

THE MYSTERIOUS COLOR OF *APIS DORSATA* WORKERS

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SUMMARY: The color of *Apis dorsata* workers presented in different publications differs very much. Therefore investigations were undertaken to describe body and pubescence color diversity. *Apis dorsata* workers and brood combs were collected in five Asiatic countries. The thorax of workers is black. Six types of abdominal body color patterns were found concerning 1.5, 2, 2.5, 3, 3.5 and 4 black terminal terga. Types 3 and 3.5 were the most frequent. The pubescence covering the scutum is black. Abdomen of emerging workers is covered with bi-colored pubescence, pale clay-yellow and white. No any bands are visible. Most of the workers in the curtain have three-colored pubescence. First tergites are ochraceous, tergites IV and V are pale yellow and the last tergite VI is white gray. An intermediate grade has the three first tergites ochraceous, tergites IV and V brown-gray and the last tergite white gray. Workers in the mouth of the curtain and foragers collected on flowers are bi-colored. The first tergites are ochraceous and three to four last are black. The structure of hairs covering the abdomen was described. We suggest that all the pubescence color changes are age related.

Keywords: *Apis dorsata* / honeybee body color/ honeybee hair color

INTRODUCTION

Five color photos of *Apis dorsata* workers are presented in Ruttners' book (1992). The color of each worker bee differs very much. In photo No 13.1 the abdominal posterior terga are black, and in No 13.14 they are yellow and white. According to Maa (1953) the integument is shining black, and Ruttner writes that it is covered with heavy pubescence. *Apis dorsata* was described by Fabricius (1793). He gives only short description of its color "A. hirta nigra thorace postice abominisque dorso testaceis. Thorax hirtus, niger, postice subtetaceus. Abdomen atrum dorso villosa, testaceo ano tamen nigro".

Further descriptions of the color of *A. dorsata* workers are presented in chronological order. Latreille (1804) described the same bee as *Apis nigripennis*. Klug (1807) recorded *Apis bicolor*, with terga I-II honey-yellow and III-VI black in strong contrast. Guerin (1833) and Smith (1859) described a black bee *A. zonata*, from Philipines and Celebes. Smith (1958) reported from Borneo *A. dorsata* var. *testacea* whose "abdomen was pale testaceous and densely clothed with short yellowish-white pubescence". Smith (1871) found also *A. laboriosa* with black abdomen living in Himalayas.

According to Bingham (1897), the head and the thorax of *A. dorsata* are more or less pale and fuscous on the hinder part of the thorax and on abdomen; the basal three tergites of the abdomen are honey-yellow, pubescence fuscous on thorax in front and apical tergites of the abdomen, pale, ochraceous yellow on the hinder parts of the thorax and the base of the abdomen. Koschevnikov (1900) reports that two extreme color types exist: - yellow bees and black bees and all intermediate forms between them.

Buttel-Reepen (1906) recognized three variations:

1. *A. dorsata* var. *zonata*; abdomen black with whitish bands on tergite 3-5.
2. *A. dorsata* var. *dorsata*; abdomen tergites 5-6 dark brown to black, often also tergites 3 and 4.
3. *A. dorsata* var. *testacea*; all abdominal tergites covered with pale testaceous pubescence.

He pointed that workers similar to var. *nigripennis*, through *bicolor* to *testacea* can be found in one colony. Therefore he excluded var. *bicolor*. Later on, he (1915) found that the pale testaceous abdomen of *A. d. var. testacea* is characteristic for the young workers of *A. dorsata* var. *dorsata*. Cockerell (1906) proposes the name *Apis dorsata binghami* for *Apis zonata*. According to Roepke (1930) most of the workers crating the outside curtain of a comb display a clay-yellow colored abdomen. Small part of the workers show rufous and black color characteristic for adult colored individuals.

Out of *Apis zonata*, Maa (1953) split out *Megapias (Apis) breviligula* which color-pattern is similar to that of *M. binghami* (abdomen black). It lives in the Philippines and can be discriminated from var. *binghami* by some morphological differences. According to Maa integument of *M. dorsata* workers is shining black,

abdomen with terga 1-2 honey-yellow turning gradually to dirty black towards abdominal apex. Pubescence on abdominal terga yellowish brown, that on 3 and following usually gradually turning to black towards abdominal apex.

Otis *et al.* (1990) reported that the pale yellow abdomen changed into orange and black when the workers become foragers between age of 15 and 35 days.

According to Morse and Laigo (1969) head, thorax and abdomen of *A. dorsata* workers are generally piceous, brownish hairs prominent on thorax and first abdominal tergite. Minor differences in color pattern do not merit distinct speciation. Undoubtedly Morse and Laigo observed *A. dorsata zonata (A. dorsata breviligula)*.

Many times it is not clear whether the author described the color of the body or of the pubescence. Once the discrimination factor of the verity is the color of the body and other time of the pubescence.

It is evident, that within *A. dorsata* species, two main types of abdominal pubescence color can be found: 1. those with black abdomen - 1a. the bee of the northern distribution border *A. dorsata laboriosa* and 1b. bees of the eastern distribution border *Apis dorsata zonata (A. dorsata binghami)* and *A. dorsata breviligula* and 2. those with first abdominal tergites of ochraceous color and terminal black ones - *A. dorsata dorsata*.

Nobody reported that one day old workers were examined. The detailed pattern of abdominal color of *A. dorsata dorsata* was not presented, nor the variation was quantitatively estimated.

The purpose of the paper was to describe in details body and pubescence color diversity in *A. dorsata*, to establish a standard for body color classification, to investigate the color frequency distribution in a population in order to get some genetic explanation of the variation, and to explain

the differences in color of the pubescence covering the abdomen.

MATERIALS AND METHODS

At first the diversity of body and hair color was investigated independently of genetic characters and environmental conditions. For that purpose J. Woyke collected and examined *A. dorsata* workers in the following places. In India, Pune (1974), all workers from two colonies were examined, 6 other colonies hived in three wall hives were observed during one month. Young workers were obtained from pieces of two brood combs in an incubator. In Thailand, Bangkok and Chiang Mai (1988 and 1992) foragers were collected from flowers. Young workers were obtained from one comb in an incubator. In Malaysia, Padu Lake (1995) several hundred foragers collecting water were investigated as well as young workers from one brood comb. In Vietnam, Ca Mau (1996) several hundred bees were collected from curtains of four nests. The frequency distribution of different patterns of body and hair color types was not recorded.

After a scale of body color patterns was established, quantitative investigation on the frequency distribution of particular body color types were investigated. For that purpose, *A. dorsata* workers were collected from 7 nests in Chitwan district, Nepal. In 1998, 554 workers were collected from the curtain of one colony in a tree at the garden of Narayani hotel in Bharatpur. In 1999, per about 300 workers were collected from four colonies in a water tank tower in the cancer hospital in Bharatpur and from two colonies in the University campus in Rampur. The localities of those nests are, about 5 km apart. The workers were killed by ether or ethyl acetate and preserved in 75% alcohol. Together body color of 2334 worker was classified.

The color of pubescence was examined on fresh workers collected from different places of the curtain. Pubescence color was identified according to Pantone color scale. The diversity of color variation was recorded, however the frequency distribution was not recorded. To obtain young workers of known age, pieces of seven brood combs were collected. Workers were allowed to emerge in an incubator and their color was determined. Young bees were kept for several days in the incubator. Several hundreds forager were collected on flowers or collecting water.

The pattern color of workers located under stereo microscope in alcohol was drawn with the aid of Zeiss drawing adjustment. Next it was scanned into computer, and worked out with CorelDRAW 9.0 software. To examine particular hairs, they were scratched from different terga, put into drop of water on a glass slide, cover with cover glass and located under translucent microscope. They were measured with the aid of an eye piece micrometer. Next, they were photographed with a digital camera, transferred into computer and worked out with the same software program.

RESULTS

Body Color

Examination of workers collected in different localities revealed a broad diversity in body color. The three tergites of thorax, the pro-, meso- and metathorax are black. The scutellum is mostly black. However, in some workers the posterior margin is dark brown. The protodeum is black with two honey-yellow areas near the petiole (Fig. 1).

The main colors of abdominal tergites are honey-yellow and black. The anterior terga are honey-yellow, and the posterior are black. Six types of abdominal colored

workers were found according to the number and amount of black colored posterior terga: (Fig. 1). 1. one and one-half (1.5), 2. two (2), 3. two and one-half (2.5), 4. three (3), 5. three and one-half (3.5) and 6. four (4). Variation was noticed in the proportion of black and honey-yellow color in the bi-colored terga. The black color covering tergites in workers of type 2.5 varied from 2.25 to 2.75 and in type 3.5 from 3.25 to 3.75. Looking from the top, black-brown corners are visible on both sides, near the posterior margin of each honey-yellow tergum except tergum I. Additionally, two small brown-gray elliptical areas are present, each on a side near posterior margins of those tergites. In type 1 workers, those ellipses are not visible on tergite II and sometimes also on tergite III.

Looking sideways, a black-brown area is visible on each light colored tergite except tergite I (Fig. 2).

In dissected terga, brown-gray margin is visible in the light colored terga (Fig. 3). The margin may be interrupted in tergum II. All the body color pattern types were found in *A. dorsata* workers collected from different countries. Most frequent were types with 3 and 3.5 black abdominal terga. However, other types were also found. Fig. 4 shows that in workers of one of the most frequent body color type 3.5, black areas are present on all sternites except I. Black areas are also present on the same sternites in workers of the other color types. The patterns of the black areas on sternites IV – VI are almost the same in all body color types of workers. However, those areas on sternites II and III are smaller and lighter in type color 1.5 and 2.5, and larger in type 4. The patterns of body color types presented above shows the range of body color diversity, which could be the results of both, genetic and environmental

influence. Exact frequencies of various body color types of workers examined in different countries were not recorded, except in bees investigated in Nepal.

After the scale of body color patterns was established, quantitative investigation was conducted. To be able to determine the influence of genetic characters, bees were collected from one region in Chitwan, Nepal. Fig. 5 shows, that the frequency distribution of different types of body color patterns was unequal in particular colonies. All six types of body color patterns were found only in two colonies out of seven investigated. Five darker pattern types were found in 3 colonies, and 4 types in 2 colonies. The most frequent were types 3 (in 3 colonies) or type 3.5 (in 4 colonies). Either of them presented more than 60% of worker populations in 4 colonies. The lightest types (1.5 and 2) did not exceed 4% in 6 colonies whereas type 2 reached 12.1% in colony No 0. The darkest type 4 exceeded 20 % of population in 3 colonies. In the overall number of 2334 workers, types 3 (36.3%) and 3.5 (40%) presented together 76.3% of the population. The lightest types 1.5 and 2 each presented less than 3.5% and types 2.5 and 4 were present in about 10% each. The overall percentage frequency distribution (Type 0 – 6) was shifted toward the darker types. The skewness was -0.75.

Pubescence color

As it was already published, the pubescence covering the scutum is black. The other thoracic parts like prothorax, scutellum, metathorax and the propodeum are covered with yellowish-brown pubescence (Panthone E 21-5, CMYK 0/20/65/30).

We found that abdomen of young workers which emerged and were reared for several days in incubator in India,

Thailand and Nepal is covered with dense pubescence. The pubescence is so compact that no body color is visible through it. Table 1. shows, that the abdomen of emerging workers, examined with naked eye is be-colored. It is covered with pubescence of gold yellow color, except the last tergite VI covered with white pubescence (Fig. 6a). No any bands are visible observing the worker perpendicularly to its body surface. However, under diagonal observations the three bands on tergites III – V can be detected. Their color is the same or similar to the rest of pubescence covering particular tergites

Under stereo microscope, particular hairs of the pubescence are visible. The abdomen is covered with decumbent pubescence. However, differences exist in the structure of the pubescence. Denser pubescence can be detected on anterior part of terga III, VI and V, where the bands are present. Strong erected spine-hairs are loosely disposed between the pubescence on all terga. However, the spine-hairs are absent on the three bands on terga III-V.

Table 1. shows, that the pubescence is little darker in already 1 day old workers. Now differentiation in pubescence color occurs. The two to three first tergites are darker, then the two to three next ones.

Three to five days old workers are still darker. The three first tergites are already light ochraceous and the last tergite VI is white gray. The three light bands on tergites III – V are visible under diagonal, oral or anal observations.

According to Roepke (1914) the bees in the curtain have clay yellow abdomen which turns into gray or blackish toward the tip, while the pygidium looks whitish. According to Otis *et al.* (1990) the callow bees in the curtain have pale yellow

abdomen. We found that bees collected from the curtain nests in India, Vietnam and Nepal were not one- or be-colored, but were three-colored (Fig. 6b) and some color variations can be distinguished (Table 1). The lighter workers have the three first tergites of light ochraceous color, tergites IV and V are pale orange yellow and the last tergite VI is white gray. Pubescence color of those workers is similar to that identified above for bees 3 - 5 days old. The darker workers have three first tergites of ochraceous color, tergites IV and V are clay yellow and tergite VI is white gray. The three light bands become visible.

In the mouth and on flowers or drinking places, we found an intermediate grade between the callow curtain bees and the bi-colored, ochraceous-black foragers (Fig. 6c). The three first tergites are ochraceous and tergites IV and V are brown-gray. In the next grade, only two first tergites are ochraceous and tergites III – V are brown-gray. The last tergite VI is white gray. Frequencies of those intermediate grades are rather low.

As it was already described by many authors, workers in the mouth and foragers collected on flowers are of the bi-colored type ochraceous - black. Three to four black terminal tergites were recorded. However, we found one more variation. Some foragers have only one, the last but one tergite V black. Those workers are characteristic by having the last apical tergites VI covered to some extent with gray white pubescence (Table 1, foragers type I). We found variation in the color pubescence on the two or three first abdominal tergites. Workers with three last tergites IV - VI black, have the three first tergites covered with ochraceous pubescence (foragers type II). The darkest foragers with four last tergites III – VI black, have also the two frontal tergite

covered with the darkest pubescence of intensive ochraceous color (foragers type III). The three light bands on tergites III – V are visible. They are of the light yellow color of the young workers.

There does not exist any relationship between the number of tergites covered with black pubescence and the number of black colored body tergites. Foragers exist whose body color of four last tergites is black, but only two last tergites are covered with black pubescence. Contrary, foragers are found whose body color of only two last tergites is black, but four last tergites are covered with black pubescence.

Older foragers lost the pubescence to some extent, and the pattern of body color is visible. Abdomen of such bees is shining. However, under stereo microscope some remaining pubescence hairs can be detected.

Table 1 shows that pubescence on the three first abdominal tergites changes the color very early. The gold yellow color of emerging workers changes into light ochraceous already in few days old bees. Then, it changes through ochraceous into intensive ochraceous in the darkest foragers. Pubescence on tergites III – V changes the different yellowish grades through brown-gray into black when the bees become foragers. Pubescence on the last tergite changes the whitish color into black as the latest, already in the forager stage.

Structure of Pubescence Hairs

Under translucent microscope, it appeared that not only the density of the pubescence differs, but also the morphology of particular hairs. Hairs of pubescence covering most parts of tergites I-V (Fig. 7a) are about 0.15 mm long. They are pinnate with bifurcate terminals of branches. The branches are 0.011 mm apart

one from the other. Hairs of the pubescence covering the three bands on tergites III-V (Fig. 7b) are shorter being about 0.10 mm long. They are denser, their bifurcate branches are only 0.0065 mm apart one from the other. The hairs of pubescence on the last tergite VI are longer, about 0.18 mm. Their branches are 0.015 mm apart one from the others and their terminals are pointed (Fig. 7c). On tergite I, except the pubescence covering all tergites (Fig. 7a), the longest hairs, 0.85 mm, with pointed branches are present (Fig. 7d). The spines present on different tergites, (except the three light bands) are 0.09 mm long (Fig. 7 e). However, spines on the last apical tergite are much longer reaching about 0.33 mm.

DISCUSSION AND CONCLUSIONS

The pattern of abdominal body color of *A. dorsata* workers was not described till now. A series of six different types was described. This may serve as a standard for comparative investigations on frequency distribution of different body color pattern in different localities. For the first time, pubescence color was identified according to an international color scale. Pubescence color of just emerged workers and of the intermediate forms was not described till now. We described also for the first time three-colored *A. dorsata* workers. The fourth color is that of the light bands. In the literature, sometimes is not clear whether the author describes body or pubescence color. Already Butel-Reepen (1915) and Roepke (1930) found that the pale testaceous abdomen is characteristic for the young workers, and the be-colored ochraceous black for the older *A. dorsata* var. *dorsata* bees. Otis *et al.* (1990) reported that the pale yellow abdomen changed into orange and black when the workers become foragers between age of 15 and

Table 1. Pubescence color of *Apis dorsata* workers.

Workers	Tergites		
	I-II, I-III	III-V, IV-V	VI
Just emerged	E 18-4, 0/25/80/0 gold yellow	E 18-4, 0/25/80/0 gold yellow	white
1 day old	E 22-2, 0/35/85/0 light orange yellow	E 22-4, 0/25/60/0 light yellow	white
3-5 days old	E 32-1, 0/50/100/0 light ochraceous	E 22-4, 0/25/60/0 light yellow	white gray
Curtain light workers	E 32-1, 0/50/100/0 light ochraceous	E 22-3, 0./30/70/0 pale orange yellow	white gray
Curtain darker workers	E 46-2, 10/60/100/0 ochraceous	E 46-4, 10/40/70/0 clay yellow	white gray
Intermediate form	E 46-2, 10/60/100/0 ochraceous	E 316-8, 40/40/50/ brown gray	white gray
Foragers type I	E 46-2, 10/60/100/0 ochraceous	IV E 316-8 V black	white gray
Foragers type II	E 46-2, 10/60/100/0 ochraceous	IV-V black	black
Foragers type III	E 53-2, 15/70/90/0 intensive ochraceous	III-V black	black

Fig. 1. Six types of abdomen body color in *A. dorsata* workers. The numbers indicate number of black tergites.

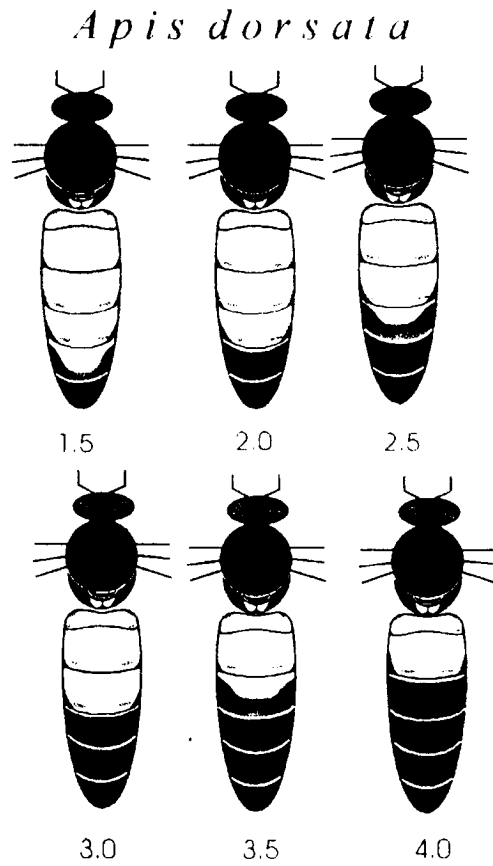


Fig. 2. Lateral view of abdomen color in *A. dorsata* worker type 2.5.

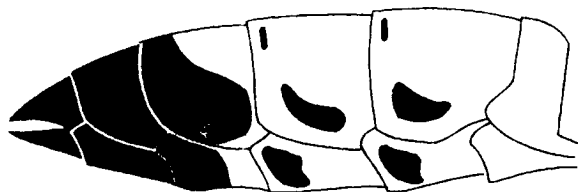


Fig. 3. Tergites of six types of abdomen body color in *A. dorsata* workers. The numbers indicate number of black tergites.

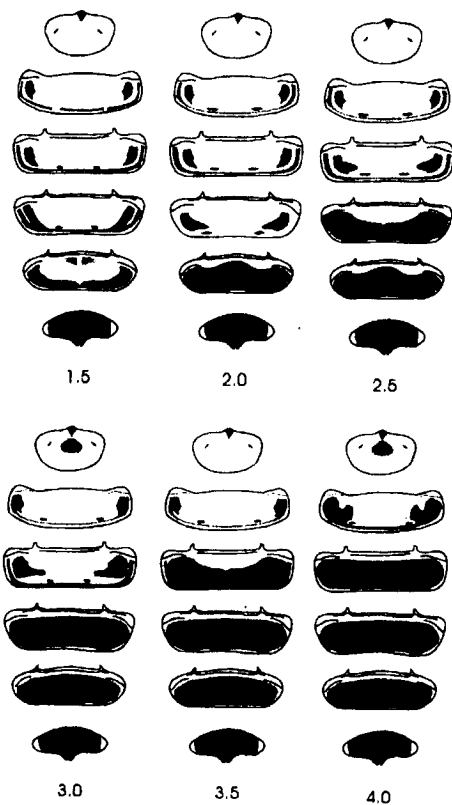


Fig. 4. Sternites of worker type 3.5.

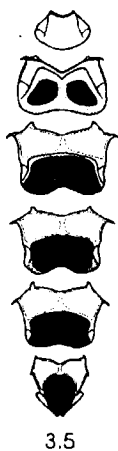


Fig. 5. Frequency percentage distribution of six body color types in seven *Apis dorsata* colonies No 0 to No 6, and in the whole population No 0 – 6. All colonies were located nearby in Chitwan region, Nepal. The colonies are arranged from the lightest to the darkest.

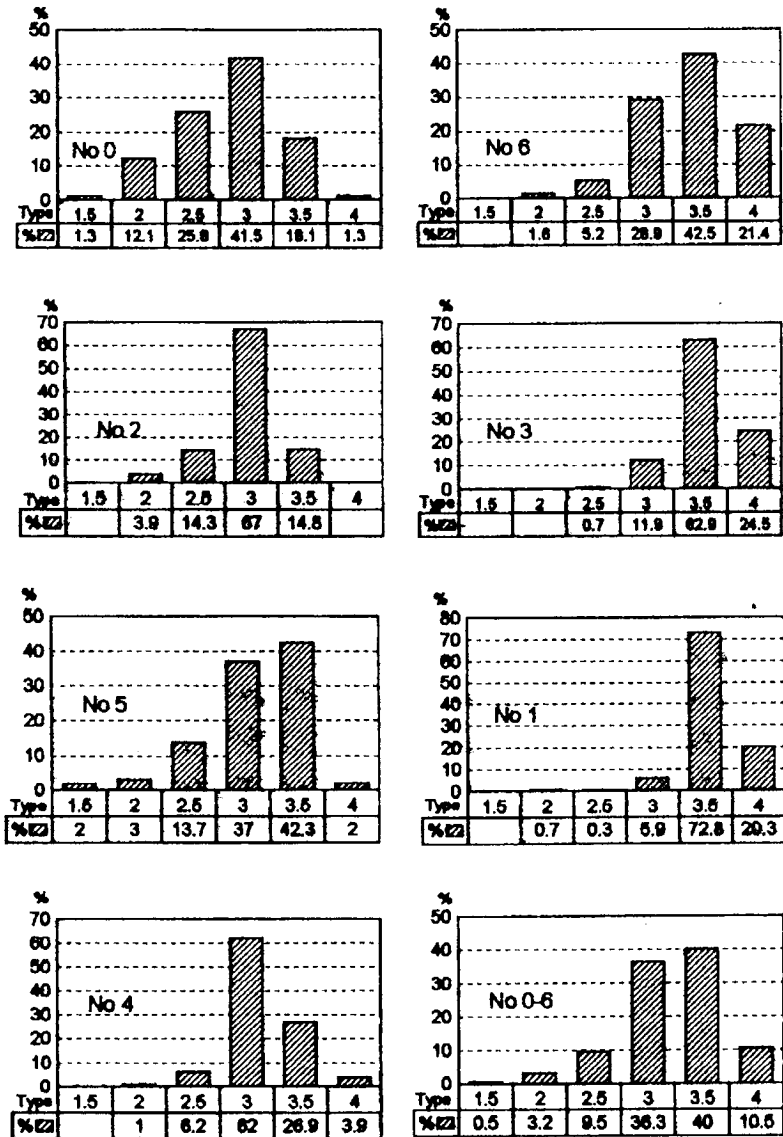


Fig. 6. Color of pubescence covering *A. dorsata* body.

Apis dorsata workers

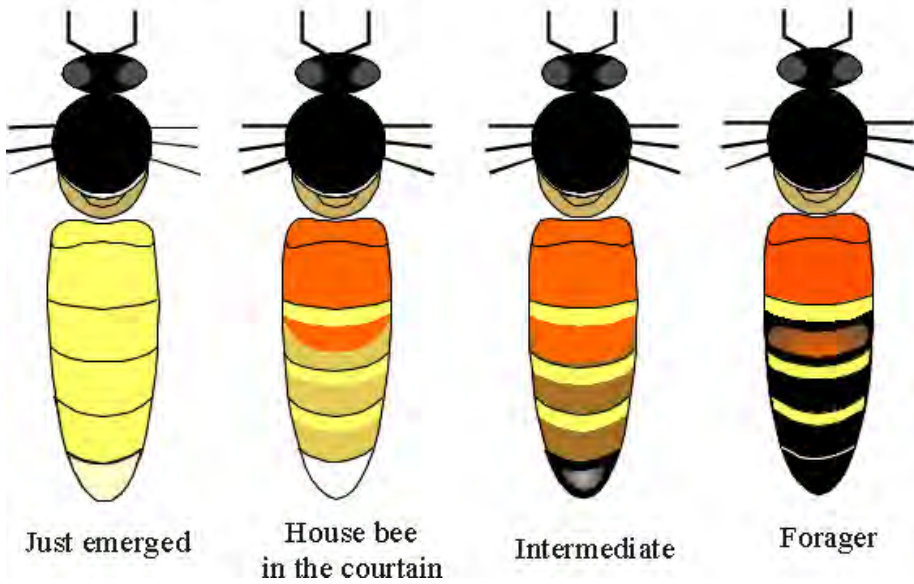


Fig. 7. Pubescence and spines covering abdomen of *A. dorsata* workers.

a – pubescence covering tergites I – V, b – pubescence covering the three light bands on tergites III, IV, and V, c – pubescence on tergite VI, d – long hairs on tergite I, e – spines on tergites II – VI.



35 days. We found foragers with black pubescence covering only one, the last but one tergite V. Foragers which have the last three or four tergites covered with black pubescence were recorded by almost all authors. However, nobody suggested that these are age-related forms. We did not find any correlation between the number of black colored body tergites and the number of tergites covered with black pubescence. We suggest that not only the pale and the be-colored abdomen are age-related (Buttel-Reepen 1906, Otis *et al.* 1990, and Roepke 1930) but that all the variations in pubescence color are not characteristic for permanent worker groups. These are age related successive steps in pubescence color changes from the lightest - bi-colored, gold yellow and white, through the median - three-colored, ochraceous, light yellow and white gray, and the intermediate three-colored ochraceous, brown-gray and white black to the darkest - bi-colored, ochraceous and four last abdominal tergites black. The shining abdomen of the foragers is the result of losing part of the pubescence by older foragers.

Workers whose body color pattern was classified quantitatively were collected from colonies in the same region. Similar environmental conditions influenced those colonies. Thus differences between those colonies must be attributed mainly to genetic variation. The distribution of the pattern of body color variation indicates that some poligenes are involved in the variation, similarly like it occurs in *A. mellifera*, Woyke (1977). Since the distribution is unimodal, the poligenes must be treated as modifiers of the one major body color gene *Do* described by Woyke (1997, 1998) which causes that the workers are yellow and black colored and queens and drones are brown.

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