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BIOLOGICAL NOTES ON *EVYLAEUS VILLOSULUS* (K.) (HYMENOPTERA, HALICTIDAE), A BIVOLTINE, LARGELY SOLITARY HALICTINE BEE

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SUMMARY

The biology of *Evylaeus villosulus* was studied both in the field and in experimental rearing cages.

Field caught females and their summer daughters were compared using multivariate discriminant functions analysis. The variables used were two size, two colouration and four sculpturing characteristics. All variables show significant differences between generations but the colouration and sculpturing characters did not show allometric variation within generations and may be environmentally induced.

Sex ratio data are available for the summer generation for both field and laboratory reared brood. Both gave female biased sex ratios approximating 4:1. Data for the overwintering generation were obtained only from the laboratory, the sex ratio was slightly female biased — 1.2:1. Pupal weights differed significantly between generations for females but not for males. Using the appropriate values, the investment ratio is female biased in both generations; approximately 5:1 in the summer and 2:1 in the autumn.

Overwintered females frequently survive long enough to forage simultaneously with their earlier emerging offspring. Cohabitation between females of the same generation occurs with low frequency but no division of labour results, rather communal colonies are formed. Similarly, when a mother and a daughter shared a nest, an eosocial colony resulted. Thus, *E. villosulus* would appear to be a basically solitary species despite exhibiting reduced size in the daughters of overwintered females and a female biased sex ratio, both of which are characteristics frequently associated with eusociality.

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RESUME

Biologie d'une abeille halictine bivoltine probablement solitaire :
Evylaeus villosulus (K.) **(Hymenoptera, Halictidae).**

Nous étudions la biologie d'*Evylaeus villosulus* dans la nature et dans des cages d'élevage.

Nous comparons des fondatrices et leurs filles, femelles d'été, récoltées dans la nature, en utilisant les fonctions discriminantes d'une analyse multivariate. Les variables utilisées sont deux caractères de taille, deux de couleur et quatre de sculpture. Toutes les variables indiquent des différences significatives entre les générations mais les caractères de couleur et de sculpture, ne montrant pas de variation allométrique entre les générations semblent être induites par l'influence de l'environnement.

Les données concernant la sex ratio de la génération d'été proviennent d'adultes prélevés dans la nature et d'autres issus de couvain élevé au laboratoire. Chez ceux-ci, comme chez ceux-là, la sex ratio est à majorité femelle dans le rapport d'environ 4:1. Les données concernant la génération hivernante proviennent uniquement d'adultes produits en élevage ; la sex ratio est à faible majorité femelle dans le rapport d'1.2:1. Les poids nymphaux diffèrent significativement entre les deux générations pour les femelles mais non pour les mâles. En utilisant les mesures appropriées, le rapport d'investissement est d'environ 5:1 en été et de 2:1 en automne mais ce dernier chiffre doit être confirmé à partir d'Insectes prélevés dans la nature.

Les femelles hivernantes survivent à l'éclosion du couvain d'été et récoltent du pollen en même temps que leurs premières filles. La cohabitation de plusieurs femelles de même génération survient parfois sans qu'il ne s'instaure de division du travail : des nids communaux se forment alors. De plus une mère et sa fille ont partagé un même nid éosocial.

Ainsi, *E. villosulus* serait une espèce bivoltine fondamentalement solitaire bien qu'elle présente deux caractères généralement associés à l'eusocialité : la taille réduite des femelles de la génération d'été et la sex ratio de la génération d'été, fortement décalée vers le sexe femelle.

INTRODUCTION

The simplest colony cycle of a social halictine involves two brood rearing phases: the spring brood in which the workers are produced and the summer brood which results in reproductives. Many halictine species have this type of social organisation which is particularly common in the "carinate" *Evylaeus* species (PACKER and KNERER, 1985). The two brooded nature of this colony cycle indicates that the ancestral solitary condition may have been bivoltinism (SEGER, 1983; for a summary of other views see MICHENER, 1974). For this reason, it is important to investigate the biology of bivoltine solitary species. To date, no detailed studies of such bivoltine solitary halictines have been carried out. The present report is an attempt to fill this gap in our knowledge as it concerns a bivoltine population of the largely solitary species *Evylaeus villosulus*. Females of the two generations exhibit significant differences in size and certain morphological characteristics (PLATEAUX-QUÉNU and PLATEAUX, 1985). That the small summer daughters produce the larger overwintering females has been proved by laboratory rearing (PLATEAUX-QUÉNU and PLATEAUX, 1986).

E. villosulus nests are typical of those found in solitary halictines, with cells located at the ends of long lateral burrows (PLATEAUX-QUÉNU and PLATEAUX, 1981). These laterals are plugged with earth immediately after the egg is laid on the pollen ball and are never reopened before eclosion of the enclosed offspring (this has been confirmed by direct observation in the laboratory). The presence of long, closed laterals between the main burrow and the brood cells prevents any contact between mother and the developing immature stages and may inhibit social evolution, as suggested by KNERER (1969).

In this paper we present further analyses comparing the females of the two generations and suggest that at least some of the observed differences are environmentally induced. Data on the sex and investment ratios of the two generations are provided. Most importantly, we provide additional information on two features of social significance:

1) longevity of overwintered females and,

2) data that strongly suggest that this species is solitary but that on the infrequent occasions when multi-female nests are established, no division of labour occurs.

It should be noted that *E. villosulus* is not very closely related to the "carinate" *Evylaeus* species, most of which are eusocial, and neither is it closely related to any of the "non-carinate" species whose biology has been investigated (EBMER, 1976).

METHODS

Field work was performed on the university campus at Jussieu, Paris, (*fig. 1, a to e*), on sparsely vegetated slopes surrounded by high buildings. The university campus is isolated in the centre of Paris; the bees foraged only on the grounds of the campus, there being no suitable areas for foraging in the surrounding built up areas. This population is probably quite isolated from others.

Several nests of *E. villosulus* were found aggregated in the ground, some entrances being situated in cracks in the dry soil surface. Bees were collected from flowers from June 22nd to July 29th 1982, killed with ethyl acetate and mounted on pins. Three sorts of bees were in flight during this period: young males and females of the summer generation and still active overwintered females. Summer and overwintered females were distinguished as follows. The hair on the mesothorax and head of older, overwintered foundresses was sparse or absent and their mandibles and wings were more heavily abraded. Additionally the wings of newly emerged females were greyish whereas those of the aging foundresses were distinctly yellowish. As a result of these differences it was easy to separate overwintered foundresses from summer females when they were flying simultaneously. Similarly, it was possible to separate aging summer females from the pre-hibernation, overwintering females later in the year.

The number of overwintered females progressively declined during the sampling period whereas their male and female offspring increased in abundance (*fig. 2*). At the end of july, 221 overwintered females, 381 summer females (of which 200 were released) and 80 males had been censused.

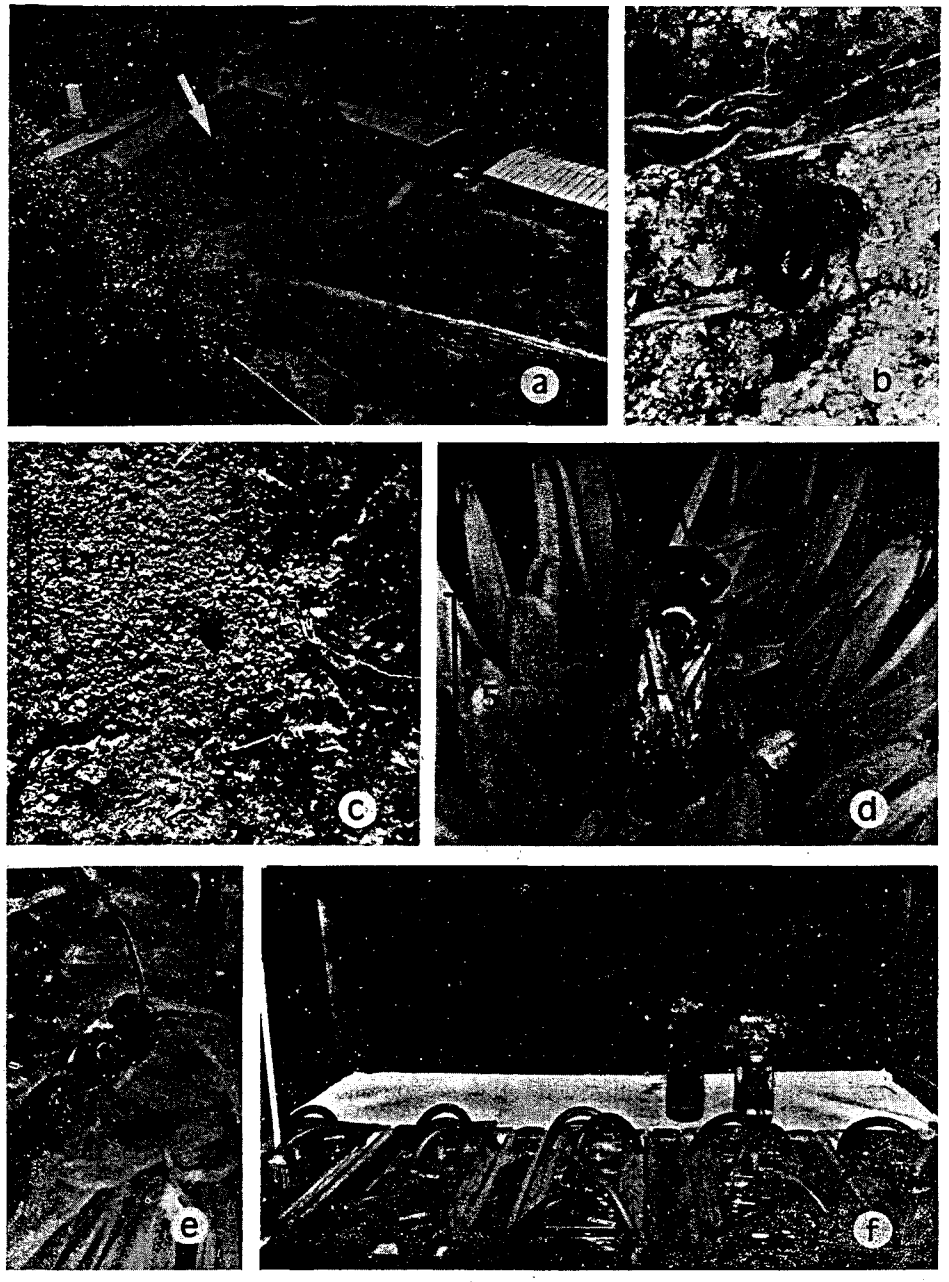

Fig. 1. — *a* to *e*: *E. villosulus* in the field. *a*: nesting site (↓) in the university campus at Jussieu; *b*: overwintered female entering her nest; *c*: entrance of a nest in a crack in the soil surface; *d*: summer female; *e*: summer male; *f*: laboratory rearing cage.

Fig. 1. — *a* à *e*: *E. villosulus* dans la nature. *a*: site de nidification (↓) dans le campus universitaire; *b*: fondatrice rentrant au nid; *c*: entrée d'un nid sur une fissure du sol; *d*: femelle d'été; *e*: mâle d'été; *f*: cage d'élevage, vue de la surface intérieure.

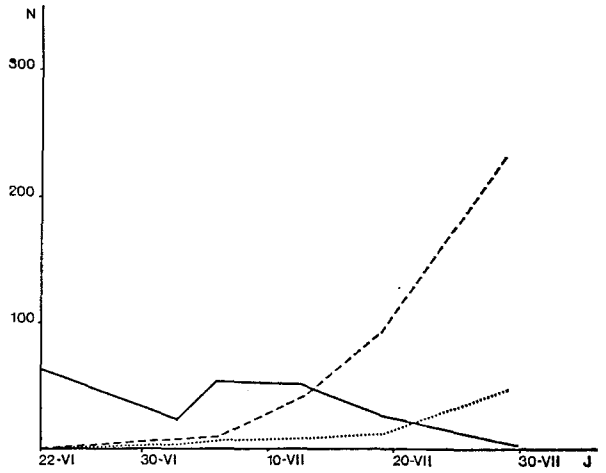

Fig. 2. — Changes in the numbers of individuals of both sexes in Jussieu, from June 22nd to July 30th 1982. ——— overwintered females; — — — summer females; ..... summer males. *J:* days and months; *N:* number of individuals.

Fig. 2. — Evolution du nombre d'individus des deux sexes à Jussieu, du 22 juin au 30 juillet 1982. ——— fondatrices ayant hiverné; — — — femelles d'été; ..... mâles d'été. *J:* jours et mois; *N:* nombre d'individus.

Eight morphological characteristics were used to compare the two successive generations of females:

— two size characters,
- head width
- wing length

— two colouration characters,
- colour of the ventral surface of the flagellum
- colour of the stigma

— four sculpturing characters,
- striation of the mesoscutum
- punctation of the mesoscutum
- depth of the median mesoscutal groove
- extent of the striae on the dorsal area of the propodeum.

The statistical procedure used here for the multivariate comparison of the two generations was the SAS programme CANDISC.

The sex ratio studies were done in the field, at Jussieu, by collecting bees of the two sexes over a period of 38 days (described above) and in the laboratory (*fig. 1* and *fig. 3*), by obtaining females from the field and rearing their offspring to adulthood in 1985 using techniques described elsewhere (PLATEAUX-QUÉNU and PLATEAUX, 1986). In 1986, sex ratios were obtained from laboratory reared brood censused in the pupal stage, this also allowed us to calculate investment based upon pupal wet weights. One female pupa was weighed each day from May 30th when it was at the white eyed stage, until June 10th when the adult eclosed. The weight of this individual remained constant until afer the integument became entirely pigmented on June 8th, thereafter she lost

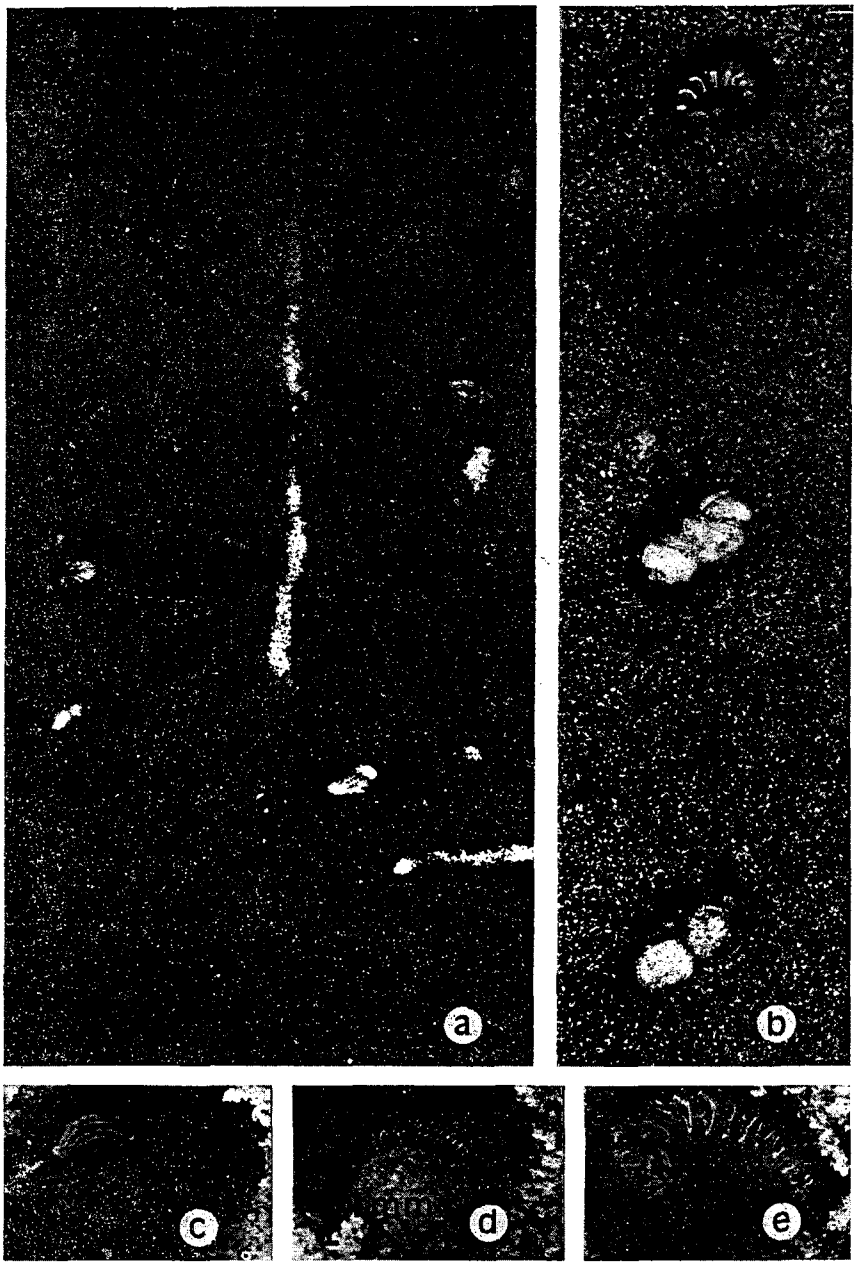

Fig. 3. — Laboratory work on *E. villosulus*. *a:* nest dug in a three millimetre thick layer of earth (the lower female pupa belongs to a nearby nest); *b:* detail of the left part of nest *a* showing, from top to bottom: larva, an emerging male and two female pupae; *c:* egg on pollen ball; *d:* small larva feeding; *e:* fully grown larva.

Fig. 3. — Elevage d'*E. villosulus* au laboratoire. *a:* nid construit dans 3 millimètres d'épaisseur de terre (la nymphe femelle située tout en bas appartient à un nid voisin); *b:* détail de la partie gauche du nid *a* montrant, de haut en bas, une larve, un mâle en train d'éclore et deux nymphes femelles; *c:* œuf sur un pain d'abeille; *d:* petite larve se nourrissant; *e:* larve ayant fini de s'alimenter.

weight. Because of these results, investment calculations are based only upon pupae weighed when they had a white integument.

Temperatures in the field in 1982 were obtained from the National Meteorological Office. They were taken in the shade 2 metres above the ground. Temperatures recorded in the laboratory in 1985 were taken from the soil surface and also at a depth of 6 cm beneath the soil surface, both before light and at 18.00 hrs.

Additional observations of the summer generation were made in the laboratory in 1986 and 1987 and some field data were collected in autumn 1987. Unfortunately, by the latter date, the nest site had become so overgrown with vegetation that the aggregation had almost completely died out. No detailed information concerning the autumn generation sex ratio has been obtained from the field.

RESULTS

1. Size and morphological differences between Females of the Two Generations

Field caught overwintered and summer females were compared using multivariate discriminant functions analysis. As can be seen from *table I*, almost 80% of the 402 females could be separated on the basis of these variables and size and colouration (particularly of the stigma) were important in discrimination between generations (*fig. 4*).

Table I. — Canonical discriminant scores for variables used to separate spring and summer females of *E. villosulus*.

Tableau I. — Valeurs canoniques discriminantes concernant les variables utilisées pour séparer les femelles de printemps et d'été chez *E. villosulus*.

Characteristic	Canonical score
Head width	0.389
Wing length	0.681
Flagellar colouration	0.024
Stigma colouration	0.651
Striation of scutum	0.204
Punctation of scutum	0.225
Depth of scutal groove	0.176
Propodeal sculpturing	0.124

Variation in size of the two generations is shown in *fig. 5*. The difference in mean size between generations is highly significant (overwintered female mean width = 1.71 mm, SD = 0.05, N = 221, summer females = 1.59, SD = 0.07, N = 181; t = 19.9, P << 0.001). Thus, it is possible that the other discriminating characteristics have their effect as a result of allometric variation. However, no such variation was detectable within generations, suggesting that generational differences in colouration and sculpturing may be environmentally induced. This could occur as a result of differences

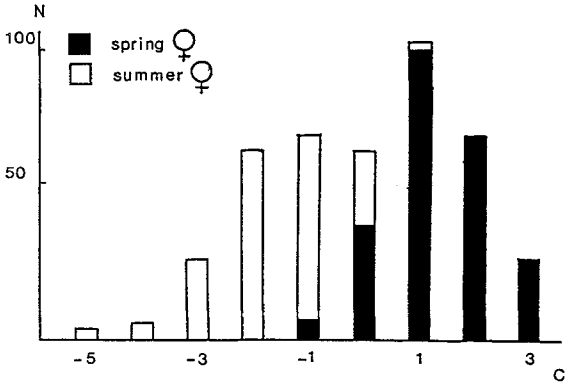

Fig. 4. — Separation of spring and summer females as a result of canonical discrimination by the eight characteristics described in the text. *N:* number of females; *C:* canonical score.

Fig. 4. — Séparation des femelles de printemps et d'été par l'analyse canonique discriminante portant sur les huit caractères décrits dans le texte. *N:* nombre de femelles; *C:* valeurs canoniques.

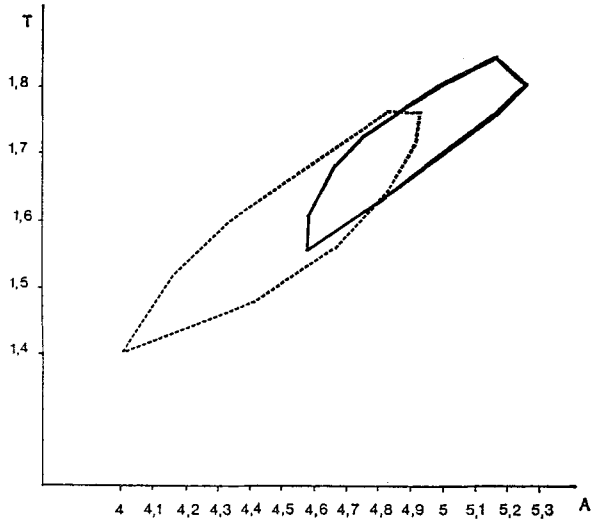

Fig. 5. — Size variation of the females of the two successive generations. ——— overwintered females (n = 221); — — — summer females (n = 181). *A:* wing length; *T:* head width (in millimetres).

Fig. 5. — Variation de taille des femelles de deux générations successives. ——— fondatrices ayant hiverné (n = 221); — — — femelles d'été (n = 181). *A:* longueur de l'aile; *T:* largeur de la tête (en millimètres).

in humidity or temperature experienced by the developing larvae and pupae at the two times of year. For example: the mean temperature during the spring provisioning phase in Jussieu in 1982 (i.e. during the foraging activity of the overwintered females) varied from 13°C to 23°C. During July, when the provisioning phase of the summer daughters began, the mean temperature varied between 17°C and 27°C. That this kind of temperature variation may affect the phenotypes of the females produced is supported by laboratory rearings (*fig. 6*) as described below.

The stigma colouration of field caught and laboratory reared spring and summer females was divided into two categories: pale (states 1 and 2) and dark (states 3 to 5). Pairwise comparisons were made of the 4 female classes taking appropriate modifications of the error rate into account by

using a chi squared test with Sidak's multiplicative inequality (ROHLF and SOKAL, 1981, pp. 101-107). The data, expressed as percentages, are shown in *fig. 6*. Comparisons of laboratory reared and field caught females revealed significant differences in stigma colouration in both generations. Overwintering females reared in the laboratory had paler stigmas than wild caught females ($\chi^2 = 17.05$, $P < 0.01$) whereas reared summer females had darker stigmas than wild summer individuals ($\chi^2 = 28.15$, $P < 0.01$). Spring and summer wild caught females also differed significantly ($\chi^2 = 104.7$, $P << 0.01$), however, spring and summer laboratory reared females did not ($\chi^2 = 4.84$, $P > 0.05$). These results coud be interpreted as indicating that the laboratory

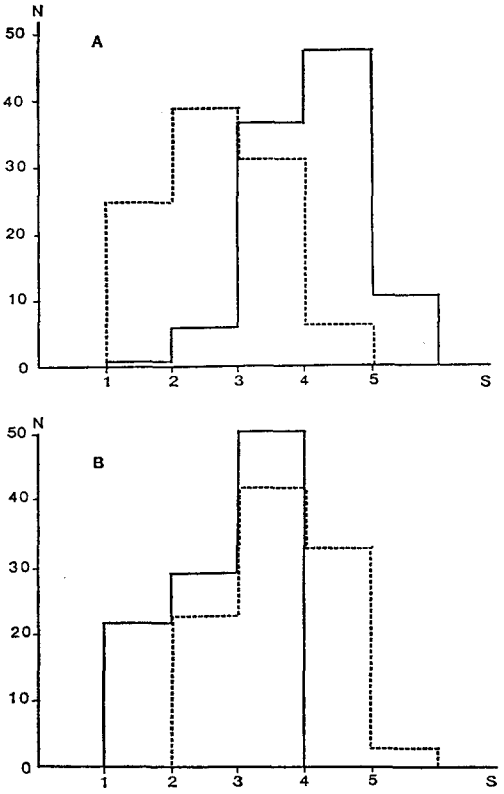

Fig. 6. — Histograms showing variation in stigma colouration in females from both summer and autumn generations collected in the field or reared in the laboratory.

A: field collected females. ——: foundresses = 2nd generation (n = 221); — — —: summer females = 1st generation (n = 181).

B: cage reared females. ——: future foundresses = 2nd generation (n = 14); — — —: summer females = 1st generation (n = 40).

N: percentage of females. *S:* stigma colouration. *1:* yellow; *2:* very light brown; *3:* light brown; *4:* brown; *5:* dark brown.

Fig. 6. — Histogrammes montrant la variation de couleur du stigma chez des femelles d'été et d'automne récoltées dans la nature ou élevées au laboratoire.

A: femelles naturelles. ——: fondatrices = 2de génération (n = 221); — — —: femelles d'été = 1re génération (n = 181).

B: femelles d'élevage. ——: futures fondatrices = 2de génération (n = 14), — — —: femelles d'été = 1re génération (n = 40).

N: pourcentage de femelles. *S:* couleur du stigma. *1:* jaune; *2:* brun très clair; *3:* brun clair; *4:* brun; *5:* brun foncé.

rearing conditions were intermediate to those experienced by developing bees of summer and autumn generations in the field. This resulted in an intermediate expression of stigma colouration in laboratory reared females in comparison to either of the wild caught generations. *Table II* presents the temperature data from the laboratory. Although differences in methods used to measure temperature in laboratory and field preclude detailed comparison, it does seem that laboratory conditions were more constant than those experienced by bees in their natural environment.

Table II. — A summary of temperature variation experienced by laboratory reared *E. villosulus.*

Tableau II. — Résumé des températures extrêmes relevées dans les cages d'élevage d'*E. villosulus.*

	Temperature (°C)			
	At ground surface		At a depth of 6 cm	
	Early am	Late pm	Early am	Late pm
	(min-max)	(min-max)	(min-max)	(min-max)
First generation	21-25	30-34	20-25	26-31
Second generation	18-21	28-35	18-20	20-22

2. Sex ratio studies

A sample collected at Jussieu in 1982 (see above) included 381 summer females and 80 males, i.e. 83% females and 17% males, a ratio of about 4:1. This is an estimate of the sex ratio obtained during the sampling period. However, differential catchability of the sexes may have contributed to this result and therefore laboratory rearing was performed.

Results obtained in 1985 for reared adults gave 40 females and 8 males in the summer generation (5:1) and 18 females and 20 males in the autumn generation (~ 1:1) (PLATEAUX-QUÉNU and PLATEAUX, 1986). In 1986, pupal counts gave 63 females and 19 males in the summer generation (3:1) and 28 females and 17 males (~ 1.5:1) in the autumn generation. The three estimates for summer generation sex ratios did not differ significantly ($\chi^2 = 0.81$, $P > 0.1$) ; neither did the two autumn generation estimates ($\chi^2 = 1.9$, $P > 0.1$). Pooling laboratory data across both years gave female biased ratios for both generations, 3.8:1 in summer and 1.2:1 in autumn.

Summer female pupae weighed significantly less than those that produced overwintering females (ANOVA, *Table III*), a result expected from the head width differences between generations. Males of the two generations did not differ significantly in weight but both were less heavy than either category of female (*table III*).

Table III. — Results of analysis of variance of wet weights of male and female pupae of summer and autumn generations.
SD: Standard deviation.

Tableau III. — Résultats de l'analyse de variance des poids humides de nymphes mâles et femelles appartenant aux générations d'été et d'automne.
SD: écart-type.

Category	N	Wet weight	S.D.	Significance * 1	2	3
1. Summer male	19	11.76	1.47			
2. Autumn male	17	12.01	1.88	n.s.		
3. Summer female	62	17.05	2.85	.01	.01	
4. Autumn female	28	20.96	2.24	.01	.01	.01

When the laboratory reared pupal weights and sex ratios from 1986 were combined to give investment ratios, the following results were obtained. Overwintering females invested in female offspring disproportionately in a ratio of 4.8:1. Their daughters raised a brood that was also female biased, in a ratio of 2.1:1.

3. Longevity of the overwintered foundresses

As stated previously (PLATEAUX-QUÉNU and PLATEAUX, 1981), the foundresses which appear in April pullulate on flowers in May, June and early July, then become scarce and, in the field, are progressively replaced by females and the less common males of the summer generation. This gives two peaks of activity for females, the first due to overwintered bees, the second due chiefly to their summer daughters. In 1982 at Jussieu, the simultaneous flight period of the two female generations lasted about one month and did not extend beyond the end of July. On July 29th, 3 old foundresses were collected along with over 200 new females (*fig. 2*) indicating that the overwintered females had mostly died off by this time. These last old foundresses were damaged with nicked wings that were reduced in surface area. The newly emerged daughters did not stay in their natal nests but dispersed and excavated new burrows in the vicinity of the old ones. That new nests were excavated was clear from the observation of hundreds of new tumuli, each day more numerous, and the direct observation of unworn new females looking for suitable places before digging their own nests. Moreover, a sample of 50 young females collecting pollen in July 1984 and 1985 showed spermathecae full of sperm and well developed ovaries (see PLATEAUX-QUÉNU and PLATEAUX, 1981, *fig. 1 a-c* for examples). There was no difference between ovarian development of these young females and those of the old foundresses that were still active at this time.

In rearing cages, where environmental conditions are much more constant than in the field and the "weather" is fine every day, the first generation

began emerging (in 1987) on June 12th. Twenty marked foundresses, out of 57 caged bees, were still alive in the middle of July. A marked foundress, whose first daughter emerged on June 30th, collected pollen until July 16th, and died on July 24th. During this time her first two daughters provisioned their new nests elsewhere.

This leads us to believe that at the end of their provisioning phase, the spring foundresses do not only disappear (they could stay in their natal nests as do overwintered females of primitively eusocial species) but they die. What remains certain is that at least some of the foundresses are still alive when their first sons and daughters emerge and that the two generations are found active together in July.

4. Cohabitation of various females

It was observed that some nests in the field contained more than one female. This was also noted occasionally in the laboratory. We used experimental frames with a thin layer of earth (3 millimeters) squeezed between two panes of glass (*fig. 3 a*) to observe the behaviour of marked bees inside their nests. Six examples of cohabitation were observed out of a total of 129 nests, 82 excavated by overwintered females and 44 by summer bees (it should be noted that different numbers of bees were placed in different laboratory cages).

Example 1. This concerns two overwintered females. Female R had provisioned 12 cells in nest A on June 27th. Then female V, which had completed 17 cells in nest B in another cage, was transferred into the same cage as R. V was first observed in nest A on the 29th of June. On July 2nd each female provisioned her own cell, never taking a pollen load into the cell of the other individual. However, when R finished her collecting trips for the day, she plugged the nest entrance with earth, even though V was still foraging on flowers. When V returned to the nest with her pollen load, she had to dig through the plugged nest entrance. The two females were seen together until July 6th when V died, R was removed from the cage and killed on July 20th.

Example 2. Eight overwintered females were placed in the same cage on May 29th. Four females were later seen in the same nest (3 on June 4th, 4 on June 11th). All foraged and a complex set of burrows and laterals was constructed. Each female constructed its own brood cells and collected its own pollen. This communal society lasted until July 8th.

Example 3. This example also concerns overwintered females. The earth in one frame of a cage became too dry and so was removed and replaced by a new frame with moist earth on June 9th. The bee that had constructed a nest in the old frame produced a new nest and was joined by two other females. The three females provisioned their own brood cells from June 17th until July 14th when the nest was destroyed.

Example 4. A foundress started provisioning her nest on June 3rd. A daughter emerged on July 1st, stayed in the natal nest and began provisioning on July 7th. On July 12th, mother and daughter both provisioned but deposited their pollen loads in different cells. On july 27th, the mother died, the daughter was killed and dissected the

same day: she showed well developed ovaries and was inseminated. These two females, belonging to different generations (mother and daughter), behaved indifferently to one another. The mother was the larger bee with a head width of 1.64 mm and a wing length of 4.75 mm. These measurements for her daughter were 1.52 mm and 4.33 mm respectively.

Example 5. Three separate experimental nests were constructed. Summer generation pupae were placed in these nests: 6 females and 2 males in nest A, 10 females and 3 males in nest B and 4 females and 3 males in nest C. No overwintered females were in the cage at this time. Each surviving female initiated a nest solitarily except for two individuals which shared a nest but worked independently within it, provisioning their own cells. The female which completed its foraging for the day earliest would close the nest entrance irrespective of whether the other bee was still foraging or not. They cohabited from July 5th until the 11th.

Example 6. Four summer females, observed from July 15th to the 30th, provisioned the same nest as follows (each x represents a cell provisioned):

	Female			
July	a	b	c	d
15	X			
16				
17	X	X		
18				
19		X		
20	X		X	
21				
22				
23	X		X	
24				
25			X	
26	X		X	X
27			X	X
28				
29	X		X	X

Each female worked independently provisioning its own cells.

5. **Summer generation**

Behaviour of the males.

Newly emerged males go through orientation flights that enable them to locate and return to their natal nest. Some marked males confirmed this: they could be observed going back into their own nest in the early afternoon and staying there at night for as long as one month after eclosion (PLATEAUX-QUÉNU and PLATEAUX, 1986). These males, flying in the morning, enter nearby nests very actively and come out cleaning their antennae. These behaviours are very similar to those observed in *E. marginatus*, males of which enter open nests in autumn to mate (PLATEAUX-QUÉNU, 1959). This suggested to us

that mating took place within the nests where new summer females were emerging, but mating was also directly observed on flowers and on the walls of the cages.

The males of *E. calceatus*, reared under similar conditions, never locate their natal nests but mate with females above ground, in their cages. When the temperature is extremely hot, they may enter any nest to shelter but during the night they usually stay on the ceiling or a wall of the cage, keeping in touch with one another and forming a sleeping cluster as has been described in the field by TORKA (1913) and SCHREMMER (1955).

Such location of the natal nest by males nevers occurs in the various other species we have reared in the laboratory and would appear to be a rather rare phenomenon in halictids.

Sequence of emergence

The sequence of emergence, studied in the laboratory, indicates that there is no clear protandry or protogyny.

Nest	# of cells	order of emergence
1 (20b1)	7	ffffmff
2 (15 1)	13	mmffmfffffffmf
3 (14 1)	14	mfmffmfffffffmf
4 (12 1)	17	mmmffffffffffffmf
5 (20 1)	18	fffffffmmfffmfffffm
6 (16 1)	19	mffmfffffffmffmfmmff

In the social species *E. calceatus*, the first brood consists of workers and a small percentage of males. A study of 22 societies reared in cages (PLATEAUX-QUÉNU and PLATEAUX, 1980 b) leads to the conclusion that when males are present in the worker brood, they are produced first, before any diploid eggs are laid, and result from smaller pollen balls. Conversely, in the second brood, made up of large males and future foundresses, the males and females are mixed as shown above for *E. villosulus*.

DISCUSSION

E. villosulus has two generations a year in Paris. Both are composed of males and females, with the second generation resulting mostly from the labours of the first, although it is possible that some may be produced from the aging overwintered females still active in July. All females mate but the summer females become active immediately whereas the autumn females overwinter until the following spring. No males can be observed in spring: they must die in autumn after mating.

In both spring and summer, the nests are mostly inhabited by single females; multi-female nests are uncommon.

Nests are generally aggregated in sparsely vegetated soil. Such aggregations are found in solitary and social species. This could result from attraction to a limited ecological factor. Alternatively, in a bivoltine species such as *E. villosulus*, the excavation of new summer nests in the vicinity of the old ones increases the density of nests in a given area. Such aggregation may favour the formation of multi-female nests.

It is assumed, for most solitary species, that the females die before the emergence of their progeny. In *E. villosulus*, a bivoltine species, the overwintered females survive the emergence of their offspring and some are still active when their daughters are provisioning their own independently excavated nests. The period when both generations may be found foraging simultaneously lasts about one month. STÖCKHERT (1923), working at Erlangen (Germany), thought that individuals of this species emerging in autumn were partly produced by overwintered foundresses still active in August. Even if some Parisian overwintered females still lay eggs at the end of July, these will turn into adults about one month later and be able, if the weather is fine, to produce a new generation which will emerge in October. In 1987, 21 females with unworn wings were seen on November 18th, a hot sunny day, feeding at flowers near Paris and, many of them, carrying pollen loads on their hind legs. It is highly unlikely that these new autumn females of the second generation (which may be easily distinguished from the last individuals of the summer generation as described in the methods section) were provisioning their nests in November before overwintering. SAKAGAMI and FUKUDA (1973) recorded many females of *Ceratina japonica* and *C. flavipes* with pollen loads on their hind legs late in the year. These females were both old worn individuals and young, recently emerged bees captured on flowers after the cessation of brood rearing. That these pollen loads were eaten by other adults in the same prehibernating nest was confirmed by SAKAGAMI and MAETA (1977). These authors noted that such pollen intake by prehibernating *Ceratina* adults is in contrast to the rarity of pollen exchange between adults in halictine bees, newly eclosed females of which also take flight before hibernation. As the emergence of the summer generation was very late around Paris in 1987 (it was not yet in flight even at the end of July!), the appearance of the second generation was delayed and newly emerged females were observed collecting pollen very late in the autumn. We do not know for certain whether this pollen is eaten by younger individuals within the natal nest. However, the last emerging individuals from these nests would certainly have difficulty feeding before winter. Of 21 females collected from flowers in November, 3 had crops which contained abundant pollen grains. It appears that pollen is introduced into the nest after the cessation of brood rearing in this species as well as

in some *Ceratina*. It is of interest to note here that trophallaxis between prehibernating adult females has been described recently within the communal nests of an Australian halictine bee (KUKUK and SCHWARZ, 1987).

The size and morphological differences between the females of the two generations deserves emphasis. The summer generation is composed of females that are smaller, lighter in various colour characteristics and have a less heavily sculptured body surface than the overwintering individuals of their parental and offspring generations. The colour (and probably also the sculpturing) differences between generations are not a result of allometric variation, they appear to be environmentally induced.

The size difference between generations, based upon wing length, was 7.4% in the Jussieu population. LIN and MICHENER (1972), working on size variation in females of solitary, semisocial and primitively eusocial bees, wondered whether caste size differences might initially be based upon the variability found in non-social ancestral populations. They noted that variance in female size increased with the establishment of eusocial behaviour and concluded that this might result more from social species producing offspring throughout the season, and therefore under variable environmental conditions, rather than being a result of sociality and morphological caste differences. We would suggest that the variation in size between generations in the solitary *E. villosulus*, in which bivoltinism results in a period of foraging activity as long as is found in eusocial species, supports these authors' suggestion.

It is known that increased temperature may result in a decrease in the size of bees produced (KAMM, 1974 for *Dialictus zephyrum;* PLATEAUX-QUÉNU and PLATEAUX, 1980a for *Evylaeus calceatus* — both of which are social species). Additionally, increased day length may result in smaller offspring (KAMM, 1974). Overwintered females of *E. villosulus* foraged from May until late July, encompassing a wide range of temperatures and day lengths. The smaller summer females foraged during July and August, the hottest months of the year, yet they produced the larger overwintering females at this time. It seems unlikely, therefore, that temperature and day length affect the size of offspring in this species in the same way as it does in the aforementioned social halictines. Nonetheless, laboratory experimentation is required to verify this.

Under laboratory rearing conditions we did observe that the summer generation required higher temperatures than did the overwintered females for activity to be initiated. The temperature varied between 23.5 and 34°C at the soil surface and 23 to 30°C at 6 centimeters in depth in our laboratory cages in spring. Under these circumstances the summer generation, emerging at the end of June in 1985 (PLATEAUX-QUÉNU and PLATEAUX, 1986), fed but did not do anything else. The addition of light bulbs placed on the tops of the cages increased both the temperature and light intensity experienced

by the bees. The temperature at the soil surface was raised by between 3 and 4°C and the summer females immediately began collecting pollen. Does this indicate that the summer females have a somewhat different physiology than the overwintering females? In the Parisian climate, this generation does not overwinter. We do not know if it is able to do so with the correct environmental stimuli or if, in contrast, the overwintering generation is ever able to avoid this diapause.

The small number of males in the summer generation is surprising. Sex ratio data are available for both field and laboratory reared brood. Both gave female biased sex ratios approximating 4:1 with investment ratios of about 5:1 in favour of females.

The examples of cohabitation, observed in the laboratory, showed that even if two or more overwintered females inhabited the same nest, they provisioned their own brood cells and no division of labour occurred. The same results were obtained if females of the summer generation shared a common nest. In both cases, we have a communal colony (MICHENER, 1969, 1974) — whereby a group of females of the same generation uses a single nest, each making and provisioning her own cells and ovipositing within. Communal societies are not very common in halictines but do appear in several lineages such as the New World genus *Agapostemon* (ABRAMS and EICKWORT, 1980) and some Australian groups of the genus *Lasioglossum* (KNERER and SCHWARZ, 1978; KUKUK and SCHWARZ, 1987). It has also been reported, but only in unusual circumstances of prolonged drought, in *Lasioglossum* (*Sphecodogastra*) *galpinsiae* (BOHART and YOUSSEF, 1976).

Example number 4, dealing with the simultaneous presence of a mother and her daughter in a nest, is different as it concerns two females of different generations sharing the same nest. Even here no division of labour was observed. The term *eosocial*, introduced by SAKAGAMI and MAETA (1977) working with the anthophorid genus *Ceratina*, applies to the coexistence of mother and daughter without caste differentiation. Perhaps this term fits our example where no behavioural caste differentiation could be observed.

We believe that *E. villosulus* is a solitary species in which communal and eosocial nests occur at low frequency. When these multi-female nests arise, the cohabiting individuals construct and provision their own brood cells and oviposit within them: all females are egg layers.

It is interesting to note that a female biased brood of smaller individuals is found in the first generation of this solitary species: only 21% of summer generation individuals were male and the average size difference between overwintered and summer females was 7.4%. Both of these characteristics are generally associated with social rather than solitary species. Indeed both the first generation sex ratio and mother/daughter size differences are very similar to those found in some social species, *E. laticeps* for example (PACKER and KNERER, 1985).

Further research will centre on the overwintering generation for which insufficient field data are available at present.

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