii queens compared with those of two queenless workers found to be secreting 9KDA.

Table 3. 10. Amounts of eight chemicals in head extracts of groups of newly queenless A. n. adrenpdi workers of various ages and ovarial development stages (February 1979) (mean ± S.E.) Raw data Appendix Table 3

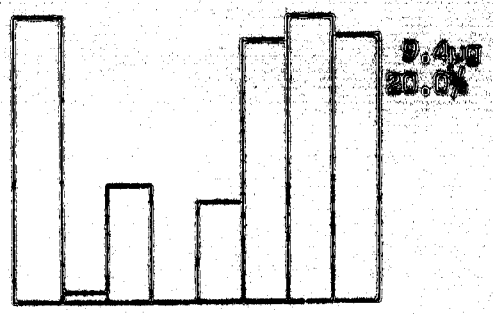
Age (days) Range	Mean	Sample size	Ovariole development	Total chemical (µg/head) DAA	pHBA	6HDAA	9KDA	4EHPE	9HDA	10HDAA	10HDA	Total
5-14	6	9	0/0	1.7 ± 0.88	1.5 ± 0.31	4.2 ± 0.59		4.8 ± 0.70	8.6 ± 1.18	4.2 ± 0.63	10.2 ± 2.13	35.2 ± 4.03
15-64	28	15	0/0	10.2 ± 1.83	0.3 ± 0.13	4.5 ± 0.80		3.9 ± 0.58	9.0 ± 0.97	10.4 ± 1.67	9.4 ± 1.11	47.7 ± 4.45
5-10	9	5	0/1/1	0.6 ± 0.30	0.4 ± 0.17	1.7 ± 0.52		2.0 ± 0.63	4.8 ± 0.82	4.6 ± 1.45	5.7 ± 1.73	19.8 ± 3.53
15-40	28	5	0/1/1	8.2 ± 2.98	0.7 ± 0.46	6.0 ± 1.76		6.5 ± 1.85	10.1 ± 2.25	13.3 ± 3.84	25.2 ± 8.51	70.0 ± 14.1
10-40	27	7	1/2/2	4.4 ± 1.39		8.4 ± 0.89		6.7 ± 0.78	8.6 ± 0.59	13.5 ± 2.51	8.2 ± 1.33	49.8 ± 6.54
8-13	10	6	2/3/3	1.7 ± 0.36	0.2 ± 0.25	4.7 ± 0.97	11.4 ± 7.59	5.2 ± 0.60	9.4 ± 1.17	8.1 ± 1.69	8.7 ± 2.67	49.4 ± 7.25
15-28	20	3	2/3/3	2.1 ± 1.15		3.1 ± 1.68		5.4 ± 0.95	7.0 ± 0.91	10.5 ± 1.10	7.6 ± 0.82	35.7 ± 5.39
8-57	34	7	3/4/4	3.8 ± 1.42	0.5 ± 0.98	5.7 ± 1.47		4.4 ± 0.92	7.1 ± 1.05	14.6 ± 2.79	13.0 ± 4.28	48.8 ± 10.6
43-57	48	8	1/2/3	15.2 ± 2.20	1.3 ± 0.25	7.5 ± 0.71	0.6 ± 0.39	4.8 ± 1.23	12.7 ± 2.15	14.0 ± 1.51	11.9 ± 3.11	69.9 ± 10.7
28-69	37	11	–	5.1 ± 0.98	0.6 ± 0.16	4.8 ± 0.89	2.4 ± 0.44	4.2 ± 0.72	10.6 ± 1.59	10.9 ± 1.67	10.5 ± 1.65	49.8 ± 15.1

Table 3.11. Percentages of eight chemicals in head extracts of groups of newly queenless *A.m.adansonii* workers of various ages and ovarial development stages (February 1979) (mean ± S.E.) Raw data Appendix Table 4

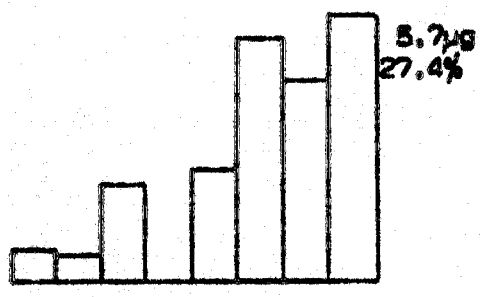
Age (days) Range	Mean	Sample size	Ovarial development	Percentage chemical DAA	pHBA	8HDAA	9KDA	43HPE	9HDA	10HDAA	10HDA
5-14	6	9	0/0	4.8 ± 0.88	4.6 ± 0.92	11.9 ± 0.94		12.9 ± 1.23	25.7 ± 2.77	12.3 ± 1.67	27.9 ± 3.91
15-54	28	15	0/0	21.5 ± 3.68	0.7 ± 0.25	8.8 ± 0.98		7.6 ± 0.90	19.7 ± 1.66	21.6 ± 2.14	20.0 ± 1.75
5-10	9	5	0/1/1	3.0 ± 1.11	2.8 ± 1.81	10.0 ± 3.55		11.2 ± 3.03	25.0 ± 3.24	20.6 ± 4.36	27.4 ± 5.35
15-40	28	5	0/1/1	11.5 ± 2.89	0.9 ± 0.55	8.0 ± 1.56		8.5 ± 1.67	14.8 ± 1.46	21.4 ± 4.62	34.9 ± 8.05
10-40	27	7	1/2/2	8.4 ± 1.65		17.3 ± 0.69		13.8 ± 1.32	18.1 ± 1.49	26.1 ± 2.57	16.3 ± 1.03
8-13	10	6	2/3/3	3.7 ± 1.03	0.6 ± 0.65	9.7 ± 1.89	19.0 ± 12.11	11.0 ± 1.60	20.1 ± 3.03	17.6 ± 3.85	18.1 ± 4.37
16-28	20	3	2/3/3	4.9 ± 2.49		8.1 ± 3.37		15.1 ± 0.93	20.2 ± 0.33	29.9 ± 1.60	22.1 ± 3.63
8-57	34	7	3/4/4	5.7 ± 1.59	0.8 ± 0.39	10.9 ± 1.56		9.3 ± 1.38	18.4 ± 4.71	30.7 ± 3.18	24.3 ± 3.73
43-57	48	8	1/2/3	23.9 ± 2.16	1.8 ± 0.36	11.5 ± 0.95	0.4 ± 0.4	6.6 ± 0.53	18.5 ± 1.02	21.4 ± 1.78	16.0 ± 1.51
28-60	37	11	–	12.4 ± 0.56	1.4 ± 0.29	9.5 ± 0.55	5.6 ± 0.92	8.4 ± 0.56	21.5 ± 1.60	30.6 ± 1.31	20.4 ± 1.57



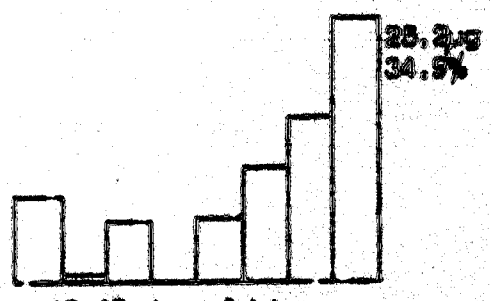
5-14 day old bees
Ovarial dev. 0/0



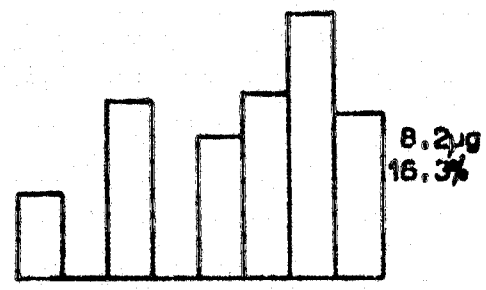
15-54 day old bees
Ovarial dev. 0/0



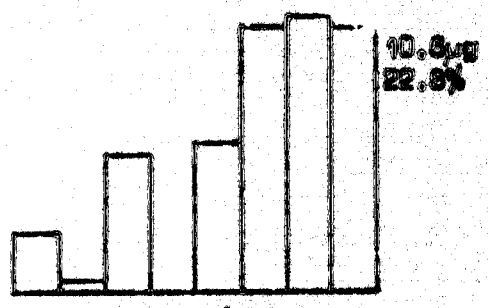
5-10 day old bees
Ovarial dev. 0/1/1



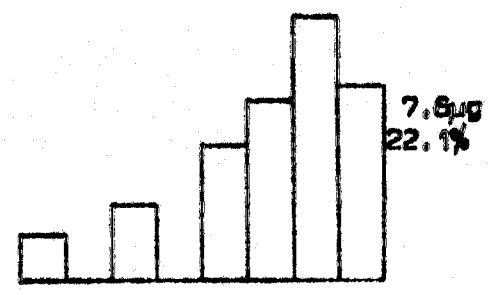
15-40 day old bees
Ovarial dev. 0/1/1



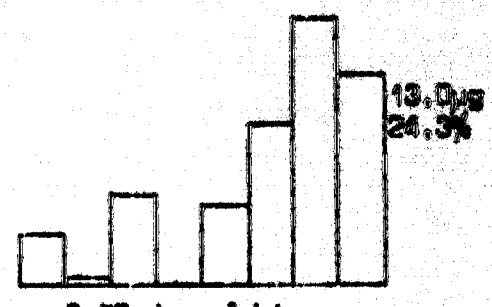
10-40 day old bees
Ovarial dev. 1/2/2



8-13 day old bees
Ovarial dev. 2/3/3



16-28 day old bees
Ovarial dev. 2/3/3



8-57 day old bees
Ovarial dev. 3/4/4

Figure 3.6. Head extracts of groups of newly queenless A.m.adansonii workers of various ages and ovarial development stages

reduction in queenless bees whose ovaries have developed may be related to the fact that queens secrete very little DAA. Differences found to occur in the secretion of DAA by queenright and queenless workers were closely associated with age and with changes in ovary development (Figures 3.7 and 3.8). Ten to twelve day olds whose ovaries had developed to some degree were found to be secreting only half the amount of DAA than were bees whose ovaries were inactive. Six week old bees whose ovaries had developed slightly experienced a 30 per cent reduction in the secretion of DAA, while bees with well-developed ovaries experienced a reduction of just over 70 per cent to levels slightly higher than those found in queen secretions.

The age of bees at the time the queen was lost was found to be an additional factor influencing the degree to which workers were able to undergo changes in glandular activity. Bees that eclosed from a week prior to loss of the queen to a week after loss of the queen experienced the greatest changes in secretion (Table 3.12). Ten of the twelve samples found to contain 9KDA were of bees less than a week old at the time the queen was lost. Workers of this age group also had significantly lower quantities of DAA, and were the only age group which had on average more 9HDA than 10HDA (Table 3.12 and Appendix Table 3).

It appeared that workers that had been queenright during the first two to three weeks of life were largely incapable of undergoing radical changes in their mandibular gland secretions, and this was particularly so with regard to 9KDA. As younger bees appeared to be able to change their secretions, it must be concluded that this ability is lost with time and may well be associated with the length of exposure to queen signals (Figure 3.9).

<u>The mandibular gland secretions of queenless A.m. adansonii workers with different degrees of ovarial development (queenless period in excess of two months)</u>.

The assumption that the bees of this colony had been queenless for over two months was based upon two observations. In the previous study (in the dequeened observation hive) workers did not lay eggs during the first six weeks of queenlessness. Similar observations have been made by Hastings (pers. comm.) which suggest that this is a normal phenomenon of queenless <u>A.m. adansonii</u> colonies. The second observation was that the present colony had a considerable number of stunted drones which

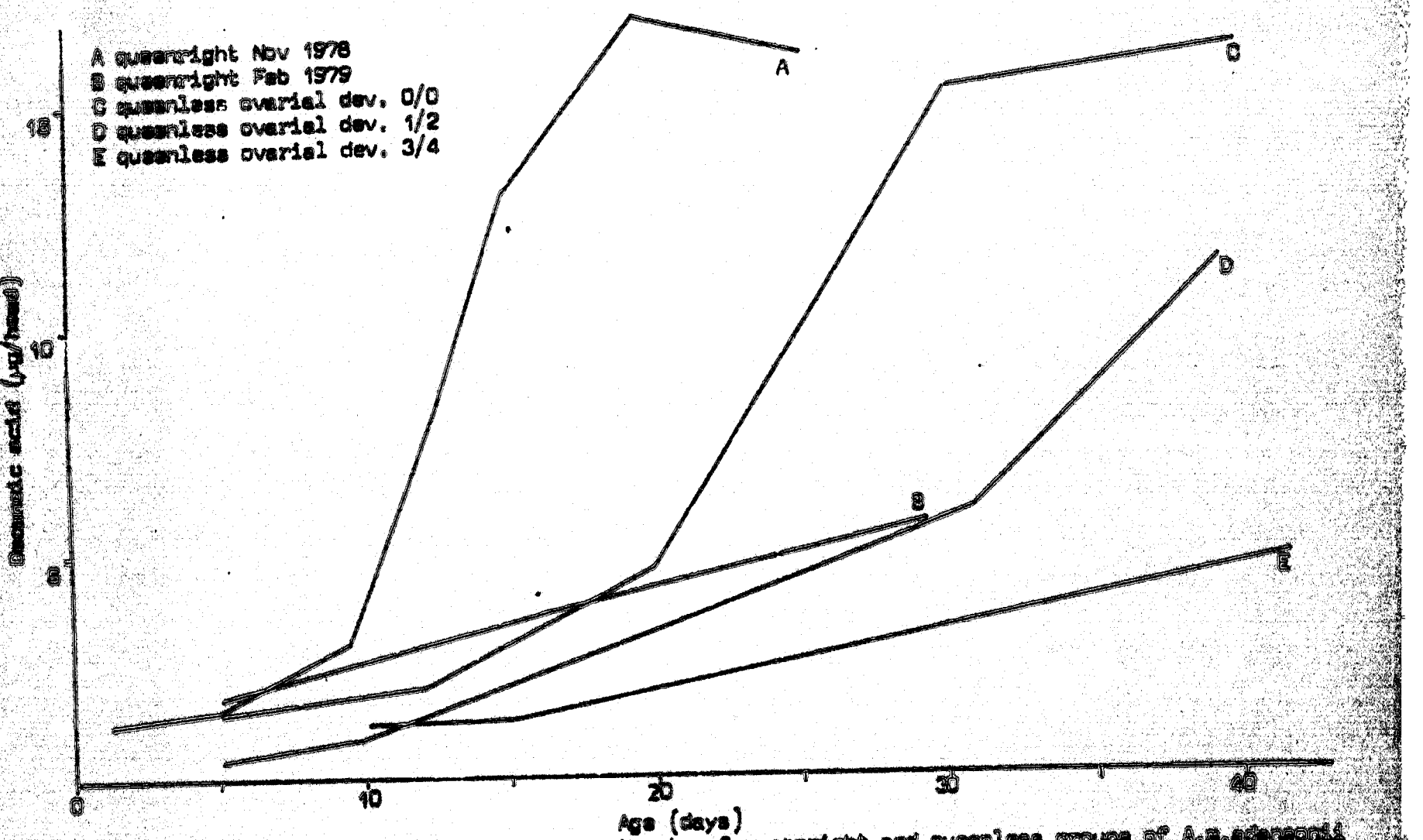


Figure 3.7. Amounts of decanoic acid in head extracts of queenright and queenless groups of A. m. adansonii workers of various ages and different degrees of ovarial development

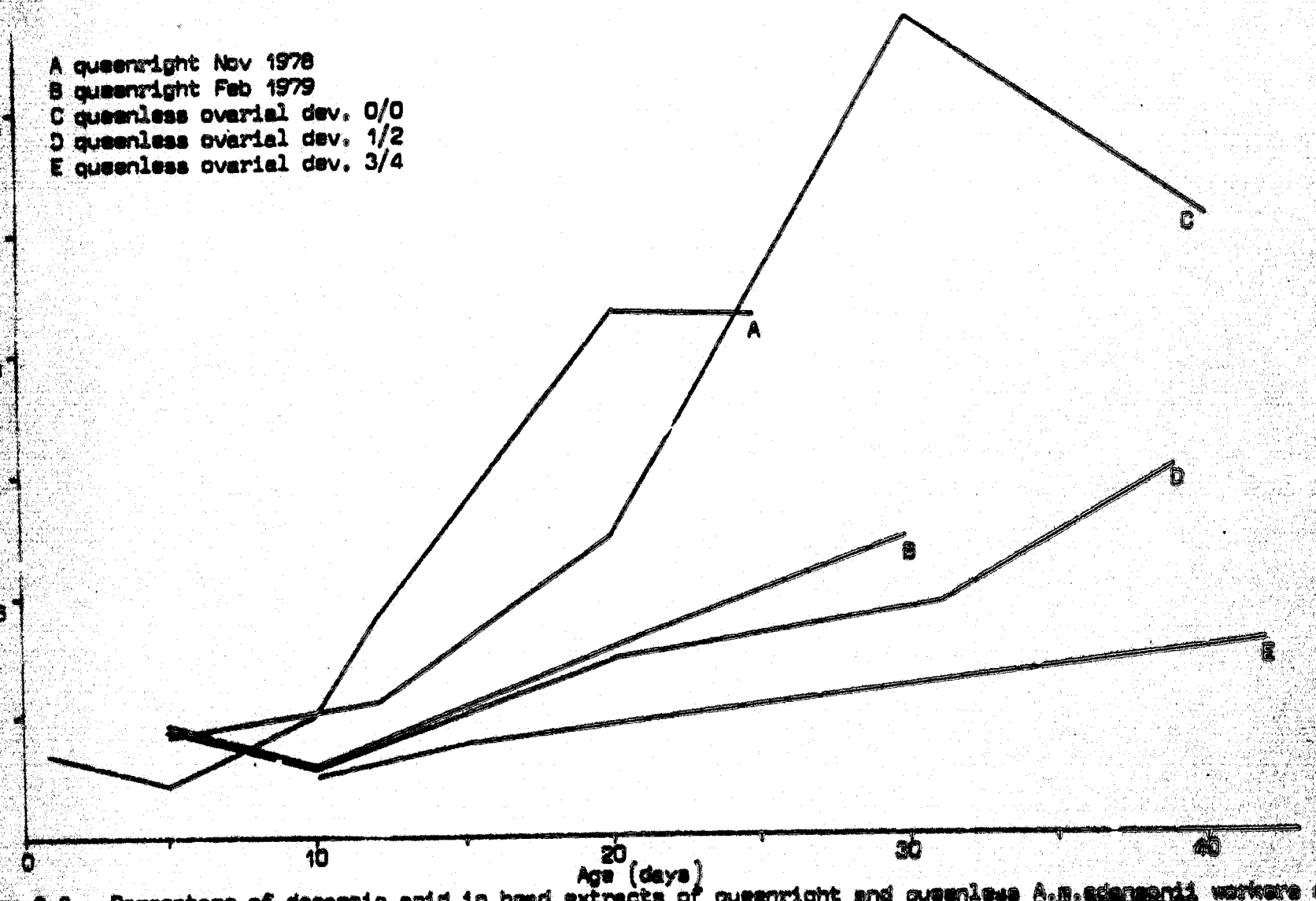


Figure 3.8. Percentage of decanoic acid in head extracts of queenright and queenless A.B.adersonii workers of various ages and different degrees of ovarial development

Table 3.12. Amounts and percentages of eight chemicals in head extracts of groups of queenless A. m. adansonii workers related to when they eclosed (February 1979) (mean ± S.E.) Raw data Appendix Tables 3 and 4

Day of eclosion	Sample size	Age (days) Range	Age (days) Mean	Ovarial development	Total chemical (µg/head) DAA	pHBA	9HDAA	9KDA	4X:PE	9HDA	10HDAA	10HDA	Total
2-9 days after dequeening	19	5-36	19	1	3.1±0.47	0.8±0.18	3.8±0.32	1.2±0.36	4.4±0.44	8.6±0.95	7.0±0.90	11.2±2.24	40.1±3.80
0-7 days before dequeening	25	5-43	19	2	4.8±1.30	0.7±0.23	4.4±0.52	2.7±1.97	4.1±0.40	8.2±0.63	8.1±0.87	8.3±0.95	41.3±3.64
8-16 days before dequeening	13	20-60	32	1	7.6±1.80	0.6±0.21	6.6±0.97	0.7±0.47	6.0±1.12	12.2±1.71	15.0±1.94	14.6±3.26	63.5±9.15
>17 days before dequeening	19	20-67	39	1.5	10.7±1.57	0.4±0.15	6.4±0.84		4.8±0.59	8.8±0.74	13.7±1.61	11.0±1.74	55.8±4.92
					Percentage chemical								
2-9 days after dequeening	19	5-36	19	1	8.0±1.09	1.9±0.49	9.9±0.57	3.0±0.85	11.3±0.76	21.9±1.21	18.1±1.56	25.8±2.69	
0-7 days before dequeening	25	5-43	19	2	9.6±2.24	2.1±0.53	10.8±1.03	4.6±3.18	10.4±0.95	22.0±1.77	19.6±1.68	21.1±1.78	
8-16 days before dequeening	13	20-60	32	1	11.2±1.35	0.7±0.23	10.7±1.14	0.8±0.44	8.7±1.04	19.9±1.20	25.5±2.05	24.3±3.22	
>17 days before dequeening	19	20-67	39	1.5	19.2±2.92	0.8±0.24	11.0±1.15		8.4±0.91	16.9±1.43	24.8±2.01	19.1±1.82	

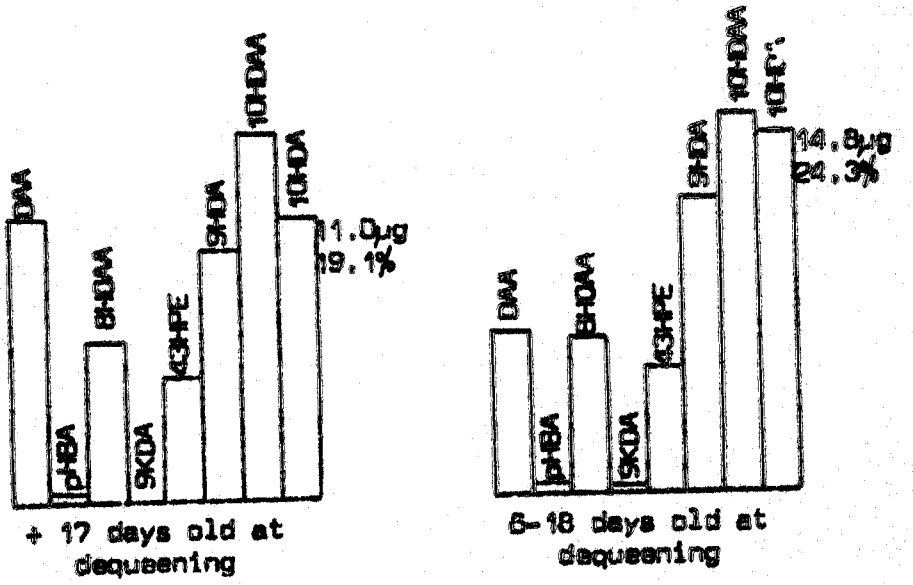


Figure 3.9. Head extracts of queenless A.m.adansonii workers who eclosed at different times

had been derived from eggs laid by workers in worker cells. Adding the developmental period of drones (which is twenty four days, Anderson et al, 1973) to the initial six week period, it can be estimated that the colony had been without a queen for at least two months. Bearing in mind that the queen may have laid eggs on the day of her loss and that the developmental period of workers is between nineteen to twenty days, the youngest bees analysed in this study were probably older than six weeks.

The entire colony was sampled over a period of four weeks. Eighty four bees were analysed individually, while the remaining eighty six were killed en masse and analysed in groups of between three and eight. The most striking feature regarding the mandibular gland secretions was the absence of individuals producing large amounts of 9KDA (Appendix Tables 5 and 6). Having identified two individuals among newly queenless workers that were exhibiting queenlike mandibular gland signals, it was expected that bees with similar signals would be present here. However although nineteen of ninety seven samples were found to have 9KDA, the extracted quantity amounted to only 15.9 micrograms, less than 5 per cent the total amount extracted from only twelve samples of newly queenless bees. Of ten bees sampled individually and found to have 9KDA, six had well-developed ovaries (stages '3' and '4') and only one had undeveloped ovaries (stage '0'). (Table 3.13).

A second feature of these bees was that on average DAA, 10HDA, 10HDAA and 2-heptanone constitued over 95 per cent of the mandibular gland secretion, the levels of the other components, including 9HDA, being extremely low (Tables 3.13 and 3.14, Figure 3.10). The latter was found to be absent from 17 per cent of samples and the highest 9HDA : 10HDA ratio was only 2: 10 (Appendix Table 5). In addition, bees with well-developed ovaries did not have higher percentages of 9HDA as might have been expected.

Of interest were the relationships between DAA, 10HDA, 10HDAA and 2-heptanone. While DAA and 2-heptanone decreased with ovarial development (2-heptanone markedly so), 10HDAA and especially 10HDA increased (Figure 3.11 and Table 3.15). In newly queenless bees only DAA had been found to alter directly with ovarial development (Figure 3.8) although there was some evidence that 10HDAA and 10HDA were also influenced. 2-heptanone was not analysed.

Nearly 60 per cent of workers with well-developed ovaries (degree '3')

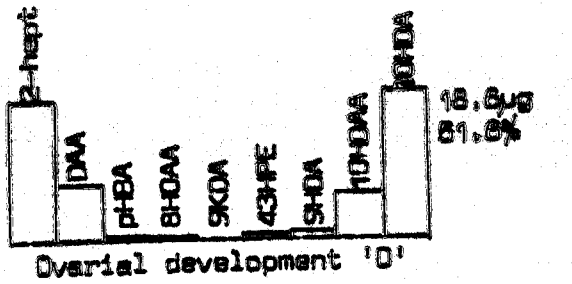
Table 3.13. Amounts of eight chemicals in head extracts of groups of A.m.adamsonii workers with different degrees of ovarial development that had been queenless for two months (November 1979) (mean ± S.E.) Raw data Appendix Table 5

Ovarial development	Sample size	Total chemical (µg/head)									
		DAA	pHBA	6HDAA	9KDA	4,9-PE	9HDA	10HDAA	10HDA	Total	2-heptanone
0	9	7.4 ± 1.11	0.6 ± 0.18	0.5 ± 0.32	tr 1*	0.8 ± 0.34	0.9 ± 0.37	5.8 ± 0.94	18.6 ± 3.05	34.6 ± 4.85	17.9 ± 4.98
1	10	8.3 ± 1.69	0.5 ± 0.27	1.0 ± 0.31	tr 1	1.3 ± 0.35	1.4 ± 0.38	7.9 ± 1.53	26.9 ± 3.70	47.3 ± 5.99	13.2 ± 3.47
2	20	4.6 ± 0.74	0.6 ± 0.17	1.8 ± 0.34	tr 2	1.4 ± 0.34	1.6 ± 0.35	10.1 ± 1.59	33.8 ± 5.75	53.9 ± 7.81	5.6 ± 1.86
3	29	6.2 ± 0.80	0.4 ± 0.10	1.2 ± 0.22	tr 3	1.6 ± 0.31	1.6 ± 0.26	10.3 ± 0.87	38.4 ± 5.26	59.7 ± 6.41	2.3 ± 0.70
4	12	4.1 ± 0.80	0.5 ± 0.21	1.7 ± 0.57	tr 3	1.3 ± 0.42	1.5 ± 0.42	8.8 ± 1.55	28.5 ± 4.31	46.4 ± 7.10	0.4 ± 0.22
Average secretion	80	5.9	0.5	1.3	tr	1.4	1.5	9.2	32.1	51.8	6.0

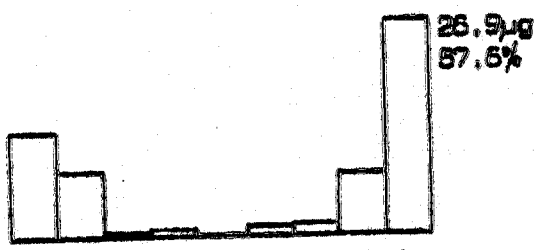
* S.E. of mean replaced by the number of individuals secreting 9KDA

Table 3.14. Percentages of eight chemicals in head extracts of groups of A.m.adamsonii workers with different degrees of ovarial development that had been queenless for two months (November 1979) (mean ± S.E.) Raw data Appendix Table 6

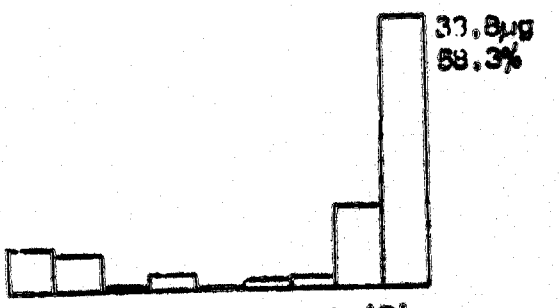
Ovarial development	Sample size	Percentage chemical							
		DAA	pHBA	6HDAA	9KDA	4,9-PE	9HDA	10HDAA	10HDA
0	9	24.1 ± 3.74	2.1 ± 0.79	1.2 ± 0.61	tr 1	2.4 ± 0.97	2.2 ± 0.62	16.4 ± 1.11	51.6 ± 3.62
1	10	19.0 ± 4.11	1.4 ± 0.85	1.7 ± 0.51	tr 1	2.2 ± 0.58	2.4 ± 0.59	15.6 ± 1.31	57.6 ± 4.43
2	20	13.5 ± 2.51	1.5 ± 0.52	2.4 ± 0.54	tr 2	2.9 ± 0.72	2.7 ± 0.36	16.6 ± 1.10	58.3 ± 2.46
3	29	12.5 ± 2.11	0.8 ± 0.20	2.0 ± 0.33	tr 3	2.9 ± 0.55	2.6 ± 0.30	16.2 ± 0.65	61.0 ± 2.17
4	12	10.4 ± 2.46	0.8 ± 0.42	3.0 ± 0.84	tr 3	2.1 ± 0.58	2.8 ± 0.55	16.9 ± 1.48	62.0 ± 1.64
Average secretion	80	14.6	1.2	2.1	tr	2.6	2.6	17.7	58.3



Ovarial development '0'



Ovarial development '1'



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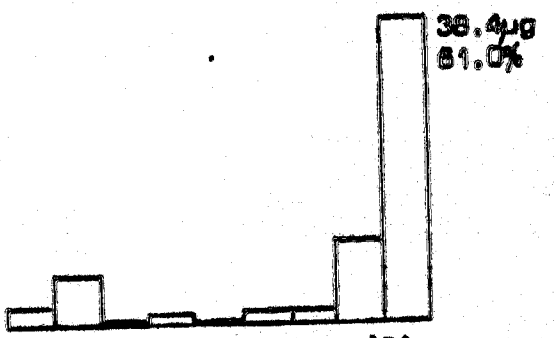
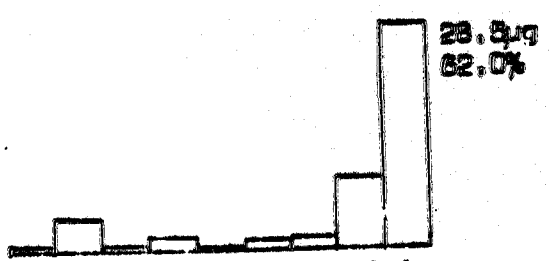


Ovarial development '3'



Ovarial development '4'

Figure 3.10. Head extracts of A.m. adansonii workers with different degrees of ovarial development that had been queenless for two months.

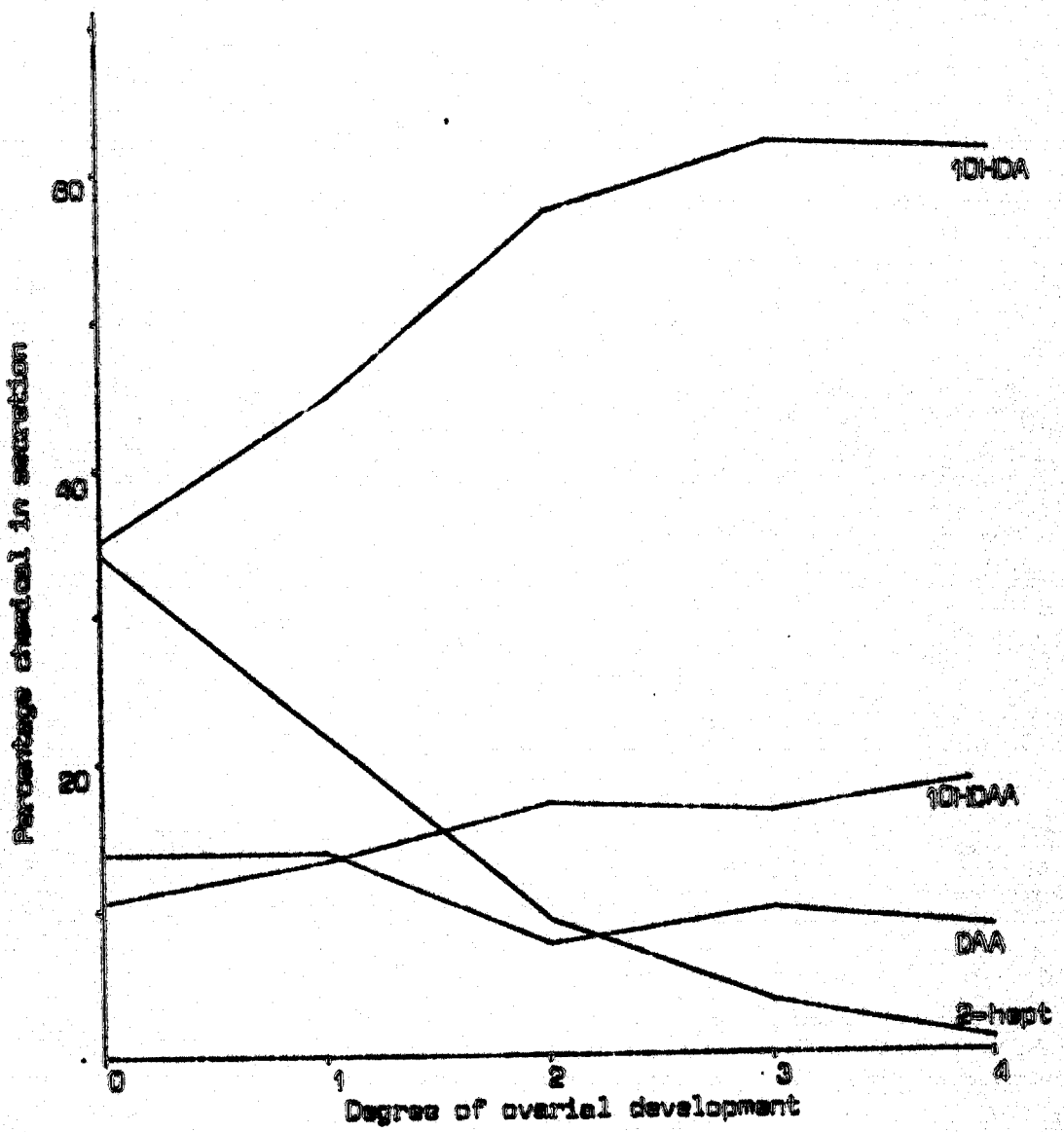


Figure 3.11. Amounts of DAA, 10HDAA, 10HDA and 2-heptanone in head extracts of queenless A.m.adansonii workers related to the degree of ovarial development

had less than one microgram of 2-heptanone in the mandibular gland secretion, while over 60 per cent of those with fully-developed ovaries (degree '4') had no 2-heptanone at all: the situation that exists in the queen secretion. In contrast, less than 30 per cent of workers with slightly-developed ovaries (Degrees '0', '1' and '2') were found to have less than a microgram of 2-heptanone (Appendix Table 5). The fact that certain individuals with little or no ovarial development were not secreting 2-heptanone clearly demonstrates that ovary activity and mandibular gland activity are not synchronous. Certain bees experienced considerable changes in secretion before their ovaries became active and vice versa. Ignoring the degree of ovarial development, it was found that the levels of 10HDA, 10HDAA and 2-heptanone were very closely related to levels of DAA (Figure 3.10 to 13), low levels of DAA being associated with low levels of 2-heptanone and with high levels of 10HDA and 10HDAA (Table 3.16). Tables 3.17 and 3.18 show that a number of the minor components of the mandibular gland secretion, including 9HDA, were also related to the level of DAA, the amounts of all but pHBA increasing as DAA decreased. The tables also indicate that, on average, bees that experienced a reduction in the secretion of DAA, had undergone greater ovarial development and possibly had more ovarioles per ovary than bees that were secreting large amounts of DAA.

The mandibular gland secretions of small groups of young queenless *A.m. adansonii* workers

The mandibular gland secretions of ten small groups of queenless workers (five bees in each group) were analysed and related to the number of ovarioles, the degree of ovarial development and the social behaviour of each individual (Appendix Tables 7 and 8). The summaries of social activity between the members of each group are presented in Appendix Tables 9a-9j.

Under the conditions tested the mandibular gland secretions could not be related to social activity as defined. There were no significant differences between the secretions of the bees with different levels of social activity. The 9HDA :10HDA ratio was found to be about 1:10 on average, although there was some indication that it tended to be higher in more active groups. This was not true for individual bees however.

Three workers found to be secreting small quantities of 9HDA had an average 9HDA :10HDA ratio of 2:10. It was expected that these individuals would be dominant (as defined) members of their groups as

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