Mandibular gland pheromone contents in workers and queens of *Apis mellifera adansonii* (Latr.)

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Short title: Mandibular gland pheromones of *Apis mellifera adansonii*

Abstract

Secretions from the mandibular glands of honeybees have been studied extensively, with those of queens dominated by ώ-9 fatty acids and ώ-10 fatty acids dominating those of non-laying workers. *Apis mellifera adansonii* is one of the widely distributed sub-species of African honeybees. However, its mandibular gland pheromones have not been analysed previously. Using gas chromatography, we analysed the composition of mandibular gland pheromones in workers and queens of *A. m. adansonii* from Nigeria. Qualitatively workers and queens have similar pheromone profiles to those previously reported in other African sub-species of honeybees. We found 9-ODA and high amounts of its precursor 9-hydroxy-2*(E)*-decenoic acid (9-HDA) in workers, thus showing that they produce queenlike signals under queen right conditions. We also found geographic variation in the pheromone profiles and morphometric characters of these workers suggesting different pheromone and morpho-clusters from the different ecological and climatological regions inhabited by *A. m. adansonii* in Nigeria.

Keywords: **African honeybees/queenright/mandibular gland secretions/pheromone clusters/morphoclusters**

Introduction

Social organisation driven by reproductive dominance is a key characteristic of eusociality in insects. In honeybees, this is mainly controlled through effective communication between the queen and workers in the colony. The honeybee colony provides a locale in which cues ranging from the temperature produced by active workers in the brood nest (Basile et al., 2008) to brood pheromones emitted by the open brood (Le Conte et al., 1990) and glandular secretions (especially those from the mandibular glands relating to reproductive dominance Crewe and Velthuis, 1980), can interact and determine responses by the members of the colony.

Queen pheromone components function primarily to keep the colony in a queenright condition by signalling the presence of a viable queen to other workers throughout the colony or over short distances. Chemical signals from the mandibular gland secretions of honeybee queens (*Apis mellifera*) have been implicated in the control of behavioural and physiological activities such as eliciting retinue behaviour among workers (Slessor et al., 1988), inhibiting emergency queen rearing as well as ovary activation in workers (Butler, 1959). The constituents of this gland have been studied in detail, and the active components identified as methyl *p*-hydroxybenzoate, 9-oxo-2*(E)*-decenoic acid (9-ODA) (which constitutes about 80% of the secretions in mated queens), 4-hydroxy-3-methoxyphenylethanol (HVA), (*R*,*E*)-9-hydroxy- 2-decenoic acid (9-HDA), (*S*,*E*)-9-hydroxy- 2-decenoic acid (9-HDA), 10-hydroxy-decanoic acid (10-HDAA) and 10-hydroxy-2*(E)*-decenoic acid (10-HDA) (Slessor et al., 1988; Winston et al., 1989). 9-ODA, the two enantiomers (*R* and *S*-9HDA), and the two aromatic compounds (HOB and HVA) are referred to collectively as the queen mandibular pheromones (QMPs) reviewed in Pirk et al. (2011). These five compounds act together to maintain reproductive dominance and elicit worker retinue behaviour. Keeling et al. (2003)

identified four other components that are not of mandibular gland origin, namely methyl (*Z)*-octadec-9-2-en-1-ol (methyl oleate), *(E)*-3-(4-hydroxy-3-methoxyphenyl)-prop-2-en-1-ol (coniferyl alcohol), hexadecane-1-ol, and (*Z9, Z12, Z15*)-octadeca-9, 12, 15-trienoic acid (linolenic acid) which act in synergy with QMPs to elicit retinue behaviour. This lead to a terminology shift from QMP to queen retinue pheromones (QRP) (Slessor et al., 2005). In addition, the mandibular glands of non-laying workers produce large amounts of (*E*)-10-hydroxy-2-decenoic acid (10-HDA) known as worker substance, which is also found in royal and worker jellies and has been implicated in queen determination in larvae (Spannhoff et al., 2011).

Africa is home to ten (Hepburn and Radloff, 1998) or eleven (Ruttner, 1988) morphoclusters (sub-species) in the *Apis mellifera* complex that have distinct geographic distributions and morphologies. However, we only know the composition and spectrum of the mandibular gland fatty acids from four sub-species i.e. *capensis*, *scutellata*, *intermissa* and *saharensis* (Crewe, 1982; Crewe and Moritz, 1989; Hepburn and Radloff, 1996). This knowledge has shown that the composition of pheromone bouquets from queens and workers are sub-species specific, especially in relation to the ability of *capensis* workers to become facultative social parasites and mimic the pheromone bouquet of queens in their host colonies (Crewe and Velthuis, 1980). Such variation in the composition of an important primer and releaser pheromone which affects both worker and queen behaviour in the hive needs to be understood not only in races of African honeybees, but also in other races. An understanding of these differences is important for the management and conservation of different races of honey bees on which sustainable apicultural practises will be dependent.

The West African honeybee *A. m. adansonii* (Latreille) is found along the West African coast of Senegal, Chad, Nigeria, far south Cameroon (Hepburn and Radloff, 1998), the Congo basin as well as Angola to the South (Ruttner, 1988). This makes it one of the two sub-species with the widest geographic range amongst African sub-species. However, despite its wide distribution and being the first African subspecies described by Latreille (in Ruttner (1992)), there is no information on the chemistry and composition of mandibular gland pheromones in *A. m. adansonii* from any of its native range.

To gain an insight into its chemical signals and how these vary along an ecological gradient, we compared the mandibular gland pheromone composition of *A. m. adansonii* queens and workers from different regions of Nigeria. We sampled workers from three regions and queens from two regions of Nigeria (North West (Sudan savannah), North Central (Guinea forest savannah) and South West (rainforest) to examine the variation in mandibular gland pheromone composition as well as bee morphology across these ecological zones. We hypothesise that, mandibular gland pheromones in both workers and queens of *A. m. adansonii* will be the same to those of the A branch of the *mellifera* complex (Ruttner, 1998) especially *A. m. scutellata*. In addition, we expect that there will not be any geographic variation in the proportions and amounts of *A. m. adansonii* mandibular gland pheromones and morphometric characters across Nigeria.

Materials and methods

Honeybees

Workers: Workers (total = 383) of *A. m. adansonii* were sampled from frames in 30 queen right colonies from apiaries at Jaja (11°15′N, 07°39′E), Tashar Fulani (11°17′N, 07°40′E), Shika Dam (11°08′N, 07°40′E), Unguwan Dan Asabe (11°09′N, 08°20′E), Zabi (11°18′N,

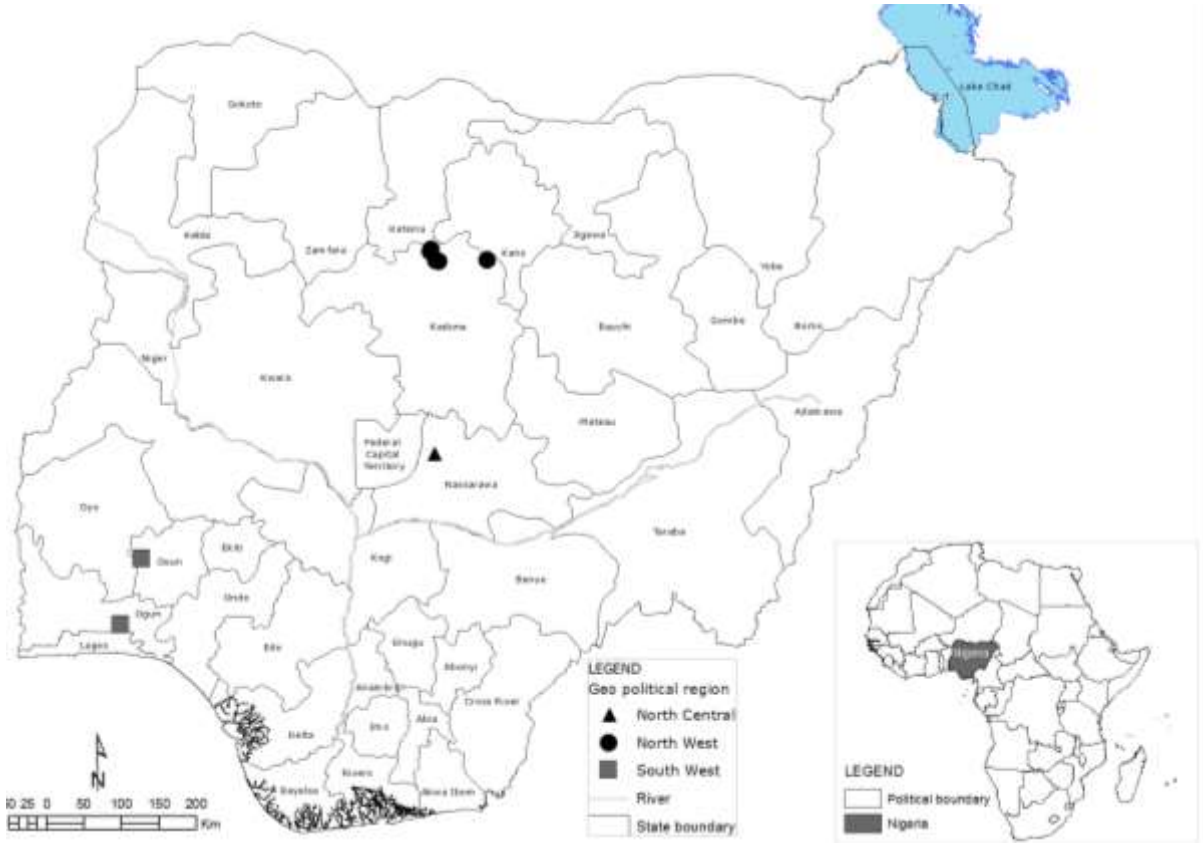

Fig. 1 Map of Nigeria showing apiaries where samples were collected from Jaja, Tashar Fulani, Unguwar Dan Asabe in Zaria Kaduna state North West (circles), Roguwa, Keffi Nasarawa state North Central (triangle), Iwo Osun state and Ijebu-Ode, Ogun state South West (squares) Nigeria.

07°43´E) in Zaria, Kaduna State (North West), a commercial apiary in Roguwa (08°49´N, 07°43´E) Keffi, Nasarawa State (North Central), and two apiaries from Iwo (07°33´N, 04°11´E) Osun and Ijebu-Ode (06°46´N, 03°56´E) Ogun States (South West), Nigeria (Fig. 1) between December 2011 and January 2013. The colonies sampled were from sites, characterised by stationary smallholder operations. The workers were randomly sampled, placed in clean labelled, perforated plastic vials, and immobilised on ice. The heads were then decapitated and placed into a clean 2ml vial containing dichloromethane (ChromSolv® grade for HPLC Sigma Aldrich, St. Louis, MO, USA), and refrigerated until analysis.

Queens: Naturally mated, egg laying queens were sampled from 17 colonies (13 from North West, and 4 from North Central), in apiaries where queen rearing and controlled mating were not practised. Unfortunately, no queens were collected from apiaries in the South West since the beekeepers were reluctant to sacrifice their queens. Queens were collected from colonies and the heads sampled in a similar way as described for workers above.

Extraction of Mandibular gland pheromones from heads

Heads were extracted in 200µl of dichloromethane (HPLC grade, Sigma Aldrich) following the methods described in Dietemann et al. (2006) and Zheng et al. (2010). Prior to gas chromatography, each extract was divided into two and half was stored as a back-up for further analysis or confirmation (if need be). The other half (~100µl) was transferred into a vial and evaporated to dryness under a gentle stream of charcoal filtered nitrogen gas. The residue was then re-dissolved in 10µl of internal standard solution (containing 1mg of octanoic acid and 1mg of tetradecane in 4ml dichloromethane) to which 10µl of bis-(trimethylsily) trifluoroacetamide (BSTFA) was added to derivatise the fatty acids.

Gas-chromatography

One micro litre of the derivitised extract from above was injected in splitless mode into an Agilent 6890 gas chromatograph fitted with a 25 m × 0.20mm × 0.33 µm HP1-methyl silicone coated fused silica capillary column and an FID detector. The carrier gas was helium at a flow rate of 1ml/min; and oven temperature programmed as follows: 50˚C at 50˚C/min to 100˚C, then increased at 3˚C /min to 220 ˚C, and then held at 220 ˚C for 10 min (modified after Simon et al., 2001; Dietemann et al., 2006; Tan et al., 2012). Chromatograms were recorded and the peak areas quantified using Chemstation® software. Six of the major components from mandibular glands of honeybees had been shown to elicit both behavioural

and physiological responses, (namely; methyl p-hydroxybenzoate, 9-oxo-2(*E*)-decenoic acid (9-ODA), 4-hydroxy-3-methoxyphenylethanol (HVA), 9-hydroxy-2(*E*)-decenoic acid (9-HDA), 10-hydroxy-decanoic acid (10-HDAA) and 10-hydroxy-2(*E*)-decenoic acid (10-HDA)) were identified based on comparison with retention times of synthetic standards. Quantification was achieved by comparing the relative mass ratios (RMR) of each of these compounds in a standard solution mixture (containing ~1mg of each in 4 ml dichloromethane) relative to the RMR of tetradecane. We did not separate the enantiomers of 9-HDA in our analysis, and therefore reported both enantiomers together through out the study. As a check for shifts in retention times of compounds between GC runs, standard mixtures were run before each batch of head extracts and then after every 25 to 30 samples. All standards and reagents with the exception of 9-ODA and 9-HDA (PheroTech Inc.) were obtained from Sigma Aldrich.

Morphometrics

Ten worker bees (not those used for pheromone analysis) were sampled from each of the 30 colonies used in this study. The bees were preserved in 90% ethanol and later dissected according to the methods of Ruttner (1988). The following seven morphometric characteristics based on the Oberursel standard list (Ruttner, 1988) were measured: forewing length (FL), forewing width (FW), hindwing length (HL), hindwing width (HW), proboscis length (PL), number of landmarks on the forewing (NF), and number of landmarks on the hindwing (NH) were measured. All together, 300 worker bees were analysed and the values expressed in mm except for NF and NH which were count data.

Statistical analyses

Since data for both percentage composition and amounts were not normally distributed, non-parametric statistical tests were applied. Kruskal-Wallis ANOVA (KWA) and median test with multiple comparisons was applied to determine differences in the individual as well as total MDG components in workers between regions, with regions as the independent (grouping) variable and the MDG components as dependent variables. Since no queens were sampled from South west, a Mann-Whitney U test (MWU) was used to test for significance in MDG components between queens from North central and North West. Cluster analyses (Johnson and Wichern, 1998) were performed using the means for both proportions and amounts of pheromones in order to determine the linkage/Euclidean distances between workers from the three regions. Multivariate statistical analyses, including principal component analysis, canonical analysis and linear discriminant analysis were employed on the morphological characters to classify colonies in relation to their geographic region. The population means between groups were compared using Wilks' lambda test and its distribution approximated by the *F*-distribution (Johnson and Wichern, 1998). Results unless otherwise stated are presented as means ± the standard errors of the mean. All statistical tests were carried out using the software STATISTICA 12 (StatsSoft Inc. USA), and α was set at 0.05.

Results

Proportion of MDG components in workers and queens

The proportions of the major MDG components from the head extracts of workers and queens of *A. m. adansonii* are presented in Fig 2 A(i) and A(ii) respectively. All the major components with the exception of HVA were present in workers, with 10-HDA and its

precursor 10-HDAA dominating the profile at 43.49 ± 1.22% and 23.99 ± 0.87% respectively, followed by HOB, 9-HDA and 9-ODA respectively. The average proportions of 9-ODA and 9-HDA that are the major components of queen signals was 17.26 ± 0.83% (Fig. 2A (i)).

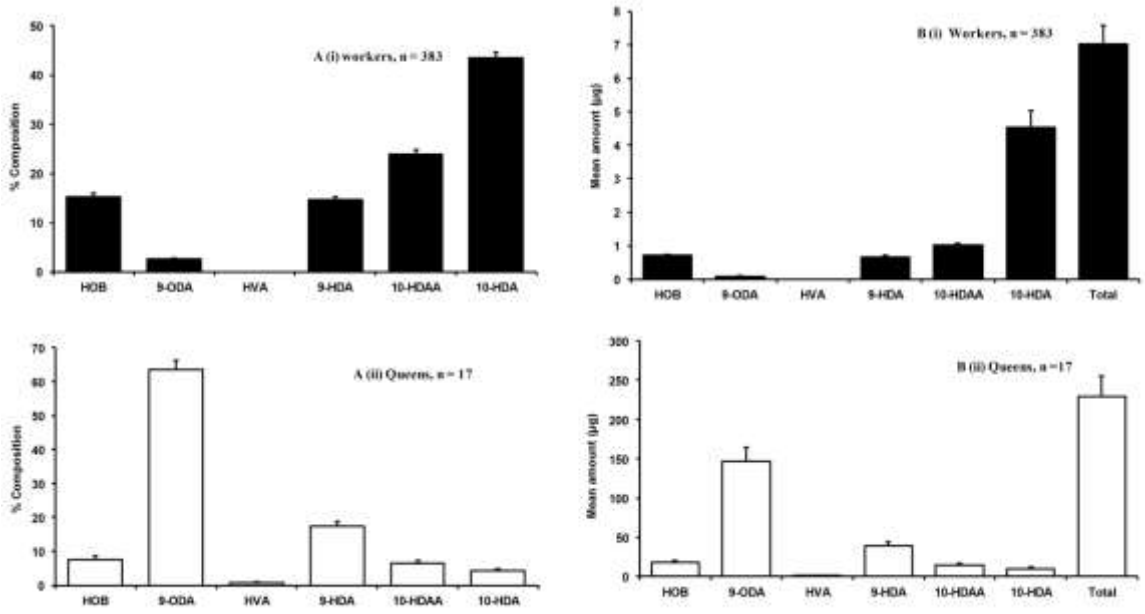

Fig. 2 Percentage composition (A) and amount (µg) (B) of mandibular gland pheromones in workers (black bars) and queens (open bars) of *Apis mellifera adansonii*. Please note the differences in scales for workers and queens of the amounts in µg. Mandibular gland components are: HOB = p-hydroxybenzoate, 9-ODA = 9-oxo-2*(E)*-decenoic acid, HVA = 4-hydroxy-3-methoxyphenylethanol, 9-HDA = 9-hydroxy- 2*(E)*-decenoic acid, 10-HDAA = 10-hydroxy-decanoic acid and 10-HDA = 10-hydroxy-2*(E)*-decenoic acid. Error bars are standard errors of the means, n= number of samples.

In the case of the queens, all six major and behaviourally active MDG components were present. The 9-ODA was the most abundant at 63.64 ± 2.74 %, followed by its precursor 9-HDA, HOB, then the two worker components 10-HDAA and 10-HDA, with HVA as a minor component (Fig 2 A (ii)).

Absolute amounts of MDG components in workers and queens

Absolute amounts were very variable in the mandibular glands of workers (Fig 2B(i)) with total absolute amounts averaging 7.0 ± 0.55µg per bee. Even though the worker component 10-HDA was highly variable at 4.53 ± 0.49 µg, it was the most abundant component in the mandibular glands of workers. This was followed by its precursor 10-HDAA (1.03 ± 0.06 µg), HOB (0.71 ± 0.04 µg) and some queen substance 9-ODA (0.09 ± 0.01 µg) and its precursor 9-HDA (0.66 ± 0.05 µg).

In queens, the total absolute amount was 229.37 ± 25.54 µg per queen with 9-ODA at the level of 146.44 ± 17.74 µg, its precursor 9-HDA (39.17 ± 5.09 µg) with HVA as the minor component at 1.54 ± 0.28 µg (Fig 2 B(ii)).

Regional variability in the pheromone profiles of workers

Results from the three regions (North Central, North West and South West Nigeria, Table 1)

Table 1. Relative proportions (mean ± SE) of mandibular gland pheromones from head extracts of *Apis mellifera adansonii* workers and their differences between the North central (NC), North west (NW) and South west (SW) regions of Nigeria.

Compounds	**North Central (NC)** Mean proportion ± SE	**North west (NW)** Mean proportion ± SE	**South west (SW)** Mean proportion ± SE	**Kruskal Wallis ANOVA H, *p***	**Differences**
HOB	13.24 ± 1.49	15.9 ± 1.10	15.04 ± 0.91	H = 2.103, *p*= 0.3495	None
9-ODA	3.83 ± 0.72	3.30 ± 0.40	1.27 ±0.15	H = 43.470, *p*= 0.0000	NC, NW, SW
9-HDA	15.65 ± 1.52	16.8 ± 0.90	11.52 ± 0.74	H = 18.607, *p*= 0.0001	NC, NW, SW
10-HDAA	23.95 ± 1.84	28.7 ± 1.40	17.75 ± 0.93	H = 24.460, *p*= 0.0000	NC, NW, SW
10-HDA	43.33 ± 3.26	35.3 ± 1.70	54.42 ± 1.62	H = 58.950, *p*= 0.0000	NC, NW, SW

HOB = p-hydroxybenzoate, 9-ODA = 9-oxo-2*(E)*-decenoic acid, HVA = 4-hydroxy-3-methoxyphenylethanol, 9-HDA = 9-hydroxy- 2*(E)*-decenoic acid, 10-HDAA = 10-hydroxy-decanoic acid and 10-HDA = 10-hydroxy-2*(E)*-decenoic acid.

showed that, the proportions of 9-ODA, 9-HDA, 10-HDAA and 10-HDA were different (KWA: H (2, N = 383), $p < 0.05$) while that of HOB were not significantly different (KWA: H (2, N =383), $p = 0.3495$) irrespective of the region sampled. Profiles of workers from South west had the most (54.42 ± 1.62 %) 10-HDA (workers substance) in comparison to those from North central and North west respectively (Table 1). Interestingly, those workers from the North west and North central had proportionally more of the queen substance 9-ODA and its precursor 9-HDA compared to those from the South west (Table 1). Based on the mean proportions of their pheromones, workers from North central and North west formed a distinct pheromone cluster from those in the South west (Fig. 3).

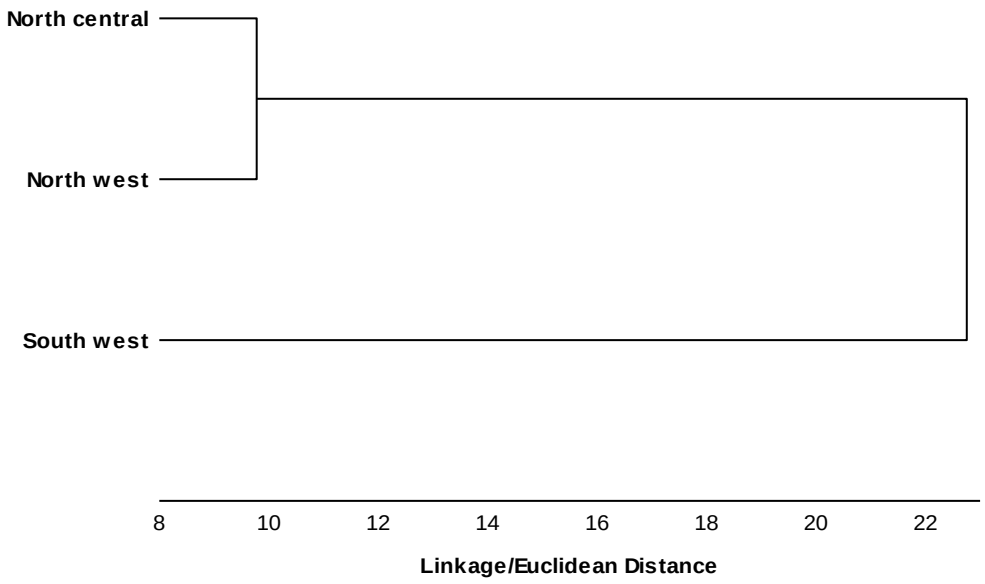

Fig. 3 Dendrogram from Cluster analysis of the workers from North central, North west and South west Nigeria based on mean proportions of each chemical from the head extracts showing the two pheromone clusters (one for workers from North central and North west, and the other for workers from South west). The Euclidean distances are 9.8, 13.7 and 22.8 for North central, North west and South west respectively.

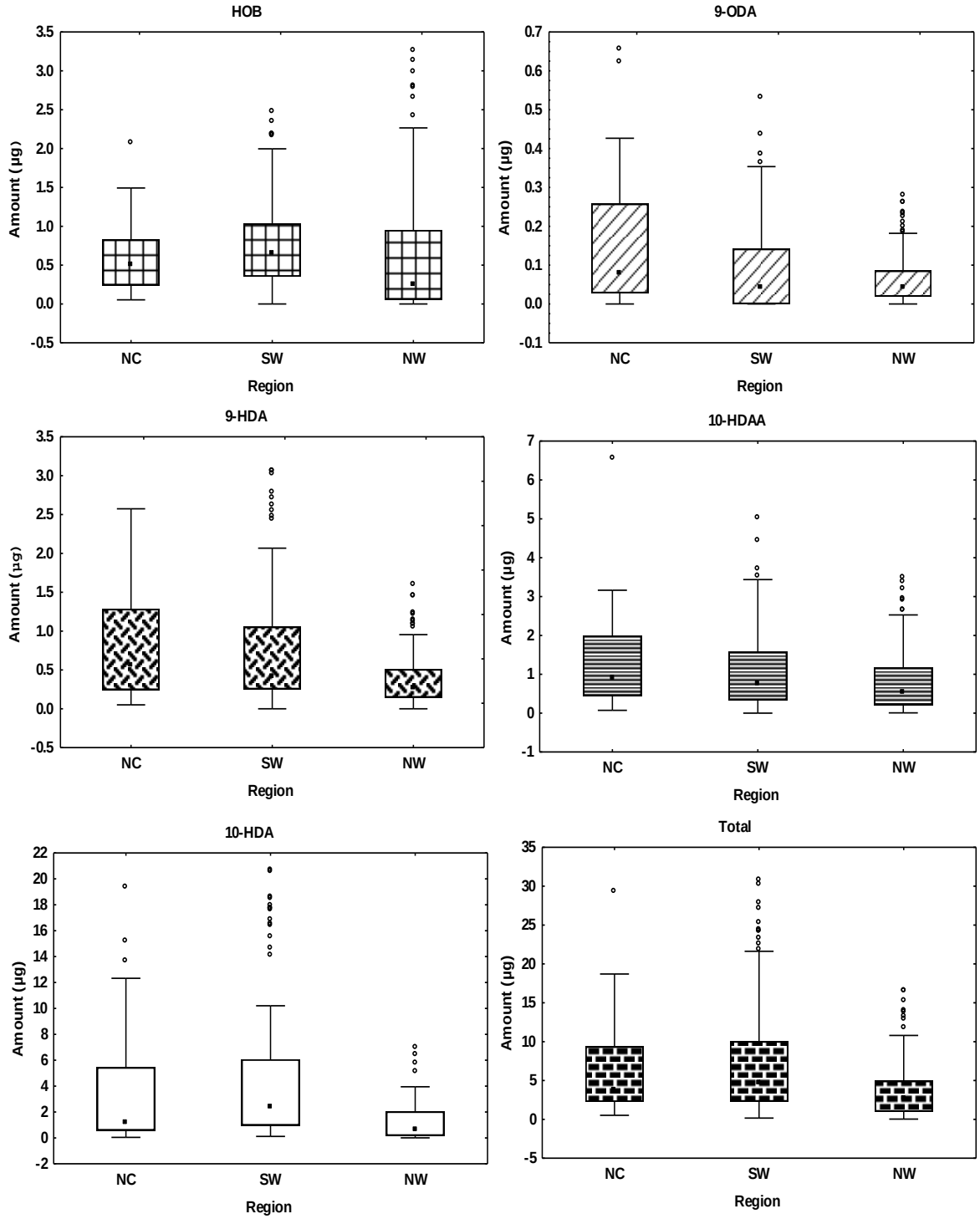

Fig. 4 Amount of five major components of the mandibular gland pheromones (HOB, 9-ODA, 9-HDA, 10-HDAA, 10-HDA) and Total absolute amounts in workers of *A. m. adansonii* from three geographical regions of Nigeria. NC = North Central (n=45), SW = South West (n=145), NW = North West (n = 193). = Median, = 1st and 3rd quartiles, = non-outlier range, (minimum and maximum), = outliers.

The variation in absolute pheromone amounts among *A. m. adansonii* workers from the three regions is shown in Fig. 4. This was significantly different between the regions for all the worker components as well as the total absolute amount (KWA: H (2, N = 383), $p < 0.05$). The pattern of variation shown is in the following order North Central > South West > North West for the amount of 9-ODA, 9-HDA, and 10-HDAA. While the amount of HOB, 10-HDA and total amounts decreased in amount in the following order: South West > North Central > North West respectively (Fig. 4), thereby exhibiting an upward trend, increasing altitudinally as one travels through the hinterland (i.e. inwards from the South to the North (see Fig. 1). Mean total absolute amounts were 9.33 ± 1.15 µg per bee for South West, 6.93 ± 1.18 µg for North Central and 5.31 ± 0.73 µg per bee from North West respectively. A cluster analysis on the absolute amounts grouped the workers into two distinct pheromone clusters similar to the one for proportions (Fig. 5).

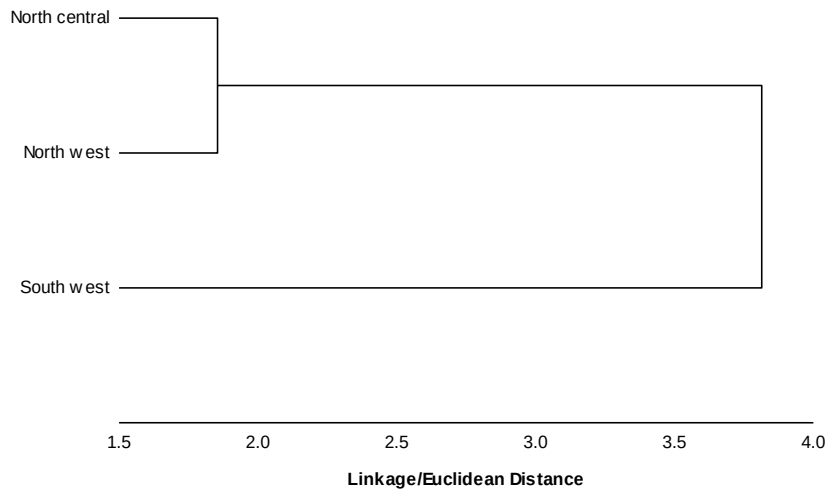

Fig. 5 Dendrogram from Cluster analysis of the workers from North central, North west and South west Nigeria based on mean absolute amounts (µg) of each chemical compound and the total amounts from the head extracts showing the two pheromone clusters (one for workers from North central and North west, and the other for workers from South west). The Euclidean distances are 1.85, 3.81 and 5.29 for North central, North west and South west respectively.

Regional variability in the pheromone profiles of queens

Proportional compositions of the MDGs in *A. m. adansonii* queens were not significantly different (MWU test $p > 0.05$) between those queens from the North Central and North West (Fig, 6). The queen substance 9-ODA, which dominated the pheromone profiles was 70.12 ± 9.42 % and 61.6 ± 2.2 % in queens from North Central and North West respectively (Fig. 6A).

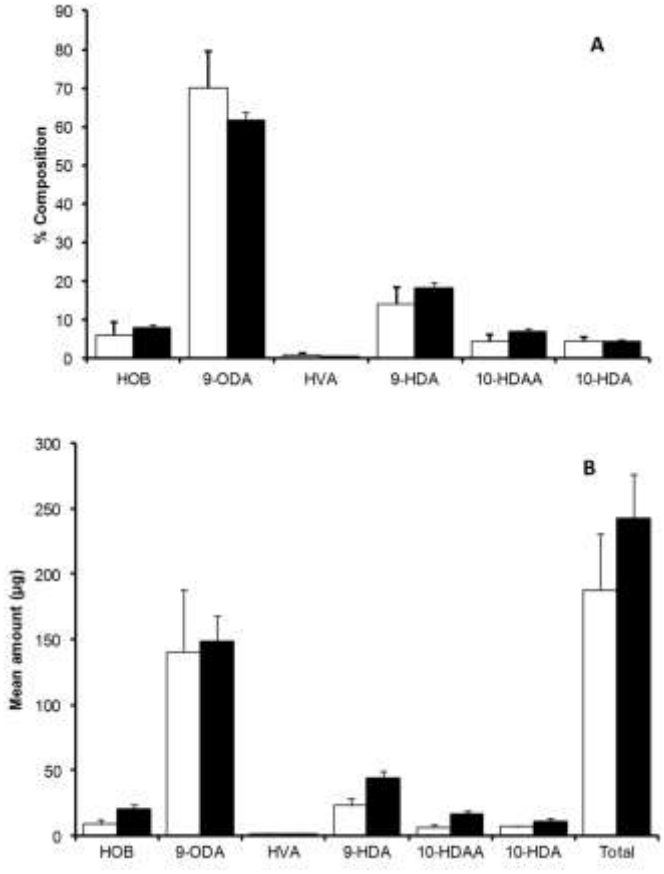

Fig. 6 Percentage composition (A) and mean amount (µg) (B) of the individual mandibular gland pheromones from heads of queens of *Apis mellifera adansonii* from North Central (open bars) and North Western (closed black bars) Nigeria. Mandibular gland components are: HOB = p-hydroxybenzoate, 9-ODA = 9-oxo-2*(E)*-decenoic acid, HVA = 4-hydroxy-3-methoxyphenylethanol, 9-HDA = 9-hydroxy- 2*(E)*-decenoic acid, 10-HDAA = 10-hydroxy-decanoic acid and 10-HDA = 10-hydroxy-2*(E)*-decenoic acid. Error bars represent standard errors of the means.

Absolute amounts of the MDG components in queens from the two regions are shown in Fig. 6B. No marked differences in the absolute amounts were observed between the queens from different regions. Overall comparisons between the queens showed that 9-HDA (the precursor of the queen substance 9-ODA) was slightly different (MWU test, Z = -1.9814, p = 0.047). However, comparatively queens from North West possess more MDG pheromones (Fig. 6B) with a mean total of 242.44 ± 33.96 µg per queen to 187 ± 43.21 µg per queen from the North Central.

Morphometric analysis

The measured morphometric characters are shown on Table 2. Length of forewing (FL) and the number of landmarks on both fore and hind wings were not different between workers from all regions (KWA, p =0.119, 0.2117 and 0.06 respectively). Width of forewings (FW), length of hind wings (HL) and proboscis (PL) were different across all regions (KWA, p = 0.0001, 0.0001 and 0.0002 respectively). By contrast, width of hind wings (HW) only differed in workers from the North West (KWA, p = 0.0007).

Table 2 Means and standard errors of measured morphometric characters (lengths are in mm) of *A. m. adansonii* workers from North Central (NC), North West (NW) and South West (SW) Nigeria.

Character[#]	**North Central (NC)** Mean ± SE	**North West (NW)** Mean ± SE	**South West (SW)** Mean ± SE	**Kruskal Wallis ANOVA H, *p***	**Differences**
	N = 40	N = 180	N = 80		
FL	6.83 ± 0.06	6.90 ± 0.03	6.98 ± 0.04	H = 4.38, p= 0.1119	None
FW	2.17 ± 0.03	2.45 ± 0.05	2.27 ± 0.04	H = 21.11, p= 0.0000	NC, NW, SW
HL	4.68 ± 0.04	4.98 ± 0.01	4.83 ± 0.03	H = 18.37, p= 0.0001	NC, NW, SW
HW	1.32 ± 0.02	1.37 ± 0.03	1.31 ± 0.02	H = 14.62, p= 0.0007	NW
PL	2.62 ± 0.02	1.79 ± 0.05	1.46 ± 0.12	H = 16.88, p= 0.0002	NC, NW, SW
NF	13.00 ± 0.00	13.00 ± 0.00	13.00 ± 0.00	H = 3.05, p= 0.2117	None
NH	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	H = 5.68, p= 0.06	None

[#]Morphometric characters were explained in the text. N = number of workers

The principal components analysis of the morphometric characters for the worker honeybees isolated these five factors; FL, FW, HL, HW, and PL with eigenvalues > 1 which accounted for 79.59% of the variance in the data. A stepwise discriminant analysis using the colony means of the morphometric characters separated the workers into three clusters (Fig. 7). From South West 87.5% of colonies were classified correctly, with posterior probabilities of p = 1.0 for 7 colonies and 0.08 for 1 colony (which was incorrectly classified). All colonies from the North Central and North West (100%) were classified correctly with posterior probabilities of p = 1.0. Mahalonabis distance D^2 between the clusters were 132.29 for South West, 66.53 for North Central and 37.93 for North West. Standard co-efficient of Canonical variance were 95.10, 14,29 (Wilk's Lambda = 0.0126, F (10, 46) = 36.611, $p < 0.0001$).

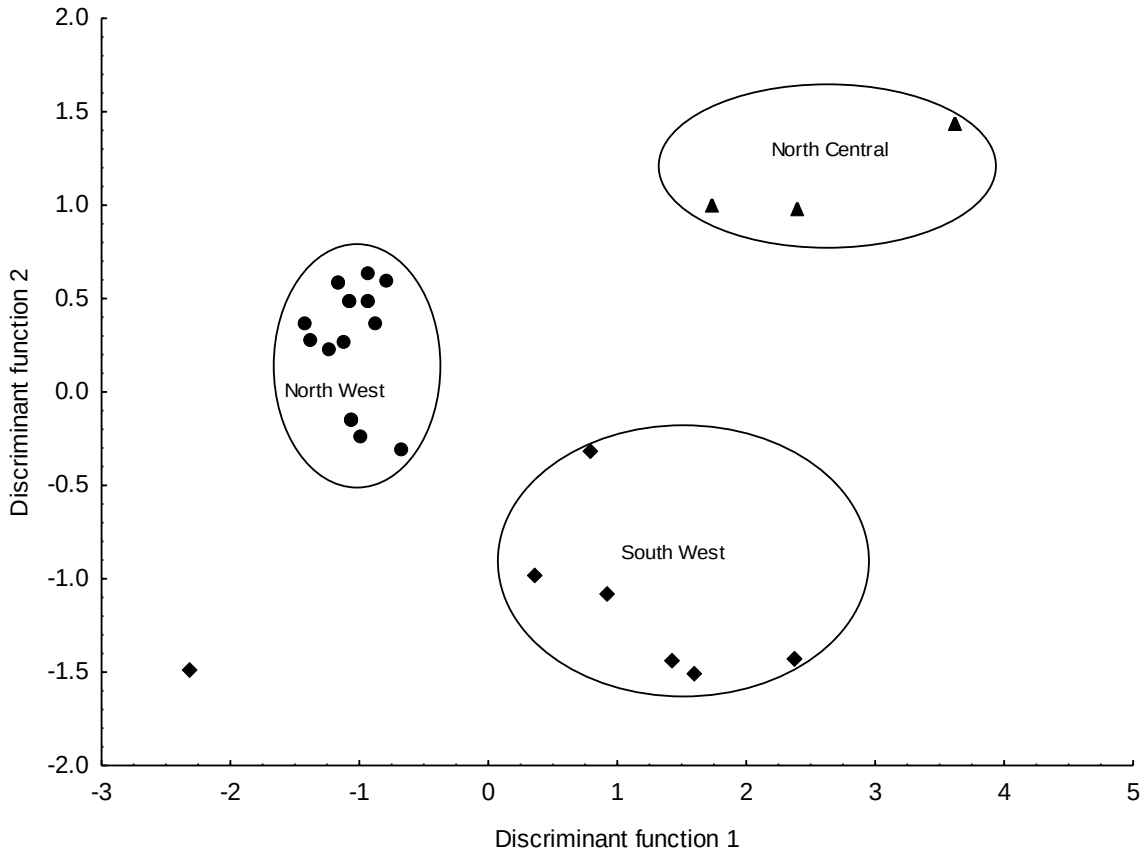

Fig. 7 Discriminant analysis plot showing discriminant functions 1 and 2 using the colony means of the morphometric data. Triangles, squares and circles represent workers from North central, South west and North West Nigeria. Confidence eclipses are drawn at the 95% level.

Discussion

One of the main findings here is that the mandibular gland pheromone profiles in workers and queens of *A. m adansonii*, a sub-species of African honeybees with a wide distribution range on the African continent, have a high degree of variability. We found in these head extracts all the main and behaviourally active compounds reported earlier from the mandibular glands of different castes in honeybees (Butler, 1959; Slessor et al., 1988; Plettner et al., 1997; Slessor et al., 2005).

The profiles of *A. m. adansonii* workers were dominated by 10-HDA and its precursor 10-HDAA (Fig. 2) similar to sub-species of the A sub-group such as *A. m. scutellata* (Zheng et al., 2010), *A. m. intermissa* (Crewe and Moritz, 1989) and *A. m. saharensis* (Hepburn and Radloff, 1996). Interestingly, the amounts of the queen substance (9-ODA) and its precursor (9-HDA) in workers of *A. m. adansonii* were higher than those reported for *A. m. scutellata*

Table 3 Percentage composition of the major components from head extracts of *Apis mellifera adansonii*, *A. m. capensis*, *A. m. intemissa*, *A. m. saharensis*, and *A. m. scutellata* workers. Values are means ± SD* from the mean.

	% composition of major components				
Sub-species	**9-ODA**	**9-HDA**	**10-HDAA**	**10-HDA**	**Reference**
A. m. adansonii	2.6 ± 4.9	14.7 ± 11.3	24.0 ± 16.9	43.5 ± 23.9	This study
A. m. capensis (laying workers)	76.2	7.7	0.5	5.5	Crewe & Velthuis (1980)
A. m. intermissa	1.2 ± 1.4	4.2 ± 3.4	0.1 ± 0.3	60.2 ± 11.3	Hepburn & Radloff (1996)
A. m. saharensis	1.7 ± 1.1	2.6 ± 0.8	0.4 ± 1.1	44.0 ± 10.0	Hepburn & Radloff (1996)
A. m. scutellata	Trace	4.7 ± 2.7	25.0 ± 5.0	70.0 ± 7.0	Zheng et al. (2010)

HOB = p-hydroxybenzoate, 9-ODA = 9-oxo-2*(E)*-decenoic acid, HVA = 4-hydroxy-3-methoxyphenylethanol, 9-HDA = 9-hydroxy- 2*(E)*-decenoic acid, 10-HDAA = 10-hydroxy-decanoic acid and 10-HDA = 10-hydroxy-2*(E)*-decenoic acid.

*Values are presented mean ± SD to ease comparisons with previous studies

(Zheng et al., 2010), *A. m. intermissa*, *A. m. saharensis* (Hepburn and Radloff, 1996) but lower than those for laying workers of *A. m. capensis* (Crewe and Velthuis, 1980) (Table 3) and Asian honeybees *A. andreniformis*, *A. florea*, *A. dorsata* (Plettner et al., 1997), *A. cerana* and *A. nigrocincta* (Keeling et al., 2001). Prior to this study, the ability of workers in African honeybees to produce substantial amounts of 9-ODA under queenright conditions had only been reported in *A. m. capensis* (Zheng et al., 2010) and *A. m. intermissa* (Crewe and Moritz, 1989) workers and had been implicated in the ability of workers of these sub-species to rapidly develop into laying workers (Ruttner and Hesse, 1981). If we compare the amounts of 9-ODA produced by workers of *A. m. adansonii* with those of both *capensis* and *intermissa*, we infer that *adansonii* workers are likely to develop into laying workers more slowly than those of *capensis* but faster than those of *intermissa* and *scutellata* respectively. However, this needs to be verified experimentally in the case of *adansonii*.

The presence of substantial amounts of 9-HDA in the head extracts of *A. m. adansonii* workers is also of interest since 9-HDA is a precursor of 9-ODA and high proportions of the two components are referred to as queen-like (Moritz et al., 2004). 9-HDA together with 9-ODA plays an active role in eliciting retinue responses (Slessor et al., 1988), maintaining swarm clusters (Winston et al., 1982), inhibiting queen rearing (Butler and Callow, 1968), and attracting drones (Brockmann et al., 2006). Workers producing queen-like signals in the presence of a queen could pose a threat to social organisation in colonies of other sub-species as seen in *capensis* workers when they invade colonies of other sub-species, but function effectively in their own colonies (reviewed in Neumann and Hepburn (2002). At this point we do not know if workers of *adansonii* will exhibit similar behavioural traits.

Queens of *A. m. adansonii* are similar in both the composition and absolute amounts of mandibular gland components as those reported for other African subspecies like *capensis*,

intermissa, and *scutellata* (Crewe, 1987), with 9-ODA and 9-HDA as the main components. Slightly different from the results on Africanized honeybees which on average only had around 100 µg of 9 ODA (Pankiw et al 1996). This is very important and shows how conserved pheromones of the mandibular glands are in queens compared to those of workers. Recently Van Oystaeyen et al. (2014) proposed that a conserved class of queen pheromones (mainly long chained hydrocarbons and esters in ants, wasps and bumble bees) were responsible for stopping worker reproduction. The expression of the chemical components of the queen pheromone in the different castes needs to be explored. Since if workers produce some 9-ODA and 9-HDA under queenright conditions this may be a way to reduce the inhibitory effect of the queen pheromones on themselves and increase the effect on other workers (Moritz and Crewe, 2005), enabling these individuals to get a head-start in the reproductive race if the colony becomes queenless.

Morphological characters revealed three morpho-clusters that put workers from the North much closer to each other than those from the South West. The morphometric data also provided support for the pheromone data showing workers from the North West closer to those from the South.

We found differences in both proportions and absolute amounts from the head extracts of the mandibular gland pheromones; and in morphometric characters of workers from the three regions sampled, with workers from South West being distinct from North Central and North West respectively. There are two possible propositions for this distinction based on pheromone profiles and morphometric characters. One is that, there are possibilities that the workers from the South West are *adansonii* while those from the North Central could be hybrids between *adansonii* and *jemenitica*. Both our pheromone and morphometric data supported earlier work by Hepburn and Radloff (1998) which used morphometric and

multivariate analysis to group honeybee workers from West Africa into three morphoclusters based on ecological and climatological characteristics. Thus; *jemenitica* in the sahelian and dry tropical zones, *adansonii* occurred in wet tropical and equatorial zones and hybrids between *adansonii* and *jemenitica* were found in the savannah. However, this does not explain the differences we found between the pheromones of workers from the North central and North West. Since the vegetation in Northern central is guinea savannah whilst that from the North West is Sudan savannah (Rabiu et al., 2011).

Our second proposition is that all the workers are *adansonii* and the differences we observed in the pheromone profiles and morphometrics are as a result of altitudinal, climatological as well as ecological differences between the regions. Indeed, Ruttner (1992) related the morphological differences among African subspecies to altitudinal, climatological as well as ecological parameters. Earlier work (see Mutsaers, 1991; Mbah and Amao, 2009; Ebenezer and Olugbenga, 2010) had also revealed different bee forage plants each for the South west, North West and North Central, Nigeria.

We have reported here a detailed account of the mandibular gland profiles from head extracts of *A. m. adansonii* workers from three ecological regions in Nigeria and have shown that these are similar qualitatively to those of A-group sub-species. We further revealed compositional and quantitative differences in pheromone profiles as well as morphometric characters among honeybee workers from the different regions. We hypothesize, based on the pheromonal data, that pheromonal development and ovarian activation would be faster in the Northern populations compared to the southern ones. Also that the values of both parameters will lie between that of *A. m. capensis* and *A. m. scutellata*, two neighbouring subspecies to the East and South. Our results also provide more insight into the nature, and to some extent, the variability present in the products from the exocrine glands of honeybees, thus supporting

earlier evidence in Crewe (1982) on how compositional variability serves as a key to social signals which cannot be ignored due to their importance in apiculture especially when the sustainable use of honeybee populations is a key requirement for food security.

Acknowledgements

The authors acknowledge Alhaji Idris Barau and Adamu Usman of the Beekeeping Extension Society, Zaria Nigeria for facilitating the sampling in Nigeria. Femi Kesington of Iwo Apiaries, Babatunde Oreyemi of Oyinladun Beekeepers Society Ijebu-Ode, Management of Roguwa Farms and other beekeepers for allowing us access to their apiaries. Habibu Aliyu, Ejuailo Dominic and Yusuf Shuaibu of the Department of Biological Sciences Ahmadu Bello University Zaria, assisted with the morphometric measurements and logistics. Funding for this research was provided in part by the South African National Research Foundation (NRF) and University of Pretoria (2011 and 2012) and funding to AAY from the DST/NRF SARChI Chair in Mathematical Models and Methods in Bioengineering and Biosciences (M^3B^2) University of Pretoria (2013).

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