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Passage of Pollens through Proventriculus in Honey Sacs of European Bee *(Apis mellifera carnica L.)*

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ABSTRACT

During the transfer of pollen grains in sugar solution throught the proventriculus, a selection of pollen grains took place according to their size and shape, which altered the relative proportions of the diffrent pollen grains. After feeding, amounts of pollen grains in honey sac were remarkedly reduced. Pollens of rape *(Brassica napus L.)* which are small (about 25 micron) and have smooth exine could not be completely filtrated by the filiform-hair of the proventriculus. One hundred thirty five minutes after feeding, they remained in the honey sac. Pollens of pine *(Pinus sylvestris L.)* which have two air-sacs (wing) floated on the upper side of honey sac. Pollens of aster *(Aster sp.)* and dandelion *(Taraxacum officinale Web.)* are relatively bigger and more spiny than rape pollens. They could be easily filtered from the fluid in honey sac by the filifrom-hairs of the comb and passed into the midgut in 95 and 100 min., respectively.

INTRODUCTION

It is not yet possible to identify the botanical and geographical origin of honey by chemical uses. However, the microscopical examination of pollen in honey can be applied. Honey-Pollen analysis is one important method which was used for along time for this purpose (**Louveaux** *et al.*, 1978; **Sawyer,** 1981; **Sturm,** 1988). But difficulties are still to be faced because the appearance of the pollen in honey and in honey sac depends on not only kinds of plant which secretes nectar but also alimentary structure of the honeybees. Proventriculus is a sphincter-like valve of honey sac which regulates flow rate of food being passed into the midgut and prevents backflow of food from the midgut when digestion and absorption take place (**Barker** and **Lehner,** 1972; **Snodgrass** 1935). Although suggested function of the bee's proventriculus have long been reported, the question how pollen grains are fittered or reduced is still unclear. **Whitcomb** and **Wilson** (1929) and **Todd** and **Vansell** (1942) found that there was a reduction process of the amount of pollen between in nectar and in honey sac. **Bailey** (1952) showed that some pollen grains were eliminated during the ripening process. They found that proventriculus trapped pollen grains from then crop and passed them from the crop into ventriculus (midgut or stomach)

This experiment was designed to study the passage of pollen grains of different sizes and forms through the proventriculus in honey sac of the european bees.

MATERIALS AND METHODS

5% pollen solution from rape *(Brassica napus L.)*, dandelion *(Taraxacum officinale Web.),* pine *(Pinus sylvestris L.)* and Helicanthus-form pollen *(Aster sp.)* collected from bee hives were prepared by using 50% sugar solution (w/v). The pollen solution were transfered in petridish and then were placed on the comb in honeybee hive. The bees took up the pollen solution. Until about 200 bees came to gather

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this syrup a small cage were carefully put on petridish and were brought finally from bee house to laboratory. In laboratory the bee fed the solution until full. The solutions were then removed from them. Every 5 minutes samples of 3 bees were collected and each bee was quick-frozen by dropping into a liquid nitrogen (−196°C). After thawing the abdomen of each bee was opened by using hand forceps and the honey sac was removed. All 3 honey sacs were pooled, crushed and mixed thoroghly. Amount of pollen grains in honey sac content was determined by use of a haemocytometer (**Soehngen** and **Jay**, 1974).

Statistical analysis of experimental data to fitting model equations were made with **Lsmlmw** computer program package from **Spain** (1982) and **Harvey** (1989) by using least square method. The curve fitting procedures were based on equation which has the minimum value for the sum of deviations square. Least Significant Difference (LSD) was used for testing the mean of the result (**Essl**, 1987). Each plotted point in Figure 1 represents the mean of 4 replications (12 honey bees).

RESULTS AND DISCUSSION

Figure 1 shows amounts of pollen in honey sac of the bee after feeding which were significantly different among the 4 types of pollen ($P < 0.001$). LSD-Test analysis showed that amounts of pollen grains in honey sac of the bee fed with 5% pollen solution of pine was highly different from those fed with 5% pollen solution of rape, dandelion and aster ($P < 0.001$). In comparison with the last 3 groups the pollen of pine lay at maximum and took an average of 17.9 ± −15.9 grains/ml honey sac content. Further, the bee fed with dandelion pollen had higher pollen content in honey sac than those fed with dandelion and aster.

Linear regression coefficient between amounts of pollen grains and the time after feeding with pine, rape, dandelion and aster were −12.65, −14.36, −18.65 and −23.60, respectively. The coefficient of multiple linear correlation between amount of pollen grains

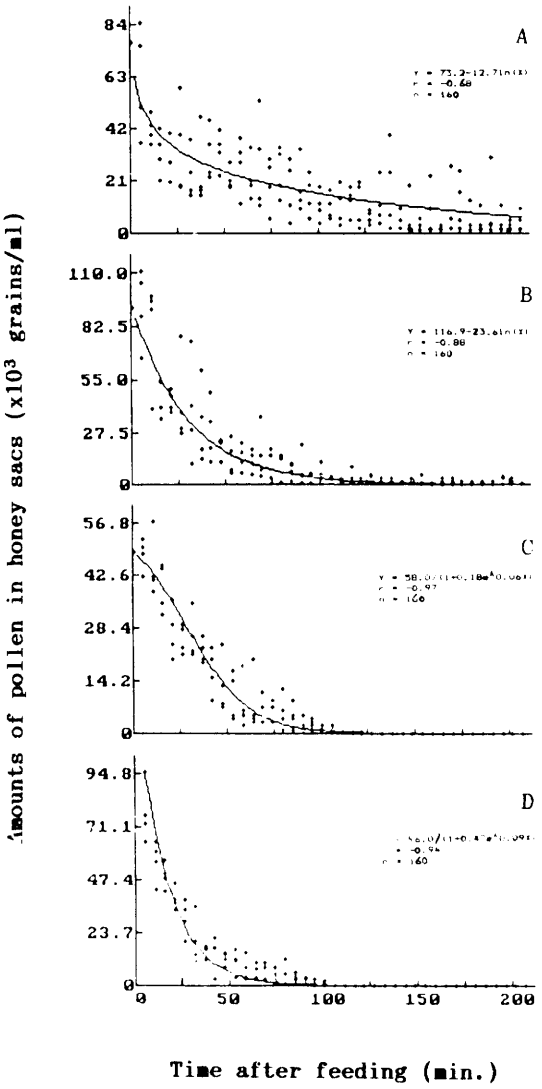

Figure 1 Changes of pollen in honey sacs of bee *(Apis mellifera I.)* after feeding with 5% pollen solution of pine (A), rape (B) dandelion (C) and aster (D).

in honey sac after feeding (as independent variable) and honey sac weight, trocken substance content, and time after feeding with pine, rape, dandelion and aster were 0.70, 0.72, 0.65 and 0.77, respectively.

The studies demonstrated that size and form of pollen play an important role in determining what kind of pollen can be utilized by bees as food sources. **Maurizio** (1949) showed that during the passage of syrup into

midgut there was no selection of pollen according to their size. However, a mutual result is found here. Passage of these four pollen types were different from others. Pollen grains of pine were found during 3 hours after the beginning of the experiment. In contradiction to the passage of pine pollen the pollen grains of rape, dandelion and aster were passed into midgut after feeding in 135, 100 and 95 minutes, respectively (Table 1). The results agreed with **Verbeke** *et al.* (1984) who reported that larger particles were more easily filtered and formed a bolus much quicker. Figure 2 shows morphological structure of the four pollen types. Form of pine pollen was vesiculate with two air sacs. Average size was about 50 micron (**Erdtman,** 1969). Because they floated on the upper site of the honey sac, so they could not be easily caught by the hairs.

Pollen grains of aster and dandelion are similar in form and shape. There was no difference of tarry duration of pollen from aster and dandelion in honey sac.

Pollens of rape have smooth exine. They could not be filtered easily with the borsten of the proventriculus like pollen of aster and

Table 1 External morphology of pollen grains from aster, dandelion, rape and pine in connection with tarry duration after feeding.

Species	Size (μm)	Form	Tarry duration (min.)
Aster	30	Tricolporate with spine	95
Dandelion	33	Fenestrate with spine	100
Rape	25	Tricolpate without spine	135
Pine	50	Vasiculate with 2 air sacs	>180

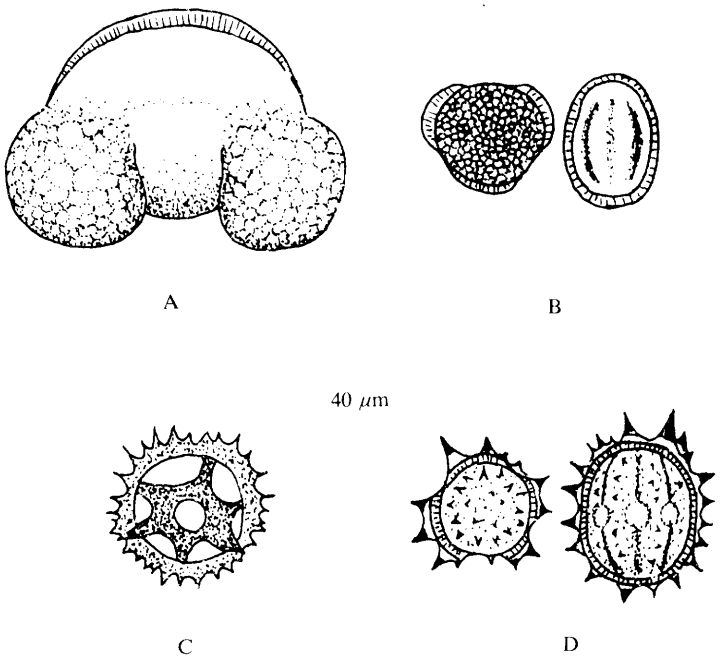

Figure 2 External morphology of pine pollen, vesiculate with 2 air sacs (A); rape pollen, tricolpate (B); dandelion pollen, fenestrate with spine (C); and aster pollen, tricolporate with spine (D).

dandelion. **Peng** and **Marston** (1986) mentioned that small particles filter through the hair and return back to the fluid.

CONCLUSION

This is important for the determination of the botanical and the geographical origin of honey by analysing the pollen spectrum in honey sac as well as in honey. Because pollen of different forms, sizes and exine structures can be variably reduced during the ripening process both in honey sac and in honey, therefore, pollen picture under microscopic field may not correspond with the source of nectar plant.

LITERATURE CITED

Bailey, L. 1952. The action of the proventriculus of the worker honeybee, Apis mellifera L.-J. Exper. Biol. 29, 310-327.

Barker J.R. and Y. Lehner. 1972. A look at honey bee gut functions.-American Bee Journal 112, 366-338.

Erdtman, G. 1969. Handbook of Palynology.-Haufner Publish Co., New York.

Essl, A. 1987. Die statistische Methode in der Tierproduktion.-Oesterreichischer Agrarverlag Wien, 316 S.

Harvey, W. 1988. Mixed Model Least-Squares and Maximum Like-lyhood Computer Program (Lsmlmw).-Ohio State University.

Louveaux, J.; Maurizio, A. and G. Vorwohl. 1978. Methods of Melissopalynology.-Bee World 59(4), 139-157.

Maurizio, A. 1949. Pollenanalytische Untersuchungen an Honig und Pollenhoeschen.-Beihefte zur Schweizerischen Bienenzeitung, Band 2, Heft 18., 455 S.

Peng, Y.S. and J.M. Marston. 1986. Filtering mechanism of the honeybee proventriculus.-Physiological Entomology 11, 433-439.

Sawyer, R. 1981. Pollenidentification for Beekeepers.-University College Cardiff Press, U.K.

Snodgrass, R.E. 1956. Anatomy of the honey bee.-Cornell University Press, New York.

Soehngen, U. and C. Jay. 1974. Studies on the honey-sac contents and pollen loads of honeybee. 1. Honey-sac contents of foraging bees.-J. Apic. Res. 13(3), 199-206.

Spain, D. 1982. Basic Microcomputer Models in Biology.-Addision-Wesley Publishing Company, London.

Sturm, J. 1988. Beispiele zur Methodik der Honigpollenanalyse (Melissopalynologie) und die Pollenspektren einiger oberoesterreichischer Honige.-Dipl. Arbeit, Universitaet fuer Bodenkultur Wien, 77 s.

Todd, F.F. and G.H. Vansell. 1942. Pollen grains in nectar and honey.-J. Econom. Entomol. 35, S. 728.

Verbeke, M.; Jacobs, F.J. and P.H. de Rycke. 1984. Passge of various particles through the ventriculus in the honey bee (*Apis mellifera* L.).- Am. Bee J. 124, 468-470.

Whitcomb, W. and H.F. Wilson. 1929. Mechanics of digestion of pollen by adult honey bees and the relation of undigested parts to dysentery of bees.- Agr. Exp. Sta. Univ. of Wisconsin, Madison, Res. Bull. No. 29.