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ORIGINAL RESEARCH ARTICLE

Presence or absence of drones in 'drone' dusk mass flights performed by *Apis dorsata* forager bees

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Summary

Apis dorsata drones perform dusk mass flights (DMF's) shortly after sunset. Bees performing DMF's were caught near the nests with insect nets. Presence of drones within captured bees was determined and the colour of worker bees was examined to classify them in age-colour categories. A total of 15,160 individual bees were examined. It was found that in the spring, drones were practically absent in arriving swarms. The percentage of drones, which participated in dusk mass flights, increased to 19% after the colonies stayed at the site for six weeks. A correlation was found between the duration of time the colonies stayed at the place and the percentage of drones, which performed those flights. In autumn, when environmental conditions deteriorated and the colonies migrated, drones were absent practically in all the colonies at the site. Of 4,450 bees captured in autumn, only 2 drones were found during 30 DMF's performed by 7 colonies. Thus, worker bees performed 'drone' DMF's despite the absence of drones in the colonies. Surprisingly, 90% of bees participating in the 'drone' dusk mass flights were foragers contrary to the diurnal periodic mass flights performed by 75% of the young curtain and intermediate bees. Thus, dusk mass flights and diurnal periodic mass flights are performed by different categories of worker bees.

Keywords: *Apis dorsata*, drone flights, dusk mass flights, periodic mass flights, colour of bees

Introduction

Apis dorsata colonies perform short (8 min) daily periodic mass flights (PMF's) ranging in number from 0 to 6 (Woyke *et al.* 2004). PMF's look like swarming. The flights play a role in the cleansing and orientation of young bees. Shortly after sunset, the colonies also perform longer (about 20 min) dusk mass flights (DMF's). According to Koeniger and Wijayagunasekara (1976), Koeniger *et al.* (1988), Rinderer *et al.* (1993), Koeniger *et al.* (1994), Otis *et al.* (2001) and Woyke *et al.* (2001b) those flights are performed by *Apis dorsata* drones. The queens also fly during such events (Tan *et al.* 1999). However, Woyke *et al.* (2005b) found that not only drones, but also worker bees, participate in DMF's. Almost nothing is known about 'drone' DMF's performed by worker bees. It is very important to understand the DMF's activity performed daily by all *A. dorsata* colonies.

The proportion of the number of drones to the number of workers performing DMF's may vary during the year. However, it is not known when *A. dorsata* drones are present in the colonies. According to Koeniger and Wijayagunasekara (1976), drones were present in colonies on large combs. Thus, it would be important to find out whether a correlation exists between the size of combs the colonies occupy and the number of drones that participate in DMF's.

Woyke *et al.* (2005b) found, that among bees performing DMF's, drones represented on the average only 11% of that population, with 68% as the maximum. The question is whether worker bees also perform 'drone' DMF's in unfavourable environmental conditions when drones are absent in all the colonies. If they do, the next question would be whether DMF's performed in presence of drones (Woyke *et al.* 2005b) differ from those performed in the absence of drones. This was one of the key questions of the present investigation. The presence of worker bees in DMF's indicates that worker bees

perform two types of mass flights: the dusk mass flights (DMF's) and the day-time periodic mass flights (PMF's). Kastberger *at al.* (1996) descried in details one PMF and Woyke *et al.* (2004 and 2005a) many PMF's. It is known that the duration of DMF's performed by worker bees is longer, but their intensity is lower than that of PMF's (Woyke *et al.* 2005b). However, it is not known whether the total number of bees, which perform both types of flights, is similar, or if the difference is only in the extended duration of DMF's.

It is known that PMF's are performed by young curtain bees. However, it is not known whether the same young worker bees also perform DMF's. Otis *et al.* (1990) recorded that young *A. dorsata* workers have pale yellow abdomens, which become orange and black at the time the bees began to forage. Woyke *et al.* (2000) described several age-colour categories of worker bees. Therefore, it should be possible to examine which age categories of bees perform DMF's, and which PMF's.

It would be also important to know whether internal colony conditions such as presence or absence of brood have any influence on the characteristics of PMF's and DMF's performed by worker bees.

Thus, the purpose of this investigation was: a) to determine if drones and worker bees are absent or present in all DMF's and in which proportion b) to compare DMF's and PMF's performed by worker bees of the same colonies, c) to determine the status/ size of the colonies and age of bees performing those flights, d) to answer the question 'why worker bees perform the 'drone' DMF's in the absence of drones'.

Materials and Methods

The observations were made in Bangalore, India (lat. 12° 15' N, long. 77° 35' E, alt. 930 m) from March 10 to 21, 2002, and from November 20 to December 3, 2005. In the spring of 2002, at the beginning of the observation, there were 19 colonies on the southern balcony of the Polytechnic Building. Seven swarms arrived during the period of observation increasing the number of the colonies to 26. The weather was fine and the environmental conditions were favorable for the bees. Evidently, this was the time of arriving of new swarms and development of the colonies. Investigations were conducted on 13 colonies, to which direct access was possible.

In the autumn of 2005, only 4 colonies were present at the Polytechnic, and 4 at the Agricultural University building, although in the spring 2002, we conducted here investigation on 82 colonies (Woyke *et al.* 2005a). One month before the present investigation, the monsoon, with its heavy rainfall, occurred. The bees could not forage, and as a result, many colonies emigrated. Evidently the environmental conditions were unfavourable for the bees and this was the period the colonies emigrated. Investigation was conducted now on 4 colonies at the Polytechnic and on 3 at the Agriculture University.

We investigated the size of the combs and the quantities of brood in them to determine the duration of the time the colonies were present at the site. For that purpose the bees covering the combs were gentle smoked, to uncover part of the combs after which the length (horizontal) and height (vertical) of the combs and the radius of the area of unsealed and sealed brood were measured with a scale ruler (Woyke *et al.* 2005a). It was difficult to determine the area occupied by eggs and young larvae. Therefore, the combs were examined ten days later. The presence of sealed brood verified the presence of eggs and larvae during the previous examination. The area of the comb was calculated as half ellipse.

To characterize DMF's and PMF's the colonies were observed from a distance of 2 m. The time of start and end of DMF's and PMF's (suddenly very many bees are flying) was recorded. After the flights started, the flying bees were captured with insect nets with a diameter of 30 cm. In 2002, the bees were caught during 1–2 min periods. The captured workers and drones were counted and then released. This procedure was repeated several times until the end of the flights. In 2005, the bees were caught during 1min periods, and then released. Bees participating in the day-time PMF's were caught without an interval break. After one person captured the bees, the other person counted the number of captured bees. In the meantime, the first person was catching the bees again. DMF's are performed by all colonies at about the same time. Therefore, due to a lack of observers, the bees were caught every second minute. After one person captured the bees, the same person counted them during the next minute, and then repeated the capture. The numbers of bees captured every second minute was doubled for statistical comparison with the numbers of bees captured every minute during PMF's.

An experiment was conducted to verify whether *A. dorsata* foragers participate in DMF's. For that purpose, foragers were caught with an insect net near the nest at daytime. They were marked on the thorax with acetone lacquer through the screen net, after which they were released. Two hundred workers from each colony were marked with different coloured paints. During the evening of that day (December 2), worker bees, which participated in DMF's, were caught with insect nets and counted, and the number of marked bees was determined. In total 15,160 individual bees were examined: 2,417 in 2002 and 12,743 in 2005.

Local time in Bangalore is UTC/GMT + 5.30 h. The time of sunset was determined from data presented by four internet sunrise and sunset databases: CNN, Time and Data, USNO Astronomical Applications, and Weather Underground.

ANOVA was used to compare variances and significant differences between particular means were detected using the Duncan Multiple Range Test at $P< 0.05$. Arcsine transformation was applied to percentages.

Results

Comb size and the duration of time the colonies stayed at the site

The 13 colonies examined on March 10 occupied white or light brown combs of different sizes and contained different quantities of sealed and unsealed brood (Table 1 and Fig. 1A). The results enabled the determination of the duration of the period in which the colonies stayed at the observed site. On this basis the colonies were divided into four groups. Group 1: Four colonies had small white combs without any sealed brood (Fig. 1A, Gr.1). They could stay in the place up to about eight days before the

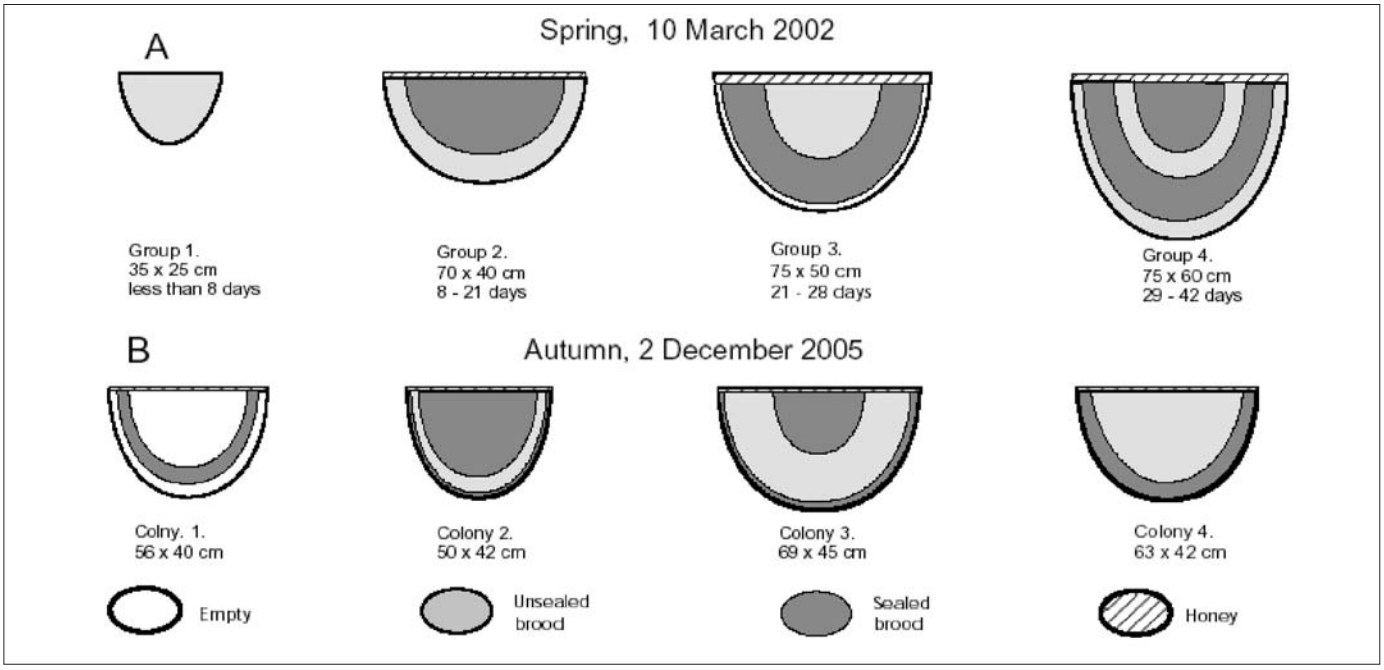

Fig. 1. Sealed and unsealed brood in colonies in the Spring (A) and in Autumn (B). The relationship and place of sealed and unsealed brood in the combs in Spring enabled the determination of the duration of time the colonies stayed at the site.

examination (eggs, 3 days + larvae, 5 days). After that time they would have some sealed brood. Group 2: Two colonies had larger white combs with sealed brood in the upper central part (Fig. 1, Gr. 2), which indicated they had arrived about eight to 21 days before the examination. After that time some workers would have to emerge. Group 3: In three colonies, workers emerged from the upper central part of the combs, and no new sealed brood was present there (Fig. 1A, Gr. 3). This revealed that the swarms had arrived about 21 to 28 days before the examination. Group 4: In four colonies, the second generation of sealed brood was present in the upper central part of the combs. It was separated by a belt of unsealed brood from the rest of sealed brood of the first generation located in the lower and marginal part of the combs (Fig. 1A, Gr. 4). Swarms of such colonies were supposed to have arrived between 29 and 42 days before the examination.

Table 1. Duration of time *A. dorsata* colonies stayed at the site, in Bangalore on March 10 2002 (see Fig. 3a) and relationship between comb size and percentage of drones flying during dusk flights from four groups of differently developed colonies (2,417 bees captured).

Group No	1	2	3	4		
No Colon.	4	2	3	4		
	Number of days the colonies stayed at the site					
Range	up to 8	8 – 21	21 – 28	29 – 42		
Mean	4	15	25	36		
	Comb size, length x height cm					
Min-Max	15x10 – 40x30	90x30 – 70x50	60x45 – 80x50	75x55 – 80x60		
Mean	30x21	80x40	72x48 76x59			
	Comb area dcm2					
Range	1.2 – 9.4	21.2 – 27.5	21.2 – 31.4	32.4 – 37.7		
Mean	5.6 A	24.4 B	27.2 B	35.2 C		
Date: March	**Percentage of drones participating in dusk flights, range and mean**					**Overall 13 colonies**
12	0.0-2.1 0.8 a	0.0-5.2 2.6 a	0.0-2.9 1.5 a	0.0-16.7	5.1a	2.6 a
18	3.6-6.7 5.2 b	3.8-8.1 6.0 a	8.3-9.7 9.2 a	11.8-55.9	27.2 b	13.0 b
20	0.0-8.3 4.8 ab	3.4-4.3 3.9 a	2.9-54.0 20.4 a	3.8-52	24.5 ab	14.3 b
Overall %	0.0-8.3 3.6 A	0.0-8.1 4.1 A	0.0-54.0 10.4 AB	0.0-55.9	18.9 B	10.0

Different letters after the means indicate significant differences P<0.05; small letters concern columns (different days), capitals concern rows (different groups).

All the combs were longer than higher. The largest combs in group 4 were on average about 6 times larger than the smallest (Table 1), and contained about 6 six times more brood than the smallest. However, surprisingly, the area of unsealed brood in the largest colonies was only about twice as large as in the smallest (Fig. 1A). Queen cells were not found in any of the colonies.

Percentage of drones, which participated in dusk flights in the spring

Bees, which participated in DMF's, were caught in three days within the period from March 12 to 20, 2002. Among the 2,417 bees captured, 304 were drones. The overall mean for 39 DMF's (13 colonies x 3 days) showed that only 10% of the bees, which participated in those flights, were drones (Table 1). However, a large variation of drones from particular colonies which participated in DMF's on different days, was detected (from 0 to 55.9%).

Table 1 shows that in group 1, the mean of 0.8% of drones which participated in DMF's on March 12 in all 4 colonies increased significantly to 5.2% six days later. On the first day of examination (March 12), drones were not found in DMF's performed by 5 colonies out of 13. Thus, drones were absent in 38.5% of the colonies. In other 7 colonies, a very low percentage of drones (0.5 - 5.2%, mean 2.4%) was present. A higher percentage (16.7%) was found only in colony #5, from group 4. As a result, the mean percentage of drones participating in DMF's the first day was 2.6%. No new drones could emerge in colonies, which stayed at the site for less than 8 days. This suggests that drones from other colonies migrated to colonies of this group and participated in their DMF's. A similar phenomenon was observed in most colonies of groups 2 and 3. This suggests that the colonies arrived with very few drones or, perhaps, without any drones.

Table 1 shows that the overall mean of 2.6% of drones participating in DMF's performed by all 13 colonies on the March 12 increased 5.5 times (14.3%) eight days later (March 20). Obviously, new drones emerged in the meantime, and joined in the flights. The overall mean of 3.6% of drones participating in DMF's performed during all three days (March 12 to 20) by all colonies of group 1, which had arrived recently, was in colonies of group 4, which stayed at the site for about six weeks, 5.3 times higher (18.9%), (Table 1). The 18.9% of drones in colonies of group 4 was significantly higher than the 3.6% and 4.1% of drones in colonies of groups 1 and 2, respectively, which had arrived recently. Thus, both percentages–those concerning the short period of eight days only (March 12 to 20), as well as those of the longer period of six weeks–showed that the percentage of drones which participated in DMF's increased with the prolongation of the time the colonies stayed at the site.

Correlation between the duration the colonies stayed at the site and percentage of drones participating in DMF's

Significant correlation was found between the mean number of days the colonies stayed at the site (Table 1) and the percentage of drones from the 13 colonies, which participated in DMF's (r = 0.64, df = 12, PMF's = 0.02). Also significant correlation was found between the four group means of the number of days the colonies stayed at the site and the four group means of the percentage of drones participating in DMF's (r = 0.96, df = 3.0, P = 0.04). Thus, the percentage of drones, participating in DMF's was related to the duration of time the colonies stayed at the site.

Correlation between the size of the combs the colonies occupied and the percentage of drones participating in DMF's

Table 1 shows that, colonies, which stayed at the site longer, had larger combs. Significant correlation was found between the size of the combs and the percentage of drones from the colonies, which participated in DMF's, (r = 0.63, df = 12, P = 0.02). This appeared when all the colonies of all four groups were taken into account–the smallest, most recently arrived with few or no drones and very small combs (group 1) and the established colonies with more drones and large combs (group 4). However, when the recently arrived colonies of group 1 were omitted, then the correlation was on the edge of significance (r = 0.67, df = 8, PMF's = 0.049). However, no significant correlation was found between the four group means of the size of the combs and the group means of the percentage of drones which participated in DMF's (r = 0.82, df = 3, P = 0.18).

Comb size and brood in the colonies in autumn

An examination of the colonies carried out on the November 23, 2005 revealed that the size of combs varied from 50 x 42 cm to 69 x 45 cm (Fig. 1B). The combs were brown, which indicates that several generations of bees developed in them. Thus, they had to stay for a long period of time. Different quantities of sealed and unsealed brood were found in the combs of the 4 colonies. On December 2, unsealed brood was absent in colony #1 (Fig. 1B).

Table 2. Comparison of 9 periodic mass flights (PMF's) and 9 dusk mass flights (DMF's) performed by the same three A. dorsata colonies, during the same three days: 26. 28 and 29 November 2005 (Total 10,647 bees captured and examined).

Trait	PMF's		DMF's		Ratio PMF's/DMF's*	
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Start DMF's h			17.58–18.08	18.05±3.16		
Duration min.	7–9	8.00±0.52	15–25	19.08±3.19	0.3–0.6	0.4±0.1
No bees/flight	638–1188	806±162	156–692	377±181	1.1–7.6	2.9±2.2
No bees/min	71–149	103±23	10–29	19±6.6	3.8–14.9	6.5±4.1

* - Calculated from individual 9 PMFs and 9 DMFs.

Absence of drones in DMF's in the autumn

Bees which participated in DMF's performed in autumn (November 20 to December 3, 2005) were thoroughly examined as several investigations were conducted, e.g. frequency distribution of the number of bees participating in DMF's, determination of body color of bees participating in DMF's or examination of the presence of marked bees. Among 4,450 bees captured during 30 DMF's performed by the 7 colonies only 2 drones were found, one at the Polytechnic, and the other at the Agriculture University site. Thus, drones were practically absent in all the colonies. Despite the absence of drones, worker bees performed the 'drone' DMF's. This was an unexpected result.

Comparison of DMF's in which drones participated or were absent

Drones were present in DMF's performed in the spring and were absent in DMF's performed in autumn. Therefore, DMF's performed in spring and autumn were compared. In the spring, sunset in Bangalore occurred on March 10, 2002, at 18.30 h. The DMF's started at 18.43 h, i.e. 13 min after sunset. The duration of these flights lasted 28–31 min, giving a mean of 29 min 30 sec ± s.d. 58 sec (n=30). In the autumn, sunset occurred on the November 30, 2005 at 17.50, h and the DMF's started at 18.05 h, i.e. 15 min after sunset. The DMF's lasted 15–26 min, giving a mean of 19 min 55 sec ± s.d. 3 min 26 sec (n=13). Thus, DMF's in the spring, when drones were present in the colonies were 9 min 35 sec, or 1.5 times longer than in the autumn, when drones were absent.

Comparison of DMF's and PMF's performed on the same day by the same colonies

Table 2 shows, that the duration of DMF's (19 min 8 sec) was 2.4 times longer than that of PMF's (8 min 00 sec; colony 4 was excluded from this comparison because a reflector lamp illuminated that colony at dusk, which failed and terminated DMF's). The intensity, in terms of the number of bees, participation in 1 min of flight, was 6.5 times higher in PMF's than in DMF's. The total number of bees, which participated during the whole period of those flights, was 2.9 times higher in PMF's than in DMF's. Thus, despite the fact that duration of DMF's was longer than that of PMF's, more bees participated in PMF's than in DMF's.

The frequency distribution of the number of bees, which participated in successive minutes over the duration of the 9 PMF's, showed that more bees flew during the first half of the duration of flights, than during the second one (Fig. 2). The peak of the number of flying bees occurred 2 times, 2 min after the start of flights and 7 times, 3 min after the start. The mean peak occurred 2 min 47 sec ± s.d. 26 sec (n = 9) after the start of those flights. However, the distribution of the 9 DMF's varied (Fig. 3). The peak occurred 5 to 15 min and the mean 8 min 20 sec ±

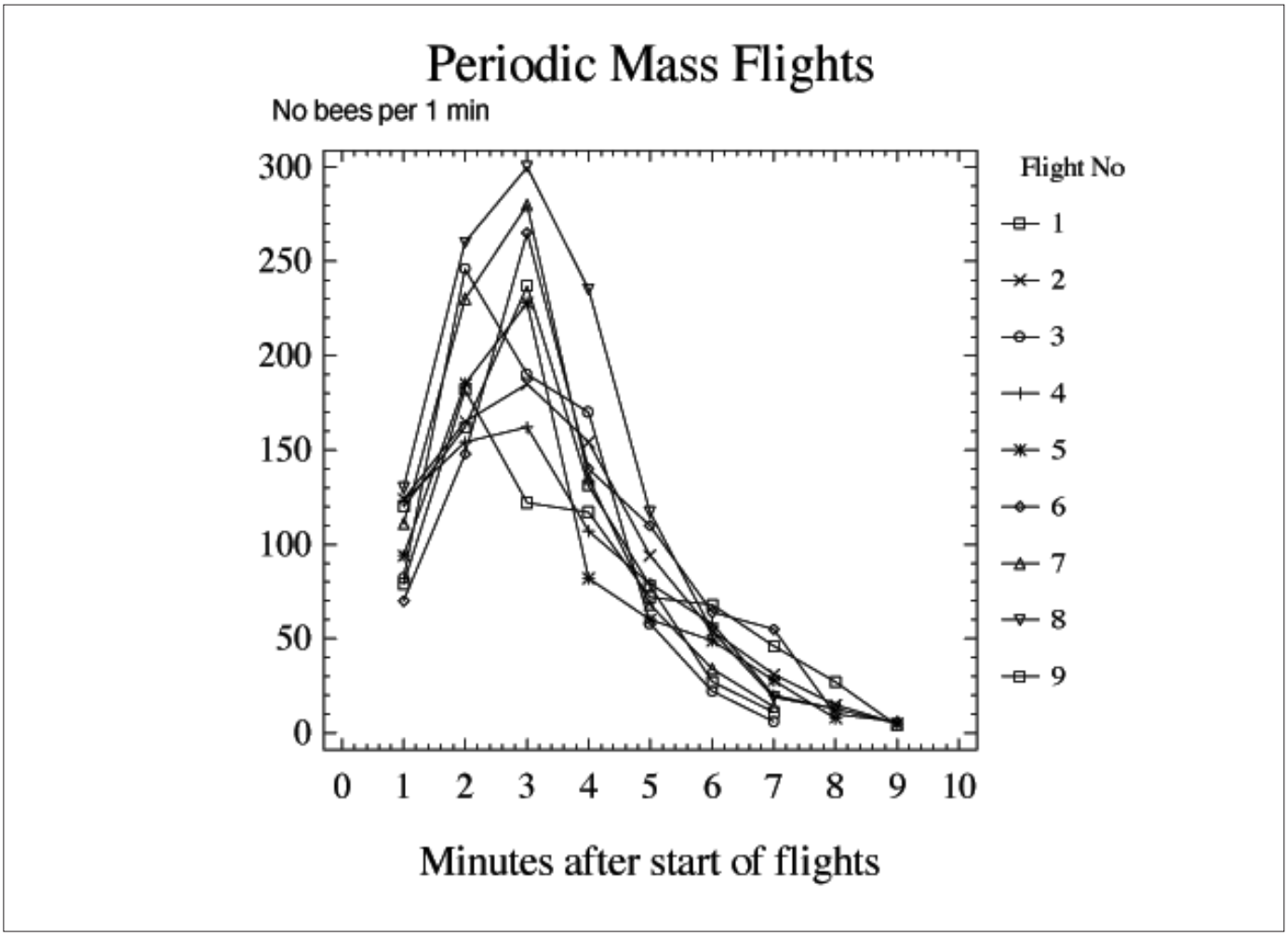

Fig. 2. Frequency distribution of the number of *A. dorsata* worker bees which participated in successive minutes of 9 periodic mass flights performed by 3 colonies during 3 days.

s.d. 3 min 28 sec (n = 9) after the start of the flights. The frequency distribution of different PMF's was quite uniform, while it varied in different DMF's.

Comparison of the distribution of PMF's and DMF's shows considerable difference (Fig. 4). In PMF's, the bees started to fly at once in high numbers, while in DMF's, the number of flying bees increased gradually The mean height of the peak, of the number of bees participating in 1 min of PMF's (232 ± s.d. 47.2, n = 9) or in 1 min of DMF's (33 ± s.d. 11.5n n = 9) was 7.8 ± s.d. 3.4 (n = 9) times higher in PMF's than in DMF's. Thus, PMF's and DMF's differed not only in the intensity and the total number of bees participating in those flights, but also in the distribution of bees performing those flights. This indicates that both, present different types of flights.

Effect of the absence of open brood on PMF's and DMF's performed by worker bees

It was interesting to find factors, which might cause differences within both types of mass flights (DMF's and PMF's). On December 2, 2005, open brood was absent in colony # 1. Only a belt of sealed brood 4 cm wide was present along the edges of the comb (Fig. 1B). Evidently, that colony was preparing for migration and it emigrated on the December 11, leaving an empty comb. This indicates that open brood was absent in that colony 21 days earlier, i.e., from November 20. Continuous observations conducted during 5 days between November 29 and December 3 showed that the colony performed only 1 PMF per day. The other three colonies, which had open brood, performed also 2 or 3 PMF's per day (respective–3 means: 1.8 ± s.d. 0.45, 1.6 ± s.d. 0.55, and 2.4 ± s.d. 0.55 (n = 5 each mean). All differed significantly from the 1 PMF performed by colony # 1. Thus, colonies with open brood performed more PMF's than those without it. However, the mean duration of PMF's (6.7 min) performed by colony # 1 without brood did not differ significantly from that (6.6–7.7 min) of the 3 other colonies.

Concerning DMF's, the mean duration of flights (23 min) performed by colony # 1 without brood was significantly longer than those (19 and 16 min) of the two other colonies. The mean number of bees captured daily during DMF's performed by colony #1 (551) was similar or higher than those (410, 172) in the two other colonies. Thus, despite the absence of open brood and preparation to migrate, colony # 1 performed longer DMF's, in which a similar or higher number of the bees participated compared to other colonies with brood in the same site. Thus, while the absence of brood in the colony affected PMF's, decreasing their number, it did not decrease either the duration or the number of bees, which participated in DMF's.

Age-colour categories of worker bees, which participate in various types of flights

It is interesting to know whether the same bees perform both types of mass flights (DMF's and PMF's). The age categories of worker beers can be determined by their colour (Fig. 5). The pubescent of emerged and young workers (Y) is yellow on 5 abdominal segments and white on the last segment. No light-coloured segmental bands are visible, because they are of the same colour as the abdomen. However, the light segmental bands are visible on abdomens of the next three colour types. The curtain bees (C) have 2–3 first segments of ochraceous (orange) colour, the 6th and 7th segments are clay yellow and the last one is grey-white. The intermediate coloured bees (I) have 3 first segments ochraceous, the 6th and 7th segments are brown-grey and the final one is grey-white. There are 4 types of foragers (F_1 – F_4). The foragers have orange-coloured initial segments and, according to the age type, 1–4 black final abdominal segments. F_1 are the youngest and F_4 the oldest forager types.

The percentage of differently coloured age categories of worker bees, which participated in the three types of flights:

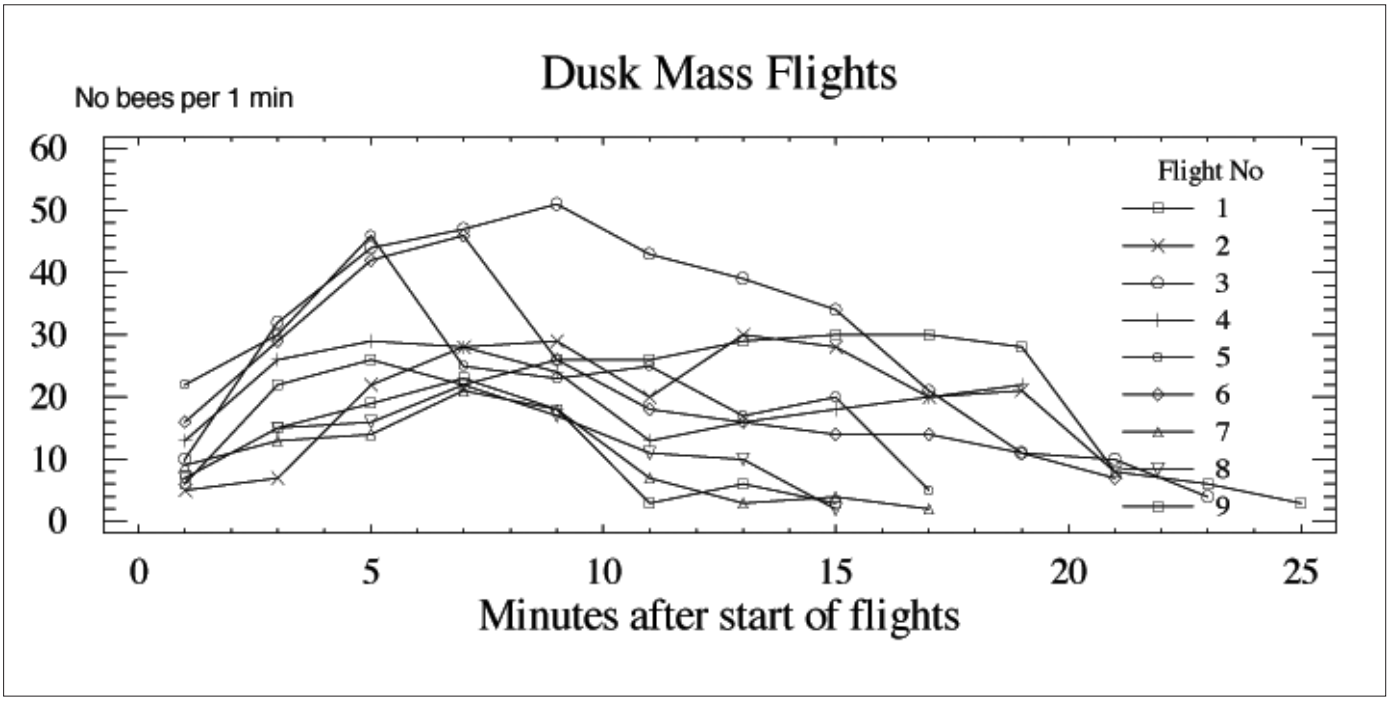

Fig. 3. Frequency distribution of the number of bees which participated in successive minutes of 9 dusk mass flights performed by 3 colonies during 3 days.

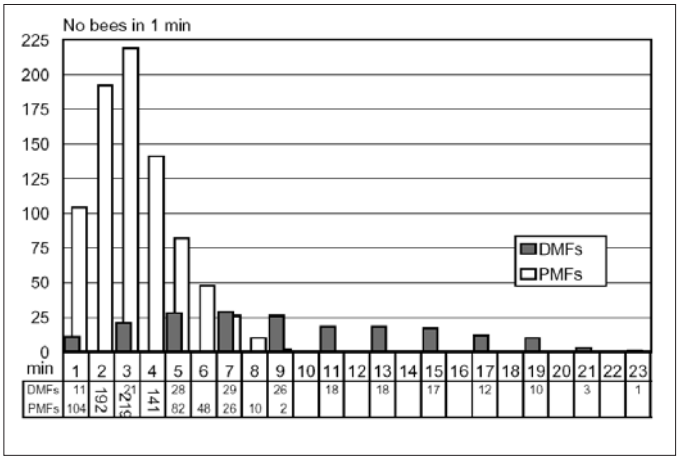

Fig. 4 Mean frequency distribution over time of the number of worker bees, which participated in 9 dusk flights (DMF's) and 9 periodic mass flights (PMF's) performed by the same 3 colonies during 3 days. X – axis indicates minutes after the start of the flights.

foraging, PMF's and DMF's varied (Table 3). The means for the 400 bees captured during foraging flights performed by workers of the four colonies at the Polytechnic showed that only 2.5% of curtain (C) and intermediate (I) bees and as many as 97.5% of foragers (F_{I-4}) performed those flights (Table 3, column 1, bottom). The high percentage of F bees, which performed foraging flights, verifies the credibility of that test. However, as many as 75% of curtain and intermediate bees (C+I) and only 25% of F bees participated in PMF's (Table 3, column 2 bottom). Among the 75% of C and I bees, the percentage of I bees (44%) was higher than that of C bees (31%). It is interesting to note that some F bees (25%) also participated in PMF's. Surprisingly, only 16.3% of C+I bees and as many as 83.7% of foragers participated in DMF's (Table 3, column 3).

The PMF's presented above were performed between 10:00 and 15:00 h. However, it happened twice that colonies #1 and #2 performed PMF's very late; with the flights ending only 3 to 13 min before the start of DMF's. It was interesting to check whether the late PMF's are similar to daytime PMF's or to DMF's. Table 3 (column 4), shows that 69.5% of C+I bees and 30.5% of F bees participated in those late PMF's. Although more foragers participated in the late PMF's (30.5%) than in the daytime flights (22.0%) performed by the same 2 colonies, the t-test ($t = 0.65$, $P = 0.58$) did not detect statistically significant difference between those two means. Thus, the daytime and the very late PMF's were performed by similar age categories of bees.

Colour of bees, determined in the 3 colonies which performed DMF's at the Agriculture University site, showed that only 5.7% of intermediate (I) bees, and as many as 94.3% of forager (F) bees, participated in those flights (table 3, column 6). The overall means for all the results collected at the Polytechnic and at the Agricultural University concerning 705 bees, which participated in 9 DMF's performed by 7 colonies, showed that as many as 87.7% of foragers participated in DMF's. Among the rest of bees performing DMF's (12.3% of C+I), the majority (11.0%) were intermediate bees. Thus, DMF's were performed by a percentage of foragers 7 times higher (87.7%) than that of young (C+I) bees (12.3%). Also, 3 times more foragers (87.7%) participated in DMF's than in PMF's (26.8%). It is worthy to note that no young bee with all yellow abdomen (Y) was found participating in any flight type.

Marked foragers in DMF's

Eight hundred foragers (200 per each of 4 colonies) were marked at daytime. An examination of bees, captured the same day during DMF's, revealed the presence of 2.2, 2.0, 2.4 and 2.3% of marked foragers in the four colonies, # 1 to # 4, respectively. Among the total of 1,074 bees captured during this experiment, 24 were marked, which indicates the presence of 2.2% marked foragers. This confirms the results presented above, according to which foragers participate in DMF's

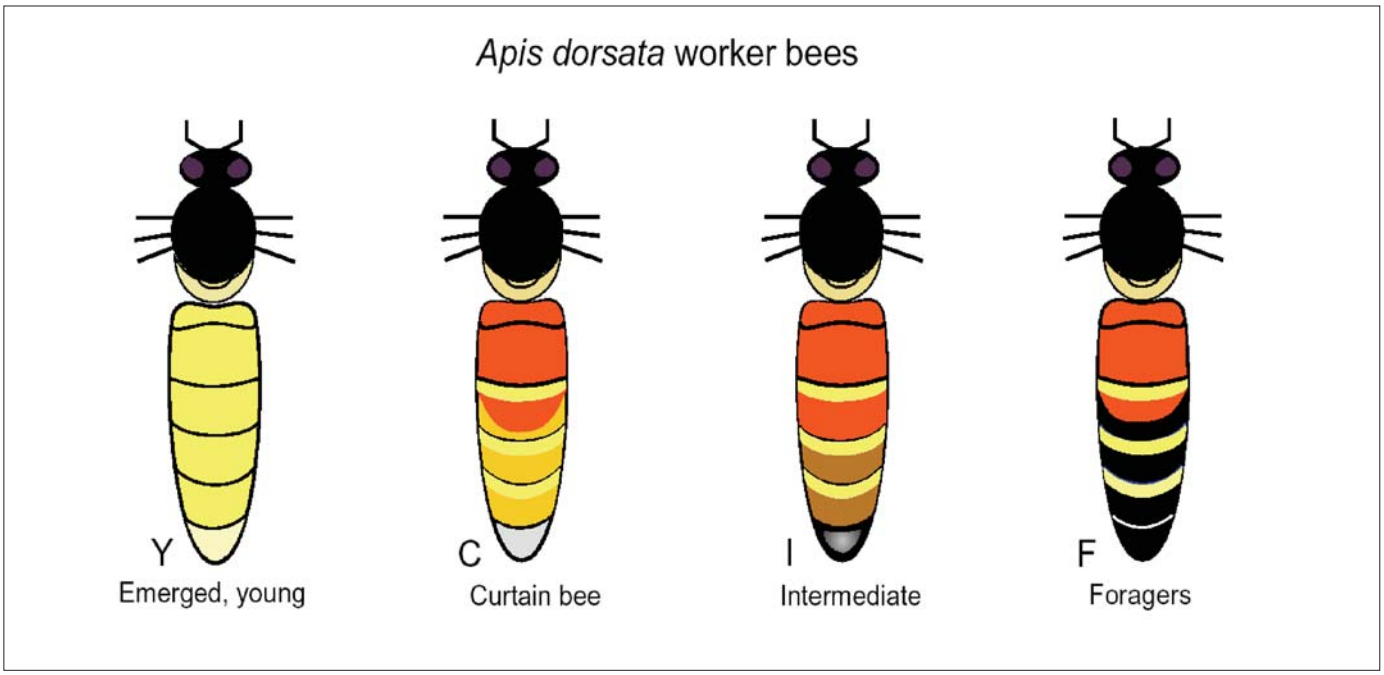

Fig. 5. Colour of four age-colour categories of *A.dorsata* worker bees

Table 3. Percentage of worker bees of different age-colour categories, which performed different types of flights: [foraging (Forag.), periodic mass flights (PMF's), and dusk mass flights DMF's)]. Colours according to Fig. 3. Colour was determined in 100 bees in each colony at Polytechnic and in 35 bees in each colony at Agricultural University.

	Polytechnic, normal PMF's			**Polytecnic, late PMF's****		**Agric. Uni**	**Overall**		
No. Colonies	4	4	4	2	2	3	4	6 fligts	9 flights
Flight types	Forag.	PMF's	DMF's	PMF's	DMF's	DMF's	Forag.	PMF's	DMF's
Age-colour categories of worker bees									
Curtain bees	0.5	31.0	1.5	19.0	3.0	0.0	0.5	27.0	1.3
Intermediate	2.0	44.0	14.8	50.0	11.5	5.7	2.0	46.2	11.0
Foragers$_{1\#}$	1.5	3.5	5.2	6.0	6.0	9.7	1.5	4.3	6.9
Foragers$_2$	35.0	17.3	51.2	15.5	41.0	64.3	35.0	16.7	53.3
Foragers$_3$	59.8	3.3	26.5	9.0	37.5	20.3	59.8	5.2	26.9
Foragers$_4$	1.3	1.0	0.8	0.0	1.0	0.0	1.3	0.7	0.6
All Curt+Interm.	2.5	75.0	16.3	69.5	14.5	5.7	2.5	73.2	12.3
All Foragers$_{1-4}$	97.5	25.0	83.7	30.5	85.5	94.3	97.5	26.8	87.7

*Colour of bees, which performed all 3 types of flights, was determined in days when PMF's were performed at mid-day, between 10:00 - 15:00 h. **Colour was determined in days when PMF's were performed very late, terminating 3-13 min before the start of DMF's at 18.05 h.
#Foragers 1–4 four types of foragers, F_1 - the last one abdominal segment black - the youngest foragers, F_4 - four segments black - the oldest.

Discussion

Until now, it is not known when drones are present in *A. dorsata* colonies, and which factors determine their numbers. Koeniger and Wijayagunasekera (1976) suggested a relation between comb size and the presence of drones, because drones were observed in all colonies on combs larger than 0.5 x 1.0 m. However, we found many drones in colonies in smaller combs 0.5 x 0.8 m or 0.6 x 0.8 m. According to Venkatesh and Reddy (1989), the comb sizes of colonies 30 days after settlement varied from 80 x 90 cm to 189 x 198 cm, being related to the size of the swarms at the time of arrival. We did not find correlation between the mean size of combs and the percentage of drones participating in DMF's, or the correlation was on the edge of significance in individual colonies, which stayed at the site longer than 8 days.

However, in the present investigation, a strong correlation was found between the duration of time the colonies stayed in the site and the percentage of drones, which participated in DMF's. The new method, to determine the duration of time the colonies stay at the place (Woyke *et al*. 2005a) specifies the time quite accurately during the first several weeks after settlement. We estimate by our method, that the colony in the comb presented by Joshi and Gurung (2005) stayed at the site between 29 and 42 days. After longer stay of the colonies at the site, estimation may be more difficult.

Until now, nobody has described absence of drones in all established *A. dorsata* colonies present at the site. However, we proved the absence of drones in all established *A. dorsata* colonies being present in unfavourable environmental conditions. Results presented here showed, that few drones were present in the colonies shortly after the swarms arrived, and drones were absent in the colonies when environmental conditions deteriorated and the colonies were ready to migrate to new sites. However, this concerns only the conditions described in this paper. In Nepal, we were catching drones for instrumental insemination during the swarming season when queen cells were present in the colonies (Woyke *et al*. 2001b). We found many drones within bees caught with an insect net. Unfortunately, we did not determine the number of drones and worker bees. When the colonies swarmed, some swarmed totally out in such a manner, that after the last swarm flew out, only empty comb was left (Woyke *et al*. 2001a). Obviously drones were present in such swarms.

Those results enable to reconstruct the drone situation in *A. dorsata* colonies during the season. Our data suggest that those few drones found in the spring in DMF's performed by colonies, which stayed at the site for only about one week, drifted from other colonies, which stayed at the site for a longer period. This indicates that all the swarms arrived without drones. Larger numbers of drones participating in DMF's were found in all colonies, which had stayed at the site for longer than 4 weeks. During the swarming season many drones are present in the colonies. The drones swarm together with worker bees. Therefore, the drones are present in swarms, which settled in the new site. However, when, environmental conditions deteriorate, and the colonies prepare to emigrate, drones are absent in all colonies. Therefore, drones are absent in new site in swarms, which emigrated from previous sites due to unfavourable

conditions. These findings enable to determine whether arriving swarms origin from swarming colonies (presence of drones) or they are colonies absconded from previous site due to unfavourable environmental conditions (absence of drones). In the last colonies, a higher number of drones can be found only after more than 4 weeks of presence at the new site. Thus, neither colonies on large combs nor colonies stayed longer period at the site assure the presence of drones in them. Hence, it is possible that some earlier publications, which described 'drone' DMF's, in fact describer DMF's performed only by forager bees. This shows the importance of applying our method of catching flying bees with an insect net to determine the presence and percentage of drones participating in DMF's.

The lack of drones in *A. dorsata* colonies in unfavourable environmental condition is similar to the same phenomenon in *A. mellifera* and *A. cerana*. However, it was believed that drones are present in *A. dorsata* colonies all the time, because when the environmental conditions deteriorate the bee migrate to sites with favourable conditions. Absconding swarms are very rare in *A. m. mellifera*. However, this phenomenon occurs in the migratory A. m. adansonii. The lack of drones in arriving swarms of *A. m. adansoni* in Ghana permitted to determine that one of the two periods supposed to be swarming seasons was the time of absconding and migration of the bees (Woyke 1989).

We showed in this paper that the duration of DMF's performed by *A. dorsata* colonies in the spring, when drones were present, was longer than in the autumn when drones were absent. DMF's started in the spring 13 min, and in the autumn 15 min, after sunset. Woyke *et al*. (2001b) reported, that DMF's started in Nepal 1 min 40 sec before sunset. However, we discovered now that the sunset time presented by CNN and used by Woyke *et al*. (2001b) is 15 min later than the time presented by the three other sunset databases used now. This is due to CNN applying the full 6 h time zone instead of 5.45 h East UTC/GMT for Nepal. When the 15 min correction was made, it appears that the DMF's started in Nepal 13 min 20 sec after sunset. This is similar to data now presented for India.

Woyke *et al*. (2005b) found for the first time that not only drones participate in the 'drone' DMF's, but also worker bees. However, drones were present in all colonies. Now, we found that despite the absence of drones in all colonies, worker bees performed 'drone' DMF's even when open brood was absent in the colonies. This was an unexpected result. Since our previous (Woyke *et al*. 2005b) and present investigations indicate that worker bees perform both types of mass flights: DMF's and PMF's, it was interesting to compare them. Woyke *et al*. (2005b) pointed out that the duration of DMF's performed by worker bees is longer, but the intensity is lower than those of PMF's. However, no specific data were presented. It is also not known whether the total number of worker bees, which participated in both flights is different or similar. A comparison of DMF's and PMF's performed on the same days by the same colonies presented in this paper shows that both, the intensity of flights as well as the total number of bees participating in those flights are higher in PMF's than DMF's. Thus, it is not true that the total number of workers participating in both types of flights is similar, and the only difference is that the DMF's are extended over longer period of flight. In the present paper, it was found that PMF's and DMF's differ not only in the duration and number of bees participating in them, but also in the frequency distribution over the flight time. The frequency distribution of the number of bees visible in successive video frames (0.04 sec) of a movie recording 1 PMF's (Kastberger *et al*. 1996) was similar to the distribution of the number of bees/1 min participating in 9 PMF's investigated by us.

Although Woyke *et al*. (2005b) described the presence of workers in DMF's, it was not known whether the same bees perform DMF's and PMF's. We applied now a new method, which enabled a mass determination of age-color categories of worker bees participating in different types of flights. We discovered, unexpectedly, that DMF's were performed by foragers, and not curtain and intermediate bees, which perform the daytime PMF's. A question arises, why foragers and not curtain bees perform DMF's? Woyke *et al*. (2005a) found, that bees which participated in DMF's defecated. However, we must add that many of the fecal spots were not large (of 5–7 mm diameter), like those found during day-time PMF's, but were small, about 2 mm. At that time, it was not known that worker bees, which perform DMF's are foragers and not curtain bees, which defecate during diurnal PMF's. Defecation cannot be an important reason for performing DMF's by foragers, which can defecate during foraging at daytime. Another explanation would be as follows: PMF's are performed by young curtain bees during daytime. However, neither *A. laboriosa* (Woyke *et al*. 2003) nor *A. dorsata* (Woyke *et al*. 2004) perform PMF's during cloudy days, or in hours when the sky is overcast. The situation may be similar after sunset. But, we observed many times, that foragers also forage long after sunset. We suggest that, curtain bees, which still have not much experience in orientation in the surroundings, have difficulties in orientating after sunset. Therefore, foragers, which are experienced in dusk and night flights, perform also the DMF's.

A second question arises is why worker bees perform 'drone' DMF's in the absence of drones? We found that worker bees perform DMF's not only when their own drones are present in the colonies, but also when the drones drifted from other colonies. The greatest surprise was the finding that foragers also perform 'drone' DMF's when drones are absent in all the colonies, and even when open brood is absent in the nest. It is believed that worker bees are performing DMF's in order to facilitate orientation of drones and queens when they are present in the colonies, and help to protect them against predators such as bats. We observed many bats flying near the nests after sunset, when the foragers stopped flying. If the queens were flying alone at that time, the baths would easily catch them. The duration of the seasonal period with unfavourable conditions, in which drones are totally absent, must be relatively short in the yearly life history of the colonies. When living conditions are unfavourable in one site, the colonies emigrate to other, more favourable places. Therefore we suggest that worker bees extend the long-lasting seasonal period of DMF activity in the presence of drones over the relatively short time without drones. This would indicate that the protective DMF's performed by worker bees at the time when drones and queens are normally flying is a heritable trait, which is carried out independently of the number and presence of drones in the colonies.

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Databases of sunset and sunrise
CNN Weather: www.cnn.com/WEATHER/
Time and Data:
www.timeanddate.com/worldclock/astronomy.html
or: www.timeanddate.com/worldclock/city.html
USNO Astronomical Applications Department:
http://aa.usno.navy.mil/AAmain.html
Weather Underground: http://www.wunderground.com/global/

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