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Bionomics and Sociology of the Summer Matrifilial Phase in the Social Halictine Bee, *Lasioglossum duplex*[1)2)]

By

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(With 35 Text-figures and 25 Tables)

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1) Bionomics of the social halictine bee, *Lasioglossum duplex*. IV (=Continuation of "Biology of the primitive social bee, *Halictus duplex* Dalla Torre. I-III").

2) Contribution No. 803 from the Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, Japan.

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Introduction

In a previous paper, the annual cycle of the primitively social halictine bee, *Lasioglossum (Evylaeus) duplex* (Dalla Torre), was briefly outlined (Sakagami and Hayashida, 1958), followed by descriptions of nest architecture (1960) and activities in the spring solitary phase (1961). After the end of spring activities, each nest of this species enters an inactive phase until reactivation in summer. Each summer nest is mostly occupied by a nest foundress or mother, surviving since spring, and her newborn daughters. A division of labor appears among them: the mother acting principally in oviposition and the daughters, mostly uninseminated with undeveloped ovaries, more in foraging. But there are some deviations from this general pattern, as discussed in the subsequent sections. The present paper is a comprehensive monograph of this summer matrifilial phase, which is the most interesting part of the life cycle in this species and in halictine sociology in general. The observations were started in 1956 and are still not finished. But during this decade, our knowledge of halictine sociology has been greatly enriched. Through the successive publications of detailed studies in both hemispheres, it has now firmly been established that these bees represented one of the most fascinating groups in the study of comparative animal sociology, because of the occurrence of diverse social patterns. Therefore, it may be appropriate to publish our results so far obtained, leaving still unsolved problems for the future, in order to facilitate comparative studies of other species.

Before going further, we express our cordial thanks to all members of the staff of the Botanical Garden, Hokkaido University, for the free use of nest aggregations, and to Dr. Kiyoki Moriya, who helpled us in the field observations. Our sincere thanks are expressed to Prof. Charles D. Michener, Department of Entomology, University of Kansas, who read through the manuscript, for his continuous stimulation and suggestions for our study during the past ten years.

Most observations were carried out with nest aggregations in the Botanical Garden, Hokkaido University, Sapporo, partly with those on the University Campus. The methods adopted are mostly those given by Linsley, MacSwain and Smith (1952) and Michener *et al.* (1955). Some special procedures will be explained in each section. For convenience's sake, the reference to, and discussions on the previous information will be given in each section. The species names are cited with abbreviations of generic names, or if designated, of subgeneric names, with corresponding changes of terminations of specific names.[1] The full names are listed here, together with the reference to the publications, which are often not given in the text unless necessary. The list also includes the names of localities where the sutides were made, and the names used by the authors, when different from our usage[2].

Al. persimilis. Augochlorella persimilis (Viereck). Ordway, 1965, '66, Kansas.

Al. striata. Augochlorella striata (Provancher). Ordway, 1965, '66, Kansas.

As. sparsilis. Augochloropsis sparsilis (Vachal). Michener and Lange, 1958 b, S. Brazil.

D. imitatus. Lasioglossum (*Dialictus*) *imitatum* (Smith). Michener, 1958, Kansas, *Lasioglossum* (*Chloralictus*) *stultum* (Cresson); Michener and Wille, 1961, Kansas, *Lasioglossum* (*Chloralictus*) *inconspicuum* (Smith); Knerer et Plateaux-Quénu, 1967 b, Ontario.

D. rhytidophorus. Lasioglossum (*Dialictus*) *rhytidophorum* (Moure). Michener and Lange, 1958 c, S. Brazil, *Chloralictus rhytidophorus.*

D. zephryus. Lasioglossum (*Dialictus*) *zephyrum* (Smith). Batra, 1964, '66, a, Kansas.

D. versatus. Lasioglossum (*Dialictus*) *versatum* (Robertson). Michener, 1966 a, b, Kansas.

Ev. calceatus. Lasioglossum (*Evylaeus*) *calceatum* (Scopoli). Fabre, 1882, France, *Halictus cylindricus* (Fabricius); Vleugel, 1961, Holland, *H. calceatus*; Plateaux-Quénu,1963, '64, France, *H. calceatus*; Bonelli, 1965 a, Italy, *H. calceatus.*

Ev. cinctipes. Lasioglossum (*Evylaeus*) *cinctipes* (Provancher). Knerer et Plateaux-Quénu, 1967 a, Ontario, *Evylaeus cinctipes.*

Ev. duplex. Lasioglossum (*Evylaeus*) *duplex* (Dalla Torre). Sakagami and Hayashida, 1958, '60, '61, Japan, *Halictus duplex.*

Ev. malachurus. Lasioglossum (*Evylaeus*) *malachurum* (Kirby). Stöckhert, 1923, Germany, *Halictus malachurus*; Legewie, 1925, Germany, *H. malachurus*; Aptel, 1931, France, *H. malachurus*; Noll, 1931, Germany, *H. malachurus*; Bott, 1937, Germany, *H. malachurus*; Bonelli, 1948, Italy, *H. malachurus*; Knerer et Plateaux-Quénu, 1966 b, '67, France, *Evylaeus malachurus.*

Ev. marginatus. Lasioglossum (*Evylaeus*) *marginatum* (Brullé). Plateaux-Quénu, 1959, '60, '62, France, *Halictus marginatus.*

Ev. minutus. Lasioglossum (*Evylaeus*) *minutum* (Schranck). Bonelli, 1954, England, *Halictus minutus.*

Ev. nigripes. Lasioglossum (*Evylaeus*) *nigripes* (Lepeletier). 1965 a, b, France, *Evylaeus nigripes.*

Ev. ohei. Lasioglossum (*Evylaeus*) *ohei* Hirashima et Sakagami. Sakagami, Hirashima and Ohé, 1966, Japan.

1) This does not mean the acceptance of these subgenera as genera.

2) The list includes many but not all halictine species, the biology of which was so far studied.

Ev. pauxillus. Lasioglossum (*Evylaeus*) *pauxillum* (Schenck). Stöckhert, 1923, Germany, *Halictus pauxillus*; Knerer et Plateaux-Quénu, 166 b, '67, a, France, *Evylaeus pauxillus.*

Ev. trisipinis. Lasioglossum (*Evylaeus*) *trispine* (Vachal). Sakagami, unpub., Japan.

H. latisignatus. Halictus (*Halictus*) *latisignatus* Cameron. Sakagami and Wain, 1966, India.

H. ligatus. Halictus (*Halictus*) *ligatus* Say. Knerer et Plateaux-Quénu, 1966 c, *Halictus ligatus.*

H. maculatus. Halictus (*Halictus*) *maculatus* Smith. Knerer et Plateaux-Quénu, 1966 b France, *Halictus maculatus.*

H. scabiosae. Halictus (*Halictus*) *scabiosae* (Rossi). Fabre, 1882, France, *Halictus scabiosae*; Quénu, 1957, France, *H. scabiosae*; Knerer et Plateaux-Quénu, 1966, b, France, *H. scabiosae*; Batra, 1966 b, Switzerland, *H. scabiosae.*

H. sexcinctus. Halictus (*Halictus*) *sexcinctus* (Fabricius). Bonelli, 1965 b, Italy, *H. sexcinctus.*

H. quadricinctus. Halictus (*Halictus*) *quadricinctus* (Fabricius). Verhoeff, 1891, Germany, *Halictus quadricinctus.*

L. leucozonium. Lasioglossum (*Lasioglossum*) *leucozonium* (Schenck.) Bonelli, 1954 a, England, *Halictus leucozonius.*

L. occidens. Lasioglossum (*Lasioglossum*) *occidens* (Smith). Sakagami, unpub., Japan.

Ps. divaricatus. Pseudagapostemon divaricatus (Vachal). Michener and Lange, 1958 a, S. Brazil.

S. aerarius. Halictus (*Seladonia*) *aerarius* Smith· Sakagami and Fukushima, 1961, Japan.

S. hesperus. Halictus (*Seladonia*) *hesperus* Smith. Sakagami and Moure, 1965, S. Brazil.

S. subauratus. Halictus (*Seladonia*) *subauratus* (Rossi). Bonelli, 1966, Italy, *Halictus subauratus.*

S. tumulorum. Halictus (*Seladonia*) *tumulorum* (Linné). Sakagami, unpub., Japan.

In subsequent sections, the terms *mother* and *summer daughter* (or simply *daughter*) are used more or less in the senses of queen and worker used by Michener and his school (Ordway, Batra, etc.), and the term *young* means the immature individuals from eggs to newborn adults still within the cells.

Results and Discussions

1. Phenology

The annual cycle of *Ev. duplex* consists of the following six phases: 1) Hibernating phase, 2) Spring solitary phase, 3) Presummer inactive phase, 4) Summer matrifilial phase, 5) Postsummer inactive phase (indistinct in some nests), 6) Autumn mating phase.

To give a clear picture of three successive active phases, separated by two inactive phases, Fig. 1 was prepared based upon the periodic sampling done in 1959. In this year the relative abundance and phenology of all wild bees on the University Campus and Botanical Garden were studied by sampling bees on flowers, eight hours per week (*cf.* Sakagami and Matsumura, 1967. On the procedures, *cf.* also Sakagami, Laroca and Moure, 1967). The records of *Ev. duplex*, which was one of the dominant species in the areas surveyed, were extracted from the results and given

in Fig. 1, the sexes separately, together with the degree of wear of mandibles and wings in female specimens.

The segregation of three active phases is obvious from the figure : Spring phase from late April to early June, but principally in May; summer phase, July to early August; and autumn phase late August to middle September. The figure also shows the gradual aging of females, both in spring and summer phases. The autumn females include two different types, fresh and quite worn ones, apparently representing newly born females and aged ones after continuous work since spring.

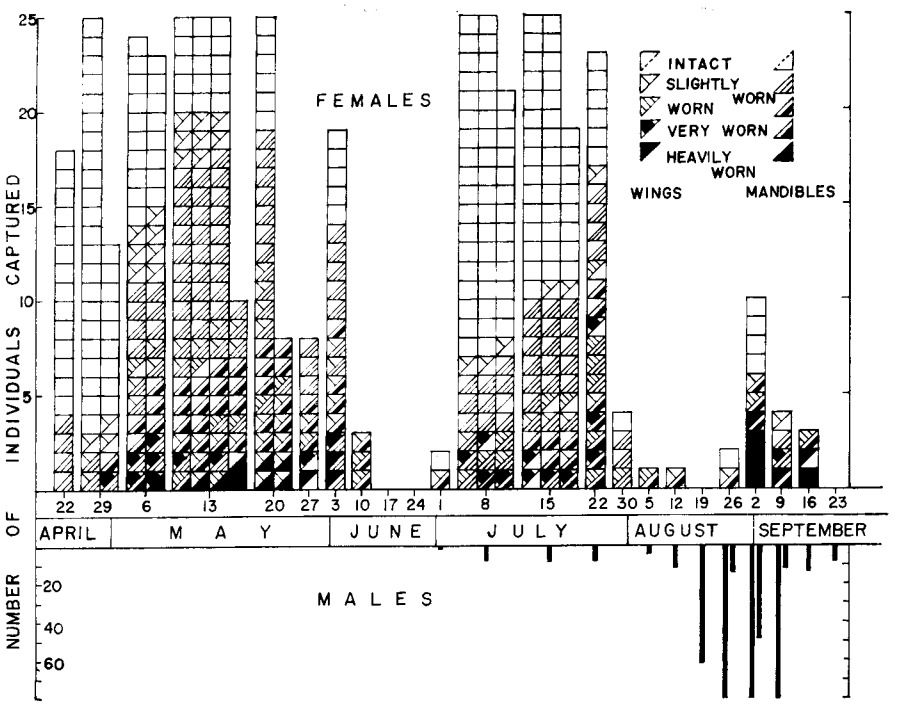


Fig. 1. Phenology of *Ev. duplex* based upon weekly sampling from flowers in 1959. Dates indicate weekly midpoint. In females, the relative age of each individual is given by wear of wings and mandibles.

Another peculiarity noticed is the larger number of females captured in spring than in summer, 129 versus 98 in the Botanical Garden, and 121 versus 71 on the University Campus, or 1:0.77 and 1:0.59, respectively (1:0.68 in total). In the previous paper, the survival of spring nests to summer was given as 22.5%, but an improved estimate gives 17.5% (*cf. 3. 3.*). In average about five daughters are produced in these successful nests (*cf. 3. 3.*), so that the number of daughter bees

captured in summer on flower is lower than that theoretically expected, provided the chance of capture is same for both seasons. The marked deviation given above from the theoretical ratio is explained as follows: 1) Effect of removal of individuals by sampling. 2) Difference in the relative ease of discovering and capturing females. (For instance, spring mothers are easily discovered and captured when visiting the European dandelion. *Taraxacum officinale*, one of the most important vernal food sources for wild bees.) 3) Shorter life span of daughters. 4) Dispersal of some daughters from original nests and resulting earlier deaths. The first two items could be classified as observational errors, whereas the latter two are intrinsic in the biology of this species as referred to later (*4.2.* and *3.4.*).

The duration of the summer matrifilial phase varies from year to year. The dates, on which about half nests began or finished foraging activities have been recorded for four years as follows:

	Activities		
Year	50% started	50% finished	Approximate duration
1957	July 10	August 7	29 days
1958	2	July 30	29 days
1959	June 29	24	25 days
1960	July 10	August 5	27 days

Delayed starts of activities in 1957 and 1960 were probably caused by adverse weather conditions. In 1960 the survival of spring nests was low (Sakagami and Hayashida, 1961), and some spring females were on the wing even on July 5. In the same paper, different developmental rates between the broods taken from the University Campus and Botanical Garden were explained by the microclimate of nesting sites. In 1959 the date of 50% activities was June 29 in the Botanical Garden, July 6 on the University Campus. A similar difference is seen even within one aggregation. In the nest aggregation occupying the beds of medicinal plants in the Botanical Garden (*cf* Fig 1, Sakagami and Hayashida, 1961), both spring and summer activities start from the northernmost area, which is most favorable in microthermal conditions. This tendency has continued during ten years without change and the date of 50% activities was June 23 in 1959 in this area.

Fig. 1 also shows the flight activities of males. The males captured during July belong to the first brood, reared together with summer daughters. The sex ratio shown in Fig. 1 is 14:98 in the Botanical Garden, 12:72 in the University Campus, or 1:6.6 in combination. The ratio of males is distinctly higher than in the samples taken from nests (1:22.8 in Sakagami and Hayashida, 1961). In the latter case, some males might already have left the nests. The ratio 1:9 or 10 may be convenient compromises. The signfiicance of these summer males is referred to later in Sections, 2, 5 and 6.

After mid-August, the number of males increases, especially in comparison with that of females; the sex ratio in young taken from nests is approximately 1:1 (*cf. 3.1.*). This fact and the lower extranidal activities of prehibernating females are characteristic of the species, especially when compared with some other

species, for instance, *L. occidens*, both sexes of which are abundantly collected on flowers in late summer to early autumn.

The discrete or periodic production of broods as seen in *Ev. duplex* is known in some other species of *Evylaeus*: *malachurus* (Stöckhert, Legewie, Noll, Bonelli), *calceatus* (Fabre, Bonelli, *cf.* also Plateaux-Quénu, 1963), *nigripes* (Plateaux-Quénu, 1956 a, b), *pauxillus* and *cinctipes* (Stöckhert, Knerer and Plateaux-Quénu, 1967 a). Knerer and Plateaux-Quénu (1967 a) regard this trait as a characteristic of the group. But it must be mentioned that not all species of *Evyaleus* produce more than one brood per year. In Sapporo, *Ev. duplex* and *Ev. trispinis* produce two broods per year, but there are at least two other species which rear only one brood per year. Even *Ev. malachurus*, which produce three broods in Southern Germany and probably four in Italy, appears to have only one brood in Northern Germany (Alfken, according to Legewie). The extent of separation of spring and summer phases also seems to be variable among species. In *Ev. duplex*, the dates of the final capture of spring females and the earliest capture of summer females are separated by an interval lasting at least two weeks, usually more. On the other hand, in *Ev. trispinis*, the interval is shorter, often less than one week, though the segregation of spring and summer phases is distinct.

Moreover, the cessation of brood rearing activity by the foundress after completing her first batch of eggs is known, though less pronounced, in some non-*Evylaeus* halictine bees: *H. sexcinctus*, *S. subauratus*, *S. tumulorum*, *Al. striata* and *persimilis*, *D. imitatus* and *zephyrus*. In *D. versatus*, Michener (966 a) did not find such cessation of activity, but he said that this did not necessarily mean that each individual did not pass through a quiescent period after laying her first batch of eggs; probably it merely reflected lack of synchronization among the numerous females in a nest. The females of *D. zephyrus* reared by Batra (1966 a) in an insectary started nests at any time of year but always ceased activity until their first progeny emerged. Therefore, the cessation of the brood rearing activity after completing the first few brood cells seems to be common in halictine bees. This trait may simply correspond to the limit of brood rearing potential in solitary Aculeata. Even in bumbelbees, the production of egg batches by the foundress queen is often discontinuous until the first workers emerge (Sakagami, unpub.). In some *Evylaeus* species, this trait has presumably become more pronounced than in other groups, resulting in a definite inactive phase. Four aspects must be considered for further studies of such discrete brood production: Laying rate of each female, duration of immature stages, time necessary for the reactivation of adult females, and synchronism of these features among nests. It is well known in many ant species that the queens rear their first batches of young very slowly until the emergence of the first workers. Probably the period until the emergence of workers represents a critical period of colony establishment in many social insects other than Apinae (honeybees and stingless bees).

Another interesting probelm, the sex ratio in the spring brood, was already discussed by Sakagami and Hayashida (1961). Recently Knerer and Plateaux-

Quénu (1967 b) referred to this problem. There is a close relation between the decrease of males in the first brood and development of social organization. As far as the ratio of males is considered, *Ev. duplex* occupies one of the lowest ranks among more or less socially developed halictine species (compare with the figures of Knerer and Plateaxu-Quénu). But such differences must be considered separately for groups with discrete and with continuous brood production. This caution is also important in the evaluation of caste difference. In the species with continuous brood production such as *Dialictus*, the body size and other features of daughters or workers would gradually change in the course of nest development.

2. Caste differentiation and related problems

It has repeatedly been noted to that mothers and their summer daughters show a slight morphological caste differentiation, but no description of the difference between them has been given. In this section, the nature of castes and related problems are discussed based upon the total samples, leaving the comparison of females within each nest in Section *5.1.*

2.1. Size difference. As an index of body size, the head width was measured in the following five samples:

Sample A: Females obtained by weekly sampling on flowers in 1959 (eight hours per week, that is, the same material as that presented in Fig. 1. Preserved as dry specimens, the internal features were not examined. Mothers taken from April 21 to June 10, daughters June 29 to August 8.

Sample B: Females peridodically captured on flowers in 1960, but without using standardized sampling as in 1959. Both external and internal features were examined. Mothers taken from April 14 to June 25, daughters July 8 to 30.

Sample C: Females taken from summer nests excavated July 10 to August 21, 1957, excluding newborn autumn daughters (=mothers of the next year). Both external and internal features were examined but the data on the wear of mandibles and wings were lost. In Table 1, *M* means inseminated females (=mothers) and *D* uninseminated and small inseminated females (=summer daughters). When more than one inseminated female were found within the same nest, the largest one was recorded as *M*, the others as *D*. These smaller inseminated females are separately given in (*f*).

Sample D: Females taken from summer nests on July 12~25, 1958, excluding newborn autumn females. The data on the wear were lost as in Sample *C*. Separation of *M*, *D*, *f* is as in Sample *C*, but besides the individuals taken by nest excavation, some guard bees picked up from nest entrances are included. In the latter case, inseminated and unin-uninseminated females are respectively classified in *M* and *D*.

Sample E: Additional data obtained by excavation of nests in 1964 (July 7) and 1956 (July 27~August 3). Both external and internal features were examined A single daughter classified in *f* had unworn mandibles and wings.

It might be argued that *M* and *D* separated in Table 1 did not always correspond to real mothers and daughters. This criticism is partly valid because spring females rarely leave the nests in summer (as to Samples *A* and *B*, *cf. 5.5.*),

and daughters are sometimes slightly larger than their own mothers (as to Samples $C \sim E$, *cf 5.1.*). But the descriptions given in subsequent sections confirm that such cases are infrequent. Therefore, the correspondence of M and D to mothers and daughters is seemingly valid for the majority of bees examined.

Another, more basic doubt might be held by the readers not familiar with the social organization of halictine bees: No direct evidence has so far been presented to verify that spring females were *actually* the mothers of the summer females. This question will be indirectly answered in subsequent sections. Direct evidence is given by the survival of the females marked while in the spring solitary phase, within summer nests together with other females (daughters). Two cases are cited, Nest nos. 1 and 4 (*cf.* Table 2, Sakagami and Hayashida, 1961). Both mothers were marked with colored paint on May 21, 1958, and found on July, 15, with daugnters, at nest excavation.

Table 1 presents the size distribution obtained. The distribution varies slightly from year to year, but except for E with a small sample size, the differences in the mean values between M and D by t-test (using Cochran and Cox's approximate

Table 1. Size difference between mothers and daughters in five samples, $A \sim E$ (Explanations in text) M: mothers, D: all daughters; (f): inseminated daughters

Size classs by head width[1] (mm)	Frequency distrbution of individuals in each sample									
	A		*B*		*C*		*D*		*E*	
	M	*D*	*M*	*D* (*f*)	*M*	*D* (*f*)	*M*	*D* (*f*)	*M*	*D* (*f*)
1.83							1			
1.88							1			
1.93		1		1	1					
1.98		3		2						
2.03	1	5	3	7 (1)	1	7 (1)	1	7	1	1 (1)
2.08	2	16	8	11 (1)	1	5 (1)	1	14		1
2.13	6	19	4	14 (3)	2	6			1	5
2.18	12	27	9	16 (2)	2	6 (2)	2	29 (1)		8
2.23	23	38	16	8	2	17 (1)	5	23 (3)	4	4
2.28	25	30	7	8	7	9 (1)				6
2.33	47	22	17	2	5	12 (4)	13	15	5	1
2.38	63	6	26		3	3	8	1 (1)	1	
2.43	46	4	8		6				1	
2.48	23		9		6		3			
2.53	7		1				3	1		
2.58					4					
2.63					1					
Mean[2]	2.35	2.22	2.30	2.16	2.36	2.16	2.32	2.19	2.31	2.26
SD	.171	.135	.131	.090	.124	.121	.125	.715	.902	.653
CV	.073	.061	.057	.041	.053	.054	.054	.326	.390	.289
N	255	171	108	76	40	67	36	97	13	27
100D/M	94.4		94.1		93.9		94.9		97.7	

1) Figures indicate class midpoints (1.83 mm=class 1.81 ~ 1,85 mm)
2) Mean, SD, etc. were calculated from all D, including f.

method in A, B and D) were statistically significant ($P<0.5$) in *A*, *B*, *C* and D,[1] and insignificant in *E*($P>0.5$). The percentage ratio of mean head width of daughters to mothers, given at the bottom of the table is 95.0% in average, and 94.3% excluding Sample *E*, or the relative size difference between mothers and daughters is 6.7%.

The size difference between mothers and their summer daughters, or queens and workers, has been described by many writers and recently summarized by Knerer and Plateaux-Quénu (1967 b, *cf.* also Knerer and Atwood, 1966). Within *Evylaeus* with discrete brood production, excluding *Ev. marginatus* with an extraordinary mode of life, the relative size difference is more than 15% in *Ev. malachurus*, without overlap, between 10–15% with slight overlap in *Ev. pauxillus*, *Ev. cinctipes*, *Ev. calceatus* and *Ev. nigripes*. Therefore, as in the ratio of males in summer brood (*cf.* Section 1), *Ev. duplex* occupies the lowest position among *Evylaeus* in this respect. The mother-daughter size difference in this species is also low when compared with the species of other groups, more or less comparable to *Al. striata*. It must also be mentioned that our data were exclusively obtained from the population inhabiting Sapporo. A larger difference might be expected if the other populations, especially southern ones, would be studied, as shown in *D. imitatus* between the populations of Kansas and Ontario (Knerer and Plateaux Quénu, 1967 b). A more conspicuous local difference is found in *H. scasbiosae*. In France, this species shows a more or less established caste differentiation accompanied with structural difference (Quénu, 1957). On the other hand, the population of Genova, Switzerland, has no definite caste differentiation (Batra, 1966a). A similar relation appears to exist between the Central American and Brazilian populations of *S. hesperus*.

2.2. Structural difference: In a superficial exmaination, mothers and daughters do not exhibit any difference in external structures. A closer comparison of many individuals reveals, however, the occurrence of some subtle differences between mothers and daughters, or exactly, between large and small females, as follows:

Character	Large females	Small females
Shape of metasoma	Relatively wide, with oval outline	Slender
Bluish enamel luster on metasomal dorsum	Stronger	Weaker
Contour of upper mrgin of head	Rather flat	More rounded

The difference in metasomal shape is relatively distinct. The difference in metasomal luster is not clear but the variation trends of mothers and daughters,

1) The difference is insignificant in Sample *D*, when calculated from the data given in Table 1, the discontinuous distribution in which was caused by the use of a different binocular microscope and the conversions of the figures to mm. The original data give a significant difference.

50 individuals of each chosen at rondom and classified into five arbitrary classes, show a signfiicant difference:

Luster	Strong	Distinct	Weak	Subtle	Obsolete
Mothers	13	18	13	6	
Daughters	4	16	12	10	8

The difference in head shape is so subtle that cannot be expressed numerically. But this difference is, together with the difference in metasomal shape, recorded by Legewie between mothers and summer daughters of *Ev. malachurus*, and relates to the head polymorphism known in certain other halictine species. Therefore the measurements of the following parts were made by using randomly chosen mother and daughter specimens, 50 individuals each: UO (Upper maximum interorbital distance), LO (Lower minimum interoribtal distance), EW and GW (Maximum widths of eye and gena seen laterally, *cf.* Sakagami and Moure, 1965),

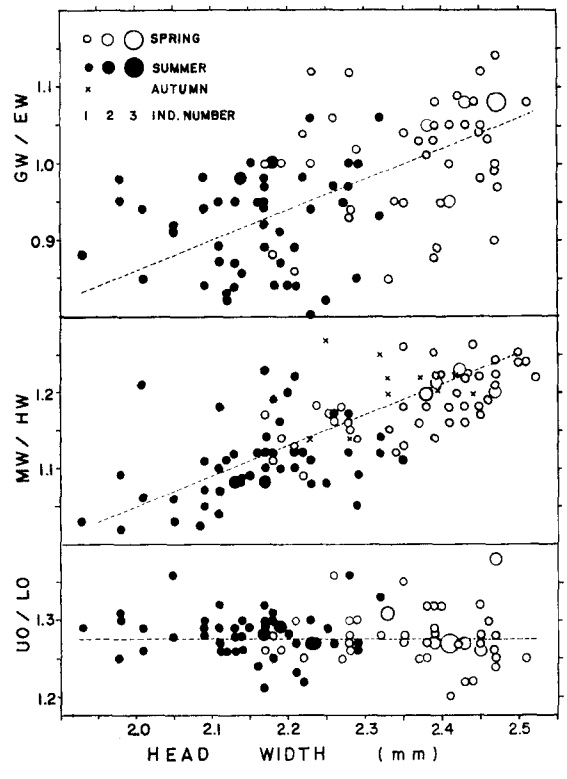


Fig. 2. Relative proportion of certain body parts in relation to body size in females of *Ev. duplex*. Explanations in text.

MW (Maximum width of metasoma). From these measurements, the ratio UO/LO, MW/HW and GW/EW were calculated for each specimen, and their distributions are presented in Fig. 2, in relation to the head width.

The distribution of values of UO/LO is isometric. There is no tendency of transverse expansion of the lower part of the head, which often appears in other species in association with a strong macrocephaly. However, the ratio GW/EW is distributed with an allometric gradient, showing the occurrence of a cryptic head allometry. The ratio MW/HW is also distinctly allometric. In this case, the larger metasomal width in larger females could be attributed to enlarged ovaries. To test this possibility, ten autumn daughters (mothers for the next year) were kept without food after emergence. After their deaths MW/HW value was measured in each specimen and the result was incorporated in Fig. 2. The values of these bees are not particularly lower than those of mothers, so that the higher value of MW/HW in mothers than in daughters is regarded as independent of the ovarian development. Summarizing, larger and smaller females exhibit a cryptic structural difference in relative growth of head and metasoma, as well as in metasomal luster. Corresponding to the difference in body size between mothers and daughters, these differences indirectly relate to the caste differentiation.

The structural difference between mothers and daughters in *Ev. malachurus* is well known. For this reason the summer daughters of this species was long recognized as an independent species, *Halictus longulus* Aurivillius. A closer inspection of the differences between mothers and daughters in *Ev. malachurus*, given by Legewie, suggests that these differences are mostly attributed to an allometric tendency, especially in the head, and the corresponding differential sclerotization. Several instances showing a similar tendency, which occasionally results in a gigantic macrocephaly, have been recorded (*H. scabiosae*, Quénu, 1957; *S. aerarius*, Sakagami and Fukushima, 1961; Certain Neotropical species, Sakagmi and Moure, 1965; *Ev. calceatus*, Bonelli, 1965 a; *H. latisignatus*, Sakagami and Wain, 1966). A cryptic size-conditioned structural differentiation in *Ev. duplex* was discovered based upon these items of information. Probably such minor difference may be found in many other species, superficially regarded as monomorphic. The occurrence of polymorphism associated with macrocephaly is seen in a much exaggerated manner in the males of some halictine species (Sakagami, Hirashima and Ohé, 1966) and in some other Aculata. Apparently this tendency is inherent in many species of halictine bees or even other Aculeata, and secondarily can be associated with the caste differentiation. A similar difference in head structures is found between queens and workers of many, probably all bumblebee species (Moure and Sakagami, 1962).

2.3. Conditions of reproductive organs (*cf.* also *2.5.*): Among various functional conditions, two features are of utmost importance in halictine sociology: Insemination and ovarian development.

As to the ratio of inseminated bees, our interest is focussed on the daughters, because mothers are nearly always inseminated throughout the summer phase. Excluding the data on nests containing newborn autumn daughters, the ratios of inseminated daughters in different years and periods are given in Table 2.

The table shows higher ratios in the last period. Using X^2-test, the difference between I+II against III was determined as significant ($P<.05$). The difference may be explained by one or both of the followings: 1) Decreased inhibition by mothers due to their senility or death developing a physiological condition in daughters which facilitates mating (*cf.* Section 6). 2) Increased number of males emerging in autumn increasing the chance of mating.

Table 2 Frequency of inseminated summer daughters (number of inseminated/total number of daughters)

Period	1957	1958	1960	1965	Total	% ratio
I ~July 15	1/26	5/41	1/21	—	7/68	*8.0*
II July 16~25	1/17	0/29	2/37	—	3/83	*3.6*
III Jl.26~Aug.14	5/22	—	4/15	1/26	10/63	*15.9*
Total	7/65	5/70	7/73	1/26	20/234	
% ratio	*10.8*	*7.2*	*9.6*	*3.8*		*8.5*

No signfiicant correlation was found between spermathecal condition and body size ($P>.05$). There was a slight tendency for more inseminated daughters to be captured on flowers (12/92) than from nests (9/251). It is plausible that foragers have more chances of mating than bees staying longer within nests. But in Samples $C\sim E$, daughters taken from nests involving both foragers and non foragers, the difference is statistically insignificant ($P>.05$).

The ovarian development both in mothers and daughters was studied with the samples explained in *2.1.* The results are summarized, all samples combined, in Fig. 3. The percentage ratios of bees with different ovarian conditions are distributed as follows (B includes BB an AB in Fig. 3):

Ovarian class	A	B	AC+BC	CC	C′	B′	A′	Total number
Mothers	8.0	6.0	2.0	64.0	6.0	5.0	9.0	100
Daughters	64.3	13.0	11.2	9.0	0.0	1.1	1.4	277

Combining classes AC, BC, CC and C' as ovarially developed females, the percentage of such bees is 72.0% in mothers and 20.2% in daughters. In daughters, annual values are respectively 9.0 (1957), 17.7 (1958), 29.6 (1960) and 30.7 (1965). (The percentage is higher in 1967, *cf. 2.5.*). The annual difference is partly dependant on the period of sampling the material. Besides the difference given above, ovaries of daughters are frequently characterized by asymmetric development. The percentage (AC+BC)/(AC+BC+CC) reaches 55.0% in

daughters, only 3.0% in mothers. The relation between ovarian development and body size in daughters was examined but no signfiicant result was obtained. The relation between ovarian development and mandibular and wing wears was examined only with the specimens taken in 1965, but the smaple size was too small to give any definite result.

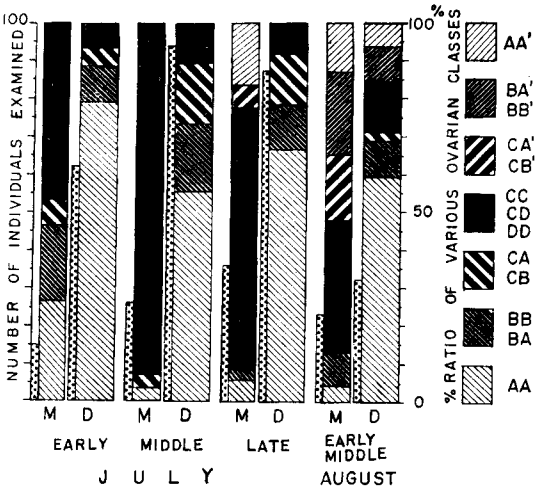


Fig. 3. Percentage ratios of various classes of ovarian development both in mothers and summer daughters. AA, Both ovaries undeveloped; AB, One ovary undeveloped, the other beginning to develop; BB, Both ovaries beginning to develop; CA and CB, one ovary well developed. the other undeveloped or beginning to develop; CC, both ovaries well developed; CA′~AA′, Corresponding to CA~AA, but indicating degeneration phases (*cf.* Fig. 5, where C is subdivided into C and D). Number of individuals examined is given by dotted vertical bars.

The relation between spermathecal and ovarian condition in daughters is as follows:

Ovaries	Undeveloped	One or both slightly swollen	One ovary well developed	Both ovaries well developed	Total number of bees examined
Spermatheca					
Uninseminated	173	33	28	21	255
Inseminated	9	6	3	4	22
Total	182	39	31	25	277

No signfiicant relation was confirmed between the two conditions ($P>.05$, using X^2-test with Yates' correction). The percentage of inseminated daughters with one or both ovaries developed is only 2.5% (7/277). From this figure, it is assumed that the contribution of daughters to the production of females of the

next generation is inconspicuous if any. The actual amount of this contribution may be lower for the reason given in Section 6 (A higher ratio of inseminated and ovarially developed daughters was obtained in 1967, *cf. 2.5.*).

Finally the relation between foraging and ovarian development in daughters was analysed:

Ovaries	Undeveloped	Slightly swollen	One ovary well developed	Both ovaries well developed	Total number of bees examined
Daughters taken					
on flowers	63	9	24	8	104
from nests	91	9	2	15	117
Total	154	18	26	23	221

When the bees with one or both ovaries developed are combined against the others, more ovarially developed daughters are found among foragers than among those taken from nets ($.05>P>.01$). In bees from nests there are more individuals with both ovaries well developed than in foragers. But it is uncertain whether this finding has functional significance or not. At least it is certain that in this species ovarially developed daughters have no particular tendency to stay in nests.

Among 73 daughters captured on flowers in 1960, only four did not carry pollen loads on the legs (all with undeveloped ovaries, AA, and uninseminated except one). The crop was nearly empty only in six individuals (ovaries AA in two bees, CB in one, BB in three and all except one uninseminated). It is certain that most daughters, including inseminated or ovarially developed ones, collect both pollen and nectar sychronously. Among three inseminated and ovarially developed daughters, two carried both pollen and nectar, the other, polen alone. Among five daughters inseminated but not ovarially developed, four carried both pollen and nectar, and the other, a stylopized one, nectar alone. The absence of pollen and nectar loads in some bees could be interpreted that they just arrived at the foraging source. Therefore, it is certain that, in contrast to mothers in summer phase, inseminated or ovarially developed daughters perform foraging activities like other "normal" daughters. The distribution of various types of daughters in each nest is given in *5.2.*

The occurrence of ovarially developed or inseminated summer daughters has been recorded in many halictine bees. But the information upon the relative abundance of their appearance is exactly known only in a limited number of species. The percentage ratio of inseminated workerlike summer daughters in *Evylaeus* with discrete brood production was given by Knerer and Plateaxu-Quénu (1966 b): 0% in *malachurus* and *pauxillus* and less than 1% in *nigripes*, all distinctly lower than in *duplex*. As to *Dialictus*, Michener and his school record the following result: *versatus* (1–2%, July~August), *imitatus* (2.1%, claculated from Tab. IX, Michener and Wille, as D/(C+D+E) in June~August), *zephyrus* (5.9%, calculated from Table 2, Batra, 1966 a, as D/(C+D+E) on June 1~August 15), and *rhytidophorus* (ca. 12%, calculated from Table 1, Michner and Lange, 1958 c, as D/(C+D+E)). On

the other hand, the percentage ratio is higher in some other groups with less pronounced caste differentiation: more than 50% in *H. scabiosae* and *H. maculatus* (Knerer and Plateaux-Quénu, 1966 b, in France. Batra, 1966 b, records much less developed caste difference of *H. scabiosae* in Switzerland, in which 23 out of 30 females were inseminated) and in *Al. striata* and *Al. persimilis*, and 80~85% in *As. sparsilis*. Increased percentage of inseminated summer daughters, found in *Ev. duplex*, is also recorded in *D. imitatus*. In *D. versatus*, Michener found 10 out of 21 workers taken on August 5 with spermatheca filled with sperms.

The percentage ratio of ovarially developed summer daughters in *Evylaeus* is recorded by Knerer and Plateaux-Quénu (1966 b) as follows: *nigripes* 61%, *malachurus* 32%, and *pauxillus* 5%. They remark that most oocytes are resorbed in *Ev. nigripes*, while they produce male offspring in *Ev. malachurus*. The percentage ratio in *Ev. duplex* is distinctly lower than in *Ev. nigripes* and slightly lower than in *Ev. malachurus*, nevertheless the latter is socially more differentiated than *Ev. duplex* in many aspects. But the percentage ratio of ovarially developed daughters appears to vary according to periods and conditions in nests. A more precise comparison based upon carefully collected samples is needed. In *Ev. malachurus*, the percentage seems quite low among the first brood, increasing in the second brood (Bonelli). Unfortunately, no numerical data are given as to *Ev. calceatus*, which is taxonomically close to *Ev. duplex*.

In *Dialictus* the precentage of daughters with enlarged ovaries is 41.8% in *D. zephyrus*, 28.5% in *D. rhytidophorus*, 17~22% in *D. versatus* and 8% in *D. imitatus* (Michener and Wille). As noted by Batra (1966 a), the caste system is most unstable in *D. zephyrus* both in the ratios of inseminated and ovarially developed workers. As to the other groups, the percentage ratio is 81% in *H. maculatus*, 48% in *H. scabiosae* (Knerer and Plateaux-Quénu, 1966, b, higher according to Batra, 1966 b, who found 10 ovarially developed bees among 30 females examined in Switzerland). In *As. sparsilis*, about 25~50% of the females examined during December to Febraury (=active season in Southern Hemisphere) possessed well developed ovaries, but Michener and Lange write that most females of this species with very incipient caste differentiation can develop their ovaries. It is interesting that ovarially developed workers are very few in *Al. striata* and *Al. persimilis*. According to Ordway, only one ovarially developed female exists in summer nests of these species, neverthless more than 50% of females in summer nests are inseminated.

2.4. Some other problems in summer phase: Besides the condition of the reproductive organs, some additional relations in the summer phase are briefly commented on:

2. 4. 1. Wear of mandibles and wings according to period: The gradual wear of both mandibles and wings in 1959 is given in Fig. 1. Five degrees of wear in Fig. 1 were converted to numerals, 1~5, and the arithmetic means of wear in each period were calculated (Table 3). The graudal increase of wear is slightly irregular for wings, while clear for mandibles. Unfortunately the results for 1957~58 were lost; the results for 1965 are given:

	Wings		Mandibles		Number of individuals	
	mothers	daughters	mothers	daughters	mothers	daughters
July 26	1.50	1.10	4.00	1.50	2	10
August 23	1.50	1.47	4.10	3.50	10	15

The number of individuals examined is insufficient but gradual increase of wear is traced except for wings of mothers. The difference between mothers and daughters is clear. Further, the greater difference in daughters than in mothers between the two dates indicates the active participation of daughters, both in foraging and subterranean activities.

Table 3 Wear of mandibles and wings in different periods

	Spring (mothers)				Summer (daughters)		
	April 19 ~May 2	May 3~9	May 10~16	May 17 ~June 13	June 28 ~July 11	July 12~18	July 19 ~August15
Wings	1.14	1.61	1.47	1.54	1.36	1.32	1.97
Mandibles	1.25	1.68	2.10	2.33	1.30	1.44	2.03
Number of Individuals	56	45	85	63	73	69	28

2. 4. 2. Body size and wear of mandibles and wings: The relation between body size and degree of wear was examined in Sample *E*. As the sample size was not large enough, the relation was not determined among daughters. As expected, the wear is greater in mothers than in daughters (Table 4). The difference is highly significant in mandibles ($P<$

Table 4 Wear of mandibles and wings in mothers and daughters from summer nests

Degrees of wear	Mandibles		Total	Wings		Total
	1~3	4~5		1	2~3	
Mothers	2	10	12	4	7	11
Daughters	17	9	26	20	5	25
Total	19	19	38	24	12	36

Table 5. Relation between body size and wear of mandibles and wings in summer daughters

Head width (mm)	Mandibular wear					Wing wear				
	1	2	3	4	Total	1	2	3	4	Total
A 1.81~2.00	3	1			4	4				4
B 2.01~2.15	26	13	1		40	36	3	1		40
C 2.16~2.30	59	28	8		95	81	5	6	3	95
D 2.31~2.45	17	7	7	1	32	21	2	4	5	32
Total	105	49	16	1	171	142	10	11	8	171

.01) and significant in wings ($P<.05$). In Sample *A*, the relation was examined in daughters with the result given in Table 5. There is a tendency for more wear in larger daughters (In X^2-test, size classes A and B, mandibular wear 3 and 4 and wing wear $2\sim4$ were combined). The tendency is significant both in mandibles and wings ($.05>P>.01$). This might be explained by the longer life span and resulting greater amount of work of larger daughters. But there is no direct evidence to prove this assumption.

2. 4. 3. Stylopization: The ratio of stylopized daughters was quite variable among samples: 10/97 (10.3%) in *D*, 3/171 (1.8%) in *A* and 4/76 (5.3%) in *B*. The following observations were made with these specimens:

1) Ovaries were undeveloped in all bees. 2) Only one bee was inseminated. 3) Dates of capture ranged from July 5 to 21, with no particular concentration at definite period. 4) Eight daughters taken from nests possessed empty crops. Among seven daughters in sample *B* captured on flowers, three had crops full of pollen grains. Among 72 non-stylopized foragers taken on flowers in the same smaple, only one had the crop full of pollen, two had crops with a few pollen grains and the remainder lacked pollen. Although the number of individuals examined is insufficient, stylopized bees appear to take more pollen than uninfested ones. 5) Among the seven bees taken on flowers, three had pollen loads on legs. This indicates that some stylopized daughters can forage for pollen, although it is unknown whether collected pollen is used for larval food or not. 6) A frequency distribution of head widths of stylopized daughters is: 1.83 mm (1 bee), 1.98 (1), 2.03 (3), 2.08 (3), 2.13 (2), 2.18 (6), 2.28 (1) and 2.33 (1). Comparing these figures with those in Table 1, stylopized individuals are on the average smaller than uninfested ones. In infested bees, tergal hair bands often becoming inconspicuous, but no definite tendency of sex reversal, as seen in *Andrena*, was observed.

From the results given in Sections *2.1.*$\sim$*2.4.*, it is now clear that mothers and daughters in summer nests are different both in morphology and physiology. Mothers are statistically larger, and structurally slightly different on the average, nearly always inseminated, mostly with developed ovaries and their mandibles and wings are much more worn. In daughters, these items are opposite in most cases. At the same time, however, it must be mentioned that none of these characters is sufficient to distinguish the castes categorically. The frequency distributions of these characters often show some overlaps between mothers and daughters. Consequently we can recognize two castes definitely but occasionally there are some bees, which cannot be properly placed in either. It is still premature to determine the place occupied by *Ev. duplex* in the scale of halictine caste differentiation.

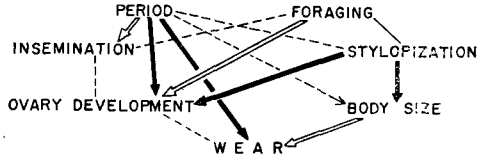


Fig. 4. Interrelations of various factors in summer daughters.
Broken line, No significant relation; Solid white line, Relation plausible but statistically insignificant; Solid black line, Statistically significant relation. Arrows indicate unilateral relations.

Apparently this scale is not simply one-dimensional. *Ev. marginatus*, for instance, has no structural difference between mothers and daughters, nevertheless their functional difference is very large. The comparison of various halictine groups with respect to the relative differentiation in caste system is not always easy. Judging from various characters, however, *Ev. duplex* can be regarded as a species possessing a distinct but mildly differentiated caste system.

Among summer daughters, the interrelations of various features or factors are summarized in Fig. 4. Except for four statistically significant relations, reasons for which are easily understood, the interrelations are not well recognized, suggesting the absence of definite sub-castes.

2.5. Additional data from early summer nests: After preparing the data given above, the lack of sufficient data on early summer nests was noticed. To fulfil this deficiency, about 30 early summer nests were excavated during July 10~14, 1967. The result of examination of 165 females obtained from these nests is

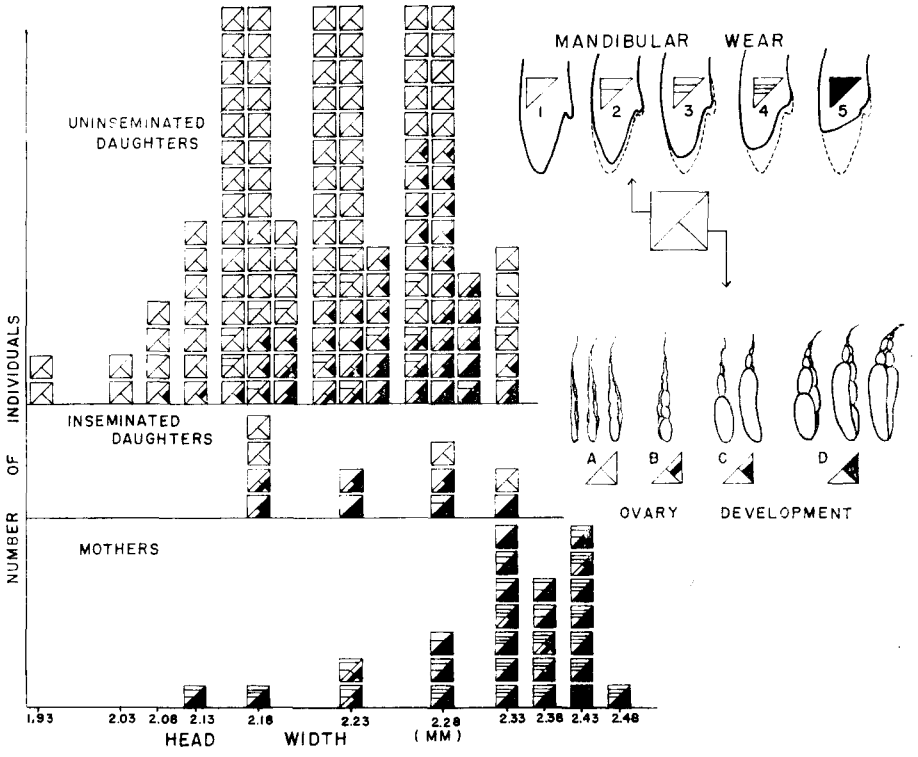


Fig. 5. Distributions of body size, wear of mandibles and ovarian development in mothers and daughters taken from early summer nests in 1967.

summarized in Fig. 5. In this sample, the distinction of mothers and inseminated daughters was more definitely made, using not only mandibular wear but also the occurrence or not of yellow spots in the ovaries, which indicated previous ovarian activity. The result in general coincides to those given in *2. 1.*~*2. 4.* The mean head width in mothers and daughters is respectively 2.34 mm (SD 0.0988, CV 0.042) and 2.22 mm (SD 0.0778, CV 0.035). The difference is highly significant ($P<.01$ by F-test). Inseminated and uninseminated daughters do not differ much in body size but the smallest daughters are mostly uninseminated.

The percentage ratio of inseminated daughters is 8.0% (11/138), approximately corresponding to the mean of the total individuals and of early July individuals given in *2.3.* The ovarian development in these bees is indicated in Table 6. The percentage ratio of ovarially developed bees is 34.8% (43/127) in uninseminated

Table 6. Ovarian development in females taken from nests in early July, 1967.

Ovarian class	Undeveloped	Developed					Total
	AA+AB	AC+BC	BD	CC	CD	DD	
Mothers		1	1		3	22	27
Uninseminated daughters	84	31		4	5	3	127
Inseminated daughters	4	1			3	3	11
Total	88	33	1	4	11	28	165

daughters, 63.6% (7/11) in inseminated daughters, or 36.3% in all daughters combined, and 100.0% in mothers. The percentage of bees with well developed ovaries (CD+DD) is respectively 18.6% (uninseminated daughters), 85.8% (inseminated daughters) and 92.6% (mothers). The percentage ratio of ovarially developed daughters is higher than in any of previous samples. Another aspect worth mentioning is the relatively high percentage, about 5.1%, of inseminated daughters with developed ovaries (7/138). Using X^2-test with Yates' correction, no signfiicant difference in the percentage ratio of ovarially developed bees was found between uninseminated and inseminated daughters ($P>.05$). The higher ratio of ovarially developed daughters suggests that this ratio is quite variable according to year, period and other conditions.

3. Brood development and related problems

This section deals with brood development, sex ratio in summer brood, number of cells produced, etc. The composition of adult population within each summer nest will be described in Section 5.

3.1. Development and sex ratio in summer nests: The periodical examination of summer nests was made regularly in 1957 throughout the season. Fig. 6

presents the seasonal change of the mean numbers per nest of cells, adult daughters, and various immature stages. There is a considerable difference, from year to year, so that incomplete data on the number of cells and adult daughters taken in 1958 and 1965 are also provided.

The figure clearly shows the gradual change of the relative abundance of various immature stages. Corresponding to the active foraging period, the number of cells per nest increases until the end of July, then keeps the same level. The pollen balls and eggs are mostly confined to this period. Foraging activity may reach the peak on about July 15, which approximately corresponds to the result of sampling bees on flowers (Fig. 1). The pupae begin to appear in late July, and, though not shown in the figure, mostly emerge in August. Summarizing, Fig. 6

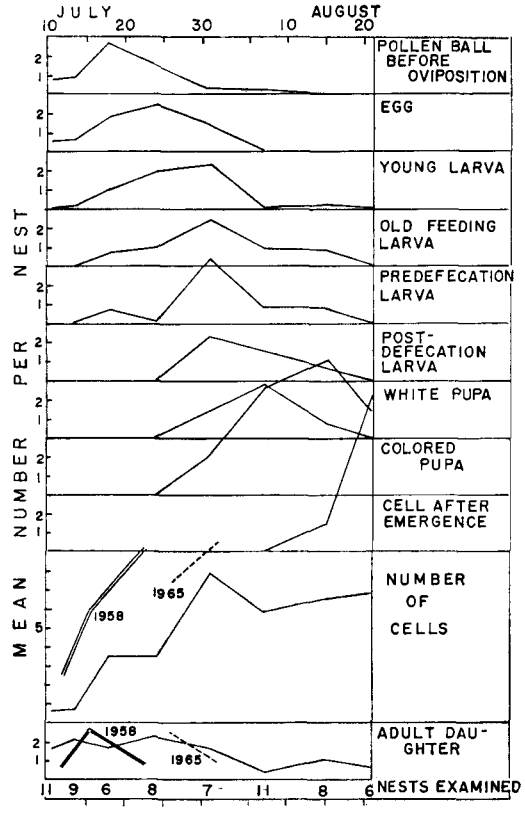


Fig. 6. Seasonal change of mean number per nest of cells, adult daughters, and various immature stages in summer nests in 1957, with additional data taken in 1958 and 1965.

well reflects the discrete brood production characteristic to this species and other related species of *Evylaeus* (*cf.* Section 1).

The exact sex ratio in summer nests is difficult to determine. In earlier nests, most immature individuals are still so young that sexes are unknown, and they are difficult to rear until pupation under artificial condition. In later nests, some bees have already emerged and the males, once leaving nests, mostly do not return. Therefore, the sex ratio was approximately determined from the pupae found in nests and old larvae reared in laboratory until pupation. In 1957, the percentage ratio varied in the course of the season as follows:

Date	July 31	August 7	August 14~16	August 21
Total number examined	41	109	63	29
% males	58.5	63.2	45.8	27.6
females	41.5	36.8	54.2	72.4

The result shows the proterandric tendency common to most Aculeata. The sex ratio obtained from the total individuals is ♂ 54.2: ♀ 45.8 (n=245) in 1957, and ♂ 59.3: ♀ 40.7 (n=123) in 1958 (July 21~24). The ratio, 56:44, when both that are combined, or, approximately 1.27:1.00, shows a slight numerical predominance of males. This figures shows a distinct contrast to that from the bees captured on flowers in later summer to early autumn (Fig. 1), which shows the absolute predominance of males. The scarcity of autumn females on flowers is characteristic of *Ev. duplex*, observed every year. The sex ratio obtained from autumn individuals taken on flowers indicates species or group specificity in halictine bees in Sapporo. In many species of *Evylaeus* with carinate propodeum, the females are scarcely found on flowers. On the other hand, both sexes are abundantly collected in *L. occidens*, *S. tumulorum*, etc.

The decrease of male production is regarded as an important moment in social evolution of insects (Michener and Lange, 1958 d, Knerer and Plateaux-Quénu, 1967 b). But the percentage ratio of males produced releates to the three different figures in females: 1) summer daughters (=workers), 2) mothers (=queens), and 3) total females produced. The decreased male production is apparent when considered in relation to summer daughters or total females but not always to mothers or queens. In halictine bees, the decreased male production in earlier brood has repeatedly been stressed (Sakagami and Hayashida, 1961; Knerer and Plateaux--Quénu, 1967 b), but the sex ratio in the final brood is usually not given with numerical data. Noll records the sex ratio in the final brood in *Ev. malachurus* as approximately 1:1, which coincides with our result in *Ev. duplex*. He assumes that most males in the final brood are produced by ovarially developed summer daughters. The participation of certain ovarially developed summer daughters in male production is probable, but there is no direct evidence to support the assumption by Noll of the predominant role of such daughters in male production (*cf.* also Michener and Wille).

In the groups with continuous brood production, the accurate sex ratio in the final brood is much more difficult to determine, especially when the caste

differentiation is still poorly developed. Michener (1966) records the maximum level of male ratio attained in several *Dialicti* as follows: *versatus* 26.8%, *zephyrus* more than 30%, *rhytidophorus* more than 40%, and *imitatus* more than 50%. But these figures could not always be regarded as the real ratios between males and future mothers.

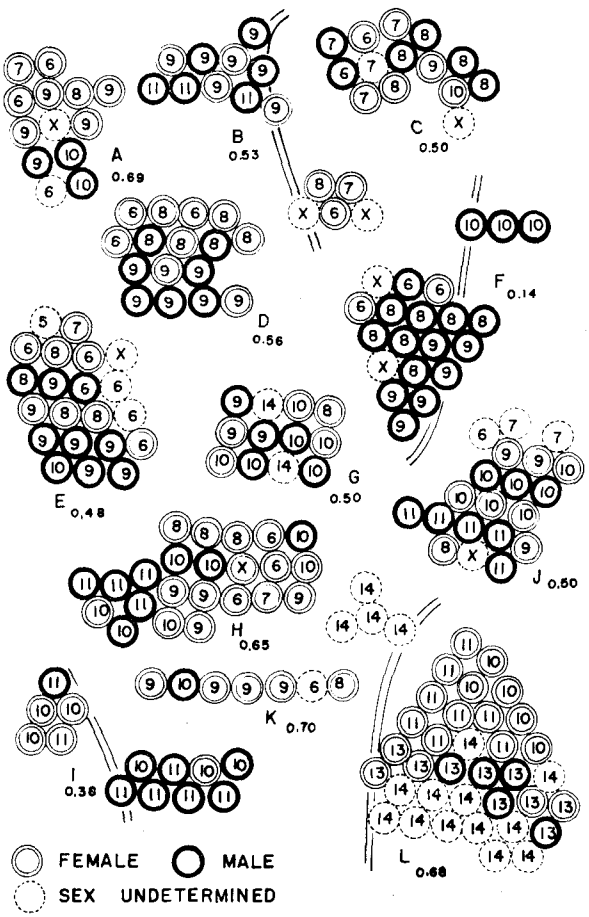


Fig. 7. Arrangment of sexes within cell clusters. Numerals within each circle, representing brood cell, indicate developmental stages. 5, Large larva still with food; 6, Pre-defecation larva; 7, Post-defecation larva; 8, White pupa; 9, White pupa with colored eyes; 10, White pupa with black eyes; 11, Pigmented pupa; 12, Pupa immediately before emergence; 13, Adult within cell; 14, Empty cell after emergence; X, Content damaged. B, F,I,L are nests with two cell clusters. Percentage of females produced given in each figure was calculated from 2(F+U)/(2T), where F=female young; T=total young and U=sex unknown young.

The arrangement of both sexes within the nests, or, in *Ev. duplex*, within the cell cluster, is also difficult to know exactly for the reason given above. Several relatively adequately known examples are illustrated in Fig. 7 (all nests with mothers). The mean female percentage in these nests is 52.5%. The female percentage given in each figure is mostly slightly higher than 50%, with two distinct exceptions (F and I). This results suggests that the role of ovarially developed daughters for male production is, under normal condition, not conspicuous. But it is possible that such daughters sometimes contribute to the male production, as is probable for nests F and I (both with such daughters).

Ev. duplex usually makes the cell cluster from the base upwardly, though the irregularity occurs frequently (Sakagami and Hayashida, 1960). This general trend is confirmed in most cell clusters in Fig. 7, notably in B,D,E,F,L. Correspondingly, the male young occupies relatively lower positions, especially in D, E,F,L, which concides with the proterandric tendency noted above. Of course, there are many exceptional instances. Interestingly the individuals of the same sex are often distributed in patches, not forming a rondom mosaic of both sexes, as if suggesting an oviposition rhythm which tends to cluster the offspring of the same sex.

3.2. Reproductive efficiency: Michener (1964) compared the reproductive efficiency per colony and per female in various social insects. The reproductivity measured by the numbers of young, cells, etc. increases parallel to the increase of female population. On the other hand, the reproductive efficiency per female shows, in most cases, a steady decrease parallel to the increase of female population. In order to know whether or not a similar trend is found in *Ev. duplex*, the number of the sum of pollen balls, eggs and young larvae in each nests was plotted

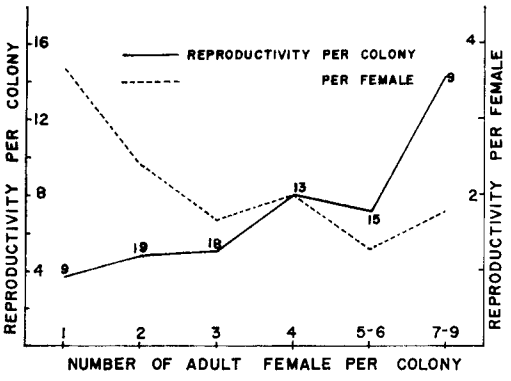


Fig. 8. Mean reproductivity per colony, based upon numbers of provisioned cells, eggs and young larvae (solid line) and mean reproductivity per female (broken line). Numerals beside the points of the solid line indicate the number of nests upon which each point was based.

in Fig. 8 against the number of females found in the respective nests, as done by Michener.

In general, the two trends pointed out by Michener are confirmed in *Ev. duplex*, too. But the curves are more irregular than most of the curves drawn by him. A correction was attempted by giving arbitrary weights of 0.2 or 0.5 to the ovarially developed daughters, when such were found, and the reproductivity was divided by the weighted female number. But this correction did not essentially alter the curves. For the time being, this irregularity cannot be explained adequately, though the rapid production of summer brood and frequent disappearance of some daughters from nests, both characteristic to the species, might contribute to such irregularity to some degree.

3.3. Number of cells produced, with additional data on spring nest survival:

Table 7. Number of cells produced and brood survival in nests excavated in inactive phase (June)

Number of cells per nest	Number of Nests examined															
	1958					1967										
	With mother	Without mother				With mother						Without mother				
		Total	Nests with *n* young survived			Total	Nests with *n* young died					Total	Nests with *n* young survived			
			0	1	5		0	1	2	3	4		0	1	2	3	5
0	1	many	—	—	—	—	—	—	—	—	—	many	—	—	—	—	—
1	1	4	3	1		1	0	1				2	2				
2	3	6	6									2	0	2			
3	3	4	4			1	1					4	1	2	1		
4	5	8	8			1	0	1				4	3		1		
5	8	8	6	1	1	2	0		1		1	5	4	1			
6	5	1	1			4	0					1	1				
7	4					5	0					1	1				
8	1	1	1			3	0					4	0	2		1	1
9	1					1	0										
10	1					1	0										
11	1					2	0										
Total	33+1	32	29	2	1	21	1	2	1		1	23	12	7	2	1	1

Table 7 presents the difference in numbers of cells produced in nests with and without mothers, based upon the data obtained in 1958 and 1967, during the inactive phase in June. The mean numbers of cells produced are as follows:

	With mother			Without mother		
Year	Mean	SD	CV	Mean	SD	CV
1958	5.22	2.233	0.428	3.56	1.640	0.463
1967	6.77	2.378	0.353	4.53	3.640	0.805

Excluding nests which did not produce cells, the efficiency was higher in 1967, but in both years the difference between nests with and without mothers is statistically highly singificant ($P<.01$ in F-test). The difference may mostly be explained by the deaths of mothers before completing their reproductive activity in the spring solitary phase. Previous records of the number of cells produced by a single female in halictine bees were reviewed by Sakagami and Michener (1962, Tab. XVI). The mean number of cells in spring by *Ev. duplex*, 5~7, approximately corresponds to that in many solitary species or in the solitary phase of social species.

In 1958 the survival of young in nests with mothers was not precisely measured, but was apparently higher than in orphan nests. In 1967 the survival was 97.3% in such nests but only 18.3% in orphan nests. It is possible that in many instances the same cause operates to kill both mother and her offspring. However, because the young is protected within the cells, there may be many cases in which only the mother succumbs, leaving her offspring for the time being intact. In such cases, however, the lack of parental care appears to affect the further survival of young. In many nests excavated in inactive phase in June, the surface of the cell cluster retains a fresh appearance free from mold, provided the mother survives. The opposite condition is seen in orphan nests even if the young are still alive within cells. This suggests the partial importance of the care by the mother for the survival of young, although the cells are closed after oviposition and direct contact between mother and young is absent in this species.

The data on orphan nests in Table 7, especially for 1958, also show all young die more often than some survive. This indicates that mortality factors operate mainly at the nest level than the individual level, more or less in an all or none way. A similar mode of action of mortality factors, asserted by Hokyo and Kiritani (1964) for aggregations of eggs and first instar nymphs of a stink bug, *Nezara viridula* Linné, may be found in many nest-making Aculeata.

Besides the brood mortality in nests, the survival of spring nests was studied in 1967, by excavating nests in June, and the following results were obtained:

	Nests	
	with mother	without mother
Burrow alone		30
Cell cavity alone		7
With cells but no young alive	1	11
With young alive	14	20

The mean survival was 16.9% (14/83). In 1961, Sakagami and Hayashida published the survival obtained by nest excavation in June as 18.0 (1957), 20.4 (1958) and 14.7 (1960). The mean survival based upon all these data is 17.5%, which is distinctly lower than the survival obtained by recording summer reactivavation in 1958, that is, the percentage ratio of reactivated nests to total nests marked individually in spring (=28.9%). The difference between these two values may represent the proportion of reactivated but orphan nests in summer. The

difference in 1958 of the two values, 8.5%, could be used to approximate the percentage ratio of such orphan nests to the total spring nests, until more precise estimates are obtained (*cf. 5.3.*).

Finally Table 8 presents the frequency distribution of the number of cells produced in summer nests. Only the nests already without incipient cells were selected for tabulation. Nevertheless, the frequency distribution is very irregular. The mean cell number is 14.4 (n=98, SD=7.53), but about half of the nests possessed only ten cells or less. This irregularity reflects various fates of summer nests as explained in *3.4.* and Section 5.

Table 8. Frequency distribution of the number of cells produced in summer nests

Number of		Number of		Number of	
Cells	Nests	Cells	Nests	Cells	Nests
3	1	13	7	23	2
4	3	14	3	25	2
5	6	15	4	26	2
6	7	16	5		
7	12	17	2	29	2
8	6	18	4	30	1
9	7	19	1	35	1
10	8	20	2	36	1
11	3	21	4		
12	2	22	4	41	1

3.4. Relation between number of cells produced in the spring phase and number of daughter bees in summer nests: As described above, the number of cells produced by the mother in the spring phase is 5~7 per nest. The percentage ratio of male offspring in the spring brood is approximately 10% (*cf.* Section 1), so that on the average at least 4~6 daughters should be found in early summer nests, if the brood mortality is not particularly high. Nevertheless, the mean number of daughters per nest does not reach three even in early July (Fig. 6). The data in Table 7 do not suggest a high brood mortality during the inactive phase in June, as far as nests with mothers are concerned. For a closer analysis of this discrepancy between cell and daughter numbers, a comparison of two items was made, mainly using the data from the nests excavated in 1967 (*cf. 2.5.*), together with some other nests carefully excavated in earlier years.

In the early part of the summer matrifilial phase, the spring cavity is filled with soil, but often the number of cells produced in spring can still be counted. The cells from which adult emerged are known by fecal layer covering the upper posterior part of the inner cell walls, because once defecated, most young can successfully emerge even under artificial conditions (Sakagami and Hayashida, 1960). On the other hand, the exact number of daughters can be obtained by excavating nests in the evening or during adverse weather,

when all inhabitants certainly stay within the nests, or by excavating after continuous observation. Table 9 presents, for nests excavated earlier than July 15 which fulfiled the conditions mentioned above, the relationship between the number of cells from which bees have emerged, and the number of daughter bees captured.

Table 9. Relation between number of cells after emergence (C) and number of daughters in nest (D). Cases C<D and C=D given respectively in italique and gothic numerals

Number of cells	Number of daughters									
	0	1	2	3	4	5	6	7	8	Total
1		**1**								1
2		1								1
3		1	1	1	*1*					4
4		1	1	1						3
5	1		2	2	3					8
6		1	1	2	1					5
7		1		3		1		2	*1*	8
8			1			1	2			4
9						2	1			3
10						1		1		2
11			1					1		2
Total	1	6	7	9	5	5	3	4	1	41

Among 41 nests examined, the relation of numbers of cells after emergence (C) and of daughters (D) is as follows: D>C in two nests, indicating invasion of bees from some other nests; D=C in four nests, and D<C in the other 35 nests. In the last case, D equals to C−1 in six nests, to C−2 in 8, C−3 in 8, C−4 in 7, C−5 in 3, C−6 in 2 and C−7 in one nest. In other words, in most nests the number of daughters is only 26～75% of the number of cells from which adults successfully emerged, and the nests containing daughters representing more than 75% of cells constitute less than one fourth of the total nests examined. The mean number of daughters in these 41 nests is 3.56 (146/41, SD 2.027, CV 0.570), that is, higher than the value given in Fig. 6, but distinctly lower than the mean number of spring cells (=6.2). The total numbers of daughters obtained and of cells after emergence are respectively 146 and 254. Therefore, the mean percentage of daughters remaining in the home nest is 146/ (254-25.4)=63.9%, when the percentage of male offspring is regarded as 10%.

The result shows a so far little noticed fact, the earlier disappearance of about one third of the daughters from their original nests. This figure is too high to be attributed to the earlier deaths of daughters by external factors, both biotic and abiotic. The dispersal of a considerable number of daughters from the home nests, either spontaneously or caused by the disorientation, which is not rare in early summer, is assumed. The fate of these dispersed daughters may be one of

the following four ways; 1) Death without making burrow, 2) Excavation of new burrows without producing offspring, 3) Production of a small number of offspring, and 4) drifting into other nests as shown in two cases mentioned above. Further discussions on these possibilities will be given in *4.1*, *5.4*, and *6.2*. It is obvious that the population trend of a given nest aggregation is much complicated by such dispersal. The irregular frequency distribution of the number of cells produced in summer (*3.3.*) may partly be explained by this fact.

4. Flight activities and related problems

The activities of summer females at nest sites were observed in 1957~1960, but here the descriptions are mainly based upon the continuous observations made in 1958 with selected nests, although other data are incorporated when appropriate.

Nests observed: The observed nests from a part of the aggregation in the shrubbery of the Botanical Garden (*cf.* Sakagami and Hayashida, 1961). The area occupied by these nests was flat and well insolated, without plant cover, approximately 1.0 × 1.5 m sq. In spring 14 nests were found within this area. In summer 10 out of these nests reactivated, an unusually high survival. Besides these ten nests (A~H, K, J), three nests were started from artificially transplanted bees in the area (A′,B′, C′). Further, two nests were made each by a dispersed daughter: one of them soon disappeared and the other (D′) represented a quite abnormal situation as given in *6.2*. The spatial distribution of these nests is illustrated in Fig. 9.

Period of observation: The first summer activities of some of these nests were noticed on July 2 and some bees were marked on the same day. But the observations were

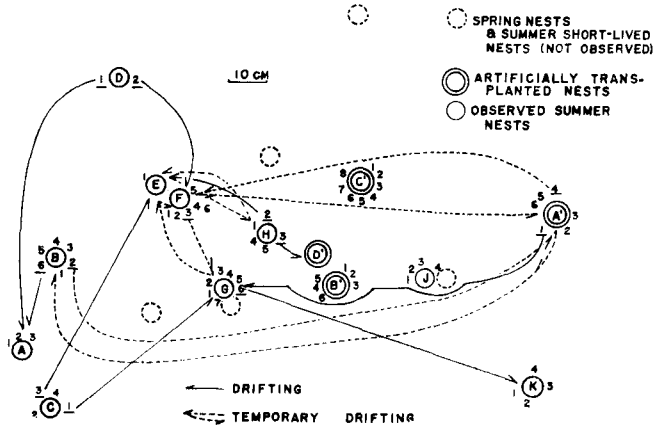


Fig. 9. Spatial distribution of nests observed in July, 1958. Nests are indicated with letters, and the inhabitants of each nest, all marked individually, with numerals (*cf.* Table 10). Dispersed individuals are underlined, and the course of drifting shown with solid and broken lines.

virtually started on July 7 and continued to July 21, until about half of the nests ceased foraging activities. On each day observations were made for 3~4 hours without interruption as follows: July 7 (10:00~12:00), 8(9:30~12:30), 9(9:15~13:15), 10 (9:23~13:00), 11 and 12 (both 9:00~13:00), 13 (not observed), 14 (9:30~13:00), 15(8:00~17:00), 16(6:00~18:00), 17 (9:23~13:23), 18(9:28~12:30), 19(7:15~11:30), 20 (rainy, not observed), 21 (9:00~12:30). On each day, marking and observation of flight activities were made as given below.

Marking: Any bees leaving or returning to the nests were, as far as possible, captured and individual marks were put with colored paints on the metasoma, and in most bees, on the face. The number of marked bees gradually increased in the course of observation, but some bees escaped from marking because they rarely made flights. After marking, each individual was pushed into the nest and the nest entrance was closed with soil. This procedure was undertaken to prevent abnormal internidal drifting, because many bees were marked at their initial flights, when memory of the nests had still not accurately been formed.

Recording: The items recorded are as follows: 1) Time of departure and return of each bee, together with individual mark, presence of pollen loads on legs, and, in case of departures, occurrence of orientation flight. 2) Occurrence of a guard at the entrance of each nest and, if possible, the guard's individual face mark. In contrast to flight activities, the appearance and disappearance of guards at entrances were not continuously recorded. 3) Opening and closure of nest entrances. 4) Weather conditions at intervals of 30 minutes.

At the peak of foraging activities, many bees departed from, and returned to nests. To avoid the omission of records, each entrance was provided with shrot glass tube, serving as an obstacle for passing bees (Michener *et al.*, 1955). At each departure or arrival, the obstacle was removed and replaced after the disappearance of the bee. On the first days, bees showed an avoidance response to such interference. Later most bees adapted to it, and, in the case of returning bees, awaited rather quietly the removal of the obstacle, hovering beside it.

The records of individual bees are summarized in Table 10, and a 12 hours' continuous observation made on July 16 are shown in Fig. 10[1]. The explanation of Table 10 is as follows:

Column 1: Nest mark. Spatial distribution is given in Fig. 9.

Column 2: Number of adults in each nest. a, Marked bees; (b), Marked bees drifted into other nests; c, Unmarked daughter bees found at nest excavation; d, Marked bees drifted in from other nests; m, Occurrence (M) or absence (X) of mother at nest excavation; e, Number of cells produced; f and g (Only in A′, B′ and C′), Number of young and mothers transplanted.

Column 3: *N*, Unmarked bees; A-1, B-2, etc., marked bees shown by nest (alphabet) and individual (numeral) symbols. Letters which do not coincide to those of the nests to which they belong are the individuals that drifted from other nests. Nests into which some bees drifted are given parenthetically (Temporary drifting in italiques). Numerical marks are given in descending order of the total number of flights (*cf.* Column 5).

Column 4: Number of flights and related observations. Italicized numerals given below each date show the duration of observation in hours. (Prolonged observations were made on July 15 and 16, but, to facilitate comparison, only the records from 9:00~13:00

1) A similar figure published elsewhere (Sakagami, 1966) includes some errors in individuals and their flight activities, which were corrected in the present figure.

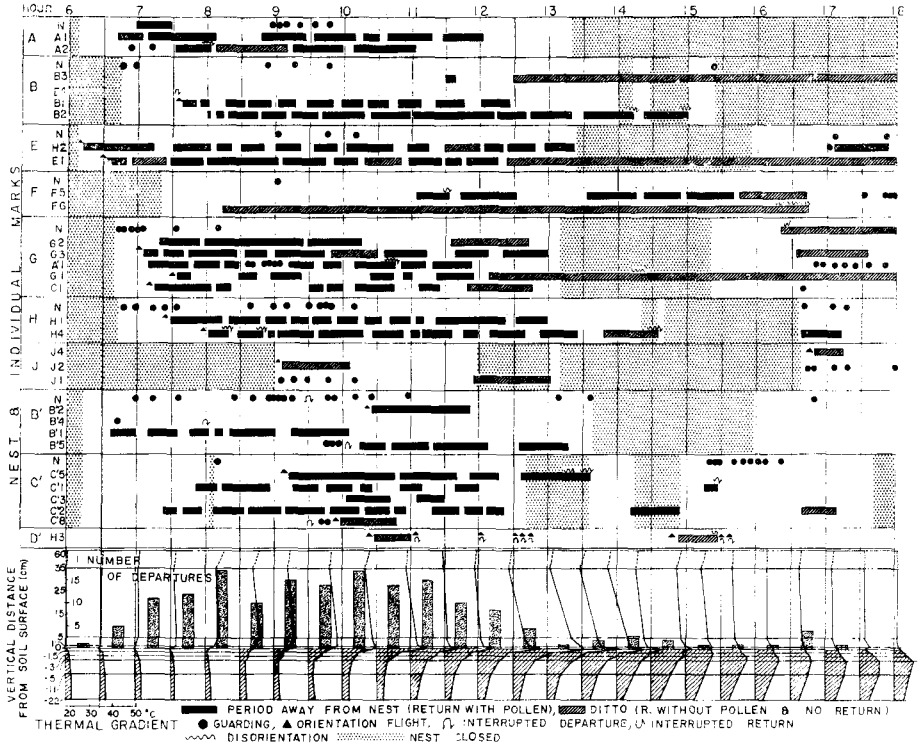


Fig. 10. A continuous 12 hours' observation of 10 summer nests on July 16, 1958 (Further explanations in text).

are cited.) In the lines for unmarked bees (*N*), x indicates the occurrence of some unmarked bees, mostly confirmed by guarding behavior and X, guarding behavior lasting more than 30 min. by such unmarked bees. In the lines for marked individuals, M denotes Marking (shown in italiques in case of bees drifted in from other nests); Numerals represent number of flights counted by the sum of returns from trips with or without pollen loads, and the final departure within the observation period (the return not confirmed); Numerals parenthetically given on July 15 and 16 show the number of flights observed during extra-hours; Italicized numerals show bees observed in two nests on the same day; x indicates brief guarding behavior; X, guarding behavior lasting more than 30 min.; Y, some other records by which the occurrence of respective bees in the nest was confirmed; d, occurrence of activities in home nests before drifting (*cf.* records in home nests); D, occurrence of activities in alien nests, after drifting (*cf.* records in the alien nests in which the respective bees drifted out); Black circle on July 21, presence at nest excavation; Double and white circles on July 21, the same but bees drifted out to other nests and those drifted in from other nests (H-3 was found as dead).

Column 5: Total number of flights of each bee during the observation periods in the home nest (M), in drifted nest (D), and the sum of M and D (=T).

Column 6: Total number of flights of each bee per day (shown only in relatively

Table 10. Flight activities of marked

1	2		3	4						
Nest	Population a(b) c d	m e	Individual mark (*Drifting*)	Date (July) and duration						
				2	7 *2*	8 *3*	9 *4*	10 *3.5*	11 *4*	12 *4*
A	3(0)	M	*N*		x	x	X	x	X	X
	0		A-1	M		1		6	5	9
	2	23	A-2	M		1	6	2	6	6
			A-3	M			1		1	
			D-1			*M*	6		3	6X
			B-6		*M*,d		d		1	2
B	6(2)	M	*N*		x	x	X	x	x	X
	1		B-1	M		2	3	4	4	4X
	0	22	B-2(*A'*,*B*)					M		*1X*
			B-3		M,1	1	6	5	8	5x
			B-4				M,1	5	2	1
			B-5				M	5	2	
			B-6(A)		M,1		2		D	D
C	4(2)	X	*N*			x	x	x		X
	0		C-1(G)		M	1	3	2	1	3
	0	7	C-2				M			2
			C-3(E)				M	D	D	
			C-4		M		1			
D	2(2)	M	*N*		x			x		
	0		D-1(A)			M	D		D	D
	0	3	D-2(F)					M,1	D	
E	1(0)	M	*N*		x	x	X	x	X	X
	0		E-1		M		1x	1		
	3	18	H-2(*H*)		*M*	1	2	*5*	6	8
			C-3				*M*	1	1	
			F-3		*M*		*1*	*1*		
F			*N*		x	x	x	x	x	X
	6(1)	M	F-1		M	3x	12	7	9	6,X
	0		F-2		M	2	4	2	6	7
	2	23	F-3(*E*,G)		M	D	D	D	D	D
			F-4		M	4	7	3	3	5,x
			F-5			M		4	3	1
			F-6						M	
			A'-4(A')		*M*		3	4	*3*	D
			D-2					*M*	3	

bees observed July 2~21, 1958

of observation (in italiques)							5 Total flights of each bee			6 Flights per day	7 Total flights in each nest
14 *3.5*	15 *4*	16 *4*	17 *4*	18 *3*	19 *3.5*	21 *3.5*	M	D	T		A B C
		X									76
8	5x	5	1				40		40	4.00	
4	4	3x	2				34		34	3.40	26
							2		2	0.40	
4	4						0	23	23		102
	(1)						3	3	6		
x	X	x			X						
5	9	7	2	1	7	●	48		48	3.69	138
5	10	7	3	3	7	●	36	1	37	3.60	
6		2	1				35		35	3.50	0
	X	Y					9		9	1.28	
							7		7		138
	D						3	3	6		
2		D			D		12	7	19	1.58	15
							2		2		
							0	2	2		0
							1		1		15
											1
D	D						23		23		0
							3	1	4		1
		X			X						22
5	4	8			3		22		22	1.83	
8	3	8x	2	4		○	1	47	48	3.69	75
							0	2	2		
1							0	26	26	2.60	98
X	X	x									
2	(0)						39		39	4.88	109
8							29		29	4.14	
D			D				0	26	26		14
							22		22	3.63	
4	2	2x					15		15	1.88	123
	3	1				●	4		4	0.44	
1	D				D		8	11	19	1.58	
							1	3	4		

Table 10.

1	2	3	4					
Nest	Population a(b) m c d e (f+g)	Individual mark (*Drifting*)	Date(July) and duration					
			2	7 *2*	8 *3*	9 *4*	10 *3.5*	11 *4*	12 *4*
G	7(1) M 0 3 30	*N* G-1 G-2 G-3 G-4 G-5 G-6(K) G-7 C-1 F-3 A′-1		x M M,2 M *M* *M*	x M,2 4 d 4	X 6 3 d *1* *M*,d	X 5 M 3 4 d *3* d	X 8 2 3 2 M d 3 d	X 7 4X 4X 1Y d 4x d
H	5(2) M 0 0 22	*N* H-2(E) H-1 H-3(D′) H-4 H-5	M	x M 2 M	x d 2 M M	x d 5 1 1X	X *1* 1 8	X d 6 4	X d 5 6 X
J	4(0) M 0 0 18	*N* J-1 J-2 J-3 J-4	M	x M M,3 4 M,1	x 4 1 1 1	x 4 2 4	x 6 3 2	x 4 3 4	X X 1
K	4(0) M 0 1 9	*N* K-1 K-2 K-3 K-4 G-6		M		x M,4 2	x 1 2	x 1 2 *M*	5 4 M,X
A′	6(2) M 0 1 23 (12+2)	*N* A′-1(G) A′-2 A′-3 A′-4(F) A′-5 A′-6 B-2		x M M M M	x 2 2 Y	x M,1 5 3 d	x 2 4 5 d	X 2 4 4 *4* M,2 *M*	X 4 6 6,X 1 1 *1x*

(Continued).

of observation							5 Total flights of each bee			6 Flights per day	7 Total flights in each nest
14 *3.5*	15 *4*	16 *4*	17 *4*	18 *3*	19 *3.5*	21 *3.5*	M	D	T		A B C
x	X	X									
7	7	5	3	2	10	1,	63		63	5.25	
3	5X	3	3	1	X	●	21		21	1.62	115
6	2	5					20		20	3.33	
							14		14	2.08	44
		(0)Y					7		7	0.88	
D	D		D				0	4	4	0.67	159
		M					0		0		
d		5			2		12	7	19		
4			4				0	26	26		
4	5	5X					9	4	23	3.28	
		X	x	x							
d	d	d	d	d		⊙	1	47	48	3.69	88
4	4	9	3	1		●	42	0	42	3.23	
4	d	d				⊙	23	3	26	2.17	0
3	4	8	5				21		21	2.33	
1							1		1	0.14	88
				x							
		1X	3	5	5X	◕	32		32	2.46	70
		1X	3	1	2X		19		19	1.58	0
							16		16	2.67	70
1		(1)					3		3	0.50	
X	x	x	x								
6	3						20		20	3.33	34
							10		10	1.67	4
			M		2	2	4		4		
							0		0		38
2	1		1				0	4	4		
	X				X						
d	d	d					9	14	23		61
							21		21	3.50	
							20		20	3.33	1
1	1				1		8	11	19		
							2		2		62
							1		1	0.17	
d	d	d	d	d	d	⊙	36	1	37		

Table. 10

1	2	3	4						
Nest	Population a(b) m c d e (f+g)	Individual mark (*Drifting*)	Date (July) and duration						
			2	7 *2*	8 *3*	9 *4*	10 *3.5*	11 *4*	12 *4*
B′	6(0) M 0 0 15 (16+3)	*N* B′-1 B′-2 B′-3 B′-4 B′-5 B′-6	M	x	x 2	x 2 M	X 2 4 M,3 M,1	X 4 6	X 4X 4 1 M
C′	9(0) – 2 0 26 (15+0)	*N* C′-1 C′-2 C′-3 C′-4 C′-5 C′-6 C′-7 C′-8 C′-9		x M	X 2 M M,Y	X 2 4 1 M,1 M	X M,1 2 3	X 2 M 3 M	x 1 X 1 Y
D′	0(0) – 0 1	H-3			M	d	d	d	d

Table 10.

Date (July)	2	7	8	9	10
I. Total number of flights		14	43	99	123
II. Frequency of daily marking	7	24	8	9	7
III. Cumulative number of marked bees survived		31	38	47	54
IV. Number of marked bees making flights		6	21	33	38
V. IV/III		19.3	55.3	70.2	70.4
VI. I/IV (mean=3.16)		2.3	2.0	3.0	3.2

active bees) = (The value T in Column 4, divided by the number of days from July 7 to the day of final observation of each individual).

Column 7: Total number of flights for each nest. A. Number of flights by bees that stayed in the home nests; B. the same by bees drifted in from other nests; C. sum of A and B.

(*Continued*).

of observation							5 Total flights of each bee			6 Flights per day	7 Total flights in each nest
14 *3.5*	15 *4*	16 *4*	16 *4*	18 *3*	19 *3.5*	21 *3.5*	M	D	T		A B C
X	X	X		X	X						
4	8	2	2	1	8		39		39	3.25	87
2	4	1			2X		23		23	2.30	
4			1				8		8	1.14	0
	X	X	2	2	2X	1	8		8	0.80	87
1	1	4X		1		●	8		8	0.62	
	Y				1		1		1	0.14	
X	X	X			X	X					
7	2	6	6	4	6X	2	37		37	3.70	117
8	3	8	8	4	6		37		37	4.62	
		2	2	3	3X	●	16		16	1.23	0
5					1		16		16	2.67	117
		4					5		5	0.62	
2							3		3	0.50	
1							2		2	0.40	
M		1x					1		1		
							0		0		
d	2	1				○	23	3	26		3 0 3

Appendix

11	12	14	15	16	17	18	19	21
140	137	144	96	115	57	33	68	6
4	2	1	0	1	1	0	0	0
56	52	45	39	36	30	23	23	14
38	34	34	25	28	20	14	17	4
67.8	65.4	75.6	73.5	77.1	66.7	60.8	73.9	28.8
3.7	4.0	4.2	3.8	4.1	2.8	2.4	4.0	1.5

4.1. Internidal translocation or drifting: The occurrence of internidal translocation or drifting was mentioned in *3.4*. At least two out of 146 summer daughters taken from 41 nests were regarded as bees drifted in from some other nests. Such drifting was found more frequently among the nests continuously observed in 1958. As shown in Table 10, the nests which neither received from,

nor sent some bees to the other nests are only three, J, B′, C′. Some internidal translocations were found in all other nests:

Nest	A	B	C	D	E	F	G	H	K	A′	D′
Number of bees											
drifted in from other nests	2	0	0	0	3	2	3	0	1	1	1
marked in home nests	3	6	4	2	1	6	7	5	4	6	0
drifted out to other nests	0	2	2	2	0	1	1	2	0	2	0

Nest D lost both marked bees while E received three. In total, 12 out of 63 marked bees, or 19% made such drifting. Actually this percentage could be higher, because the observed nests formed a part of a large nest aggregation, so that the translocations of unmarked bees between observed and unobserved nests are not inconceivable. In general the frequency of translocations between two nests may be inversely proportional to the internidal distance. Within the nests observed, however, no such relation was confirmed. (*cf.* Fig. 9, D→A, A′→B, A′→F, G→K, A′→G).

The type of drifting is diverse among bees: 1) Drifting out (in) 1–2 days after marking (C-3, D-1, D-2, G-6). These instances may be explained by the disorientation due to the weak nest memory at initial flights. These bees nearly spent their whole adult lives as the members of alien nests. 2) Ditto, with a temporary return, probably due to the weak memory to the home nest (H-2). 3) Drifting out (in) several days after marking (B-6, C-1, H-3, A′-1). These instances show the possibility of drifting and adoption a considerable time after initial flights. 4) Temporary possession of two homes (F-3, A′-4). 5) Temorary drifting with immediate return (B-2), probably caused by the temporary confusion of the nest memory.

At any rate the occurrence of drifting adds another complication to the activities of summer nests. The contribution by the drifted-in bees to the total foraging activity in each nest is variable, for instance, 24.8% in A, 27.6% in G, and reaching 77.3% in E. On the other hand, F, H and A′ lost a considerable part of foraging capacity by the drifting out of some bees and D succumbed from the loss of daughters.

Among differentiated insect societies, the internidal movements is well known in some ants, especially those making polydomous colonies. Recently Scherba (1964) gave a quantitative measure upon such movements in *Formica opaciventris* Emery, a species making discrete colonies. Another well known instance is the drifting in honeybee workers among the hives arranged closely nearby (Ribbands, 1953). This affects often the efficiency of a whole apiary. Detailed studies were recently made by Free (1954) and Free and Spencer-Booth (1961). Sekiguchi and Sakagami (1966) published the results obtained by individual marking. The percentage ratio of drifting bees ranged from 2.3 to 16.7% of the total marked bees, and, just as in *Ev. duplex*, diverse types such as drifting at various ages, temporary drifting, synchronous possession of two homes, etc., were observed. In these highly evolved insect societies, however, the internidal translocation of

workers does not usually evoke exchange of genetic elements. On the other hand, in solitary and primitively social insects, internidal exchange of individuals has a possibility of producing brood of heterogeneous origin.

Tsuneki (1965) observed in *Cerceris hortivaga* Kohl the entries of the females belonging to a nest aggregation into several nests indiscriminably, resulting in the communal possession of several nests by several females. Recently, Yoshikawa, Ohugushi and Sakagami (unpub.) found a similar relation in a primitively social Malayan wasp, *Parischnogaster striatula* (Du Buysson). In Halictinae, Vleugel (1961) found internidal translocations of some marked females in *Ev. calceatus*, and pointed out interference by ants as an important contributing factor. Michener (1966 b) recorded in *D. versatus* occasional interconnections between different nest burrows and entries of some workers into alien nests and departures from them, leaving pollen loads. The observations made on *Ev. duplex* give an additional information upon these internidal movements. Moreover, the result shows not only temporary drifting but also permanent translocation of even some relatively aged females. Except in polydomous colonies of certain ants, internidal translocation may not occupy an important position in the life mode of social insects. Nevertheless, this phenomenon is of a particular interest, because it suggests the occurrence of some, however weak, internidal flows among nests in these insects, which are usually regarded as typically 'closed' social systems.

4.2. Difference in activities among individuals and nests: As shown in Table 10, columns 4–6, flight activity is quite variable among marked bees. Some bees, B-1, G-1, H-2, for instance, show a very high and constant flight activity, whereas others are sporadically active or rather inactive. Fig. 10 also reflects such individual difference. The result is similar to that for honeybee foragers (Sekiguchi and Sakagami, 1966). The date of marking of each bee reflects, to some degree, the beginning of her flight activity. This may depened on the date of emergence, but it is possible that some bees stay longer as inside workers (cf. *4.6.* Guarding behavior). However, the ratio (Number of marked bees making flights)/(Number of total marked bees) (Table 10, appendix, V), is, except for July 2 and 21, relatively constant throughout the observation period. Although no observations were made for July 3∼6, the fact mentioned indicates the absence of pronounced age-conditioned differentiation of the adult life span into household and outdoor duties, as is well known in the honeybee. The ratio of the number of flights to that of bees making flights (Table 10, appendix, VI) is high during the period of July 9∼16, which could be regarded as the peak of foraging activities for these nests.

The number of flights made by a single bee per day (actually per 3.5–4.0 hours) is distributed as follows (mean=2.35. Observations excluded for the day of marking of each bee):

Number of flight per day	0	1	2	3	4	5	6	7	8	9	10	11	12
Frequency	115	53	65	38	46	28	25	12	15	4	2	0	1

The frequency distribution monotonously decreases from 0 to higher numbers. The lack of a bimodal tendency indicates the absence of particular differentiation of household and foraging bees. Bonelli records division of labor among summer daughters in *Ev. calceatus*, the first daughter serving as guard and making cells while the other daughters mainly forage. No such tendency was found in *Ev. duplex*.

As many marked bees disappeared before nest excavation on July 21, the relation between foraging activity and ovarian conditions and other internal features was not exactly determined. Table 11 presents the result of examination of these features in the bees still alive at nest excavation (together with some bees dissected earlier. The result in three artificially induced nests, A′, B′, C′, is given in *6.5*.).

Except for Nest C, which showed no activity after July 14 (*cf.* Table 10), some females were found in all nests examined. In each of these nests, one

Table 11. Ovarian development and other features of some bees in the observed nests (July, 1958)

Nest	Date of examination (July)	Individual mark (*mother)	Head width (mm)	Crop content		Ovarian development (*cf.* Fig.3)	Inseminated (+)or not(−)
				Nectar	Pollen		
A	21	*−	2.40	−	−	CC	+
	15	D-1	2.17	−	−	AA	−
B	21	B-1	2.25	+	−	AA	−
	21	B-2	2.17	+	−	AA	−
	21	*−	2.32	+	−	CC	+
	11	−	2.06	+	±	AA	−
D	21	*?−	?	−	+	AA	+
E	21	H-1	2.10	+	−	AA	−
	21	*−	2.25	−	−	CC	+
F	21	*−	2.32	−	−	CC	+
	21	F-6	2.32	?	?	?	?
G	21	*−	2.40	±	−	CC	+
	21	G-1	2.03	−	−	AA	−
	21	G-2	2.03	−	−	AA	−
H	21	*−	2.54	−	−	CC	+
	21	H-1	2.17	−	−	AA	−
J	21	*−	2.40	+	−	CC	+
	21	J-1	2.17	−	−	AA	− stylopized
K	21	*−	2.32	+	−	CC	+

relatively large and inseminated, unmarked female was found, which possessed, except for D, more or less developed ovaries (marked with asterisks). Apparently these are the nest mothers, alive since spring, and in summer mainly participating in oviposition and not leaving the nests. The inseminated female in D, which was relatively large (but her head was crushed), was, in all probability, also the nest mother. Her undeveloped ovaries may relate to the earlier cessation of flight activity in this nest (Table 10). All other females, except one female in Next B killed accidentally on July 11, were marked bees, indicating the participation of most daughters in foraging activities to some degree. In other words, daughters which stayed in the nest without flight activities were practically absent. The ovarian and spermathecal conditions agree to the results given in *2. 3.*

The survival of only ten out of 42 marked bees (one bee, B-2, drifted into A′ and survived there until July 21, *cf.*. Table 10) on July 21 shows the relatively short life span of summer daughters in contrast to that of mothers. Contrary to the assumption of shorter life span in active froagers, most marked bees found on July 21 were relatively active foragers, including J-1, the stylopized female.

On July 28, we excavated a nest of *Cerceris hortivaga* Kohl found near the observed nests of *Ev. duplex*. Among various halictine prey of this wasp, two marked *Ev. duplex* were discovered, though the individual marks were not determinable. The nests of this bee hunting wasp are common in the Botanical Garden, so that its influence upon the foraging ing population of *Ev. duplex* may be not neglegible. For the time being, however, we have no way to distinguish the relative importance of various factors affecting adult mortality.

Few reliable data have so far been published upon the life span of summer daughters or workers of social halictine bees. Bonelli reports the longevity of the first summer daughters in *Ev. malachurus* as probably less than three weeks and in *Ev. calceatus* about 20 days, with foraging days less than two weeks. This estimate corresponds approximately to our result. In our case, some nests showed flight activity from July 2 to 21, and the longest life span confirmed was more than 20 days in B-1 and H-1.

Michener and Wille gave the foraging period of *D. imitatus* as 11～12 days, in maximum 15 days, and the mean life span after emergence, about 21 days. They found one worker that survived for 32 days after marking, but this was for at least the last ten days the only worker left in the nest, which it did not leave during that time.

Finally the relation between foraging activity and productivity measured by the number of cells containing young in each nest is arranged in the descending order of the latter item:

Nest	D	C	K	B′	J	E	H	B	A	F	C′	G
Number of cells produced (y)	3	7	9	15	18	18	22	22	23	23	26	30
flights (x)	1	15	38	87	70	97	88	138	102	132	117	159

Nest	D	C	K	B′	J	E	H	B	A	F	C′	G
Number of bees at least once observed, including those drifted in	2	4	5	6	4	4	5	6	5	8	9	10
bees, the flights of which were observed in respective nests (z)	0	3	4	6	4	4	5	6	5	8	8	9

The relation between y and x or z are expressed by two linear regressions:
$y=0.16x+4.20$ and $y=2.87\,z+3.16$

Apparently the number of cells produced increases parallel to the number of flights observed. The reproductive efficiency obtained by dividing the number of cells by the number of bees observed making flights shows a linear increase. On the other hand, the reproductivity per female does not decrease parallel to the increase in the number of bees, as given below, keeping more or less the same level:

Number of dees	Reproductivity per nest	daughter bee
3∼4	13.0	3.71
5∼6	20.5	3.81
8∼9	29.6	3.94

4.3. Opening and closing of nest entrances: As described in the previous paper (Sakagami and Hayashida, 1961), the nest entrances of *Ev. duplex* are opened only in daytime. The nests are opened in morning earlier in summer than in spring. In spring many nests were still closed even after 9:30, for instance 7/18 on May 15 and 7/17 on May 19. In summer most nests were already open when the observation was strated at 9:00–9:30. All nests were still closed when the observation was begun at 6:00 on July 16 (Fig. 10). The number of nests opened in successive hours is 4 (6:00∼29), 3 (6:30∼59), 1(7:00∼29), 1(8:00∼29) and 1(8:30∼59). On July 19, observation was begun at 7:15 and eight out of ten nests were already open.

The times between opening of nests and first departures on July 16 are (in min.): 2, 3, 22, 25, 34, 36, 44, 51, 58, and 69 (m=34.4min.). The interval becomes longer when the nests are opened earlier. This tendency is also seen in spring but is more distinct in summer. In other words, the opening of nests becomes much earlier in summer than in spring but the first departures do not follow them at the same rate. This may partly be explained by the microclimate at the soil surface. In the morning the temperature of the air immediately above the soil surface is lower than the soil temperature (Fig. 10, 7:00∼30).

The time of nest closing begins, as seen in Fig. 10, at about 11:30 in some nests. Most nests are closed at 13:00∼14:00. At this time, the temperature gradient at and near soil surface is very steep and the temperature of the upper soil layer becomes gradually higher. No further data were available because

observations were not made after 13:00 on the days other than July 15 and 16. But it is interesting that some nests have a tendency to close earlier than others, for instance (X means closed all day):

Nest	July 8	9	10	11	12	14	15	16	17	18	21
J	11:47	13:00	11:54	12:15	12:25	10:42	X	11:58	12:00	X	X
C	–	12:40	12:25	12:46	12:25	11:15	X	X	12:09	X	X

Probably this depends on the occurrence of certain bees which are particularly sensitive to the change of the temperature gradient in the upper soil layer. The time of closing appears to become progressively earlier on later days of the summer phase. For instance, on July 8～9, only three nests were closed at 12～13:00, while on July 17～18, nine nests were already closed at the same hours, nevertheless no marked difference in soil surface temperature was recognized (*cf.* Fig. 11).

As seen in Fig. 10, nests are often temporarily closed in the early afternoon but are opened later. In some nests, for instance, Nest J, this was related to the reappearance of weak flight activities in later afternoon. In other nests, the reopening is often made by a bee which was outside of the nest when the entrance was closed by a bee within the nest (C′ ,B). The bees inside close the entrances regardless of nest mates still outside (cf. *4.4.5.*).

In spring closing of nest entrances occurred soon after the final return (12/48 within 2 min., 25/48 within 10 min.). Such relation was not found in summer nests:

	Time between final return and closing (in min.)					
	0–2	3～5	6～10	11～30	31～60	61～
Frequency	2	3	2	6	7	8

This difference may partly be explained by the occurrence of more than one bee in summer nests. In spring the entrances is closed by the solitary bees themselves, while in summer the closing by the forager is not always practiced because of the occurrence of nonforaging guarding bees.

Because of earlier opening of nests in morning and occasional reopening in late afternoon, the time during which nests are open is longer in summer than in spring. Although the available data are insufficient, the most reliable observation made on July 16 (Fig. 10) gives the following open periods (in hours): 4.5, 7, 7.5, 9, 9, 9, 9, 9.5, 10. The duration may be less early and late in the summer phase, but indubitably longer than in spring (3～4 hours, rarely more than 5 hours) at the peak of foraging activities.

4.4. Flight activities: Various results obtained as to flight activities are gathered together in this section.

4.4.1. Number of flight trips per day:

During the period July 7～21, observations of flight activities were carried out for 2～4 hours per day (*cf.* Introductory note for Section 4). Based upon the results of a continuous full day observation made on July 16, from 6 to 18 hours (Fig. 10), the mean number of trips per bee on each day was estimated as follows:

Table 12. Number of flights per day per female

n	A_n	B_n	X_n	Y_n
Date (July)	Total number of flights calculated	Total number of flying bees calculated	Number of flight trips per flying bee	Number of flight trips per surviving bee
7	33.4	6.2	5.39	1.08
8	86.0	21.8	3.94	2.27
9	154.5	34.2	4.52	3.30
10	200.0	39.4	5.08	3.70
11	214.0	39.4	5.44	3.82
12	211.0	35.3	5.97	4.05
14	247.5	35.3	6.99	5.50
15	107.2	25.9	4.13	2.74
16	*127*	*28*	6.13	4.78
17	92.7	20.7	4.48	3.09
18	66.1	14.6	4.54	2.87
19	99.0	18.3	5.41	4.32
21	9.3	4.1	2.27	0.66

$X_n = A_n/B_n = (a_{16} \cdot a_n/a_{16}')/(b_{16} \cdot b_n/b_{16}')$ and
$Y_n = A_n/c_n = (a_{16} \cdot a_n/a_{16}')/c_n$
where X_n and Y_n are the mean numbers of trips per day per flying bee and per surviving bee on each day (*cf.* Table 12, Column X_n and Y_n), a_n, b_n, and c_n, respectively the total numbers of flights observed, flying bees observed and surviving bees on day n given Table 10 Appendix (Lines I, IV and III), a_{16} and b_{16} the total numbers of trips and flying bees observed on July 16 (172 and 28 respectively) and a'_{16} and b'_{16} the numbers of flights and flying bees observed on July 16 during the interval corresponding to the observation period on day n. The mean numbers of trips per day per flying bee and per surviving bee are respectively 4.95 and 3.22, and the means excluding July 7 and 21 are 5.14 and 3.67.

Few data exist concerning the mean number of trips per day per bee in other halictine species. Bonelli gives the records of 4～9 trips in *L. leucozonium*, Knerer and Plateaux-Quénu (1966 c) 5～8 in *H. ligatus*, Batra (1966 a) 1～8, mean 3 in *D. zephyrus*, and Michener and Wille a maximum of 7 trips in *D. imitatus*. All records are lower than the mean number of trips per day per worker estimated for the honeybee (about 10 trips, Park, 1949).

4.4.2. Duration of trips and intertrip periods:

From the records of daily observations, the flying bees were divided into active foragers (those making five trips per day or more) and inactive foragers. The frequency distribution of the durations of trips and intertrip periods is as follows, in which the duration is divided into several classes, 1-5 min., 6–10 min., 11–15 min., etc. and each class is represented by the middle point (3 min. in class 1–5 min., 8 min. in class 6–10 min., etc.):

Duration (min.)	3	8	13	18	23	28	33	38	43	48	53	58	61
Trips bringing pollen													
Active foragers	7	16	23	49	30	33	32	27	25	13	11	12	22
Inactive foragers	10	29	63	75	74	46	33	20	11	7	6	2	6
Trips bringing no pollen													
Active foragers	4	4	4	5	5	7	5	3	3	5	1	1	11
Inactive foragers	6	5	6	4	5	5	3	1	1				

Duration (min.)	3	8	13	18	23	28	33	38	43	48	53	58	61
Intertrip period after return with pollen													
Active foragers	39	98	50	20	16	13	6	11	6	7	7	3	16
Inactive foragers	117	174	48	19	11	10	4	6	5	2	1	2	16
Intertrip period after return without pollen													
Active foragers	2	12	2		2	1	2			1		1	4
Inactive foragers	8	11	7	1	3	1							2

The mean trip and intertrip durations are given as follows:

		Trip bringing		Intertrip period after bringing	
		pollen	no pollen	pollen	no pollen
Active foragers	Mean (in min.)	32.5	39.6	20.5	25.4
	Variance	356.91	981.78	202.57	633.57
	Sample size	300	58	292	27
Inactive foragers	Mean (in min.)	25.2	17.9	13.7	21.5
	Variance	230.86	129.84	386.20	1638.50
	Sample size	418	36	415	34

In all figures the duration is longer for active foragers than for inactive ones. But it is dubious that this reflects the real relation, because the means are affected greatly by the occurrence of some quite prolonged trips and intertrip periods. Except for the duration of trips without pollen loads in inactive foragers, all figures are higher than the comparable figures in spring (Sakagami and Hayashida, 1961: 18.95, 21.49, 9.80 and 15.0 min. respectively). The mean duration of trips bringing pollen in summer is nearly twice as long as that in spring. This fact was partly caused by the general prolongation of trips, but partly by the frequent appearance of particularly long trips and intertrip periods.

The durations of trips (T) and intertrip periods (t) are recorded in many halictine bees: *Ev. malachurus* (t, 1～2 min., Legewie), *L. leucozonium* (T, 3–20 min., mostly 10～15 min.), *Al. striata* and *Al. persimilis* (T, minimum 20 min., mostly 60 min., shorter in early morning, gradually becoming longer). *Ps. divaricatus* (T, 9～34 min., t, 9～14 min., *D. rhytidophorus* (T, 8～30 min., t, 3～6 min.), *D. imitatus* (T, 3–105 min., mostly 8～40 min., most frequent duration, 16 min., t, 4～10 min. Similar to *Ev. duplex* both in T and t), *D. zephyrus* (T, 3～32 min., mostly about 10 min.,Batra, 1966 a). For the last species, Batra (1964), working in an insectary, records the time required for deposition of pollen as 1–2 min., of nectar 15 sec.～1 min., the bees usually staying 2～4 min. within the cell.

Besides these normal trips, some returning bees occasionally approach the nest entraces but fly away. Actual entry is often made only after considerable time. Some instances (all trips with pollen loads) are cited below. In each pair of numerals separated by comma, the first figure means the duration from departure to first return without entry, and the second one, that from the second departure to entry. The hyphen means the occurrence of intervening returns and immediate departures, and asterisks indicate the attempt to open the closed nest entrance:

8,11; 8, 12–5; 8,19–33; 16,52–23; 16, 19; 18,15; 19,24; 22, 28-1; 25,20-3; 28,4-*2-*4-50; 29,**83; 32,11-11-20; 37,12; 47,38-1.

Under high soil surface temperatures, returning bees often find a difficulty in opening

the closed nest entrances (*cf.* *4.4.5.*). This occasionally leads to departure and delayed return. But the data given above involve also cases of departures in the absence of any noticeable disturbance. The cases connected with hyphens indicate wandering of the bees, which failed to enter their nests, around the nest site. On the other hand, many bees flew off and returned after considerable time, often more than the period from initial departure to first unsuccessful return. The return from a trip may be governed by some factors acting to reduce the motivation which elicits the foraging. As the loss of the memory for the whole nest site, not for the location of their own nests, is difficult to assume, the delayed return given above suggests that the motivation reduction governing the return trip can secondarily be modified when the entry is disturbed.

4.4.3. Orientation flight:

The observation on July 16, undertaken in the later half of summer phase, shows the performance of orientation flight by 13 out of 28 marked bees, or about half, at the first departure. Eight of these 13 bees were good foragers and only six were virtually non-foragers on preceding days. Therefore even well experienced foragers daily reinforce their memory of the location of their own nests. The data on other days are scarce because summer flight activities start early in morning. Within the limits of observation hours, the orientation flight was confirmed at 12 first departures, six second departures and three third ones. Among 12 first departures with orientation flights, ten were made by good foragers of the preceding day. This result shows the frequent performance of orientation flight by good foragers, especially at initial daily flights. A similar observation is cited by Ordway, who writes, as to *Augochlorella*: "an orientation flight is made when a bee leaves its nest for the first time, the first time it leaves each day and in response to changed conditions at the nest entrance." The pattern of orientation flight is the same as described in Sakagami and Hayashida (1961) or that in other species, for instance, *D. imitatus*. Disorientation due to the changes of the microtopography at the nest entrance is frequent, contrasting to the well developed nest memory in some hunting wasps, especially those performing temporary closure of the entrance before hunting flight. The disorientation may lead to one of the following four consequences: 1) Final fining after lasting wandering. 2) Death. 3) Drifting into another nest. 4) Excavation of a new burrow.

4.4.4. Return from trip without pollen loads:

Section *4.4.2.* shows many returns from trips without pollen loads, that is, 16.2% (58/358) in active foragers and 7.9% (36/454) in inactive foragers among accurately recorded flights. These returns may be explained by one of the following three possibilities: 1) Exercise flights by young bees, 2) Nectar foraging, and 3) Unsuccessful pollen foraging.

There is no meaningful information on exercise flights. The relative abundance of nectar foraging and unsuccessful pollen foraging is difficult to determine, unless returning bees are captured and examined. Returns without pollen loads appeared between returns with pollen, at any hours, as in A-2, H-2, E-1, G-3 and H-4 in Fig. 10. For each marked bee with active flight records, the daily flight activity on each day was examined and the bees were classified according to whether returns without pollen appeared in the earlier half or in the later half of the daily activity. In 22 cases, such return appeared in the earlier half, in 41 cases in the later half.

Frequent returns without pollen in afternoon are recorded in *Al. striata* and *Al. persimilis*. Michener and Wille found the same tendency in *D. imitatus* and regarded this decrease of pollen foraging in afternoon. Probably this phenomenon depends on the floral make up near the nest site. If there are many flower species which produce pollen principally in morning, pollen foraging may decrease in afternoon.

4.4.5. Departure, daytime and soil surface temperature: The relation between flight activity and daytime is better determined by examining the time of departure than return. A frequency distribution of departures during daytime is given in Table 13. The observation hours varied from day to day, so that the total number of observed departures in each 30 min. interval were divided by the number of observations carried out at corresponding hours and such corrected frequencies are given in the final column.

Table 13. Relation between departure and time of day

Hour	Departures observed	Number of daily observations	Mean departures per 30 minutes	Hour	Departures observed	Number of daily observations	Mean departures per 30 minutes
6 00-	1	1	1.0	12:00-	101	12	7.8
6:30-	5	1	5.0	12:30-	64	11	5.8
7:00-	7	1	7.0	13:00-	4	4	1.0
7:30-	15	1	15.0	13:30-	5	3	1.6
8:00-	42	3	14.0	14:00-	7	2	3.5
8:30-	29	3	9.7	14:30-	4	2	2.0
9 00-	69	6	11.5	15:00-	6	2	3.0
9:30-	163	14	11.6	15:30-	5	2	2.5
10 00-	147	14	10.5	16:00-	5	2	2.5
10:30-	142	14	10.1	16:30-	4	2	2.0
11 00-	129	14	9.2	17:00-	1	1	1.0
11:30-	96	13	7.4	17:30-	0	1	0.0
							mean=6.0

The observations before 8:00 and after 17:00 were made only once, on July 16 (Fig. 10). The high corrected frequency at 7:30～8:00 may partly be caused by particularly intensive departure on July 16 at these hours. In general the departures are frequent in morning, especially during the period of 7:30～12:00 and decrease later. Although observed only two days, a very weak second peak can appear after 14:00. In spring the peak appears from 9:30～13:00, that is, distinctly later than in summer, and, though no systematic observation was made, never later in the afternoon.

Flight activity with a peak before noon seems to be common in many wild bees in temperate regions. In halictine bees, *D. imitatus* has the maximum activity from 8:30～11:30, decreasing to half in the afternoon. In *D. zephyrus*, the maximum appears from 9:00～12:00 and a second peak occurs from 15:00 to 16:00 (Batra, 1966 a). But the peak of flight activity can vary according to the microclimate. Bonelli found flight activity of *Ev. minutus*, nesting on a north-facing, shaded and humid bank, only in the afternoon.

The flight activity indubitably depends on various climatic conditions. Temperatures at various vertical distances above and below the soil surface measured every 30 minutes are given at the bottom of Fig. 10. From various readings, the temperature at the soil surface was chosen and its relation to the frequency of

departures and the number of open nests every 30 minutes from 9:30 to 13:00 (more extensively observed on July 15 and 16) were presented in Fig. 11. The absolute number of flights is small on July 8, gradually increasing until July 14, then decreases steadily (*cf.* Table 10. appendix, Line I). On the other hand, the diurnal rhythm of departure is similar on each day, always higher in morning, distinctly reduced after 11:00, when the surface temperature exceeds 45°C. A single exception is seen on July 12; the weather on that day was cloudy throughout the observation period. On the other days, the drop of flight intensity is inversely related to the increase of surface temperature, usually reaching the maximum at about 13:00. The observations on July 15 and 16 shows the appearance of a weak recovery of flight activities when the surface temperature becomes lower than 40°C, Probably this fact is related to the change of thermal gradient below the surface as shown in Fig. 10.

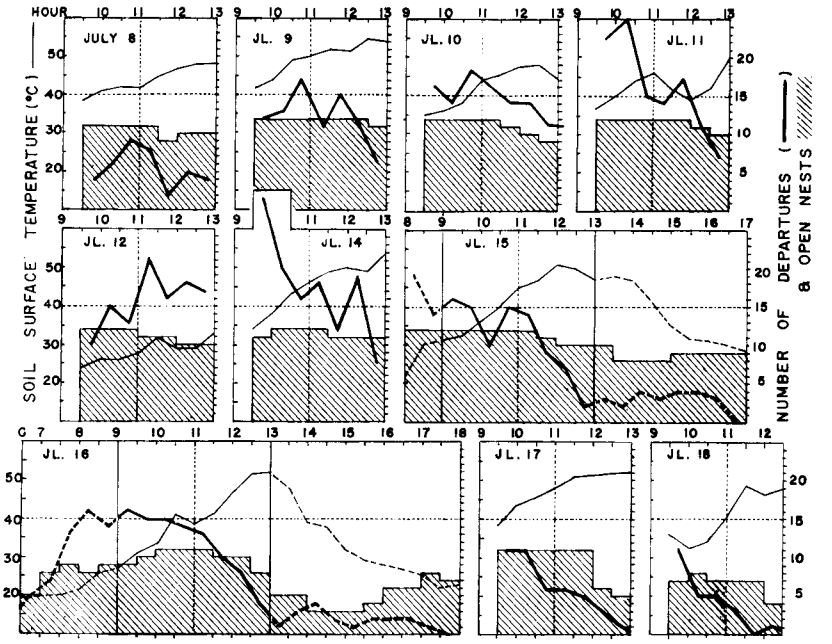


Fig. 11. Relations among departures, soil temperature and closing of nest entrances in selected nests on successive days during summer phase (*cf.* Table 10).

Fig. 10 also shows a marked thermal gradient from 13:00~14:00. At these hours, the surface temperature of the nesting site, exposed to the sun and protected from wind, fluctuates between 40~50°C, even in the nearly subfrigid climate of Sapporo. Many workers of *Formica fusca japonica* Motschulsky were found dead on tops of *Stellaria*, about 5~7 cm

above surface, growing near the nests of *Ev. duplex*. These ants climbed up the plants avoiding the blazing heat on the surface, were trapped there, and killed by scorching insolation. Flight activities are greatly inhibited by this overheating. Many nests are closed at these hours (Figs. 10 and 11), often even though some bees are out of the nests (*cf. 4.3.*). The returning bees confront a serious difficulty when the entrances are closed. They cannot continuously work to open the entrances on the blazing soil surface. Consequently they often fly away after unsuccessful efforts, returning only after a considerable time. For instance, C′-5 in Fig. 10 returned to the nest at 13:10 with pollen. She flew away after a desperate effort to open the entrance, and did not return until 13:33. G-1 in the same figure returned at 14:10, but could not open the entrance. She flew away and did not return on that day. Even in the case of successful entry, bees cannot work continuously on the ground to open the entraces. Often they stop the digging, flying up and stay in the air for a pause, then again begin to dig. (*cf.* Fig 10, B-2, 14:00~15:00; F-5, 11:30; H-4, 14:30; C′-5, 13:00-13:30). Similar discontinuous digging, interrupted by flying up, is observed in bembicid wasps burrowing in sand dune surfaces (Chapman, 1931, p. 383).

4.5. Absence of food regurgitation: Food regurgitation among adults is a widespread phenomenon in social insects, found frequently in ants, termites and various genera of social wasps. Among bees it is commonly seen in honeybees and stingless bees, and its social signfiicance is well known (Nixon and Ribbands, 1952). However, this trait is not well developed in other bee groups. Regurgitation seems to appear in Xylocopinae, though crucial evidence is still lacking (reviewed by Sakagami, 1960; *cf.* Michener, 1958). In other groups, for instance, in bumblebees, regurgitation has so far been observed only exceptionally (*cf.* Free, 1955, Sakagami and Zucchi, 1965); nevertheless, their social organization attains a level comparable to that of many social wasps. Because of their subterranean life, the intranidal behavior of halictine bees had not been observed until Batra (1964) published her observations on *D. zephyrus* made through glass.[1]. She observed no direct transfer of food among nest mates. The following observation does not confirm but makes plausible the absence of food regurgitation in *Ev. duplex*.

In July, 1957, four nests (6, 4, 3 and 2 bees each) were excavated and all inhabitants from the same nests were transferred into a vial 2 cm in diameter and 5 cm in length. Only one of them (either mother or daughter) was fed with 30% sugar syrup mixed with honey, and the subsequent behavior of bees was observed, one hour for each vial. For the vial containing two bees, the observation was repeated 13 times, during six days. In these 16 hours' observation, no single instance of food transfer was observed. Mutual touching with antennae, which appeared frequently, always released simple retreats of one or both of participants, either preceded by a mild grasping or not. Thereafter, the lower part of the clypeus of one bee was smeared with honey and the observation of behavior was continued for another hour for each vial (three hours for the vial with 2 bees). Frequently one bee extended her glossa and licked the clypeus of another bee smeared with syrup. Smeared bees usually retreated from the partner, sometimes rejected

1) Cf. also Batra (1968), which deals with the intranidal behavior of other halictine bees.

mildly the latter. In one instance, the smeared bee attacked the partner with her mandibles, but in no instance did she respond with food regurgitation.

Obviously this negative result observed under unnatural condition cannot be regarded as crucial evidence for the lack of food regurgitation. But it must be mentioned that the arousal of food regurgitation is very easy in the honeybee workers confined under a similar circumstances, even though they are often more restless (*cf.* also Free, 1956).

4.6. Guarding behavior: Guarding at the nest entrance is an ethological characteristics of many halictine bees. The constriction of the nest entrance to a width which admits passing of only one bee is also a group characteristic (Sakagami and Michener, 1962). This architectural trait, which is also seen in Ceratinini (Sakagami, 1960), is regarded as an adaptive character evolved in relation to the defense of nests from enemies (Lin, 1964). The guarding behavior in spring nests of *Ev. duplex* was described by Sakagami and Hayashida (1961), but definite and prolonged guarding appears only in summer nests inhabited by several bees.

Bees found at nest entrances stay there only briefly or for a considerable time. The occupation of entrances by some marked bees, either briefly or continuously (=more than 30 min.), is shown in Table 10, using symbols x and X; some examples in Fig. 10 also illustrate the participation of certain unmarked bees in guarding. Even active foragers occasionally participate in guarding for a prolonged time (For instance, F-1, A′-3 on July 12, G-2 on July 12 and 15, and J-1, C′-1 on July 19. *Cf.* also A′-1 in Fig. 10). In general, however, the total number of flights for each bee appears to be inversely proportional to the frequency of guarding, though statistically insignificant because the samples size is small:

Number of total flights	0~5	6~10	11~20	21~30	31~40	41~
Participation in brief guarding			4	2	2	1
Participation both in brief and prolonged guarding	1	3	1	3	2	

A similar, though less conspicuous, relation is seen between number of daily flights and frequency of guarding:

Number of daily flights	0	1~2	3~4	5~
Participation in brief guarding	4	6	4	5
Participation both in brief and prolonged guarding	7	2	2	2

The participation of unmarked bees in guarding is attributed to one of the following three possibilities: 1) Frequent performance by young bees. 2) Occurrence of certain bees, which tend to stay in nests, without foraging intensively. 3) Performance by mothers. As marking was usually made at initial flights by each bee, the frequent guarding by unmarked bees (*cf.* Table 10, line *N* in each nest, and also Fig. 10), may partly be attributed to young daughter bees still not making flights. The occurrence of certain 'inner workers', which dedicate themselves

mainly to inner work including guarding is less plausible, because most marked bees more or less showed flight activities, and only one unmarked daughter bee was discovered on July 21 at nest excavation (*cf.* Table 11). But the individual differences in the relative amounts of flight activity and inside work is suggested from the result given in *4.2*. An extreme case is G–7 in Table 10. In Nest G, consistent guarding by one unmarked be was observed from July 12 to 16, although most inhabitants were already marked. On July 16, one unmarked bee departured and was marked as G–7 but she did not reutrn to the nest. Apparently this bee participated in guarding during preceding days. However, the fact that this instance is rather exceptional indicates the scarcity of such specialized bees if any.

On the other hand, guarding by foundress mothers is confirmed in Nests A, B, C, E, F and H, by the occurrence of unmarked guards after all daughters were marked (confirmed later by comparing Tables 10 and 11). Obviously some daughters might escape marking, but the unmarked guards consistently observed in Nests A, B, E and H, several days after the final marking, indubitably indicate guarding by foundress mothers. To obtain further evidence, 12 consistent guards, staying at entrances more than 30 minutes, were captured from summer nests July 16～18, 1959. Among these females, six were inseminated with fully developed ovaries, and six uninseminated with undeveloped oaries (Classes A and B, Fig. 3). This results confirms the participation of mothers as well as daughters in nest guarding.

The previous information upon the guarding behavior of other halictine bees varies as to what kinds of females participate in this activity. Fabre reported that guarding in summer nests of *H. scabiosae* is mainly performed by mothers. Legewie denied the guarding by mothers in *Ev. malachurus* but Stöckhert recorded it in this species and in *Ev. pauxillus*. In *Ev. calceatus*, Bonelli found that guarding was mainly made by the mother and her first daughter. Out of 24 hours' intermittent observation, from July 13～29, he found the mother guarding 605 min. and the first daughter 771 min. His conclusion is interesting but probably more comparable observations with several nests are required to confirm it, although the occasional appearance of such specialzied guards is not inconceivable, as is known in the honeybee (Butler and Free, 1952; Lindauer, 1952, Sekiguchi and Sakagami, 1966). Ordway observed in *Al. stirata* and *Al. persimilis* the guarding by any types of bees: mothers, young workers and foragers, and Plateaux-Qéenu (1965 a) in *Ev. nigripes* noted occasional guarding by the mother. On the other hand, the guarding mothers are not known for *D. imitatus*, *D. zephyrus*, *D. rhytidophorus*. In these species of *Dialictus*, the guarding by young, preforaging workers is seemingly common.

Continuous observation of guarding bees in *Ev. duplex* was not systematically carried out. One instance is cited here (Obs. July 18, 1958, 15:30～16:00, G, Exposing the forebody above the entrance; g, Head at the ground level; g′. Head slightly below the ground level; O, Head not seen; c, Nest closed):

15:30	g	35	G	40	O	45	O	50	gG	55 c
31	g	36	g	41	g	46	O	51	OGO	56 c
32	g	37	g	42	O	47	g	52	gOgO	57 c
33	g	38	g′	43	O	48	g	53	O	58 c
34	G	39	G	44	g	49	OGO	54	GO	59 c

At 15:49, a worker of *Formica fusca japonica* entered in the nest; it was immediately driven away, and thereafter, the guard seemed excited, frequently exposing the fore-body out of the entrace until closing the latter at 15:55.

Although not continuous observations in the strict sense, there are the following records on the prolonged occupation of the entrance by one bee:

Duration (hrs)	0.5	1.0	1.5	2.0	3.0	3.5
Frequency	5	10	5	1	3	1

The most prolonged guarding was observed in Nest 92, on July 24, 1957. The nest contained the mother and five daughters, all uniniseminated with ovaries undeveloped. Only one bee, R, made two foraging grips, while another, B, occupied the entrace from 7:56 to 13:32 nearly without interruption.

The behavior of guarding bees does not essentially differ from the description given in Sakagami and Hayashida (1961.) The invaders, such as ants and disoriented bees of other nests, are usually immeidately pushed away. The blocking of the entrace by means of the metasomal dorsum was never observed in *Ev. duplex*. This trait has frequently been recorded in various species of halictine bees, including *Ev. calceatus* (Bonelli), which is very close to *Ev. duplex*, but the lack of this response is recorded in *All. striata* and *Al persimilis* and *H. scabiosae* (Batra, 1966 b).

4.7. Deposition of pollen loads in the same cell by more than one female:

From the results mentioned above, especially those given in Table 10 and Fig. 10, it has clearly been demonstrated that several females in the same nest performed foraging trips on the same day. Then, either each forager possesses her own cell in which she deposit her pollen loads or several females occasionally deposit the loads in one and the same cell ? No direct observation was carried out but the following records confirm the occurrence of the latter case:

No. 1. July 10, 1957. Two bees with pollen loads successively entered a nest, which was immediately excavated. Contents: four females. Mother (Head width 2.49 mm, inseminated, ovaries well developed) and three daughters (HW, 2.34, ovaries slightly developed; HW, 2.34, and 2,28, ovaries undeveloped. All uninseminated, the two last bees were foragers). Spring cell culster, seven cells (five after emergence, two still with preimagines). One summer cell, containing a pollen ball.

No. 2. July 13, 1957. Similarly the nest was excavated after two successive returns. Contents: Mother (HW, 2.45, inseminated and ovaries well developed), four daughters (HW, 2.39, and 2.34, ovaries slightly developed; HW, 2.34 and 2.28, ovaries undeveloped. All uninseminated, the two last foragers). Spring cluster, six cells (four after emergence, two with preimagines). Summer cluster, four cells (three already oviposited, one with pollen ball only).

No. 3. July 24, 1957. Similarly two bees returned with white and orange pollen loads respectively and the nest was immediately excavated. Contents: Mother (HW, 2.49, inseminated and ovaries developed), two daughters (HW, 2.28 and 2.02, uninseminated

and ovaries undeveloped). Spring cluster, eight cells (all after emergence). Two summer clusters: One with three cells (one egg and two larvae). The other with two cells (both with pollen clumps, one orange, the other a mixture of orange and white, in layers from the center, orange, white and orange).

From fragmentary observations on *Ev. malachurus*, Aptel concluded that each summer female cares for her own cell. But Legewie noted situations similar to the observations cited above, that is, more than one forager versus only one cell being provisioned, which give positive evidence for the common use of one cell by more than one female. Similar observations were made for *D. rhytidophorus*, *D. imitatus*, *As. sparsilis*, *Al. striata* and *Al. persimilis*. Crucial evidence was recently brought out for *D. zephyrus* by Batra (1964), who directly observed intranidal behavior, including common use of one and the same cell by several, up to six, females (*cf.* also Batra, 1967). Plateaux-Quénu (1963) observed in *Ev. calceatus* the preparation of a single pollen ball by 16 trips of four daughters.

In many solitary Aculeata, maternal behavior appears in a definite sequence of cell construction, lining, provisioning, oviposition and closure. The sequence can be different among groups, but, within a given taxonomic group, usually is constant and stereotyped (*cf.* Iwata, 1942). The halictine bees often show a tendency to the disintegration of this chain even when solitary (Sakagami and Michener, 1962, p. 73). In the summer matrifilial phase, the disintegration becomes more distinct and some foragers seemingly repeat only provisioning during the daytime, approaching the condition seen in more differentiated social system in the Apidae (*cf.* Sekiguchi and Sakagami, 1966).

5. Family structure in summer nests, normal and abnormal

In the preceding sections, it was established that each summer nest of *Ev. duplex* contains a foundress mother and her summer daughters. The differences between them were described in Section 2, based upon the samples as a whole. However, the adult population of each nest shows a noticeable internidal variation in number, condition of reproductive system, etc. Further, besides the normal social pattern schematicized as the association of a relatively large, inseminated and ovarially developed mother with her daughters, relatively small and usually uninseminated and ovarially undeveloped, there appear some other patterns, which are interesting as showing the appearance of various family structures. The present section deals with these problems.

5.1. *Size difference between mother and daughters in the same nests*: The result given in *2. 1.* shows a statistically significant difference of body size between mother and daughter samples. At the same time, however, a considerable overlap in frequency distribution of sizes of the two castes was demonstrated. To know how the difference is distributed among the inhabitants of the same nests, the percentage ratio of head width of each daughter to that of the mother in the same

nest was calculated in 70 nests and the frequency distribution of the relative size was presented in Table 14. Separate calculation was made for each bee when more than one daughter was found in the same nest. As indicated previously, the distinction of mothers and daughters is more reliable in later samples, so that the data in 1957~58 and 1965~67 were separately tabulated.

Daughters larger than their own mothers were only 15.2% in 1957~58, and 11.8% in 1965~67. Although the overlap range was considerable when mothers and daughters were compared as a whole, in most nests the mothers were larger than their own daughters.

Table 14. Relative head widths of daughters (D) compared to mothers (M) in the same nests

Relative size 100·D/M	Freqneucy obtained	
	1957~58	1965~67
70.0- 74.9	1	
75.0- 79.9	1	
80.0- 84.9	7	1
85.0- 89.9	17	13
90.0- 94.9	28	46
95.0- 99.9	13	37
100.0-104.9	9	11
105.0-109.9	3	2
Number of nests	42	28
daughters	79	110
Mean % ratio	92.06	90.90
SD	5.83	25.15

There are many records on the size difference between queens and workers, or mothers and summer daughters in halictine bees. But the size difference of the two castes in the same nests is known only in *D. imitatus*. In this species, the queens are usually but not constantly larger than the workers in the same nests. The percentage of nests with queens larger than workers is 77.7% in June, 65.2% in July, and 58.3% in August, suggesting gradual increase of average body size of workers. Such seasonal change is not expected in *Ev. duplex*, which produces summer daughters only once, in a batch. The percentage of nests with mothers larger than daughters, obtained from Table 14, is higher than in *D. imitatus*, reaching 91.0%.

5.2. Family structure in summer matrifilial phase:

Many summer nests were excavated and their adult population was studied. In a considerable proportion of them, it is possible that some of the adult bees were not captured. All such nests and some abnormal ones explained later were excluded and the reliable data were presented in Table 15. The data obtained in 1759~64 and 65~67 are given separately. The inhabitants of each nest are shown by combination of the following letters, each of which represents a single female; M, Ovarially developed mother; m,

Ovarially undeveloped or degenerated mother; D, uninseminated and ovarially developed daughter; d, Uninseminated and ovarially undeveloped daughter; F, Inseminated and ovarially developed daughter; f, Inseminated and ovarially uneveloped or degenerated daughter (Daughters were regarded as ovairally developed even if only in one ovariole, see class C in Fig. 5). Each combination of letters represents a single nest, unless the number of observed cases is parenthetically given. Italicized series show that one of the "daughters" is a bee drifted in from another nest. In each combination, the bees are arranged in the descending order of body size.

The Table 15 indicates the occurrence of various combinations. The percentages of nests with various types of daughters are given in Table 16. When the nest is headed by the mother, the daughters remain uninseminated and ovarially undeveloped (d) in about the half the cases (Table 16, I). But ovarially developed daughters (D) appear in about one third of the nests (Table 16, II), so that their

Table 15. Family structure in 88 precisely studied summer nests (Explanations of abbreviations in text)

Period	1957~1964				1965~67			
	M>D or d Without F, f		Others	Orphan	M>D or d Wtihout F, f		Others	Orphan
	WithoutD	With D			WithoutD	With D		
July 10~15	Md(4) Mddd(2)	MD MDdd *MDDdd* mDd	dMf	ddd dd d(2)	Md Mdd(2) Mddd Mdddd(2) Mddddddd	MD MDd *MdDdd* MdDddd MDDddDdd MdDdDdd MDDDdd MddDdDddd MDDDDdd MMDDDdD	Mddfdddd DMDDDdfD dddDM DFM ddMd	dD DDd DFDDD dDfDD Ddddddd DdDdd
July 16~31	Md(7) Mdd(2) Mddd Mddddd	MD(5) MDdd MDdD	Mdf(2) Mfd ddM	fd dddFDdd DDf ddd dddf	Md	MDdddd		dDdd
August 1-	md		mfdf Mdf Mf mf mF	df Ff	Md(3) Mdd			dFf DfD Dddd
Number of Nests	18	11	10	11	12	11	5	10

Table 16. Numbers and (in italiques) percentage ratios of nests with various types of daughters (D, d, uninseminated daughters with ovaries developed or not; F,f, inseminated daughters with ovaries developed or not; +, with, ±, with or without, —, without)

Nests with various types of daughters	I		II		III		IV		Total
	+d −D,F,f		+D ±d −F,f		+f ±D,d −F		+F ±D,d,f		
Normal	33	*49.3*	22	*32.8*	10	*14.9*	2	*3.0*	67
Orphan	5	*23.8*	6	*28.6*	6	*28.6*	4	*19.0*	21

probable contribution to the production of male autumn offspring is assumed. Inseminated and ovarially developed daughters (F) appear in normal nests only exceptionally (Table 16, IV). Their contribution to the production of the mothers of the next generation is seemingly neglegible. Under orphan condition, the percentage of nests with inseminated daughters (F, f,) increases notably, reaching 47.6% of the total nests (Table 16, III+IV), and the percentage of nests with inseminated and ovarially developed daughters becomes high (19.0%) compared with normal nests. Their contribution to the production of mothers of the next generation is unknown but may be higher than in normal nests.

The number of ovarially developed daughters found in each nest excavated during July is distributed as follows (Orphan nests in italiques):

	Number of daughters															
Number of ovarially developed daughters	1		2		3		4		5		6		7		8	
0	13	*5*	9	*4*	5	*5*	4	*1*	2						1	
1	7	*3*	2		2	*1*	1	*2*	3							*1*
2			1		1	*2*	1			*1*	1			*1*	1	
3									1	*1*			1			
4									1		1					
5										*1*			1			
Number of cases	20	*8*	12	*4*	8	*8*	6	*3*	7	*3*	2		3	*2*	1	
% ovarially developed	35.0		25.0		37.5		33.0		57.1							

The result is insufficient to give any reliable mean percentage, only indicatcating the rarity of ovarian development in more than half of the inhabitants of a nest. Moreover, the numbers and percentages given above must not be understood rigidly. The number of bees as well as the percentage ratios of the four types of daughters (D, F, d, f) varies in the course of the summer phase. We are still not in a position to present an account of the temporal changes in family structure.

The percentage ratios of four types of daughters, D, d, F, f, in normal and orphan nests are given in Table 17, according to the date of nest examination.

Table 17. Numbers and (in italiques) percentage ratios of various types of daughters in normal and orphan summer nests excavated in different periods (Abbreviations in Table 16)

Period	D		d		F		f		Total	% inseminated	% ovarially developed
Normal Nests											
Juyl 10~15	33	*28.7*	78	*67.8*	1	*0.9*	3	*2.6*	115	*3.5*	*29.6*
16~31	9	*20.5*	32	*73.0*			3	*6.5*	44	*6.5*	*20.5*
August 1~			8	*57.1*	1	*7.2*	5	*35.7*	14	*42.9*	*7.2*
Total	42	*24.4*	118	*68.2*	2	*1.0*	11	*6.4*	173	*7.4*	*25.4*
Orphan Nests											
July 10~15	12	*36.4*	19	*57.6*	1	*3.0*	1	*3.0*	33	*6.0*	*39.4*
16~31	4	*19.9*	13	*62.0*	1	*4.8*	3	*13.4*	21	*18.2*	*23.8*
August 1~	3	*22.3*	5	*35.8*	2	*14.3*	4	*28.6*	14	*42.9*	*36.6*
Total	19	*27.9*	37	*54.4*	4	*5.9*	8	*11.8*	68	*17.7*	*33.8*

The gradual increase of inseminated daughters (F+f) in later periods is seen both in normal and orphan nests, which coincides to the result obtained from total samples recorded in *2.3.* On the other hand, the monotonous decrease of ovarially developed daughters (D+F) does not follow the pattern shown in *2.3.* (Fig. 3), suggesting the variability in this phenomenon. The percentage ratios of both inseminated (F+f) and ovarially developed (D+F) daughters are significantly higher in orphan nests than in normal ones ($P<.05$), which agrees with the result mentioned above. Further comments on orphan nests are given in *5.3.*

To show various types of family structure in summer nests, data for certain examples of nests carefully excavated in early July, 1967 (*cf.* 2. 5.) are presented in Fig. 12. For each nest, the numbers of spring and summer cells found are given by the segments of two horizontal bars placed above and below the series of squares each of which represents an adult female, with records of mandibular wear, ovarian condition and body size.

Nest A~J are those headed by a mother. In most nests the number of daughters found is less than that of spring cells. Some spring cells might produce summer males (*cf.* Section 1), but most produce summer daughters. Therefore, the difference between the number of spring cells and of summer daughters found in nests, given by the number of broken lined segments of upper horizontal bar, indicates the daughters left the nest, the subsequent fate of which was discussed in *3.4.*, and will be referred to in *5.4.* and *6.2.*

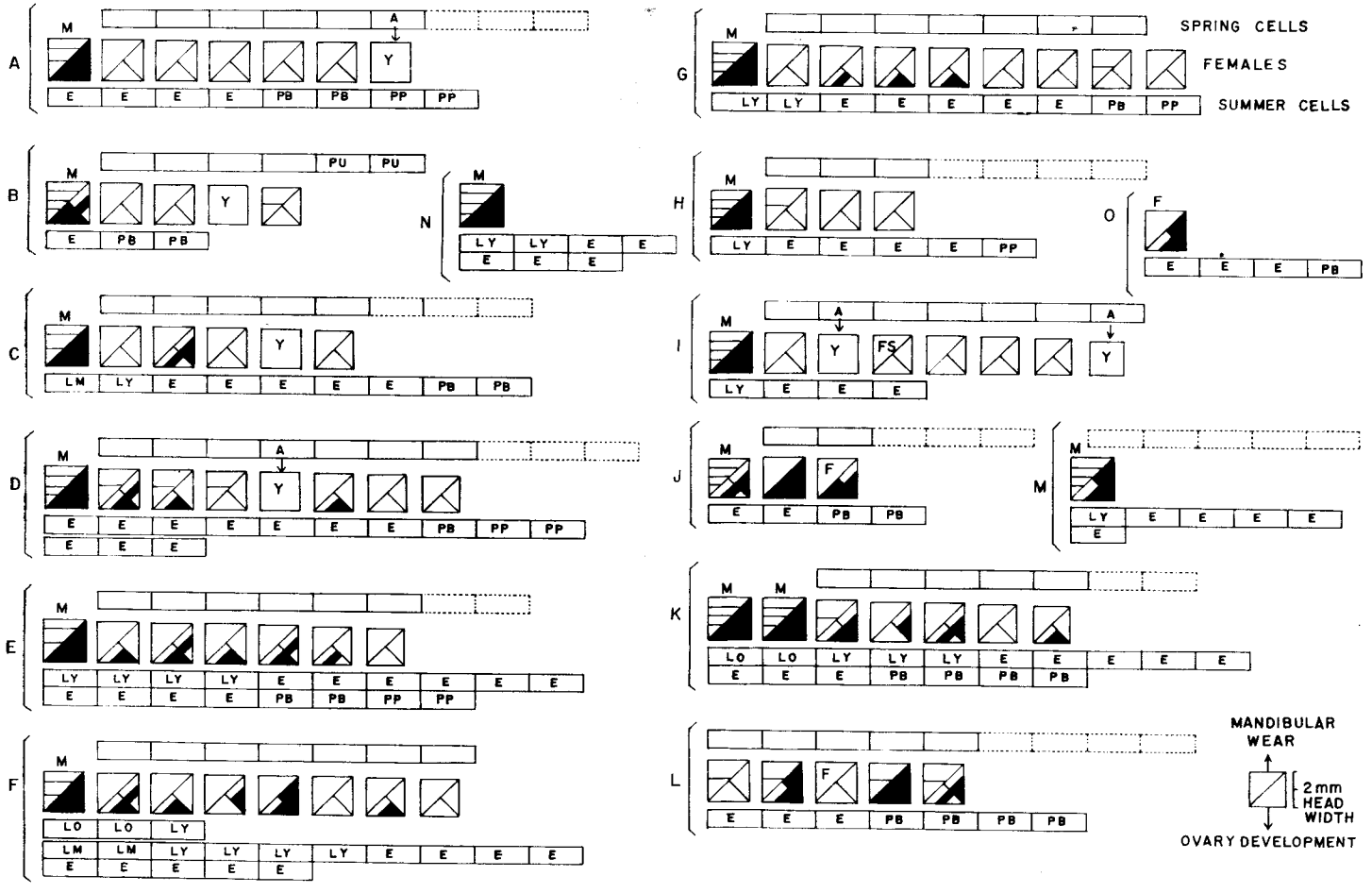


Fig. 12. Composition of adult populations in certain nests excavated in early July, 1957 (July 10, C,F,I,J,L: July 12, B; July 13, A,D,K,M,O; July 14,E,G,H,N). Each square represents a female, upper and lower halves of which give, respectively, degrees of mandibular wear and ovarian development, using norms shown in Fig. 5. The size of square gives body size (length of one side of square=head width). Squares in each nest are, except mother (M), arranged in the descending order of body size (Y, Daughter soon after emergence; F, Inseminated; S, Stylopized). Upper and lower horizontal bars indicate, respectively, the number of cells in spring and summer cell clusters found at nest excavation. Each segment means a cell and its content (Pu, Black pupa; A, Female adult soon after emergence; LO, LM and LY, Old, medium and young larvae; E, Egg; PB, Pollen ball; PP, Pollen mass before preparation). Empty segments in upper bar represent cells after emergence, and for those outlined with broken lines, the bees abandoned the nest or already died after emergence.

Nests A and B represent the standard type, that is, the mother is larger than all daughters, and no daughter is inseminated or ovarially developed. Nest A has already lost three daughters and produced eight cells, while B is in the incipient state; two daughters are still in pupal stage and only three cells are produced. Nests C~F possess some ovarially developed daughters, the number of which is large in E and F. All except F have lost some daughters, while the number of cells produced is large. D and F possessed two summer cell clusters, shown by two sepsrate lower bars. Nest G is similar to D, but contains one extra daughter in comparison with the number of spring cells, indicating a migrant from another nest. Nest H is similar to A and B, but the mother is smaller than two of her daughters. Nest I and J, have one inseminated daughter each. Moreover, in J, the mother is smaller than two of her daughters which have well developed ovaries. These examples may facilitate an understanding of various social patterns exhibited by summer nests. Nests K~O are abnormal in some aspects and referred to in subsequent sections.

5.3. Orphan nests: Some data on oprhan nests were presented in the preceding section. An example of such nests in early July is given in Fig. 12, Nest L, which lost the mother and four daghters but produced seven cells. Among five daughters in the nest, three developed ovaries and one was inseminated though the ovaries were still (?) rudimentary.

Table 15 involves 67 normal and 21 orphan nests, giving the frequency of the latter as ca. 24.0% of total nests. But it is not easy to give a meaningful ratio of orphan nests. The mother may leave the nest at various periods in the summer phase. If she were lost late, it would not affect the productivity of nest. The frequency of orphan nests in the early summer phase, July 10~15, is ca. 15.4% (Table 15). This figures seems to underestimate the real ratio, because many nests without mothers were discarded in preparing the table, nevertheless many of them were certainly truly orphan.

In *3.3.*, an estimated percentage of summer orphan nests to spring nests in 1958 was given as 8.5%. This percentage was obtained from the results of 1958 alone; the mean percentage ratio becomes 7.3% when recalculated according to the mean survival of spring nests (=nests each inhabited by the mother, 17.5%, *cf. 3.3.*). Therefore the percentage ratio of oprhan nests to total summer nests continued since spring (=24.8% of total spring nests: 7.3+17.5) becomes about 29.3% or more than one fourth at the beginning of summer phase. This figure is used as a first approximation to the relative abundance of orphan nests until more reliable estimates can be obtained, though we have an impression that the figure is a little higher than the actual one.

To what degree orphan nests contribute to the population increase of the species depends on the number of inseminated and ovarially developed daughters in such nests. If such daughters were absent, the nests which were orphan since the beginning of the summer phase apparently would have no contribution to the

production of the next generation's females. The appearance of ovarially developed but uninseminated daughters may produce males, gradsons of the lost mother. These males may affect the productivity of the next generation by inseminating some autumn females. But the real contribution of orphan nests to the next generation is achieved principally by producing autumn females, which are in this case the granddaughters of the lost mother, not the younger sisters of summer daughters as in normal nests. Under Dzierzon's rule, which governs *Ev. duplex* and all other halictine bees so far carefully studied (*cf.* Michener, 1960), the production of female offspring is possible only by insemination. The frequency of such daughter is higher in orphan nests than in normal ones (*cf. 5.2.*). But the frequency of inseminated daughters tends to increase near the end of the summer phase, probably due to the appearance of autumn males. Apparently such late inseminated daughters do not produce autumn females. Excluding such instances, the data are still insufficient to give any definite estimates. Summarizing, there are more inseminated daughters in orphan nests than in normal nests. Such nests could produce some female offspring even if not so many as normal nests. But we have still no accurate estimation of the relative role of orphan and normal nests in producing the next generation's females.

The numbers of cells produced in orphan nests with ovarially developed daughters, excavated July 10~16, is as follows: 0, 2, 2, 7, 10, 18. The mean, 6.5, is not much lower than that of normal nests at the corresponding period (Fig. 6). But this does not show the real productivity under orphan condition, because we do now know when the mother left these nests. Further results obtained from experimentally induced orphan nests are given in the final section.

Few observations exist upon the orphan nests in halictine bees. Based upon the observation on *D. imitatus*, Michener and Wille asserted that the ovarian development of workers is not a result of the lack of queens in the nests. Certainly the ovaries of some workers(=summer daughters) develop in the presence of mothers in *Ev. duplex* as repeatedly mentioned above. But as the frequency of such daughters increases in orphan nests, the assertion by Michener and Wille must not be understood as stating that there is no influence of the presence or absence of queens upon ovarian development. Plateaux-Quénu (1959) cites an orphan nest in the final year of the perennial species, *Ev. marginatus*. Among 77 "workers" in the nests, 12 had well developed ovaries and eight more or less developed ovaries. Probably this was caused by the lack of inhibition by the queen. In general we could assume higher frequencies of ovarian development or insemination among summer daughters in orphan nests. But the modes of life of halictine bees are very diverse, and one cannot now generalize. There are certain species with high frequency of ovarially developed or inseminated workers even in normal nests, such as *D. zephyrus* or the species in which the mothers are often replaced by one of their daughters as in *Augochlorella*. The problem of orphan nests may be virtually meaningless in species with very incipient caste differentiation such as *As. sparsilis*. In halictine bees we should always avoid arranging various species of

different social patterns uncritically along a unidimensional scale.

5.4. Solitary nests made by dispersed summer daughters: A remarkable discrepancy between the numbers of spring cells and summer daughters in the same nests was described in *3.4.* and illustrated in Fig. 12. Many daughters that left the nests may have died and some invade other nests and live there for a considerable time (*cf.*. *3.4.*, *4.1.* and Fig. 9, *5.2.* and Fig. 12, Nest G). Some others, however, excavate their own nest burrows and occasionally produce offspring. The occurrence of such nests is confirmed when one of the following conditions are fufiled: A . Appearance of unmarked nests in an aggregation, all nests of which had individually been marked in spring. B. Presence of only one daughter bee and absence of a spring cell cluster in the nest. (These criteria are valid only at the initial phase of summer period. Spring cell clusters are later filled with soil, so that they may be unnoticed even if present.) C. The appearance of burrows in places which had, prior to the discovery of the nests, been artificially disturbed, so that absence of any nests was confirmed. (This criterion is valid only when the soil was disturbed deeply, say, 40 cm. Otherwise lower blind burrows might remain intact after the disturbance.)

Twenty one nests, rather casually excavated on July 5～20, 1957～'67, were identified as made by dispersed summer daughters, based upon the conditions B and C above. In some nests found in previously disturbed places, the bees were observed at discovery of the nests, but not at excavation. It is theoretically possible that some such nests were made by dispersed mothers. However, the dispersal and preparation of new nests in summer by mothers is a rather rare event (*cf. 5.5.*). Most ,if not all, of nests mentioned were certainly made by dispersed daughters, and all inhabitants, when present, were confirmed as summer daughters, even if inseminated and ovarially developed, from body size, mandibular wear and absence of yellow spots in ovaries which were characteristic in early summer mothers.

The nests are classified into several groups as follows:

A. Burrow only, without adult bee and cells (six cases).

B. With cells, but without bee (Two cases). One nest had two empty cells filled with soil, indicating interrupted nesting activity. Another nest is noteworthy. The owner was not found when it was excavated on July 24, 1958, but three cells contained pupae, two males and one female. If this was made by a dispersed daughter, which is more probable than the preparation by a dispersed mother, it offers positive evidence for the production of female progeny by a solitary daughter.

C. Burrow alone, with one adult daughter (10 cases). Nine of these nests contained an uninseminated and ovarially developed daughter each. In three of them, the inhabitants were discovered on July 10, 1957, at the nest entrances. Excavation was postponed until August 7, in expectation of brood production. No cell was found on that day. These bees remained in nests nearly one month, performing no brood rearing activity. Another nest contained an inseminated daughter with slightly developed ovaries.

D. With cells and one daughter (Three cases). 1. One inseminated and ovarially

developed daughter, with four cells (three eggs and one pollen ball), excavated on July 13, 1967 (shown in Fig. 12, Nest O). 2. One inseminated and ovarially developed daughter, with one cell (pollen ball), excavated on July 14, 1967. 3. One inseminated and ovarially degenerated daughter, with four cells (all with black male pupae), excavated on July 24, 1958.

Summarizing, three out of 21 nests, or ca. 14.3% of total cases, produced brood. This percentage is close to that obtained through another approach (*cf. 6. 2.*), but further study is required to determine the real contribution of dispersed nests to the next generation.

It is not easy to give a reliable estimate of the percentage ratio of dispersed nests. The number of cells produced in successful spring nests was 5.22 in 1958, 6.77 in 1967, or 6.0 in average (*3. 3.*). As about 10% of spring brood are males (*cf.* Section 1), the mean number of summer daughters produced in each nest is ca. 5.4. From the result given in *3. 4.*, about 36.1% of the total daughters, or 1.96 daughters per nest, are assumed to leave their nests. If all these daughter make their own burrows, the total number of summer nests at the peak should be 2.96 times many as those continued since spring. (This value could become higher, because unsuccessful spring nests could also produce a small number of daughters. *cf.* Table 7.) The percentage ratio of reactivated summer nests to total spring nests is estimated as 24.8% (*cf. 3. 3*). This figure was based upon both normal and orphan nests. (The estimate must be corrected if the percentage of daughter dispersal differs between normal and orphan nests.) Therefore, if there were 100 spring nests, about 73.4 summer nests ($=24.8\times2.96$) are expected, among which 48.6 nests are made by dispersed daughters, and 24.8 being continued from spring. Obviously such high number as 48.6 dispersed nests must never be realized. Many summer daughters leaving their nests may succumb without making their own burrows. A tentative estimate of the percentage ratio of nests made by dispersed daughters was sought in another way. In 1967 a total census of the number of nests in the Botanical Garden was periodically made (Sakagami and Fukuda, unpub.). The maximum numbers of nests in spring and summer were respectively 3,729 (May 6) and 1,027 (July 17). The percentage of summer nests to spring ones is 27.5%. The difference between this figure and the number of spring nests reactivated in summer (24.8%), 2.7%, could be regarded as a first approximation to the percentage of dispersed daughter nests to total nests in spring. The percentage of the former to the nests continued since spring is therefore about 10.9 % ($100 \times 2.7/24.8$) and those producing offpsring, 1.6% ($14.3 \times 10.9/100$). The number of summer daughters excavating their own burrows is estimated as 5.55% ($2.7/48.6 \times 100$) and those successfully producing brood only 0.8% ($5.55 \times 14.3/100$) of all dispersed daughters. This estimation favors to the assumption that the contribution of dispersed nests to the productivity of the species is very low. Obviously our estimates are still not reasonably reliable. The figures basic to these percentage ratios were mostly obtained from limited observations of highly variable events. Our procedures to correlate various figures are also not free from criticism. We present these first approximations principally to call attention to a so far

relatively ignored field in wild bee studies, the bionomics and sociology in relation to population dynamics.

5. 5. Activities of mothers after the spring phase and solitary mother nests in summer: After the end of spring phase, the mothers confine themselves nearly exclusively within their nests throughout the inactive phase in June and the matrifilial active phase in July and early August. Consequently we have only fragmentary records upon their behavior. Occasional participation of mothers in guarding at nest entrances was described in *4. 6*. The other records are gathered together in this section.

The departures of mothers after the spring phase from nests is very rare. We have only the following two observations: 1) July 14, 1958. Return of a mother was observed. This bee had been marked individually on May 27, near the end of the spring phase. 2) During the period July 8~11, 1958, 29 bees departing from, or returning mostly with pollen loads, to seven particular nests were captured. These bees were classified as follows: A) Inseminated and both ovaries fully developed or nearly so (4 bees);, B) Uninseminated (25 bees), with ovaries undeveloped (18), with one ovary or both slightly swollen (3), and with one ovary more or less well developed (4). Thereafter the seven nests were excavated on July 12. No adult was found in four nests. Each of the other three nests was inhabited by the mother and one daughter. It is very probable that the four inseminated bees captured at the nest entrances, at least two of them caught bringing back pollen loads, were the nest mothers. This observation gives indirect evidence of foraging by mothers, when all daughters were removed from nests.

The departure of mothers in matrifilial nests seems to be exceptional in many halictine bees. This phenomenon was not observed in *Ev. malachurus* (Bonelli), *Ev. marginatus* and *Ev. calceatus* (Bonelli). Ordway also records the absence of pollen foraging by queens in summer nests of *Augochlorella*, and assumes probable inhibition by the occurrence of pollen foraging workers. In *D. imitatus*, only three queens among 180 females were captured in the field. The absence, or, at least, rarity of summer flight activity by mothers seems to be common in many other species, although the papers dealing with them do not always give a definite statement. On the other hand, Bonelli records the departure of mothers in *H. sexcinctus* at least in the early period of the summer phase. Moreover, according to his observations upon *S. subauratus*, only mothers forage in summer; daughters, besides guarding and repairing the nest entrances, make flights but only to feed themsevles, not bringing pollen loads. This unusual result requires further critical study. No such relation was confirmed in detailed observations on a related species, *S. tumulorum* (Sakagami, unpublished).

In *D. imitatus*, the mandibles of mothers become gradually more worn in the course of the matrifilial phase, indicating their participation in building activities within nests. Probably this is also true in *Ev. duplex*, judging from the captures of very worn mothers in autumn (Fig. 1). Seemingly the mothers also prepare pollen balls. In July, 1967, 13 bees were found striding over still small pollen balls or over amorphous pollen masses, when nests were excavated. Among these bees,

three were mothers, seven ovarially undeveloped daughters and three others ovarially developed daughters. Batra (1964) also found in *D. zephyrus* the formation of pollen balls by foragers as well as egg layers.

The observations cited above suggest flight activity by mothers in nests from which all daughters were removed. If such is the case, can the mothers perform their second brood rearing activity when they are left in solitary condition ? A few observations indicate such is occasionally possible.

No. 1: June 19, 1959. A tumulus was found at a place which had previously been artificially disturbed by us. The excavation of the nest revealed that only the upper part of the burrow was destroyed by the previous interference (Fig 13). The spring cluster was situated in intact soil, but young were all decomposed. Two newly made cells, still not surrounded by a cavity, were found at a lower level, one containing a pollen ball, and the other with a still amorphous pollen mass. The single adult female was inseminated, with fully developed ovaries and trace of yellow spots, indicating previous activity. Wings and mandibles were still only slightly worn and head width of 2.32 mm. This case is interpreted as showing the start of a second brood rearing activity by a mother which had lost her offspring. The repair of the uppermost part of the burrow might release foraging activity at the middle of June inactive phase, earlier than in the normal case.

No. 2: June 23, 1959. Entry of a bee with pollen loads into a nest was observed at 13: 00. Immediate excavation exposed a spring cluster consisted of three cells, containing one adult and two black pupae, all females, and two newly made cells, at the end of the burrow, one with a pollen ball, the other with an egg on a pollen ball. The lower blind burrow was absent (Fig. 14). The single adult female was inseminated with well developed ovaries and head width of 2.44 mm. This case clearly demonstrates the second brood rearing activity of the mother, at very early period for some unknown reason.

No. 3: July 12, 1967. Contents: Spring cluster with six cells (one black pupa, one half-black pupa, one black eyed pupa, all females; one post-defecation larva and two cells after emergence), one summer cell with pollen mass, and a single female which is distinctly the mother. It is possible but not probable that two daughters, already emerged, had brought pollen loads to the summer cell before their dispersal or death.

No. 4: July 13, 1967 Contents: Spring cluster with five cells, all after emergence, six newly made cells (five eggs and one young larva) and the mother (Fig. 12, Nest M). The case is similar to, but much more advanced than No. 3. The contribution of some daughters before dispersal is much more probable.

No. 5: July 13, 1967. Contents: Spring cluster with six cells (one black female pupa, five cells of damaged contents), four new cells (three pollen balls and one pollen mass) and the mother. The new cells are, as in *Nos. 1* and *2*, doubtless made by the solitary mother.

No. 6. July 12, 1967. Contents: No spring cell, one new cell with pollen mass and the mother. This is a newly made nest of a dispersed mother.

No. 7. July 14, 1967. Contents: No spring cell, nine new cells (five eggs and two young larvae) and the mother (Fig. 12, Nest N). This is also a new nest made by a dispersed mother, characterized by an exceptionally high rearing activity. Probably this nest indicates the limit of brood rearing ability by solitary mothers in summer (*cf. 6. 1.*).

Judging from the frequent dispersal of summer daughters from their nests, summer nests of solitary mother left behind may not be very rare. Nests made by dispersed solitary mothers may be less frequent. At any rate, these cases show

that the mothers *can* perform their second brood rearing activity in the solitary state. However, the discovery of only seven such cases out of more than about 200 nests excavated during about ten years suggests that lone second reproductive activity rarely results in the production of brood. Further discussion on this problem is given in *6. 1.*

5. 6. Nest fusion and pleometrosis: Finally two very rare social patterns found in summer phase are referred to. One is the fusion of two nests. Only two such cases have so far been discovered. One is the fusion of two main burrows, resulting in the common use of the entrance by two nests, illustrated in Sakagami and Hayashida (1970, Fig. 39). The other case is the fusion of two cell cavities, found on July 27, 1967 (This nest was obtained under semi-artificial conditions. A fuller description will be given elsewhere). In the first case, it is probable that the inhabitants of two nests behaved rather separately from each other, only using the entrance communally, as seen in the social structure of some halictine species, which have a common nest burrow in which each bee makes her private section (Michener and Lange, 1958, a; Sakagami, Hirashima and Ohé, 1966). On the other hand, the fusion of cavities might evoke complete intermingling of inhabitants of the nests. Verhoeff (1891) illustrates a similar instance of the fusion of two cavities in *H. quadricinctus* (cf. Friese, 1923; Sakagami and Michener, 1962). The fusion of nests appears to be rare in halictine bees, but according to Michener (1966, a, b), *D. versatus* is unusual in frequent interconnection of burrows of different nests.

Pleometrosis or coexistence of more than one inseminated female with developed ovaries is extremely rare in *Ev. duplex*. Sakagami and Hayashida (1961) recorded four observations which suggest the occurrence of two mothers in the same nests in the spring phase. But none of them gives evidence of more than temporary coexistence. The unique instance of pleometrotic association in summer is shown in Fig. 12, Next K. The nest was excavated on July 13, 1967. It contained two mothers and five daughters, all except one with more or less developed ovaries. Seven spring cells, all after emergence, and 17 summer cells were found. Two daughters were seemingly lost from the nest. The provenance of two mothers is unknown. One of them would certainly be the legitimate foundress. The other may be a migrant judging from the size of spring cluster, being not particularly large.

Pleometrosis has often been recorded in other halictine bees, for instance, in *D. imitatus*, *D. zephyrus*, *Al. striata* and *Al. persimilis*. Recently Knerer and Pleateaux-Quénu (1966, d) described interspecific differences of pleometrotic association in several species. Comparison of various types of pleometrosis is important in the study of social evolution in bees, or in insects in general. As far as *Ev. duplex* is concerned, however, we can point out only its rarity. Both nest fusion and pleometrisis are indubitably exceptional patterns in the formal sociology of *Ev. duplex* in summer nests. Their percentage ratio to total reactivated

nests in summer may never exceed 1%.

6. Observations upon experimentally induced summer nests

The early history of biological studies of halictine bees developed through a controversy of opinions concerning the occurrence of thelytokous parthenogenesis in this group. The opinion asserting its occurrence, held by Fabre (1882), Armbruster (1923) and Legewie (1925), was challenged by Stöckhert (1923) and objectively rejected by Noll (1931, *cf.* Michener, 1960). As one of several approaches to prove his own opinion, Noll attempted to remove the mothers from nests and to show the inability of remaining daughters in the orphan nests to produce female offspring. For this purpose, he excavated nests in the inactive phase, extracted cell clusters. Then he placed each cluster in a hole made in soil, put an inverted flower pot over it, and filled the hole with soil up to the surface level. Later some females emerged from the buried cluster and made their own cell clusters. This procedure was adopted by us to study various social patterns mentioned in Section 5 experimentally.

Procedure: Nests were excavated in June during the inactive phase. Mothers and cell clusters were extracted. A hole of ca. 10 cm in diameter and 10 cm in depth (= mean depth of spring cell cluster) was made in the ground where the transplantation was carried out (Fig. 15, A). The bottom of the hole was tightly pressed, and, in order to reproduce natural condition, a vertical burrow of about 10 cm in depth was perforated in the bottom (F). One cell cluster or more were placed on the bottom (C) and an unglazed flower pot 4 cm in bottom diameter, 6 cm in mouth diameter and 5.3 cm in depth (all outside dimensions) was inverted over the cluster (D). The mother (B) was added or not according to the aim of the experiment. When included, she was marked individually and introduced into the pot through the bottom hole. Thereafter, the hole containing the artificial cavity made by the pot was tightly filled with soil up to the level of the ground surface. Again to reproduce conditions similar to natural nests, the bottom hole of the pot and the ground surface were connected by means of a vertical burrow (E). Finally the uppermost part of this burrow was closed with soil. The result of 23 transplantations by this procedure in 1958 was unexpectedly successful. The experiment was repeated in 1959 with the following modifications.

1) In 1958, an intact cell cluster was placed on the bottom of the hole, so that the content of each cluster was not examined. As about 10% of the spring brood are males (*cf.* Section 1), it is possible that transplants involved certain males. These males might inseminate their sisters within the artificial cavity. If such were the only chance of the insemination, the daughters from the transplants not including males would remain uninseminated. To test this possibility, however presumed unlikely, each young was extracted from its cell. The post-feeding larvae were reared until pupation, and only sexed individuals were transplanted. Each pupa was inserted in a small tube made from paraffin paper, and both ends of the tube were closed (Fig. 16). Several soil blocks were placed on the bottom of the hole, then capsules containing pupae were placed upon them, in positions avoiding direct contact with the bottom of the hole. This procedure was also advantageous in preparing transplants consisting of any desired number of individuals. The sexes of transplanted pupae were recorded. Mothers were added or not according to the aim.

2) The results given in *3. 4.* and *5. 4* show the dispersal of a considerable number of daughters from nests and preparation of their own burrows by some of them. To obtain some quantitative measure of the percentage ratio of successful nests made by them, transplantation was made in ground previously free from nests of *Ev. duplex*. An area of ca. 8 m.sq., densely covered with white clover, was selected on the University Campus, because the area was completely out of the range of nest site preference of the stentopic *Ev. duplex*, and the nearest nest aggregation was ca. 300 m from the area. The root systems were completely removed and the soil surface was evenly pressed. The transplantation was made in a regular grid arrangment.

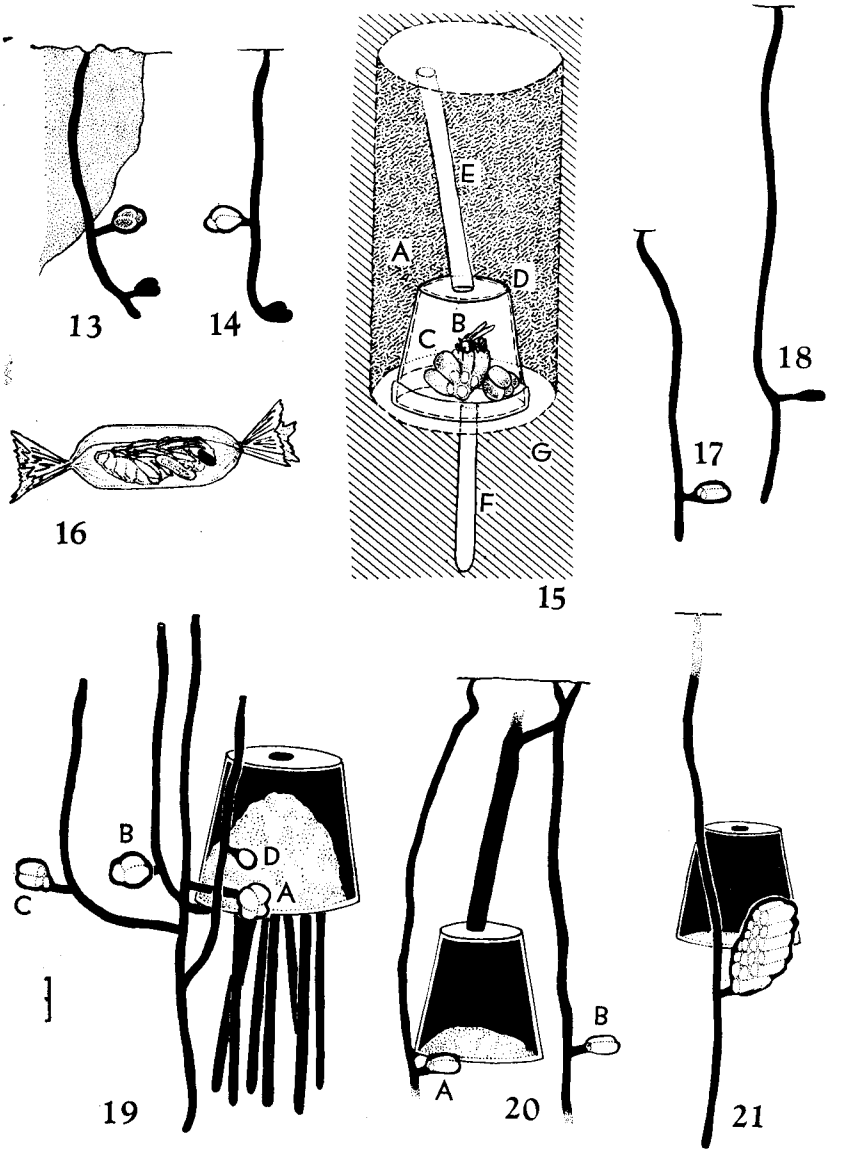
The ratio of successful nests in 1959 was less than in 1958, probably partly because of the frequent rains during the experiment. Our primary question, concerning the relation between size of transplant (=number of transplanted pupae in each case) and productivity was not definitely answered. Yet the results contain some qualitatively interesting information. Some additional transplantations were made in 1960 and 1967.

As a whole, our experiment was more successful than that by Noll. In his results, only two out of 106 experimentally induced orphan nests in 1929 and only one out of 40 in 1930 produced progeny. Explaining this low efficiency, he assumed that summer nests can develop normally only in the form of the matrifilial association. This interpretation could be valid, because *Ev. malachurus* possesses indubitably a social system more advanced than in *Ev. duplex*. But it is probable that the nest pattern of *Ev. duplex* is better for this type of experiment. The spring cluster, surrounded by an ample cavity, can be extracted with minimum damage to the contents. The use of such a cavity might match the unnatural space under the inverted pot. But all transplanted bees made their summer cell cluster outside of the artificially given space under the pot, which occasionally remained but was usually filled with soil (Figs. 19~32). Consequently the nests made by the transplants seldom produced the large tumuli characteristic of normal summer nests (Sakagami and Hayashida, 1960). The lower blind burrows and upper burrows given artificially were never used by the bees. In some nests, the main burrows were connected with the cavity under the pot (Figs. 24, 26, 28, 29, 31, 32).

In subsequent descriptions, the number of transplanted mothers and daughters are shown by the following abbreviations: 1M+3 d (=one mother and three daughters, or, three cells in 1958). The inclusion or not of males in transplants is not mentioned, because inseminated daughters were found in many nests, irrespective of whether transplanted together with males or not. This proves the extranidal mating of summer daughters. Sexes of offspring were partly recognized by rearing larvae obtained.

6. 1. Transplantation of mothers, not accompanied by daughters: Transplantation of mothers not accompanied by daughters was made 22 times as follows:

Year	Transplants	Number of cases produced		
		trials	burrows	cells
1959	2 M together	6	0	0
	4 M 〃	1	0	0
	5 M 〃	5	1	0
1960	1 M	9	3	0
1967	7 M together	1	1	1
	Total	22	5	1



Figs. 13–21. Some natural and experimentally induced summer nests. 13. Summer nest made by a solitary mother (*5. 5., No. 1.* Dotted area means the soil layer artificially disturbed). 14. Ditto (*5. 5. No. 2.* Blind burrow absent). 15. Design of artificial transplantation. A. Hole filled with soil after transplantation; B and C. Mother and spring cell cluster transplanted; D. Inverted flower pot; E, and F. Artificial upper and lower burrows; G.

The transplantations were made June 19~24 and nests were examined July 24~30. As shown above, only six out of 22 trials produced nest burrows, and only one, offspring. Probably this indicates the frequent abandonment of artificial cavities by mothers soon after transplantation. Except for the case in 1967, only in one trial with five mothers in 1959 was a mother still alive in the nest at examination. In this case the mother stayed within the nest for 45 days (June 12~July 27) with no sign of brood rearing activity. Besides these records, there is a single record of the nest made by a mother which dispersed from a transplant in 1959.

The nest was excavated on July 14. One mother with pink mark and two cells, both containing half-pigmented male pupae, were observed (Fig. 17). The mother possessed quite degenerated ovaries and heavily worn mandibles. Another interesting observation is cited here. On June 20, 1959, one open nest was discovered at a bed of medicinal plants, Botanical Garden. It consisted of a main burrow alone, inhabited by one mother, marked with blue paint, inseminated with fully developed ovaries. Clearly she was one of the mothers transplanted on the University Campus. She abandoned the artificial cavity and returned to the home site about 1 km distant from the transplanted area, across a band of fairly complicated topography, including a wood, a main street, a railway and two roads. Probably this is the limit of economic flight distance traversed by *Ev. duplex*, because the makeup of the melittofauna of the Unfversity Campus and Botanical Garden is, though naturally similar in basic pattern, quite different in some groups, indicating that the areas are fairly independent habitats for many wild bees except bumblebees (*cf.* Sakagami and Matsumura, 1967). Batra (1966, a) marked nine females of *D. zephyrus* and liberated them at a place, 0.5 miles from the nest site. Only one of them returned to the nest. This result is more or less comparable to that in *Ev. duplex*.

The difficulty of obtaining summer cells produced by experimentally isolated mothers presumably depends in part on the frequent abandonment of the artificial cavity by them. An additional trial was made in 1967 using seven mothers. They were taken from the Botanical Garden and transferred to the garden of one of us (S.F.S), surroundings of which were, at least for a radius of 500 m, and probably of 1 km, free from any nest aggregations. Seven mothers were placed in the common hole, but not directly. Seven vertical burrows were perforated in the bottom of the hole, each closed with soil after receiving one mother (Fig. 19). Several soil blocks were placed on the hole, in order to decrease the size of the empty space, which was assumed as a cause of early dispersal. Thereafter, an inverted pot was placed and the hole was filled with soil as in previous cases. The mothers were transplanted on June 19 and seven tumuli, A~G, appeared on June 23~25, that is, within one week after transplantation, A~D near the point of transplantation (Fig. 19) and E,F,G, respectively appart from the point 10, 50 and 300 cm. The activities of these nests, each with one marked mother, was recroded nearly daily. Nest F disappeared within a few days. Nest G continued activity for a while but disappeared after the final

Surrounding soil. 16. Pupa packed with paraffin paper for transplantation. 17. Nest made by a dispersed mother (*cf. 6. 1.*). 18. Nest made by a dispersed inseminated daughter (Table 18, No. 10). 19. Composite nest containing four summer cell clusters, each made by four out of seven mothers transplanted (*cf. 6. 1.*). Burrows below pot were used for transplantation (In subsequent figures, artificial upper and lower burrows are not given unless necessary). 20. Two nests produced by fission of a transplant consisted of 0M+5d (Table 20, Nos. 10a, b). 21. Nest made by a transplant of 0M+20d (Table 22, No. 3). Scale=2 cm in Figs. 13~14 and 17~32.

observation of return with pollen on July 20. Excavation on July 27 brought out only the ruined main burrow. The other five nests, A~E, excavated on July 26~27, produced cells. E was an independent nest while the other four each possessed an independent entrace and cell cluster but there was a common lower blind burrow, a nest pattern rarely found in normal nest architecture of halictine bees (Fig. 19). The contents of each cell cluster are as follows:

Nest	Number of				
	cells	young	♀	♂	mother
A	4	3	3	0	1
B	3	2	0	2	1
C	3	1	0	1	1 (dead)
D	1	1	0	0	1
E	4	3	1	2	1

Although Nests A~D were connected with one another, each cell cluster was certainly made by the mother found in her respective cavity. This fact also reflects the rarity of pleometrotic nests in this species.

Combining the results given above with those presented in *5. 5.*, we can conclude: 1) Mothers deprived of daughters can commence once more brood rearing activity in summer and occasionally can produce progeny, both male and female. Therefore the solitary mother nests are not always futile for the population productivity. 2) The efficiency of brood production in such nests is, however, very low. Only five (one dispersed mother included) out of 57 mothers used for transplantation, that is, 8.7%, produced offspring, and only two, 3.5%, produced female offspring. 3) The number of cells produced in summer solitary mother nest is, 1, 2, 2, 4, 6 and 9 under natural condition (*cf. 5. 5.*) and, 1, 2, 3, 3, 4 and 4 in experimentally induced nests. The data obtained from natural nests are not very reliable, because these nests were mostly still in an initial phase, so that addition of further cells is conceivable. Moreover, the contribution of daughters before leaving the nests is possible in these nests, except for two, made by dispersed mothers. The mean cell number in experimentally induced nests, 2.8, is distinctly lower than the mean in spring (cf. *3. 3.*). Among the six nests that produced cells, four had one empty cell each and the other nest two such cells. the reproductive activity of mothers is presumably quite low, when they are obliged to make summer nests solitarily.

6. 2. Transplantation of one or two daughters, not accompanied by mother: This type of experiments was repeated 39 times as follows:

Year	Transplants	Number of trials	cases of certain * activities observed	Cases produced cells
1959	1d	12	4	2
	2d	5	4	1
1960	1d	22	9	3
Total		39	17	6 (15.4%)

(* Formation of burrow, guarding or flight activities by inhabitants, etc.).

The number of cells produced in six nests is 2 in one 2 d nest and 1, 2, 3, 4, 4 in five 1 d nests. All these cells contained young and all of them were males. As these nests were excavated after the end of summer phase, the mean cell number, 2.66, gives an estimate for brood rearing capacity of solitary transplanted daughters. Two daughters with ovaries showing previous activity were found, one, uninseminated, in a 1 d nest with two cells, the other, inseminated, in a 1 d nest with three cells.

Besides these nests, 53 nests made by solitary daughters, dispersed from the transplanted locations after emergence, were discovered in 1959 in the experimental ground (*cf.* Introductory note of Section 6). These nests were excavated July 13~30. Burrows were detected in 27 nests (50.8%), the cells in 14 (26.4 %) and young in 9 (17.0%). The nests with cells or adult daughter are summarized in Table 18. The mean cell number, 1.8 (31/17), is very low. Some nests, for instance, Nos. 8, 9, 11, 12, might have received additional cells, if the excavation

Table 18. Nests produced by dispersed summer daughters in 1959 (only those containing adults or cells)

No.	Number of		Conditions of * reproductive organs of adult (one daughter in all nests)	Formation ** of cavity surrounding cells	Remarks
	cells	young			
1	0	—	d		
2	0	—	F_0		
3	0	—	F		
4	1	0	—	−	Cell empty, polished
5	1	0	—	−	
6	1	0	—	−	
7	1	0	F	−	Cell half-built
8	1	0	F_0	±	Pollen mass alone
9	1	1	−	−	Egg
10	1	1	F′	−	One ♂ pupa (Fig. 18)
11	2	1	F	−	Pollen ball and egg
12	2	1	F′	−	Pollen ball and larva
13	3	2	—	+	Two ♂ pupae, another cell filled with soil
14	4	2	F	+	Egg and larva
15	4	3	F	±	Two eggs and a larva
16	4	3	F′	+	Ditto
17	5	4	dead	+	4 ♂ pupae and one cell with soil. Adult with parasitic fly in metasoma

* (Abbreviations of females in Tables 18~22, d, D, D′: Uninseminated daughters with ovaries, respectively, undeveloped, developed and degenerated; f, F_0, F, F′: Inseminated daughters with ovaries, respectively, undeveloped, beginning to develop, developed and degenerated; M and M′: Mothers, ovaries developed and degenerated; s: Stylopized)

** (− not built, ± half-built, + completely built)

had been postponed. But the nests with more than three cells were invariably provided with the cavity surrounding cell cluster. Therefore, in combination with the result mentioned above, it is concluded that a single dispersed daughter rarely produces more than four cells.

It is remarkable that 10 out of 11 daughters examined were inseminated. In the present case, daughters had no contact with mothers since emergence. Probably this explains the percentage of insemination of daughters, decidedly higher, not only than that in normal nests, but also than in orphan nests found under natural conditions (*cf.* Table 17).

Using this occasion, interesting solitary behavior of a drifting daughter is referred to. This is H-3 in "Nest" D′ given in Table 10 and Fig. 10 (Section 4). After active foraging in the original nest, H, for six days, this bee suddenly left its nest and settled in the artificial cavity under the pot which was used for the bees, which later produced Nest B′. For two days she made a number of departures, using the burrow artificially added at transplantation (Fig. 23, X). This is the unique case of the use of artficial burrows. The behavior of H-3 was very erratic as follows:

July 15: 8:35, Entry without pollen; 8:40, Departure; 11:00, Return without pollen but flying away without entry; 11:01, Entry; 15:30, Departure without orientation flight and immediate return followed by flying away; 16:06, Entry: 16:08, Departure with orientation flight, followed by immediate return and entry.

July 16: 10:28, Departure with orientation flight; 11:00, Return with pollen and entry; 11:02, Departure *carrying pollen on legs*, with orientation flight, followed by immediate return and entry: 12:03, Departure, again carrying pollen on legs, followed by immediate return and entry; 12:30, As at 12:03; 12:38, As at 12:30; 12:40, As at 12:38; 14:59, Departure *carrying pollen*: 15:27, Return *without pollen*, followed by flying away without entry; 15:28, Return and entry; 15:29, Departure with orientation flight, followed by immediate return and entry: 15:30, As at 15:29.

On July 21 (=six days after drifting), the pot was excavated and the corpse of H-3 was found on the soil accumulated in the cavity by the bees of Nest B′ (Fig. 23, Y). No cell was found and the cavity was not connected with Nest B′.

6. 3. Transplantation of one daughter, with mothers: Fifteen trials of this type were undertaken, all in 1959:

Transplants	Trials	Number of cases of activities observed	Number of cases produced cells
1M+1d	10	6	3
2M+1d	5	5	1

Besides the nests that produced cells, frequent returns to a nest of a daughter bee carrying pollen loads were observed. This nest disappeared together with another one, the mother of which once made a foraging trip, by the extension of a nest of *Formica fusca japonica* nearby.

Four nests produced cells having the following contents: No. 1 (2M+1d, two empty cells), No. 2 (1M+1d, two cells both with male pupae, one adult female, escaped, probably the daughter), No. 3 (1M+1d, five cells, four empty, one pollen

and soil, one probable daughter, the mestoma of which accidentally crushed), No. 4 (1M+1d, six cells, four empty and two male pupae, one uninseminated and ovarially developed daughter and the dead mother). In general, the productivity in this series is not high, even lower than that of solitary nests cited in *6. 1.* and *6. 2.*, probably because of the dispersal of many bees, both mothers and daughters, from the transplants.

6. 4. *Transplantation of 4~9 daughters with or without mothers*: All of 14 trials of this series in 1958 produced cells, while the result was less successful in 1959 as follows:

Transplants	Number of trials	Number of case of activities observed	Number of cases produced cells
0M+5d	23	15	8
1M+5d	18	13	8
2M+5d	4	4	3

The nests that produced cells or those with adults are summarized in Tables 19 and 20. The mean numbers of cells and young produced by orphan transplants consisting of 4~5 duaghters are respectively 16.3 and 15.9 in 1958, while only

Table 19. Nests produced by transplants consisting of 4~9 daughters (all without mothers) in 1958 (Examined on July 28 and 30)

No.	Trans-plants	Number of cells	Number of young	♀/♂ in young	Number of adults * (types)	Remarks
1	4d	3	2	1/ 1	1(f)	One empty cell
2	〃	11	11	2/ 9	1(d)	
3	〃	17	17	3/12	0	
4	5d	6	6	1/ 5	1(?)	
5	〃	15	14	0/10	2(d,F)	Stages discontinuous, three eggs, others old larvae or pupae
6	〃	23	23	6/10	0	Two cell clusters in the same cavity
7	〃	26	25	4/17	3(F,F′,fs)	
8 a	〃	2	2	0/ 2	2(D′,F′)	Two separate burrows, each with a cluster
8 b	〃	27	27	1/23		
9	6d	6	6	1/ 5	2(fs,F′)	
10	〃	10	8	0/ 8	1(d)	
11	7d	4	1	0/ 1	0	One pupa. Other cells filled with soil
12	〃	6	3	0/ 3	0	Three pupae. Other cells
13	〃	17	17	3/11	1(F′)	filled with soil
14	9d	22	22	6/11	2(d,F′)	Stages discontinuos, one egg,all others pupae

* Abbreviations explained in Table 18.

Table 20. Nests produced by transplants consisting of five daughters with 0, 1 or 2 mothers in 1959 (Examined on July 14~31)

No.	Trans-plants	Number of cells	young	♀/♂ in young	adults* (types)	Remarks (all marked bees were absent when nests were examined)
1	0M+5d	3	3	—	—	
2 E	〃	—	—	—{	1(F) }	Two independent burrows (Fig.22)
N		3	2	—{	1(F′) }	
3	〃	8	6	—	3(d,d,D)	Another daughter marked
4	〃	3	1	—	2(D,F)	Four other daughters marked. Therefore, one daughter drifted in from another nest
5	〃	3	3	—	—	
6	〃	2	—	—	—	Cell cluster damaged
7	〃	4	—	—	—	〃
8	〃	4	—	—	1(F)	Three cells empty, one with pollen and soil
9	1M+5d	1	—	—	—	Cell with pollen and soil
10 a	〃	2	2	—{	1(F) }	Two independent burrows. Three other daughters marked (Fig.20)
10 b		2	1	—{		
11	〃	?	—	—	1(d)	Excavation failed. Another daughter marked
12	〃	10	10	0/3		Three daughters marked (Fig. 27)
13	〃	0	—	—	2(D,f)	Another daughter marked
14	〃	5	5	0/5		
15	〃	11	7	1/5	1(F′)	(Fig.24)
16	〃	7	2	0/2	2(D,D′)	Five cells empty
17	〃	7	4	0/2	1(d)	Three cells with young damaged
18	〃	7	6	1/5	—	One cell with soil
19	2M+5d	1	1	0/1	—	
20	〃	6	6	0/5		
21	〃	2	—	—	1(ds)	Cells damaged

* Abbreviations explained in Table 18.

3.75 and 1.88 and 1959. The transplants involving mothers in 1959 also produced less cells and young (means, 4.1 and 2.9) than orphan transplants in 1958. The percentage of nests with cells is 50% in trials with mothers, 34.8% in those without mothers. The difference is statistically insignificant.

The frequency of inseminated daughters in orphan nests is very high, 10 out of 15 nests in 1958 and 4/7 in 1959, while 3/9 in nests with mothers. In spite of high frequency of inseminated daughters, the low production of female progeny is remarkable:

	Number of young			
	Total	Sex known	♀/♂	% females
1958 Orphan	184	157	28/129	17.9
1959 With mother	44	30	2/28	6.7

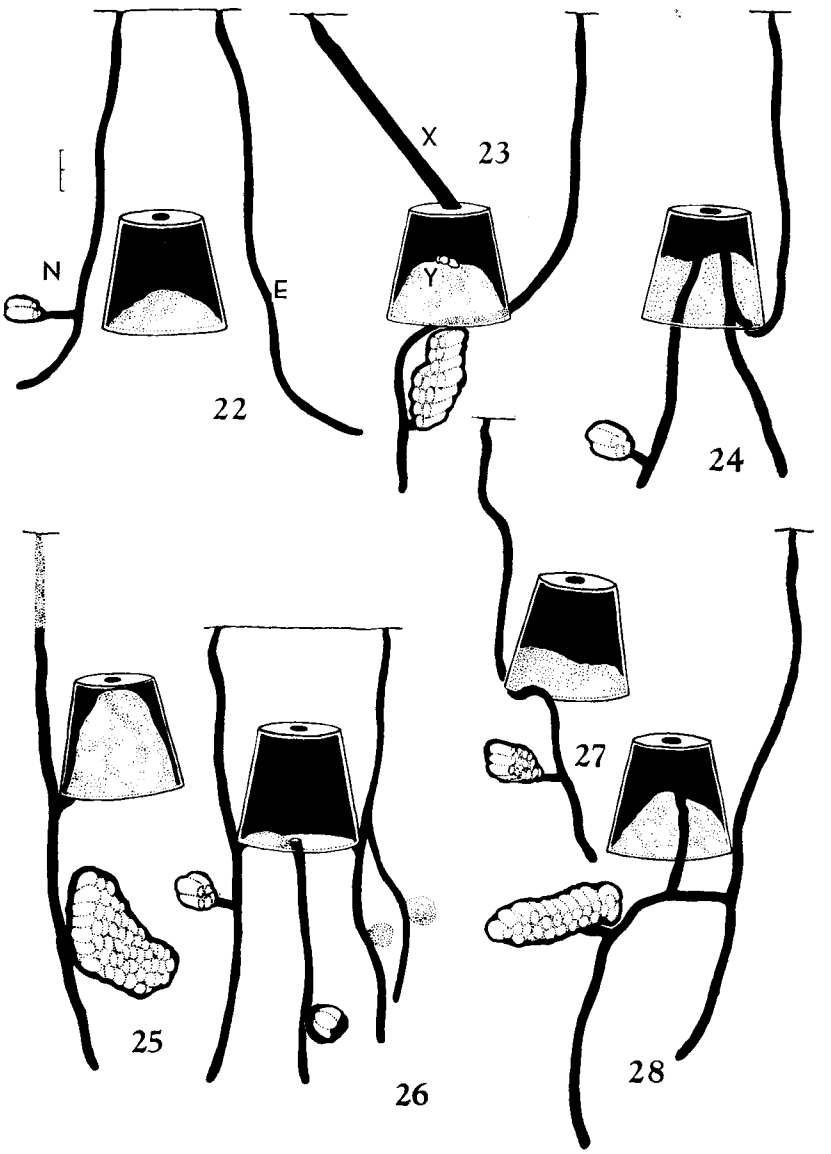
The percentage of female offspring is lower in nests made by transplants involving mothers, too. Probably many of these nests were virtually orphan by the early dispersal of mothers. But it is also assumed that mothers might produce more males than females in summer under unstable social condition, for the percentage of female offspring in solitary mother nests in summer was also low (*cf. 6. 1.*). This does not mean, however, the consumption of sperms. The spermathecae of many aged mothers obtained from normal nests excavated in late summer to early autumn still contain a considerable amount of sperms.

The flight activities in certain nests of this series were observed in 1959 by individual marking. The observations were carried out not so extensively as in 1958 (*cf.* Section 4), but individual differences in intensity and duration of foraging were again confirmed. Among 24 marked bees, returns with pollen loads were observed for 15. Some discrepancy between intensity of foraging and number of cells produced was noted. More than 15 returns with pollen were confirmed in Nest 13, but no cell was discovered at excavation. Probably cells were prepared but destroyed or thieved by ants. Internidal drifting was observed for six out of 24 marked bees, the percentage being higher than in normal nests (*cf. 4. 1.*). The individual record of 2-RB, which possessed temporarily three"homes", is cited (M, Marked; P, Return with pollen; N, Return without pollen; D, Departure; G, Guarding):

Nest	Date (July)							
	8	9	11	13	14	16	18	19
3		P		PP	P	PD	PDPD	PD
2E	MPP	DG		P				
2N	D		G		D			

In two nests, No. 2 (Fig. 22) and No. 10 (Fig. 20), the transplants divided, each part forming separate burrow. Certainly some daughters independently started their burrows from the cavity under the inverted pot. Such instances were also seen in the series subsequently described and suggest the occasional breakup of social ties among nest-mates by the occurrence of ample nest space.

6. 5. Transplantation of 10~20 daughters with or without mothers: This series, using many individuals per transplant, gave better results. All of nine trials in 1958 produced a large number of cells (Table 21). In 1959 three combinations were attempted with the following result (Number of cases produced cells/number of trials): 0M+20d (3/5), 1M+20d (4/5), 5M+20d (5/5). The results are summarized in Table 22. The number of cells produced is variable but the means are higher in 1958 (without mother 29.4, with mother 19.0) than in 1959 (without mother 9.0, with one mother 22.3, with five mother 27.2). In this series, too, the number of cells produced is likely to be proportional to the number of bees not dispersed rather than those involved in transplants. The high number of cells produced by transplants involving five mothers is probably caused by the increased chance of the establishment of one mother in the pot. The absence of pleometrotic association agrees to the result given in *5. 6.* The high percentage of inseminated daughters, not only in orphan transplants (6/15) but also in those involving mothers (6/11) may be explained by the fact that many of the latter



Figs. 22–28. Some experimentally induced summer nests (continued). 22. Two nests produced by fission of a transplant consisting of 0M+5d (Table 20, No. 2. N with three cells). 23. Nest B′ in *6. 5.* (*cf.* also Table 10 and Fig. 10) made by a transplant of 3M+16d. (Cavity under pot and artificial burrow, X, were used by a daughter, H-3, dispersed from Nest H, the dead body of which, Y, was found in the pot). 24. Nest made by a transplant

Table 21. Nests produced by transplants consisting of 10~16 daughters and 0~3 mothers in 1958 (Examined on July 24 and 30)

No.	Trans-plants	Number of				Remarks
		cells	young	♀/♂ in young	adults * (types)	
1 a	0M+10d	10	10	0/10	3(D′,D′,F′)	Two independent burrows
b		7	7	2/ 1		
2	0M+11d	21	20	0/15	2(d,D)	
3 a	0M+12d	25	25	10/5	1(F′)	Two independent burrows
b		29	29	4/24		
4	0M+13d	16	16	6/ 8		
5	0M+14d	41	40	9/28	3(F′,d,ds)	
6	0M+15d	30	30	1/26		
C′	0M+15d	27	26	—	2(F,F)	
A′	2M+13d	23	23	0/19	1(M)	
B′	3M+16d	15	15	3/ 8	2(M,fs) (Fig.23)	Two daughters and one mother, all dead in pot

* Abbreviations explained in Table 18

were virtually orphans (*cf. 6. 6.*). But there is at least one instance of the occurrence of one inseminated daughter in the presence of a mother (B′ in 1958). The sex ratio in the offspring is again characterized by a high proportion of males:

	Females	Males	% females
1958 With mothers	3	27	10.0
Without mother	32	197	13.8
1959 With mothers	28	86	24.5
Without mother	1	7	12.4

The lower proportion of females in the offspring from transplants involving mothers may be explained by the reason given in *6. 4.*

The foraging activities of three nests in 1958, A′, B′, C′ were observed together with those of some other normal nests (*cf.* Section 4, Table 10 and Fig. 10). The numbers of transplanted individuals in these nests were 12, 16 and 15, while the numbers of daughters marked individually were respectively 6, 6 and 9. Nest C′, with less dispersed daughters, produced more cells than A′ and B′. The foraging activities in these nests were similar to those in normal nests, showing considerable individual variation in frequency, duration and consistency. When the nests were excavated on July 24, nearly all makred bees had already been lost. The bees found in these three nests were:

A′: Mother with blue mark.

B′: Mother, B′-5 (Inseminated, ovarially undeveloped and stylopized. A few foraging

with 1M+5d (Table 20, No. 14. Burrow irregular). 25. Large nest made by a transplant of 5M+20d (Table 22, No. 12). 26. Four nests produced by fission of a transplant of 1M+20d (Table 22, Nos. 4a, b, All burrows with cell clusters, but only two clusters remained intact). 27. Nest made by a transplant of 1M+5d (Table 20, No. 12). 28. Nest made by a transplant of 5M+20d (Table 22, No. 9. Burrow irregular).

Table 22. Nests produced by transplants consisting of 20 daughters and 0~5 mothers in 1959 (Examined on July 28–31)

No.	Trans-plants	Number of				Remarks
		cells	young	♀/♂ in young	adults * (types)	
1	0M+20d	1	—	—	1(D)	One broken cell alone
2	〃	9	8	1/ 7	2(d,D)	
3	〃	17	11	0/ 7	—	Many cells with mold and mites. Two cells occupied by *Ponera* ant (Fig. 21)
4a	1M+20d	3	3	—	1(f)	Four independent burrows, each with a cell cluster. Two clusters broken, one with soil (4a) (Fig.26)
b		5	5	0/ 4		
5	〃	23	17	5/ 4	3(D,D′,M′)	Two clusters in the same burrow (Fig. 29)
6	〃	36	22	8/12	—	
7	〃	—	—	—	1(F′)	With ruined cell cluster
8	5M+20d	18	9	1/ 4	1(M′)	Two clusters in the same burrow
9	〃	20	14	2/ 6	1(F′)	Burrow irregular (Fig.28)
10	〃	21	21	0/18	1(D)	
11	〃	32	22	4/16	1(F′)	Two clusters in the same burrow
12	〃	45	35	8/22	3(D,D′,F′)	Four mothers in pot, all dead. (Fig.25)

* Abbreviations explained in Table 18

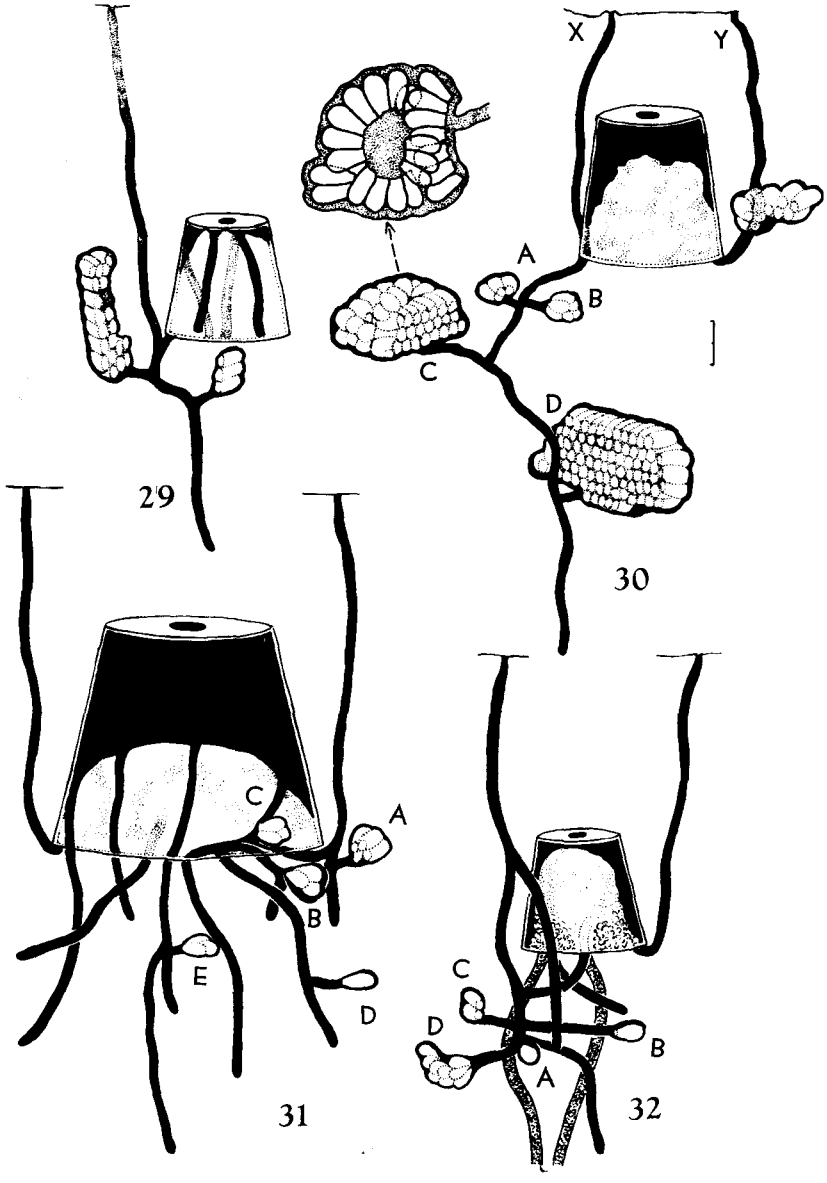
trips were observed as shown in Table 10; nevertheless she carried two parasites).

C′: C′-3 (Active forager, inseminated with one ovary fully, the other slightly developed). One daughter (Inseminated with both ovaries fully developed. No flight activity of this individual was observed, probably she behaved as if she was the foundress).

As to nest architecture, the construction of more than one cell cavity was found in three nests (Table 22, Nos. 5, 8, 11. *Cf.* Fig. 29). This tendency is also seen in natural nests containing many cells (Sakagami and Hayashida, 1960). Further the preparation of several independent burrows by bees of the same transplant, as mentioned in *6. 4.*, was found in Nos. 1 and 3 in 1958 and No. 4 in 1959.

6. 6. Transplantation of numerous daughters together: Because of the difficulty of obtaining a large number of spring cell clusters at the same time, only three trials were made in this series. The results obtained in 1958 (Nos. 1 and 2, each 0M+100d) and in 1967 (No. 3, 1M+42d) are quite different for each other.

In No. 1, the size of the pot, 6 cm in mouth diameter, was too small for 100 pupae. About 35 dead pupae were found within the pot at excavation, while virtually no such deaths were found in other trials so far described. No. 2 was transplanted by using a larger pot, 10 cm in mouth diameter, and no dead pupa was found. In both cases, two tumuli appeared a few day after transplantation on June 19 Returning bees with pollen loads were observed



Figs. 29–32. Some experimentally induced summer nests. 29. Nest made by a transplant consisting of 1M+20d (Table 22, No. 5. Two cell clusters in the same burrow). 30. made by a transplant of 1M+42d (No. 3 in *6. 6.*) resulting in fission (X and Y) and further formation of four cell clusters (A~D) in X. 31 and 32. Nests produced by fission of transplants consisting of 0M+100d (Nos. 2 and 1 in *6. 6.*), Most cell clusters are unsuccessful. Two irregularly hatched burrows in 32 are those made by earthworms.

until July 4, but no flight activity was seen thereafter. At excavation made on July 29, several small, mostly unsuccessful cell clsuters were discovered in both nests:

No. 1 (Fig. 32): A (one empty cell), B (one cell with pollen ball), C (two broken cells), D (Nine cells with damaged contents), in total only 13 cells. A few burrows were found, connected with one another in a complicated manner. Two vertical burrows of earthworms were found below the pot. Only one uninseminated daughter was captured in a burrow; she had fully developed ovaries, seriously worn mandibles and crop filled with nectar and pollen.

No. 2 (Fig. 31): Eight burrows, some of which carrying the following cell clusters. A (Four cells, two empty, two male pupae), B (Three cells, all with male pupae), C (Three cells, one female pupa, two with pollen and soil), D (one empty cell), E (Three cells, two post-defecation larvae, one feeding larvae, all males). In total 14 cells. Three adult daughters, all stylopized with undeveloped ovaries and intact mandibles, were found, one uninseminated with three parasites, one inseminated with one parasite and one inseminated with a full grown parasite and crop filled with pollen (*cf. 2. 4. 3.*).

Apparently both cases were unsuccessful for various reasons: too narrow space which caused a high mortality (No. 1), too ample space probably caused disintegration of the transplant (No. 2), interference by earthworms (No. 1), stylopization (No. 2), etc. Probably many bees emerged successfully left the transplanted spot. The remaining bees made their cell clusters independently of one another but none of them produced a sufficient number of cells.

The unique case in 1967, No. 3, was transplanted on June 20 into the garden of one of us (S.F.S.). No nest aggregation of *Ev. duplex* was found near the place, at least within the raiuds of 500 m, except a single transplant consting of seven mothers (*cf. 6. 1.*) The ground was tightly pressed. A pot of moderate size, 6.5 cm in mouth diameter, was used and the transplanted individuals (1M+42d) were carefully arranged to keep reasonable spaces between them.

The first tumulus (X) was found on June 28, the second and third (Y and Z) respectively on July 9 and 10. Flight activities were observed until July 14 in Z and July 17 in Y. The excavation of these two nests was made on July 28. No trace of cells was found in Z, eight cells in Y (Fig. 30, Y), with the following contents: One young larva, two post-defecation larvae (both males), one predefecation larva (male). one empty cell and two cells with pollen ball but smeared with soil. The number of cells was larger than the average produced by a single daughter (*cf. 6.2.*). Perhaps more than one daughter from the transplant was involved in constructing this nest.

The other burrow, X, showed flight activity to August 1 and guarding activity to August 11. On August 12, an inverted vial with a metal base was put over the nest entrance and emerging bees were daily captured until September 9. After a continuous rain on September 10–14, the nest was excavated on September 15. The nest contained four cell clusters, each with 3(A), 6(B), 47(C) and 84(D) cells, in total 140 cells, the highest cell number so far obtained in *Ev. duplex*. Therefore, the transplantation of a large number of pupae can produce a very large summer

nest, provided death and dispersal of transplanted individuals is decreased by giving favorable conditions. Most cells were already after emergence except seven, containing six female and one male pupae. Some males may have left the nest before emergence was checked by the vial. Thrity-one decomposed bodies of adult were found within the metal base of the emergence via The sexes of offspring were identified in 109 bees, 36 females and 73 males. The number of females is less than that in normal summer nests but higher than in other experimentally induced nests. As the transplanted nest was isolated from any other aggregations, the insemination of daughters by alien males is not much expected. Consequently the 36 females obtained were, in all probability, produced by the single mother transplanted. Probably some males were produced by ovarially developed daughters. But it is plausible that many males were also produced by the mother, suggesting a high reproductive potentiality of mothers under favorable conditions. The cell clsuter C had a curious arrangement as shown in Fig. 30. Behind an ordinary comb, another horseshoe-like comb was attached. Several cells were irregularly added to the comb and the cluster cavity possessed a central free area.

6. 7. Remarks on experimentally induced summer nests: The descriptions given above show that summer nest can be experimentally induced from the transplanted spring cell cluster, even without the mother, provided the conditions are favorable for nest establishment. The success of transplantation is seemingly proportional to the number of daughters remaining, rather than the number of transplanted. Consequently, the transplantation of a single bee, either mother or daughter, only rarely produces summer nests.

The percentages of inseminated or ovarially developed daughters increase remarkably in experimentally induced nests in comparison with those in normal nests (Table 23, *cf.* 5. 2., Table 17). The high proportion of such daughters in nests even with mothers are probably due to the fact that many of these nests were virtually orphan by the earlier dispersal of the mother, transplanted together with immature daughters (*cf.* 6.4.). The difference between normal and experimentally induced nests clearly indicates the role of mothers in inhibiting insemination and ovarian development of daughters. The higher percentages of inseminated and ovarially developed daughters in the nests produced by the transplants of smaller size are interesting. This might suggests a mutual inhibition among nest mates in nests with many daughters.

In spite of the frequent appearance of inseminated and ovarially developed daughters, the percentage of female offspring produced in experimental orphan nests is remarkably low as given in Table 24. The available data are still insufficient to determine the precise relation between sex ratio and size of transplants, but the lower female production is clear when the data are compared, as a whole, to the sex ratio in normal nests, not much remote from 1:1 (*cf. 3. 1.*). The production of females appears to be difficult by daughter bees, even if they are

Table 23. Numbers and percentage ratios of inseminated and ovarially developed daughters in experimentally induced summer nests

Transplants		Number of daughters of four types*				% inseminated	% ovarially developed
Mothers	Daughters	D	d	F	f		
—	1	1	1	11		84.7	92.4
—	4-9	3	6	11	3	61.0	61.0
—	10-	5	6	4	2	35.3	53.0
Total		8	13	6	3	59.6	65.4
+	+	8	4	26	5	42.9	66.6

* D, d, F, f, as in Table 17. D′ (Tables 18–22) is included in D and F_0 and F′ (Tables 18~22) in F.

Table 24. Sex ratio of offspring produced by experimentally induced summer nests

Transplants (Number of daughters)	Number of female and male offspring produced by transplants					
	without mother			with mother		
	Female	Male	% female	Female	Male	% female
0				4	6	40.0
1	0	22	0.0	0	4	0.0
4-9	28	157	15.2	2	28	7.2
10-20	33	204	13.8	31	113	21.5
42				36	73	33.1
100	1	8	11.0			
Total	62	391	13.7	73	224	24.6

inseminated and ovarially developed.

Knerer and Plateaux-Quénu (1967 b) recorded a series of observations which indubitably related to the problem presented here. The sex ratio produced by the mother is more or less species specific at a given season but can be modified experimentally by changing environmental conditions and social structure. In *H. scabiosae*, all of 23 solitary mothers produced only male offspring, whereas some pleometrotic associations produced both sexes. Fenale production is determined by the liberation of sperms from spermatheca at oviposition. Probably this mechanism is controled by environmental and social conditions. The sperm liberation might be difficult in daughters, even if they were inseminated. Knerer and Plateaux-Quénu record a large orphan nest of *Ev. malachurus*, excavated on August 17. Five among 12 daughters were inseminated. Nevertheless the number of female and male offspring were 4 and 241 respectively. This result shows an interesting similarity to the result obtained in our experiments.

The low female production in nests produced by the transplants involving mothers may partly be explained, as repeated above, by the earlier dispersal of mothers. However, it is also conceivable that the sex ratio is changed among offspring of mothers not in natural nests. Many nests produced by such transplants, especially those in 1959, were not very successful. Under unfavorable conditions, it is not improbable that sperm liberation by mothers was inhibited to some degree. From this assumption, the possibility of higher female production by daughters is reciprocally implicit, provided the conditions releasing sperm liberation are fulfiled. However, it seems unlikely that such high female production by daughters is realized under natural condition. From the standpoint of species productivity, therefore, the contribution of orphan nests to the next generation is less than that of normal nests.

Concluding remarks

Particular aspects of summer matrifilial phase in *Ev. duplex* were already discussed in preceding sections. Here are given some general remarks upon the important outcomes of the present study.

1. Position of *Lasioglossum* (*Evylaeus*) *duplex* in halictine social spectrum

At the initial phase of the present study, we believed this species to represent a very low social level among halictine bees. We are now inclining to withdraw this opinion; *Ev. duplex* lies, though certainly not occupying a high rank, at a moderate level in the social spectrum of halictine bees, as far as its caste differentiation is concerned. The difference between mother and summer daughter is small but clearly recognized and functionally more or less stable in comparison with some other species, such as *As. sparsilis* and those in *Halictus* s. str. However, the recent advance in halictine sociology brought out the occurrence of diverse social patterns, which cannot simply be placed on a unidimensional scale. Moreover, certain relations between social pattern and taxonomic grouping are little by little being clarified.

In a previous paper (Sakagami and Hayashida, 1961), we warned against premature discussions in determining the relative level of social evolution *in general*. Social evolution is a complicated phenomenon, involving diverse aspects such as caste differentiation, population size,defensive efficiency, communication, nest structure, etc. These aspects often but not necessarily evolve mutually. One species might be *higher* than another in one aspect but lower in another. We must, for the time being, separately consider these aspects before making premature generalizations. Besides this caution, we must also avoid a careless comparison of species belonging to different phyletic groups. At the present state of halictine sociology, we have still no sufficient data to compare etho-ecological characters of various taxonomic groups. For this reason, here the comparison is mainly confined to the social species of the subgenus *Evylaeus*,

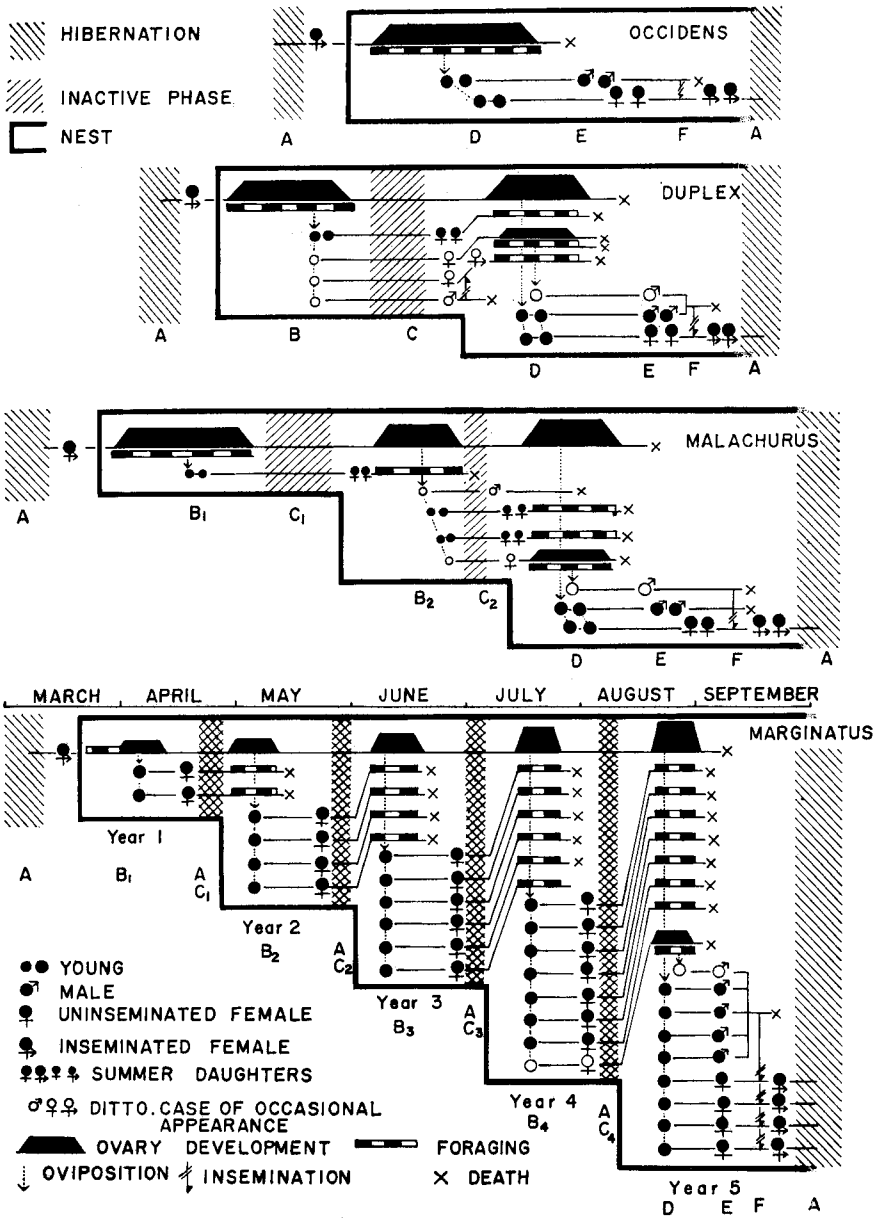


Fig. 33. Annual cycle and social pattern of three closely studied *Evylaeus* species, together with those of a Japanese solitary species, *Lasioglossum occidens*. Seasonal trends in *L. occidens* and *Ev. duplex* were taken from our own data, of *Ev. malachurus* from Knerer and

Evylaeus Robertson (in the definition by Michenr, 1944) is an enormous Holarctic group, characterized by the weak first and second r-m veins and more or less depigmented margins of metasomal terga. The subgenus is divided at least into two groups. One group involves the medium to relatively large species with carinate propodeum, and the other the medium to small species with carinaless propodeum. As far as the Japanese species observed by one of us (S.F.S.) are concerned, this distinction appears to correspond to certain ethological characters (*cf.* Sakagami and Munakata, 1966). All six Japanese species of the carinaless group, the biology of which was so far known, make nests of primitive pattern, with cells connected to the main burrow by means of narrow laterals, or Type III A, formula O(LCh)^{n}B of Sakagami and Michener (1962), as in *Lasioglossum* s. str. All six species are solitary, though one species, *Ev. ohei*, make a composite nest, consisting of several burrows, each of which is inhabited by one female, and connected to a common entrance.

The nests of at least seven Japanese species of carinate *Evylaeus* are known. All of them make comblike cell cluster surrounded with a cavity. Four out of seven have a summer matrifilial phase, distinctly separated from spring solitary phase by an inactive phase. It is this group to which *Ev. duplex* belongs, together with *Ev. malachurus*, *Ev. calceatus*, *Ev. nigripes*, *Ev. cinctipes*, *Ev. pauxillus* and a socially aberrant species, *Ev. marginatus*. Fig. 33 presents the annual cycle and social pattern of three closely studied species, *Ev. duplex*, *Ev. malachurus* and *Ev. marginatus*, accompanied with those of *L. occidens*, a solitary Japanese species. The figure shows a gradual complication of life cycle and social pattern from top to bottom. Both *L. occidens* and *Ev. duplex* start solitary nesting activity after hibernation (Phase A). In *L. occidens* this activity results in the production of larvae or reproductive individuals, males and normal females (Phase D), followed by a preautumn inactive phase (E) and an autumn mating phase (F). In *Ev. duplex* the brood produced by the spring solitary activity of the mother mostly consists of relatively small daughters (B). They emerged during inactive phase (C) and start, together with the long-lived mother, the summer matrifilial phase (D); the young produced during this phase are reproductive individuals. Therefore, the matrifilial phase (D) is comparable to the solitary phase in *L. occidens* in its significance in the life cycle, which is complicated compared with that of *L. occidens* by the insertion of two additional phases, B and C. Correspondingly, three new characters appear; prolonged life span of the mother, decreased ratio of males in the spring brood and dwarfism of summer females. In *Ev. malachurus*, the life cycle is further complicated by the duplication of B and C. The size difference between mother and her first and second daughters becomes greater, the first brood does

Plateaux-Quénu (1967 a, France) and a five years' cycle of *Ev. marginatus* from Plateaux-Quénu (1959, France). The schemata presents only essential aspects. Gradual increase of population in successive broods is given only in *Ev. marginatus*. Letters A~F mean equivalent phases among species. Vertical arrows indicating insemination do not mean endogamy as symbolized by two oblique bars.

Table 25. Ethological and sociological comparisons of certain social *Evylaeus* species

Species / Character	*duplex*	*nigripes*	*calceatus*	*cinctipes*	*pauxillus*
I. Number of brood per year	1+A* (Japan)	1+A (France, *15*)	1+A (Italy,*2*)	1+A (Canada, *6*)	1+A (S.Germany *17*; France *5*)
II. Number of cells in final brood	3~41 (m=14.4)	?	ca. 12(*2*)	25~40(*5*)	3~25 (*5*)
III. Size difference between mother and daughter in first brood	6.7%+	4~7%+ or 10~11% (*7*)	13%(*7*)+	14~15%+ (*7*)	15%(*4,7*)+
IV. Structural difference between mothor and daughter in first brood	slight	slight (*15*)	slight (*2*)	?	distinct (*17*)
V. Ratio of ovarially developed daughters in first brood	25.4%+ 20.2%+	61% (*4*)	?	?	5% (*4*)
VI. Ratio of inseminated daughters in first brood	8.5%	1% (*4*)	?	?	0% (*4*)
VII. Ratio of males in first brood	10%	5% (*4*) 5~6%(*7*)	0% (*2*) 2~3% (*7*)	0 % (*7*)	0% (*7*) a small portion (*17*)
VIII. Pleometrosis	Extremely rare	Occasionally (*15*)	Occasionally (*2,18*)	Absent? (*5*)	Occasionally (*5*)
IX. Cells open (+) or closed after oviposition(−)	−	−(*16*)	−(*14*)	+(*5*)	+ (*5*)
X. Cavity surrounding cell cluster	+	+(*15*)	+(*2*)	?	+ (*17*)

Sources of data: *1~2* Bonelli, 1948, '65a; *3*, Bott, 1937; *4~7*. Knerer and Plateaux-Quénu, *9*. Noll, 1931; *10~15*. Plateaux-Quénu, 1959, '60, '62, '63 '64, '65, '66; *17*. Stöckhert, 1923;

not involve males, and the life span of the mother is further prolonged. These phases, which appear in *Ev. duplex* and *Ev. malachurus* in the annual cycle, develop in *Ev. marginatus* over several years, resulting in a very large nest in the final year. Nevertheless, it is remarkable that this species does not possess a fixed caste system.

A comparison of several *Evylaeus* species in various etho-sociological

(Italicized numerals in parentheses refer to citations given at the bottom of the table)

malachurus	*marginatus* (*10-12*)	Main evolutional trends	Remarks
A (Germany,*8*), 2+A (S.Germany,*8,9,17*; France,*5,6*), 3+A (Italy, *1*)	perennial 4(or 5)+A (years)	Increased number of broods	*A=Final brood with ♂/♀≒1.0
60~95(*8*), 12~68(*1*), ∠120(*9*), >200(*5*)	to 548	Higher number of cells	
17~18% (*4,7*)*	0%	Larger difference	*(Without size overlap) + (With size overlap)
distinct (*8,9,17*)	Absent	Larger difference	
32% (*4*) ##	0%	Lower ratio of ovarially developed daughters	+(From nests), # (From all samples) ## (Probably both first and second broods combined)
0 % (*4*)	0%	Lower ratio of inseminated daughters	
0 % (*1,7,9,17*)	0%	Lower production of males	
Occasinally(*5, 17*) but rare (*17*)	Absent	Decrease of pleometrosis (?)	
+ (*5*)	+(*5*)	Cells open after oviposition	
−(*17*), ±,−(*9*)*, +, −(*3*), +, −(*5*) **	—	Disappearance of cavity (?)	+ Present, −absent * (=Occasional presence in spring, absence in summer), ** (=Presence in spring, absence in Summer)

1966, b, '66 c, '67 a, '67 b; *8*. Legewie 1925; *18*. Vleugel, 1961.

characters was attempted in Table 25. *Ev. marginatus* occupies an outstanding position by the possession of both very differentiated (perennial life cycle with many successive broods and a large population size in the final phase, I and II in the table) and primitive characters (Castes not fixed, III, IV, nevertheless well differentiated functionally, VI~VII). Among other species, *Ev. malachurus* is apparently more differentiated in characters I~IV and VI~VII. The percentage

of ovarially developed daughters is relatively high (V). But this figure was probably obtained based upon all summer daughters, involving both first and second broods. All authors who studied this species reported the absence of males in the first brood.

The differences among other species are not conspicuous, but in general a gradual differentiation is traced from left to right across the table. *Ev. duplex* is regarded as occupying the lowest rank within social *Evylaeus*. However, the table is by no means complete. Further studies must be undertaken in most aspects mentioned, especially as to the percentage ratios in III, V~VII, preferably using standardized procedures and reasonably large samples.

A few words must be given concerning the three characters given at the bottom of the table. The tendency to keep brood cells open after oviposition is certainly regarded as a differentiated feature (Knerer and Plateaux-Quénu, 1966 c). Whether haplometrosis or pleometrosis is more differentiated cannot easily be answered. As a limitation of the reproductive function to the unique individual, haplometrosis certainly means *a* differentiated state. On the other hand, there may be two categories of pleometrosis, the primitive type such as that from which the haplometrosis has evolved, and the re-differentiated type as found in some ants, which assures the continuation of the colony life, for instance, as in the difference between haplometrotic *Formica rufa rufa* Linné and pleometrotic *F. r. rufopratensis minor* Gösswald (*cf.* Gösswald, 1951). At the level of social differentiation attained by *Evylaeus*, we believe the haplometrosis is more differentiated than pleometrosis. Interestingly, however, *Ev. duplex*, being relatively primitive in many aspects, is nearly strictly haplometrotic (*5. 6.*). Probably the transition from pleo- to haplometrosis and gradual differentiation in other characters are still not well coordinated in *Evylaeus*, as in the discrepancy in differentiation of the caste system and annual cycle in *Ev. marginatus*.

Finally the lack of the cavity surrounding cell clusters in the two most differentiated species is noteworthy; it is always absent in *Ev. marginatus* and facultatively absent in *Ev. malachurus*. Cavity formation is *an* advanced nesting habit, evolved independently in various groups of halictine bee (Sakagami and Michener, 1962) and common to most carinate species of *Evylaeus*, the nests of which have so far been discovered. Its absence in two socially highly evolved species might therefore indicate a further differentiation, possibly appearing in connection with a difficulty of supporting numerous cells in the presence of the cavity. The different records on the presence of the cavity in *Ev. malachurus* among authors, especially Bott, Noll and Knerer and Plateaux-Quénu, according to whom the cavity is present in spring while absent in summer favors the assumption given above. A similar instance of the possible secondary disappearance of an advanced nest architecture was assumed by Michener (1961) for the comb formation in stingless bees.

The most conspicuous etho-sociological character common to *Evylaeus* is indubitably its discrete brood production. As explained in Section 1, most

halictine bees have an inactive phase after the production of first brood by solitary or pleometrotic mothers. But this tendency has become very distinct in *Evylaeus*, by which this group is distinguished from another group containing several well studied species, *Dialictus*. Such discrete brood production may amplify the size different between mother and summer daughter, or queen and worker, by the disappearance of transitional individuals. Even the difference seen in *Ev. duplex*, occyping the lowest level in social differentiation in *Evylaeus*, is not much different from that in *D. imitatus* and distinctly higher than in *D. rhytidophorus* (Knerer and Plateaxu-Quénu, 1967 b). An uncritical comparison might lead to an erroneous opinion, asserting *Evylaeus* is *in general* much more differentiated than *Dialictus*. The size difference between castes in *Ev. malachurus* is more conspicuous than in some bumblebee species, for instance, *Bombus* (*Fervidobombus*) *morio* (Swedrius) (Moure and Sakagami, 1962, Fig. 10), but no specialist on social insects would conclude that *Ev. malachurus* is socially more differentiated than any bumblebee species. The final evaluation of social organziation must be made from a synthesis of various aspects, the detailed studies of which remain for the future. Another interesting difference between *Evylaeus* and *Dialictus* is the participation of mothers in guarding. This phenomenon has been recorded in several *Evylaeus* but so far not in *Dialictus*, suggesting a possible intergroup difference.

It is premature to make further comparison of *Evylaeus* with other halictine groups. There are still many other groups of halictine bees, especially tropical ones, the biology of which is yet not accurately studied. But in general carinate *Evylaeus* and *Dialictus* involve socially more advanced species than other groups, such as *Halictus*, *Seladonia*, *Augochlorella*, *Augochloropsis*, etc, which include some more or less socially differentiated species. Certainly carinate *Evylaeus* and *Dialictus* represent two of some summits of social evolution attained by halictine bees.

A few words are added to the evolution of social system through the semisocial stage proposed by Michener (1958, *cf.* also Michener and Lange, 1958 d) for halictine and apid bees. The social patterns of *Ev. duplex* in Fig. 33 could be interpreted, when compared with that of *L. occidens*, as if supporting the evolution through a subsocial stage, characterized by the co-existence of mother and her adult daughters, which was, according to Michener, the way passed through by termites, ants, vespid wasps, and probably, xylocopine bees, than through the semisocial stage, which postulated an assemblage of individuals of the same generation. Although we have a doubt in postulating the semisocial stage as an inevitable step passed through by *all* social halictine bees, we have no idea to connect the two social patterns in Fig. 33 directly as showing a subsocial evolution. Instead, the two patterns, which represents the fact, not the presumption, can be applied to both hypotheses by inserting appropriate intermediate terms, representing co-existence of individuals still without castes. However, even if the assemblage would appear through the semisocial stage, the co-existence of the individuals of the same generation had relatively quickly to be replaced by the matrifilial co-existence by the prolongation of mother's life span, as inferred from the social patterns of the majority of socially more or less advanced halictine and apid bees.

2. Percentage ratios of summer nests representing various social patterns:

From the results so far described, an association of one mother and her uninseminated and ovarially undeveloped daughters is recognized as the social pattern basic to the summer matrifilial phase of *Ev. duplex*. At the same time, however, diverse other patterns were also discovered. A brief perspective of these patterns is summarized here in reference to the relative abundance of them as presented in Fig. 34.

At first we would like to repeat the remark given at the end of *5.4*. These percentage ratios are nothing other than first approximations, some of which had to be estimated arbitrarily. Consequently, these ratios cannot yet be directly applicable to population dynamics. Nevertheless, we believe our approach is useful and necessary. In the studies of insects with relatively simple life cycles and life modes, bionomic researches immediately open the way to population researches. In the studies of bees and wasps, their complicated social system operated by sets of elabroated behavior mechanism has attracted many students. This tendency is in itself justified but it is partly true that it has acted to retard the development of their population ecology, which is also an important and fascinating field.

Among spring nests producing broods, only 24.8% are reactivated in summer. These nests are divided into five types representing different social patterns: I. Normal, II. Orphan, III. Solitary mother, IV. Pleometrotic, and V. Fused. The percentage ratio of orphan nests, 29.3%, was taken from the results given in *5. 3.* We have an impression that the real percentage might be lower, say, ca. 20%, but the value was adopted as a first approximation until a more precise estimate would be obtained. Both pleometrotic and fused nests are exceptional in *Ev. duplex* (*cf. 5.6.*). Our estimste of 0.5% for each of them, given arbitrarily, would not deviate much from the real percentage ratios.

The percentage ratio of summer nests with soilitary mother is most difficult to estimate. The summer nests of *Ev. duplex* excavated by us exceed 200. Less than half of them were used for the preparation of Table 15 (*cf.* 5. 2.), but many of the remainder, except seven cited in 5.5., were certainly not solitary mother nests. On the other hand, not all of these nests were randomly chosen and excavated. The high frequency of daughters leaving their nests (*3. 4.*) suggests the occurrence of a not neglegible number of solitary mother nests, although many of them would disappear without producing offspring (*6.1*). We estimated arbitrarily all solitary summer nests as 5% of the total nests reactivated in summer and those producing offspring as 10% of them, or 0.5% of the total reactivated nests. We believe these estimates are not too remote from the real figures, but admit that they are the most unreliable part in Fig. 34. The percentage ratio of normal nests, 64.7%, was obtained by a simple subtraction of those ratios mentioned above from total reactivated nests set as 100%. Consequently the ratio is also affected by

arbitrarily estimations but it may certainly be more reliable than the ratio of solitary mother nests.

Besides these reactivated nests, there are nests made by mothers and daughters

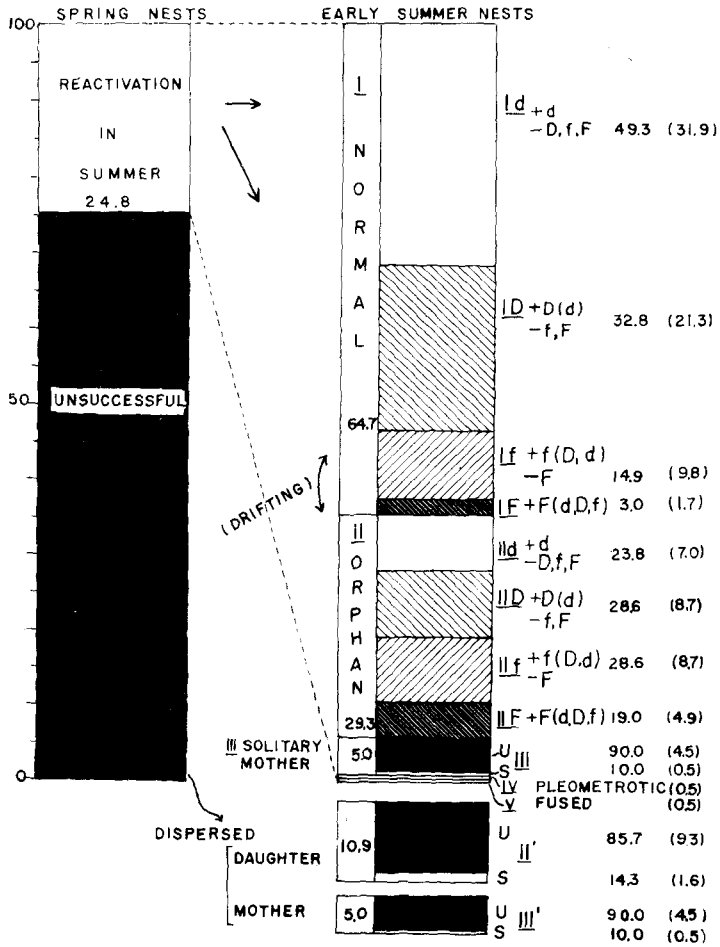


Fig. 34. Percentage ratios of summer nests representing various social patterns. The ratios to total reactivated nests (=100%) are given parenthetically in the second row at the right. Percentages in the first row give those within each major pattern. Abbreviations; D, Uninseminated and ovarially developed summer daughters; d, Uninseminated and ovarially undeveloped summer daughters; F, Inseminated and ovarially developed summer daughters; f, Inseminated and ovarially undeveloped summer daughters; +, with; —, without; U, Unsuccessful; S, Successful, D, d, f, given parenthetically indicate their facultative occurrence.

which leave the home nests. The percentage ratios of total dispersed daughter nests and of those producing offspring to total reacivated nests were estimated respectively as 10.9% and 1.6% (*5. 4.*). As to dispersed mother nests, we tentatively adopted the ratios arbitrarily estimated for solitary mother nests, 5% and 0.5%, respectively. Finally the ratios of four types of nests classified according to the inclusion of four types of daughters (Table 16) were inserted as to normal and orphan nests.

Two remarks are added to: 1) The percentage ratios of four types of nests, both normal and orphan, temporally change (*cf.* Table 2 and 17 and Fig. 3), not being stable as shown in Fig. 34, which represents the relative proportion of various patterns of nests in early summer. 2) Except solitary mother nests and dispersed mother and daughter nests, Fig. 34 is prepared as if all nests can successfully produce offspring. Actually a considerable portion of nests of any patterns would disappear without producing offspring. Especially all nests belonging to IId and IIf must succumb unless some ovarially developed daughters appear, changing to IID or IIF respectively. For certain technical difficulties, we have still no reliable estimate for the percentage of summer nests which successfully produced brood, although the percentage is certainly higher than at the transition from spring to summer phases. The percentage ratio of successful nests may apparently be higher in normal, pleometrotic and fused nests than in orphan and other ones. The percentage ratio of really effective nests, that is, those producing next generation's females, would also be highest in normal nests (*6. 7.*). Therefore, in spite of the appearance of diverse social patterns, the contribution to the species productivity would mostly be accomplished by normal nests, which constitute slightly more than half of all summer nests. Finally the complication of the social system in the summer phase is increased by drifting, which produces internidal exchange of inhabitants (*4.1.*), but its relation to diverse social patterns is difficult to express quantitatively.

It must be stressed, however, that the complicated social system due to the occurrence of diverse social patterns does not mean a social differentiation but rather a primitive character of *Ev. duplex*, mainly conditioned by a still not well fixed caste system. In socially much advanced species, such as *Ev. malachurus*, for instance, the patterns If, IF, IIF and IIf do not appear more. The decreased percentage ratio of ovarially developed daughters or workers decreases the appearance of pattern ID. A high frequency of daughters which leave their nests might also suggest a primitive character in *Ev. duplex*, indicating the remains of maternal independent nest founding. In general, increased caste differentiation may lower the contribution of patterns other than that presented by the normal pattern in *Ev. duplex* or make impossible their appearance. At the the summit of social evolution in bees, the honeybee, the pattern Id is absolutely predominant and really effective.

3. Importance of social inhibition for the appearance of the social system operated through caste differentiation

The comments given above lead to a third important outcome of the present study, the inhibition of insemination and ovarian development in daughters by mothers. The ratio of ovarially developed daughters is low and of inseminated daughters very low in normal nests. Both percentages become higher in natural orphan nests and very high in experimentally induced orphan nests deprived of mothers since emergence of daughters, as given in Fig. 35 prepared from Table 17 and 23.

During this decade the importance of various pheromones in regulating inter-individual relations in various insects has gradually been recognized (Butler, 1967). In social insects, the first discovery of "queen substance" in the European honeybee by Butler (1954) gave an impetus to the accumulation of a number of interesting contributions, especially dealing with two important regulative mechanisms,

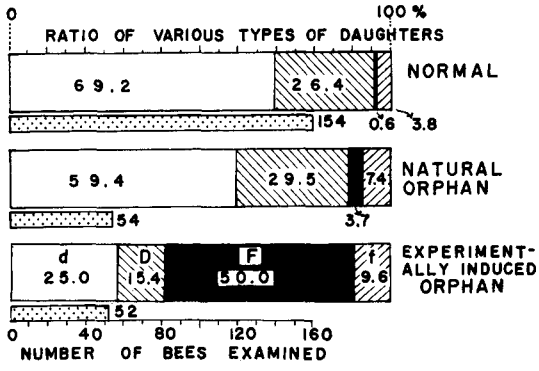


Fig. 35. Percentage ratios of inseminated and ovarially developed daughters in normal and natural orphan nests (in both those excavated in July alone) and in experimentally induced orphan nests. D, d, F, f, as in Fig. 34.

inhibitions of queen rearing and worker ovarian development by the queens in normal colonies. Many such instances in honeybees, bumblebees, wasps and ants are reviewed by Butler (1967). It is out of the scope of the present study to deal with such records known in socially higher groups. Instead we would like to stress the occurrence of inhibition, clearly recognized from Fig. 35, even in a relatively primitive social organization in *Ev. duplex* and many other halictine bees referred to in the present paper (*cf.* discussions in *2. 3.*, *5. 3.*, and *6. 7.*). This fact throws light upon the evolution of insect societies, the key character of which is the differentiation of reproductive and non-reproductive castes.

It is absurd to assume the direct and sudden appearance of such a system

from an unorganized assemblage of solitary individuals. We must theoretically admit, as a necessary step, the occurrence of an assemblage, consisting of individuals (or females in Hynemoptera) of equivalent social status, bearing complete functions for both self- and group-maintenance. Such an assemblage, either matrifilial or intersib, would virtually be confirmed when all postcallow stage adult members were inseminated and ovarially developed. Strangely enough, there are few instances which correspond to this step. Obviously such a step cannot be sought in recent ants and termites, which changed to social as a group. In social wasps, there are only two instances which probably exhibit such co-existence: *Belonogaster* wasps in Africa and stenogasterine wasps in the Indo-Malayan region. The classic work by Roubaud (1916) on *Belongsater* indicates an equivalent co-existence. At least certain species of stenogasterine wasps, for instance, *Parischnogaster striatula* (Du Buysson), possess a similar social pattern (Yoshikawa, Ohgushi and Sakagami, unpubl.). In bees, recently Batra (1966) recorded some instances of such co-existence in nomine bees, particularly in *Nomia capitata* Snith. Probably this is virtually the unique definite case of such equivalent co-existance in bees. There are some other similar records in Halictinae, Ceratinini, Euglossini, etc. But a closer examination of these records reveals that most of them belong to one of the following instances: 1) Co-existence is superficial. The bees only share the nest entrances, each possessing her "own" nest section within the same nest cavity (Some anthophorine bees, reviewed by Michener and Lange, 1958 e; some halictine bees, Michener and Lange, 1958 a, Sakagami, Hirashima, and Ohé, 1966, etc.) 2) co-existence is facultative. Usually each bee make her solitary nest, but occasionally a co-existence of more than one bee appears (In some species of Euglossini, Halictinae, Megachilinae, etc. A closer bibliographical survey not given here). 3) Co-existence is frequent but an incipient caste differentiation already begins to appear (For instance, *As. sparsilis* and species in Ceratinini, Michener and Lange, 1958 b, and Michener 1965). Even the record by Batra on nomiine bees might become one of these cases, most likely to 2), provided more extensive observations would be carried out. From these instances, we recognize that caste differentiation appears at very incipient stage of the communal life. Using abbreviations X, Y, Z, respectively for solitary, facultatively communal, and obligatorily communal, and A,B,C, for absence of castes, appearance of incipient castes and establishment of a caste system, six instances are theoretically conceiveable:

	Solitary	Communal	
		Facultative	Obligatory
Castes			
Absent	XA#	YA#	ZA±
Incipient		YB+	ZB#
Established			ZC#

We have a plenty of records upon XA and ZC in insects, some records upon YA, and ZB, very few on YB and virtually no detailed record on ZA. Further

studies might prove the occurrence of more reliable records of ZA and YB. But the paucity of information upon these two types suggests the development of inhibitory mechanisms as soon as a tendency to the communal life appears. It is open to question whether or not incipient caste differentiation in *As. sparsilis*, representing YB, is already governed by the inhibition. But even if not, such a condition would be preadaptive for the appearance of social inhibition. On the other hand, the inhibition is often partial even after the establishment of the caste system, as shown by the occurrence of diverse types of pleometrotic association. Consequently, a highly efficient inhibitory mechanisms in the European honeybee, controled by 9-oxodec-*trans*-2-enoic acid and possibly some other pheromones (Butler, 1967), is regarded as a further elaboration of a similar phenomenon found in halictine bees, indicating its very old origin, which can be traced down to the appearance of a tendency toward communal life.

Interestingly enough, the inhibition of worker ovarian development disappears at a summit of social evolution in bees. In a number of Neotropical species and at least in one Ethiopean species of stingles bees, the young household phase workers possess fully developed ovaries in the presence of the queen (Sakagami *et al.*, 1963). In many of these species, the workers oviposit into the brood cells and these worker born eggs are devoured by the queen before her own oviposition (Sakagami, Montenegro and Kerr, 1965; Sakagami and Zucchi, 1963, 1966, 1967). Instead of inhibiting ovarian development in workers, these bees adopted the use of worker born eggs as the food for the queen. It is open to the question whether or not this way is bioeconomically more efficient than inhibition. But this way is apparently more differentiated than the inhibition seen in the honeybees, which differs from that in *Ev. duplex* in degree but not in kind.

Summary

The annual cycle of *Lasioglossum* (*Evylaeus*) *duplex* (Dalla Torre) consists of six phases: Hibernating phase, spring solitary phase, presummer inactive phase, summer matrifilial phase, postsummer inactive phase and autumn mating phase. The present paper deals with activities and social structure in the summer matrifilial phase. In this phase, most nests are occupied by an association of one mother, surviving since spring, and her daughters, usually uninseminated with undeveloped ovaries. Mothers principally participate in oviposition and daughters mainly in foraging. Further details are summarized as follows:

1) As in some other social species of carinate *Evylaeus*, the summer matrifilial phase is clearly separated from the spring phase, lasting about one month, under local climate, from early July to early August. Summer males, born synchronously with summer daughters from spring broods, are estimated as about one ninth to tenth of summer daughters in number.

2) Mothers and daughters considerably overlap in body size, though significantly distinguished in mean size in most samples. Whether mothers or daughters,

larger females tend to have wider metasomata with stronger luster and cryptic macrocephaly, though not so conspicuous as in some other halictine species.

3) About 8% of the daughters are inseminated and about 20% ovarially developed, but the latter percentage is quite variable according to year and season, occasionally exceeding 35%. Inseminated and ovarially developed daughters constitute only 2.5% in most daughters. Both inseminated and ovarially developed daughters tend to increase near the end of the summer phase. Inseminated daughters are not significantly larger than uninseminated ones. Foraging is made by all types of daughters, inseminated or not, and ovarially developed or not.

4) Gradual change of young in nests shows a proterandric tendency. Sex ratio is ca 1.0 (1.3♂ : 1.0♀). Distribution of sexes in a cell cluster is not completely random. Reproductive efficiency per nest increases while per female decreases, roughly parallel to the number of bees per nest. Additional data on survival of spring nests were given. Mean number of cells in summer nests is 14.4, but the frequency distribution is very irregular.

5) A so far relatively unnoted fact, dispersal of summer daughters from nests of origin, is quite common, reaching one third of all summer daughters produced. Observations with marked bees show a considerable amount of internidal translocation or drifting of inhabitants, reaching 19% of total marked bees.

6) Intensity of flight activity is quite variable among individuals and nests. No marked differentiation of household and outdoor periods of adult life span, nor differentiation of indoor and outdoor workers, were observed among summer daughters, the mean life span of which did not much exceed 20 days. Nests are opened during the daytime, mostly from 6~7:00 to about 13:00 or earlier. The duration of open time is more than five hours, longer than in spring. Some nests reopen in late afternoon.

7) The mean number of daily flights per bee is 5.14. Mean durations of trips and intertrip periods are 32.5 and 20.5 min. in returns with pollen by active foragers and 25.2 and 13.7 min. in non active foragers, distinctly longer than in spring. Even experienced foragers frequently make orientation flights at first daily flights. Returns without pollen loads tend to increase in the afternoon. Flight activity is intense from 7:30 to 11:30, distinctly drops in the afternoon, although a low second peak may appear in late afternoon. The influence of microclimatic conditions, especially soil surface temperature, upon flight activity is very plausible.

8) Although behavior within nests was not observed, food regurgitation among nest-mates is unlikely too occur. Even active foragers occasionally guard but inactive foragers tend to guard more frequently. Young preforaging daughters and mothers guard while specialized guards not making flight activity are rare. More than one bee deposits pollen loads in one cell.

9) Although the size overlap in mothers and daughters is considerable in the total sample, daughters are larger than their own mothers in only 12~15% of the nests. Besides an association of the mother, inseminated and ovarially developed, and her uninseminated and ovarially undeveloped daughters, which

occupy about the half of the normal nests, ovarially developed daughters appear in about one third and inseminated and ovarially developed daughters in about 3% of normal nests headed by mothers.

10) A considerable portion of summer reactivated nests, estimated as more than one fourth, are orphan. The percentage ratio of ovarially developed daughters increases and that of inseminated daughters distinctly increases in orphan nests compared to normal ones. About 5.5% of summer daughters dispersed from their nests are estimated to make their own nests and a fraction of those, 14.3%, produce small number of progeny, mostly males. Tentative estimates for the percentage ratios of such nests to total nests reactivated in summer are given 10.9 and 1.6% respectively.

11) In normal matrifilial nests mothers seem to participate in inside work, but only exceptionally leave the nests. Rarely a solitary mother, either staying in her original nest or making a new nest, produces a second brood. Two rare social patterns were also discovered, nest fusion (two cases) and pleometrosis (one case).

12) Various numbers of spring pupae, accompanied by mothers or not, were experimentally buried in artificial subterranean cavities and the preparation of summer nests by them was observed. Brood rearing by solitary mothers or orphan transplants consisting of one or two daughters is possible but difficult and the number of offspring produced is very low. The percentage ratio of successful brood rearing increases, roughly parallel to the increased size of transplants, or more plausibly, the increased number of daughters staying in artificial nests. In such experimentally induced orphan nests, the percentage ratios of inseminated or ovarially developed daughters is high, attaining 59.6% and 65.4% respectively, compared to corresponding percentages in normal nests (4.4 and 27.0 %), indicating inhibition by mothers in normal nests. In spite of a high percentage of inseminated and ovarially developed daughters, the percentage of female offspring produced is very low, only 13.7%. Therefore, nests which were orphan since the beginning of the summer phase seemingly do not contribute much to the species productivity.

13) A comparison of several social species of carinate *Evylaeus*, all characterized by discrete brood production, shows that *Ev. duplex* occupies the lowest social level in this group. A closer comparison with other halictine groups was postponed in order to avoid premature generalizations. The percentage ratios of summer nests representing various social patterns were estimated. The appearance of diverse patterns was regarded as a primitive character of *Ev. duplex*. Finally, the importance of inhibition in social evolution of bees was pointed out.

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