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Studies on Honeybee-Palynology in Sapporo, 1958-1959[1) 2) 3)]

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(With 7 Text-figures)

During the recent two decades, quantitative and qualitative studies upon the honeybee-palynology were actively investigated in several countries, by Todd and Bishop (1941, U.S.A.), Synge (1947, England), Hodges (1953, England), Maurizio (1949–58, Switzerland), Hirschfelder (1951, Germany) and Louveaux (1958–59, France) etc.. Because of the lack and necessity of such works in Japan, the writer began in 1958, his palynological studies in connection with honeybee biology. The present paper is the first outcome of these. It deals with results of continuous collection of pollen pellets by the pollen trap, undertaken in 1958–59 as a joint work with Mr. Kiichi Sekiguchi, Director of Bee Culture Laboratory, Hokkaido Agriculture Experiment Station, at his apiary in Tsukisappu near Sapporo.

Before going further, the writer wishes to express his sincere gratitude to Professor Tohru Uchida and Dr. Shôichi F. Sakagami for their kind guidance. His grateful acknowledgement is also offered to Mr. Kiichi Sekiguchi for his close cooperation and aid during the course of this study.

Methods employed

The trap in the collection of pollen loads was of the type ordinary employed by many Japanese beekeepers. The cross section and screen of the trap are shown in Fig. 1. The screen is made of a piece of celluloid, 35 cm × 5 cm in size and 1 mm thickness with 120 perforations, of 5 mm in diameter; the bottom is of 3 mm wire mesh. In the ordinary apicultural procedure, two screens are inserted at intervals of several centimeters from each other, but only one in the present study, to avoid danger of too much pollen deficiency in the colonies studied.

The pollen loads were classified with naked eyes based on colour, size, shape, hardness and other characteristics. Two or three representatives of each sort were mounted on slide glasses. The material was washed first with 70% alcohol, then with xylol and 70% alcohol and fixed with glycerin jelly with a few drops of 10% basic fuchsin solution or methyl green 50% alcohol solution.

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2) Honeybee-Palynology in Hokkaido, I.

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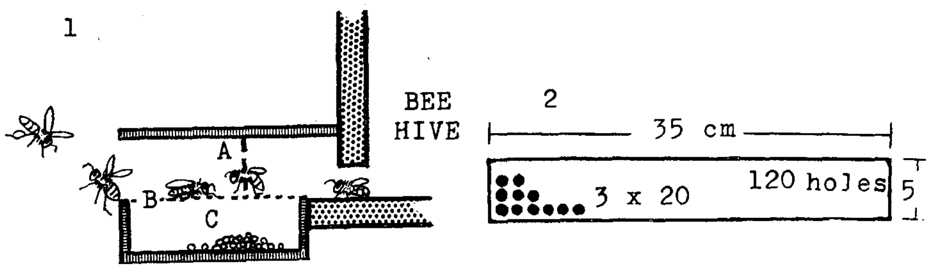


Fig. 1. Cross section of the trap (1), and the screen (2).

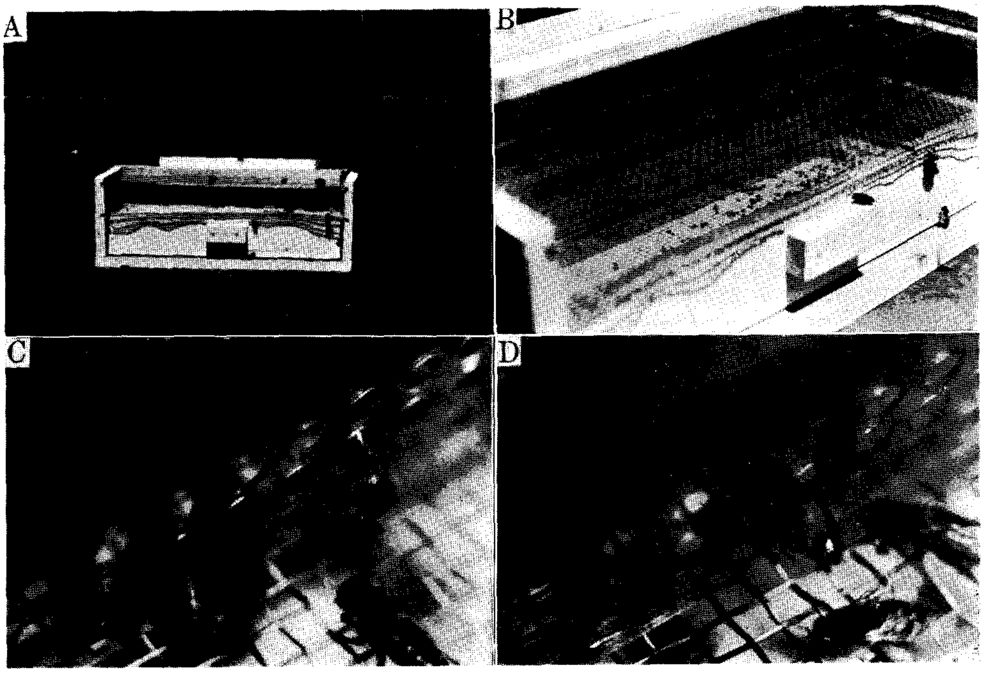


Fig. 2. A....Pollen trap in position in front of hive.
B....Close up of the pollen trap.
C, D....Bees passing the screen.

Sometimes gentian violet (0.01%) was also used for staining. In most cases identification was made by comparing the slide-samples with the slide-specimens taken directly from the flowers, of which the scientific names were accurately labelled in the Botanical Garden, Hokkaido University, Sapporo. Comparisons with figures given in previous papers, such as those of Armbruster and Jacobs (1934-'35), Erdtman (1952), Hodges (1952) and Ikuse (1956) were naturally undertaken as far as possible.

In 1958, the pollen loads were daily taken from two queen right colonies of an impure Italian race (as in most Japanese colonies of *Apis mellifera* L.). Further the population trends of the two colonies were estimated by weighing the total mass of adult bees and measuring the extent of brood area in each comb about two times per month; these values were converted to numbers for each of the two colonies studied. The total amount of daily pollen harvested was weighed by means of a 0.5 g sensitive balance every evening after foraging activities have ceased.

In order to know the principal pollen sources and their seasonal succession in the area surveyed, quantitative analyses of pollen crops were taken about weekly in 1959. In each examination, a sample of 2 g (approximately corresponding to 150 to 200 loads) was taken from the total yield which had been thoroughly mixed in advance. The number of loads in the sample was counted and the loads were classified as mentioned above.

Nature of area studied

The apiary Tsukisappu is located in hilly suburbs southeastward the city of Sapporo. The area, within circle of 2 km radius around the apiary consists of the following zones: crop fields (40%), woods (20%), meadows (20%), grass lands (10%) and flower gardens (10%).

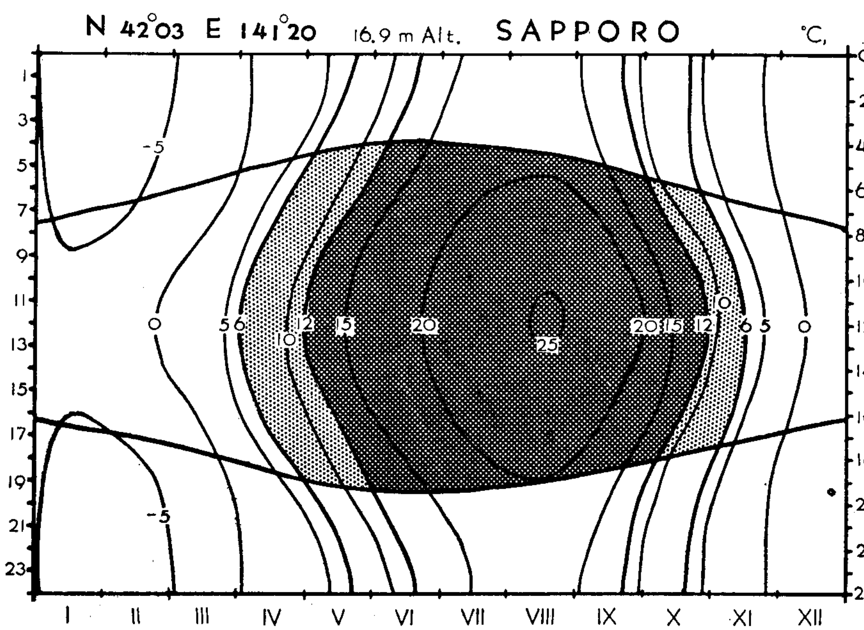


Fig. 3. Isothermoplate of Sapporo. (Data from "Climate of Hokkaido" publ. by Sapporo Region. Meteor. Observatory, 1952.)

The foraging activities of the honeybee in the area begin usually at early April but are still rather weak to early June, affected strongly by adverse weather conditions, notably in air temperature, insolation and wind velocity. From June to early September the foraging activities are brisk from early to late, the period is the most productive one through the annual cycle. From mid or late September, the foraging activities begin to decrease and quite cease in early November. Such annual and daily trends of foraging activities are illustrated in Fig. 3 as an isothermoplate. In the figure, the middle area limited by two horizontal curves shows the seasonal change of daytime in Sapporo, the lightly dotted areas the relatively unfavourable seasons and day hours for foraging, while the heavily dotted area indicates the favourable season and daytime.

Results

Population trends and amounts of daily yield of pollen (1958) : Fig. 4 gives population trends of the two colonies studied. Both colonies were sufficiently strong and kept in very good condition, but at mid July the queen of Colony 2 was replaced by a newly emerged one, resulting in the lack of subsequent broods as shown in the figure. The extent of the brood area was comparatively small in early April but grew rather rapidly to late May, and was maintained approximately in the same level during June to August and began to decline rapidly in late August or early September. The adult populations showed similar trends but the

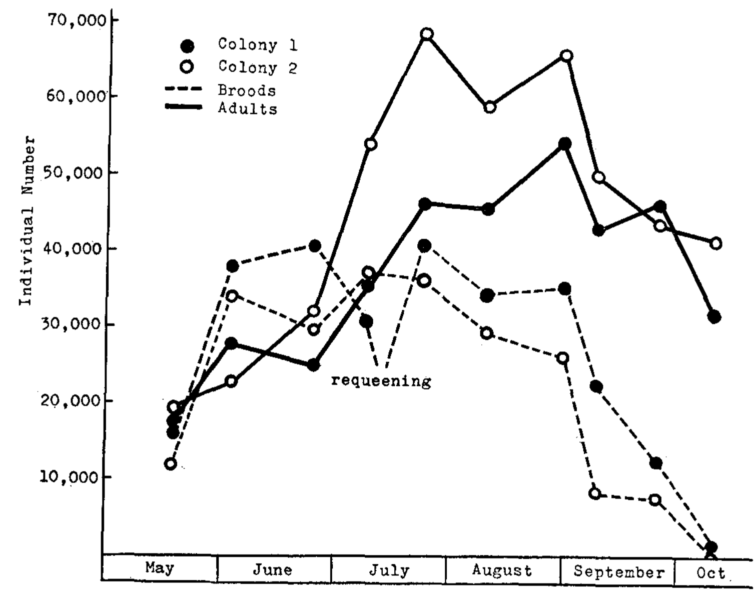


Fig. 4. Population trends of the two colonies studied in 1958 (Tsukisappu).
Notice : The colony markes for broods reverse.

attainments of maximum size showed a considerable delay in both colonies with two peaks in supersedured Colony 2 in late July and the end of August, while only one peak in Colony 1. The gap between the two peaks in Colony 2 reflects the process of colony revival by requeening. The spring constriction due to the alternation of old and new bees was distinct in Colony 1 but not in Colony 2. It was estimated from Fig. 4 that the total number of emerged bees during the course of this study, was 185,000 in Colony 1 and 190,000 in Colony 2.

Daily yield of pollen loads trapped from two colonies is shown in Fig. 5. Amounts brought in varied considerably from day to day mainly caused by weather conditions.[1] There were two peaks in pollen income, one at mid June to early July and the other at late August. The maximum yield was recorded on 15 June, 1958 as 350 g in Colony 2.

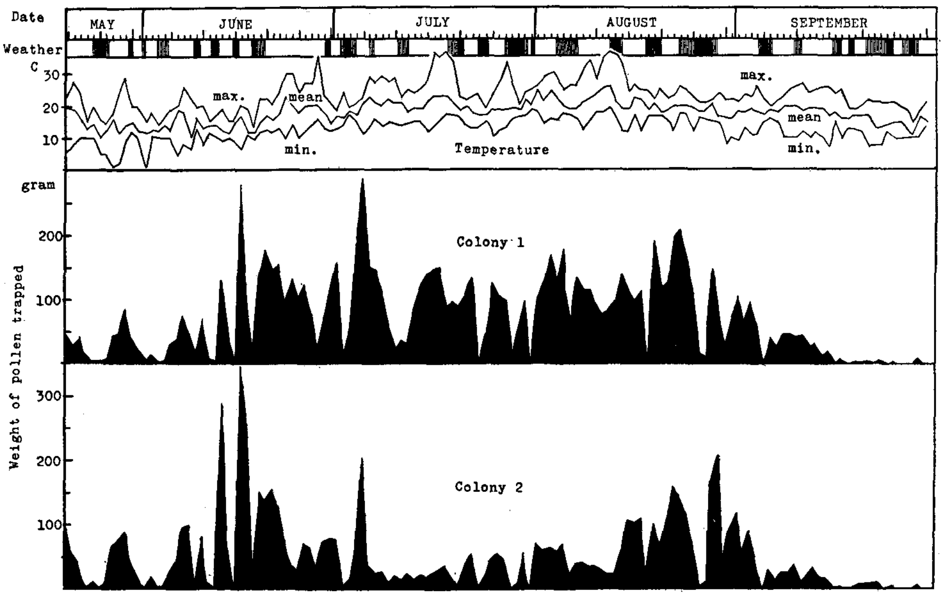


Fig. 5. Seasonal variation of pollen loads trapped, temperature, and weather in 1958 at Tsukisappu. Black bands; rainy days, Gray bands; cloudy days, Blanks; fine days.

Todd and Bishop (1941) showed in California the distinct increase of pollen income corresponding to that of sealed broods and adult population, both curves having two peaks in spring and summer respectively, while the effects of pollen abundance upon the sealed broods were less conspicuous in summer than in spring. In the present study, too, the two peaks in the curves of pollen income

1) Relationship between weather conditions and pollen yield will be reported elsewhere.

were detected but they did not always correspond exactly to the curves of the population trends. The first peak shows a correlation to the size of sealed broods in both colonies, while the second peak in late August has virtually no such relation.

Trap efficiency: Trap efficiency was tested 9 times on 10, 27, June, on 8, 22, 23, July, on 5, 29, August, and on 10, 25, September, in 1958. All test days were generally of good weather for the foraging. During 30 minutes from 8.30, 10.30, a.m., 1.30, 3.30, p.m. each, the numbers of returning pollen foragers and of trapped loads were recorded. From the results shown in Table 1, it is

Table 1. Trap efficiency shown as the percentage of trapped loads to total income.

Colony	Time	27/V,	10/VI,	27/VI,	8/VII,	22/VII,	23/VII,	29/VIII,	10/IX,	25/IX.
	8.30- 9.00	23.9	26.2	46.6	34.1	35.4	39.2	25.4	28.1	13.5
	10.30-11.00	26.2	27.6	36.1	23.7	31.1	44.5	39.4	53.1	20.7
1	13.30-14.00	30.5	28.1	42.8	51.2	20.2	—	39.4	41.9	18.6
	15.30-16.00	48.3	21.4	16.6	38.5	24.2	33.8	—	25.4	6.8
	average	32.8	25.8	41.3	36.8	27.6	39.1	34.7	37.1	14.9
	8.30- 9.00	36.3	25.3	32.4	14.0	59.3	26.6	41.2	28.3	11.5
	10.30-11.00	33.0	27.7	38.9	22.0	29.9	34.7	29.6	40.9	25.9
2	13.30-14.00	43.2	34.0	38.3	6.5	13.1	25.4	31.3	51.1	40.5
	15.30-16.00	24.9	36.6	60.0	4.2	16.7	53.6	24.6	22.4	8.4
	average	31.4	30.9	42.4	10.5	29.7	35.0	31.6	35.6	21.5

recognized that the trap efficiency varies considerably from case to case. The maximum efficiency obtained was 60 %, the minimum 4.2% and the mean 31% (31 loads trapped/50 pollen foragers). Such great variability is mainly caused by the relative ease of dropping down of pollen loads. When a pollen forager passed through the trap screen, either only one of her two loads or both of them or neither of them were removed. Removal of only one load was most frequent (See Fig. 2). Such difference depends on the size of loads and adaptability of the forager to the trap. A few days after setting the trap the foragers gained skill to pass through the screen without dropping their loads and the size of loads seems to became smaller than before setting of the trap. Precise study is required to clarify this intresting trend.

Rate of pollen consumption: From the total amount of brood reared, total weight of pollen trapped and the trap efficiency mentioned above, rate of pollen consumption can roughly be estimated as in Table 2. The values do not include winter consumption by the colonies, but there is no remakable deviation from the rates obtained by Alfonsus (1933, 145 mg), Rosov (1944, 125 mg), Haydak (1935, 3.21 mg nitrogen, approximately corresponding to 120 mg pollen) (cited from Ribbands 1953), Todd (1941, 100 mg) and Tokuda (1942, 92 mg). Further consideration will be presented elsewhere in connection with the nutritional values of pollen contents.

Seasonal succession of major pollen sources in 1958 and 1959: From analysis of pollen crops in 1958, it was clarified that the two colonies studied foraged

Table 2. Estimations of the rates of pollen consumption.

	Amount of pollen trapped	Estimated total amount of pollen, brought to the hive	Estimated total pollen consumption	Total number of emerged bees	Estimated rate of pollen consumption per individual
Colony 1	9.7 kg	29.2 kg	19.5 kg	185,000	108,3 mg
Colony 2	7.0	23.1	16.1	190,000	84.7

Table 3. Monthly amount of pollen crops and estimation of total pollen yield in gram.

Colony		May*	June	July	August	Sept.	Total
1	Trapped	402	2,277	2,915	3,431	683	9,718
	Total estimated	1,225	6,796	9,053	9,507	2,626	29,208
2	Trapped	705	2,392	1,071	2,279	618	7,068
	Total estimated	2,245	6,536	5,328	6,834	2,168	23,111

* From 19th to 31st.

about 60 species of plants, although about one half of these species are still unidentified. The seasonal succession in 1958 of the identified sources is shown in Fig. 6 as a pollen crop calendar. Further the relative importance among major sources was quantitatively studied in 1959. By the sampling procedure mentioned, the relative values among different pollen loads trapped do not always strictly correspond to the frequency of bee visits, for the size of pollen loads varies according to the species, on different days. The results may, however, serve as a first approximation to the relative importance of major pollen sources in the area studied. The mixed loads occupied only 1 % or less (mean 0.5%) of some daily amounts, hence these may be discarded from the consideration.

The pollen collection in 1959 began about half a month earlier than in 1958. First spring pollen trapped was that of coltsfoot (*Petasites japonicus* Miq.) and willows (*Salix* spp.), the former species is more abundant than the latter in early spring, but the relative weight reversed in due course. In late April, ash (*Fraxinus Sieboldina* Blume.) and pachysandra (*Pachysandra terminalis* Sieb.) appeared at the rate of 30 % and 10 % in Colony 2 and 9% and 5% in Colony 1. At the beginning of May the escaped winterraps (*Brassica napus* L.) showed the high ratios of 40% (Colony 2) and 60% (Colony 1). Dandelion (*Taraxacum officinale* Wed.) appeared synchronously and continued to mid June (Colony 2) or early July (Colony 1), with long duration of relatively low importance. Stone-fruits pollen, apple (*Malus pumila* Miller var. *domestica* Schneider), cherry (*Prunus avium* L.), bird cherry (*Prunus Padus* L.) and other species of *Prunus* were the principal pollen sources in May supplying about 30%

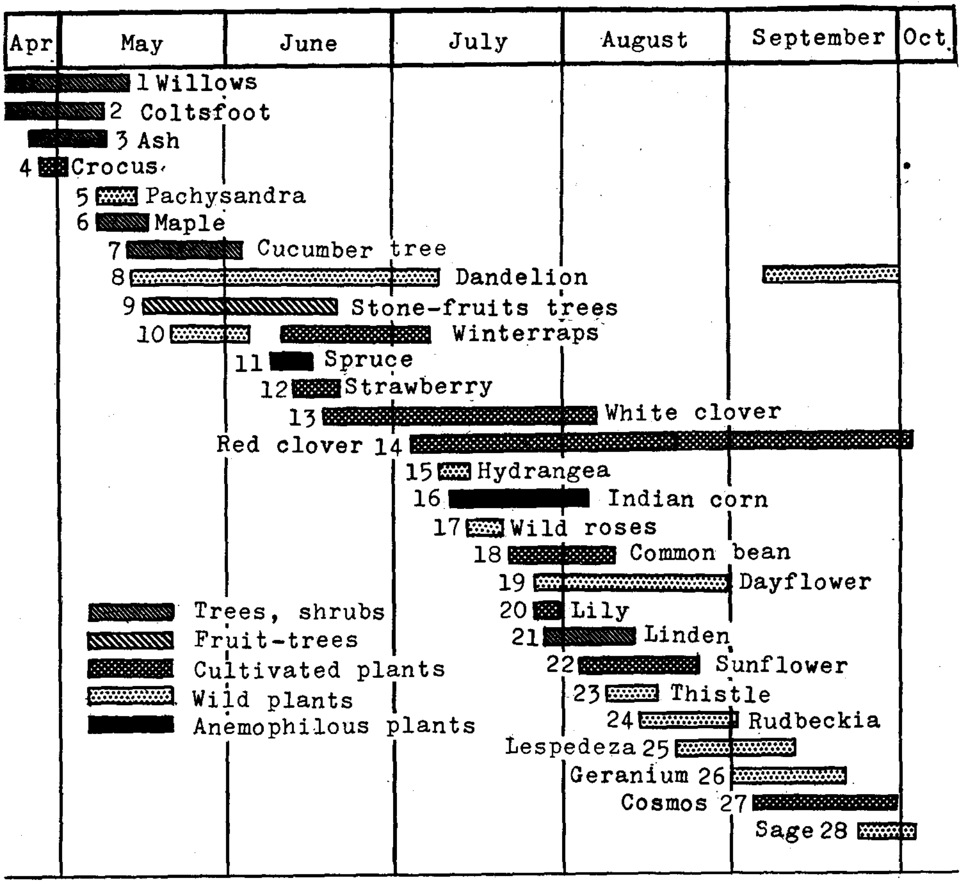
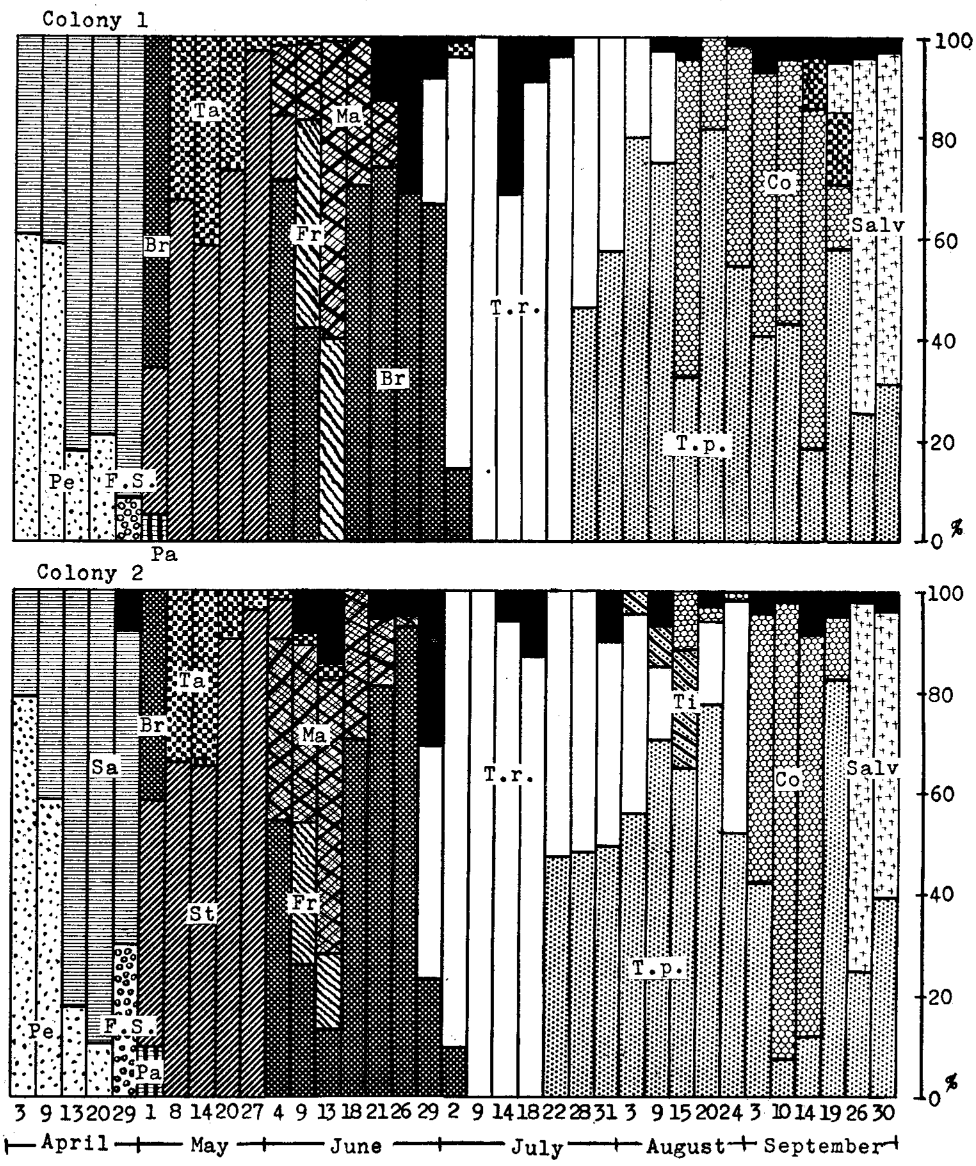


Fig. 6. A calendar of pollen loads trapped at Tsukisappu (1958).

Fig. 7. Seasonal variations of relative abundance of pollen loads trapped in the two colonies at Tsukisappu (1959). Abbreviations : Sa; willows (*Salix* spp.), Pe; coltsfoot (*Petasites japonicus* Miq.), F.S. ; ash (*Fraxinus Sieboldina* Blume.), Br; winterraps (*Brassica napus* L.), Pa; *Pacysandra terminalis* Sieb., Ta; dandelion (*Taraxacum officinale* Web.), St; Stone-fruits (mostly *Prunus* spp.) Ma; cucumber tree (*Magnolia obovata* Thunb.), Fr; strawberry; (*Fragaria chiloensis* Duch. var. *ananssa* Baily), T.r.; white clover (*Trifolium repens* L.), T.p.; red clover (*Trifolium pratense* L.), Ti; lindens (*Tilia japonica* Simk. and *Tilia Maximowiziana* Sirasawa), Co; dayflower (*Commelina communis* L.), Salv; sage (*Salvia* sp.), black; others.


Colony 1
Colony 2
Pe
F.S.
Pa
Sa
Br
Ta
St
Ma
Fr
T.r.
T.p.
Ti
Co
Salv
100
80
60
40
20
0 %
3 9 13 20 29 1 8 14 20 27 4 9 13 18 21 26 29 2 9 14 18 22 28 31 3 9 15 20 24 3 10 14 19 26 30
April May June July August September

at the beginning and nearly 90% at the end of this month. In early June, cucumber tree (*Magnolia obovata* Thunb.) pollen was trapped in rate of 14% (Colony 1) and 32% (Colony 2) and reached the maximum in mid June, with subsequent gradual decrease. Then the honeybee turned from trees to cultivated plants gradually during mid June. The cultivated winterraps (*Brassica napus* L.) was the dominant source from this period to early July; it is also one of the most important nectar sources in this area. In early to late June strawberry (*Fragaria chiloensis* Duch. var. *ananssa* Bailey) was observed to supply at a rate of 42–40% in Colony 1 and 18–25% in Colony 2, caused by a purely local condition, namely its abundance of occurrence in fields immediately in front of the apiary. Spruce (*Picea excelsa* Link.) pollen was found in this period, but it was of low importance quantitatively. In June and July the pollen sources were numerous but mostly minor sources, still unidentified. From late June, the pollen of white clover (*Trifolium repens* L.), one of the most important nectar plants of Hokkaido, appeared and with relative high importance in both colonies as a pollen source. Red clover (*Trifolium pratense* L.) pollen was trapped from early July. It has the longest incoming period from July till September, nearly end of the bee season and is of high importance. At the beginning of August pollen income from lindens (*Tilia japonica* Simk., *Tilia Maximowizciana* Sirasawa) and dayflower (*Commelina communis* L.) were observed. In September, the dayflower occupied the greater part of the total, while dandelion (*Taraxacum officinale* Web.), cosmos (*Cosmos bipinnatus* Cav.), thistle (*Crisium* spp.) were trapped as the major sources. The pollen foraging ceased during early October with pollen of red clover and sage (*Salvia* sp.) being the last to be foraged.

Discussion

Among the results described above, here are offered some remarks on the relative importance of major pollen sources alone. Other problems such as the total yield, consumption rate, trap efficiency, etc. will be considered in subsequent papers.

From the amounts of monthly pollen input and relative rate of supply from various major sources, the relative importance can be ordered from high to low as follows: 1) white clover, 2) red clover, 3) winterraps, 4) stone-fruits and 5) cucumber tree. According to Synge (1947) the important pollen sources in Rothamsted, England, were white clover, red clover, fruits, *Onobrychis sativa*, *Brassica* and *Sinapsis* quantitatively.

Louveaux (1958–'59) summarized the relative importance of various sources from the pollen census taken from 20 different localities in France as follows: cruciferous 32%, red clover 16%, chestnut 5.3%, *Quercus* 4.9%, white clover 3%, dandelion 3%, willow 3%. Comparing these results with each other, there are, of course, some minor differences reflecting geographical differences, but it is distinct that the clovers, fruits and winterraps play important parts in the maintenance of the life of the honeybee, either in the West or in the East.

This indicates that although the honeybee is qualitatively one of the most polylectic species among various bees (Bohart 1952), she obtains her protein nutrients mainly from cultivated plants and these are intimately connected with the man-made environments, in contrast to certain oligolectic wild bees. Such similarity is seen also in the seasonal succession of major sources, as ordered in Tsukisappu, Rothamsted, Bures-sur-Yvette and Bavaria in general (Table 4).

Table 4. Monthly distribution of main pollen sources in different localities.

	March	April	May	June	July	August	September
Tsukisappu n. Sapporo		*Salix* *Petasites*	*Pachyasndra* *Fraxinus* Fruits *Taraxacum*	*Brassica naps* white clover *Magnolia*	white clover red clover	red clover white clover *Commelina*	*Commelina* *Compositae*
Rothamsted Synge (1948)	*Fraxinus* *Ulmus*	*Populus* *Fraxinus* Fruits *Acer*	Fruits *Crataegus* *Vicia faba* *Ranunculus*	white clover *Onobrychis*	red clover *Onobrychis*	red clover	*Brassica* *Hedera helix*
Bures-sur-Yvette Louveaux (1958-59)	*Corylus*	*Salix* *Veronica* *Populus*	Fruits *Acer* *Brassica naps* *Ranunculus*	*Cruciferae*	*Cruciferae*	*Castanea* *Cruciferae*	*Compositae*
Bavaria in general Bauer (1958)		*Erica*	*Taraxacum* *Acer* Fruits *Brassica naps*	*Onobrychis* *Rubus* *Sinapsis alba*	*Picea* *Rubus* white clover	red clover *Centaurea* *Abies* *Melilotus*	*Cirisium* *Medicago*

The difference at the start of the season, reflected by the absence of pollen collection during March in Tsukisappu and Bavaria, is obviously conditioned by the cold climate. It is of interest that pollen of spruces, *Picea* and *Abies*, appeared in Bavaria and also that spruce (*Picea excelsa* Link.) pollen was trapped in Tsukisappu because of the bioclimatological similarity. It is supposed that absence of red clover and white clover in Bures-sur-Yvette was caused by a local condition.

A few words may be devoted here to the importance of red clover, because the necessity of an increase of seed production of this crop has been one of the important problems in pasture management. It was often believed that the red clover is not attractive to the honeybee because its tongue is too short to suck up the nectar through long collora tube of this plant. Various studies, however, suggested that the honeybee is useful for the seed production of the red clover, and many attempts were made to increase the number of honeybees visiting this plant. (cf. Butler and others 1956, v. Frisch 1947 and Thompson 1949.) There are several Japanese writers who expressed that the honeybee is less effective in the seed production of red clover in comparison with some wild bees (Suematsu and Matumoto 1949, Yamada and Ebara 1952, Hiraishi, Koike and Hirase 1954). From these papers a negative opinion as to the use of honeybee for seed production of red clover has dominated the thinking of students of pasture plants of Japan. There is no question as to the inefficiency of an individual honeybee in comparison with that of certain wild bee such as *Bombus* and *Eucera*. But these writers just referred to overlooked an important fact. The summation of indivividually the great number of inefficient honeybees can serve as powerful pollination agents. Moreover, Murakami and others (1958) recently found that the honeybee forages both nectar and pollen from red clover to serve as a pollination agent in Tsukisappu. In the present study, the pollen analysis suggests that the honeybee used enormous amounts of red clover pollen. This strongly indicates that honeybees can be useful as pollinators of red clover and their use must be developed in Japan, too.

Finally, there is a troublesome problem in the study of honeybee palynology : the colony difference within one and the same apiary. Todd and Bishop (1940) and Synge (1947) described that the pollen yields differ both qualitatively and quantitatively even among colonies set side by side. This was emphasized by Ribbands (1954) in connection with his theory of the origin of nest odour. In the present study, too, pollen yield of colonies 1 and 2 showed some significant differences in the proportion and duration of the different sources. For instance, pollen of the lindens appeared only in Colony 2. The proportion of the dayflower pollen in Colony 2 was almost always higher than in Colony 1. Also the white clover pollen collection of Colony 2 had longer duration than that of Colony 1. The colony differences are indubitably important facts to be checked in order to prepare the pollen and nectar calendar of a given area.

However, there was found no marked difference between the two colonies studied and between the two years, 1958 and 1959, at least in the succession and relative importance of the major pollen sources. The results described above may indicate approximately, though incomplete in minor details, the general features of honeybee-palynology in the area studied.

Summary

1) The pollen foraging of the honeybee was studied in 1958–59 by using pollen trap method at Tsukisappu near Sapporo.

2) Approximately 60 species of plants were used as pollen sources. The relatively important of some major sources and their seasonal succession were quantitatively determined. There quantitatively important pollen sources were white clover, red clover, winterraps, stone-fruits and cucumber tree.

3) Daily takes of incoming pollen of two colonies were continuously recorded from 19 May to September in 1958. The were two periods of heavy income, mid June and late August. The maximum harvest was recorded on 15 June as 350 g in Colony 2.

4) From the estimated total amount of emerged bees, and the trap efficiency, the amount of incoming pollen was 19 kg (Colony 1) and 22 kg (Colony 2); the total consumption, 7.8 kg (Colony 1), and 9.1 kg (Colony 2) and the consumption per individual 108 mg (Colony 1) and 84 mg (Colony 2) in fresh weight.

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