ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. XLIV, nr 2
1975

Gametogenesis and pollination processes in *Ornithopus pinnatus* (Mill.) Druce in reference to flower bud development

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(Received: May 21, 1974)

Abstract

It was found, that in *Ornithopus pinnatus* the development of microspores and pollen grains is not simultaneous in the two stamen whorls and it precedes the development of the embryo sacs. The pollen grains attain the highest germination capacity before the bud is open, when the embryo sacs are mature.

INTRODUCTION

Studies in which the flower, or rather its primordium is investigated as a whole do not extend beyond the early stages of organogenesis. In the follow-up of further steps of bud development the formation of the particular elements is usually investigated separately. It is only in the mature flower that the final result of the earlier occurring processes is seen. Works concerning the interrelations between pollen, ovules and perianth development, thus dealing with the developmental rhythm of the entire bud are so scarse up till now, that they do not allow any more general conclusions. It seems, therefore, useful to investigate from this aspect a large number of plants in order to detect eventual regularities in these processes.

Investigations of this type have partly been performed, among other plants, on two species of *Ornithopus*: *O. sativus* and *O. compressus* (2n and 4n) (B. Wojciechowska 1969, 1972). The present study concerns the species *O. pinnatus* from the subgenus *Arthrolobium*. The first two leaves of the seedlings of this plant are not pinnate, this distinguishing them from those of pinnate seedlings of other *Ornithopus* species (Fig. 1). The arrangement of the ovules in the ovary was also found to bo different than in the species examined by B. Wojciechowska (1972) (Figs 3 and 4)

Fig. 1. *Ornithopus* seedlings: on left seedling of *O. sativus*, on right of *O. pinnatus*. First leaves *of O. pinnatus trifoliate* not *pinnate* as in other *serradella* species

MATERIAL AND METHODS

Seeds of *O. pinnatus* were obtained from the Botanical Garden in Coimbra (Portugal). Most of them were sown in the glasshouse. Buds and flowers at various stages of development were collected according to the stage classification of B. Wojciechowska in her paper on *O. sativus* Brot. and *O. compressus* L. (1969, 1972). The earliest stage, however, was additionally divided into 5 substages. The successive stages denoted I—VII are as follows:

I — green bud, corolla cannot be seen through sepals

(a) petals reach ± to mid 2nd tier of anthers (Plate II, photo 1);

(b) petals stand out somewhat higher than top of 1st anther tier (Fig. 2, photo 1);

(c) petals reach ± to mid 2nd tier of anthers (Plate II, photo 1);

(d) petals reach to top of 2nd anther tier (Fig. 2, photo 2);

(e) petals almost completely developed, but not yet visible through sepals (Fig. 2, photo 3);

II — petals hardly visible between sepals (already formed but not fully grown — Fig. 2, photo 4);

III — petals and tips of sepals at one level (Fig. 2, photo 5);

IV — petals slightly outgrowing the sepals (Fig. 2, photo 6);

V — petals distinctly elongated sticking out beyond sepals (Plate III, photo 1);

VI —petals fully developed, but not opened;
VII — flower completely open.

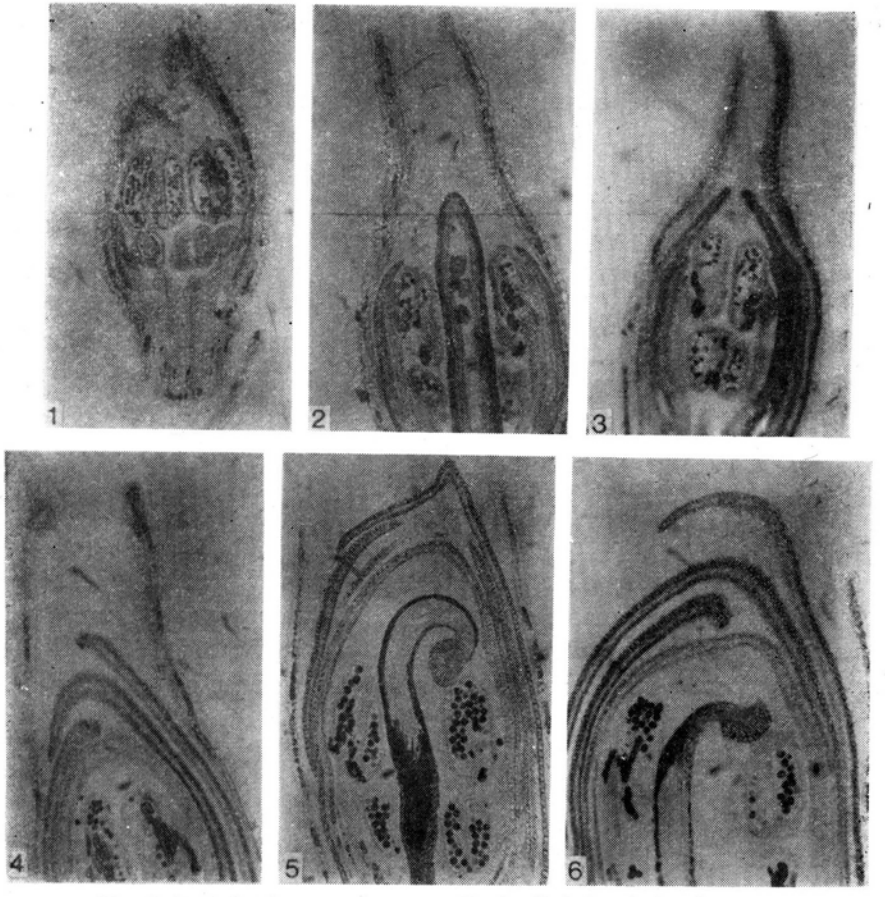


Fig. 2. Bud development stages, × 50, detailed descritpion in text, 1. stage Ib, 2. stage Id, 3. stage Ie, 4. stage II, 5. stage III, 6. stage IV

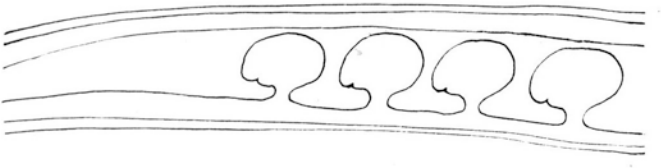


Fig. 3. *O. pinnatus* ovules pointing by micropylar part towards style (epitropic)

The material was fixed in FAA, kept in 70 per cent alcohol dehydrated through a ternary butyl alcohol gradient, embedded in paraffin and sectioned. The section thickness in dependence on the development stage was 4—15 μm. Iron hematoxylin was used for staining according to Heidenhain with counterstaining with fast green.

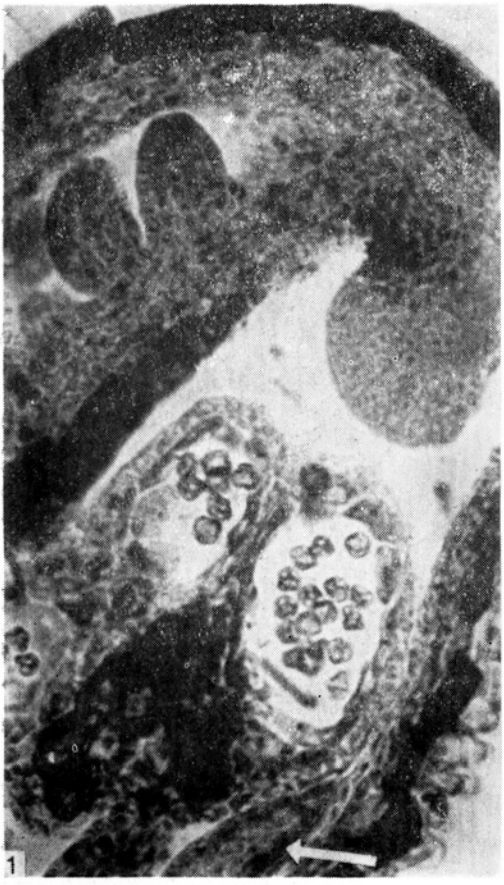
Pollen grain was germinated *in vitro* in 3 replications on 2 per cent agar gel with sucrose of various concentrations (0—40%) added. Material for observation of pollen grain germination in situ was fixed in glacial acetic acid with 2 per cent anilin blue (cotton blue) in a 1:3 ratio. Lactophenol was used after Darlington and La Cour (1960) for preparation. The stigmata were observed in stages IV—VII. At each stage 80 stigmata (40 from glasshouse and 40 from field conditions) were inspected and the per cent of stigmata with germinating pollen was calculated.

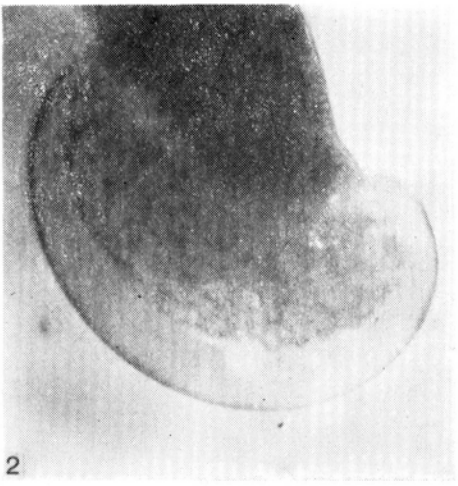
In statistical elaboration the non continuous numerical data were converted to Bliss degrees (Elandt, 1958), so that the sample would have a normal distribution, and then analysis of variance was performed. In comparison of the means after these analyses Duncan's test was used.

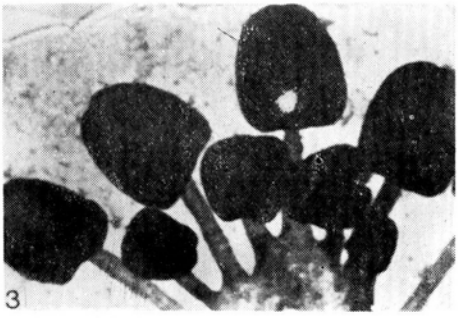
RESULTS

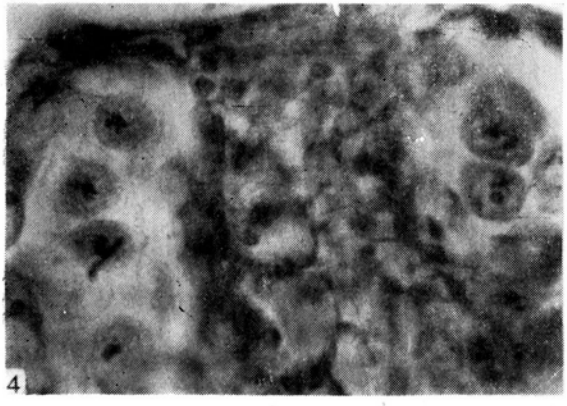
The *O. pinnatus* flower has 10 stamina, one of them is free and the others coalesce forming a tube surrounding the pistil. The stamina form two whorls. In the period of anther development the stamina in the upper whorl are as a rule larger and more advanced in development (Plate I, photo 3 and Plate II, photo 1). Microgametogenesis occurs in the material studied in the mode typical of dicotyledonous plants. Deviations from the general tendency to synchronization in the stages of meiotic division have, however, been noted rather frequently (Plate I, photo 4). The difference between the stages when synchronization fails is not large, the widest in one anther occurs within the limits from prophase I to telophase I. In the end phase of pollen formation microspores not dividing yet, microspores in various phases of mitosis I and 2-cell pollen grains were observed simultaneously in one anther loculus. Since in the period of mitosis I simultaneous divisions were never observed, it is believed that this stage either lasts but a very short time or synchronization does not occur in this period (Plate I, photo 5).

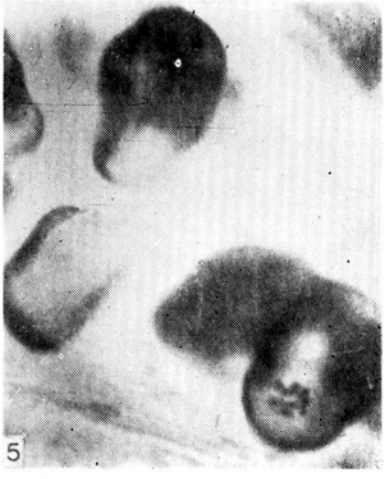
The pistil stands out above the androaecium, the long style is bent and the stigma points towards the stamina (Fig. 2, photos 5 and 6, Plate I, photo 1, Plate III, photo 4). In some buds the styles are longer by 0.5—1.5 mm than the size typical for the given developmental phase and they stand out above the perianth (Plate III, photos 1, 2). This is noted more frequently in plants growing in field than in those in glasshouse conditions. In the open flower stage part of these standing out stigmata is hidden from sight by the petals, but in the period of wilting of the corolla they again become visible. At the green bud







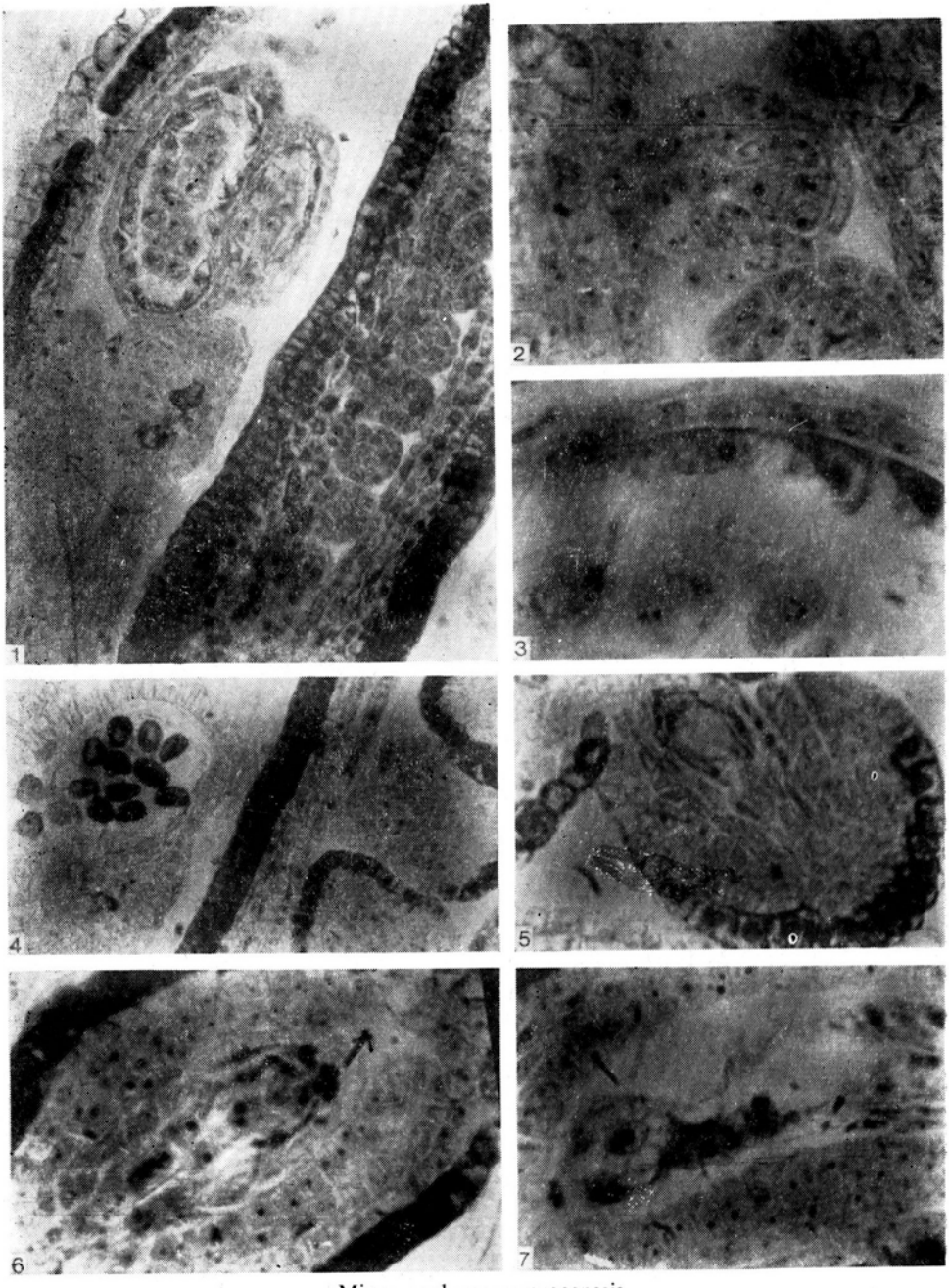




Pistil, stigma and stamina, examples of lack of synchronization in microsporogenesis

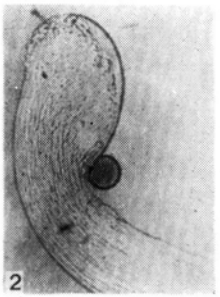
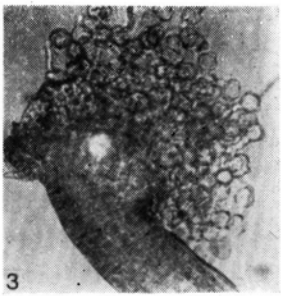
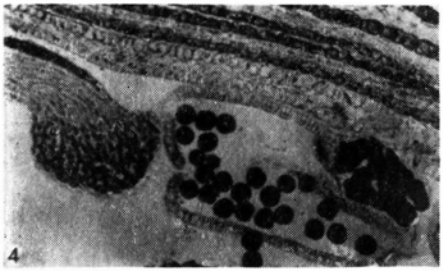
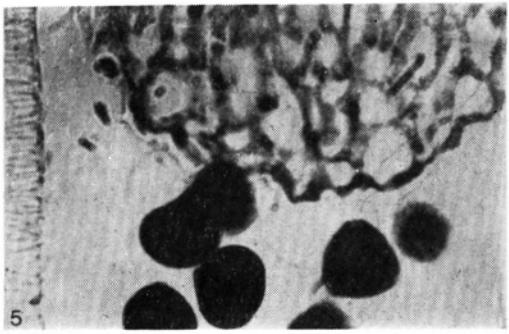
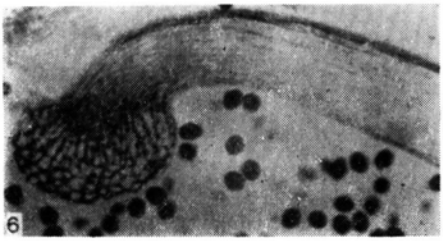
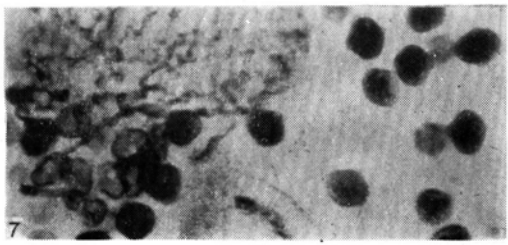
1. fragment of longitudinal section through flower bud at stage Ie — stigma still smooth without secretion; in upper part of embryo sac undifferentiated ovules are seen; in stamina of upper whorl young microspores arrow indicates corolla petal reaching mid way up 2nd whorl of stamina, × 240; 2. mature stigma — stage VII — with papillae and covered with secretion, × 100; 3. fragment of dissected tube with stamina — stages II—III; stamina of lower whorl smaller than in upper one, × 37; 4. in one pollen sac various stages of meiotic division — in left loculus metaphase in PMC, in right one prophase; 5. in one pollen sac on right pollen grain with nucleus in metaphase, on left binucleate pollen grain

Plate II



Micro- and megasporogenesis

1. fragment of longitudinal section through flower bud at stage Ia, arorw indicates corolla petal, arrangement of stamina in two whorls is seen, × 188; 2. megasporocyte surrounded by 2-layer nucellus, stage Ib × 675; 3. cross section through 3-layer microsporangium with microsporocytes in metaphase II, stage Ib, fragment of the same bud as the megasporocyte in photo 2, anther from lower whorl, x 750; 4. fragment of longitudinal cross section through bud at stage Ie, in ovule linear tetrad, in anther of lower whorl binucleate pollen grains, ×150; 5. 4-nucleate embryo sac, stage II, integuments do not surround nucellus, x 375; 6. 7-cell embryo sac surrounded by nucellar epiderm; arrow indicates inner integument not completely coalesced, stage III, × 525; 7. Zygote, first endosperm division, the traces of nucellus surrounded by endothelium layerstage VII — x 700

Pollination

1. Flower buds at stage V with stigmata sticking out, x 6; 2. enlarged sticking out stigma, pollen absent x 80; 3. mass germination of pollen on stigma not standing out, cut from open flower at stage VII, x 90; 4. fragment of cross section through flower bud at stage III, beginning of cracking of endothecium, x 90; 5 and 6. falling out pollen surrounds stigma at stage IV, x 337 and x 116, respectively; 7. germinating pollen grains on stigma just before anthesis, stage VI, x 175

stage (I) the stigmata are smooth without excrescences (outgrowths) and their surface is not yet covere dwith secretion (Plate I, photo 1). Beginning with stage II excrescences appear on the stigmata and at first a thin gradually swelling layer of secretion is seen as the flower develops (Plate I, photo 2, Plate III, photos 2, 4, 5, 6). When pollen begins to germinate on the stigma this layer is digested (Plate III, photo 7).

One ovary contains usually 12—15 ovules which are set on both the edges of the ventral suture (Plate I, photo 1 and Plate II, photo 1). Most ovules point with the micropylar part towards the style (epitropic), only in rare cases is a reverse orientation (apotropic) of the particular ovules observed (Fig. 3, 4). In young orthotropic or already slightly bent ovules one megasporocyte forms (Plate II, photo 2) from which after two divisions four megaspores arise, linearly arranged or sometimes in the shape of the letter T (Plate II, photo 4; Fig. 6). The megasporocytes are, still before the formation of integuments, surrounded by a 2—4 layer nucellus. At the phase of transformation of the chalazal megaspore into a monospore embryo sac of *Polygonum* type the process of digestion of the nucellus starts. This process ends when the embryo sac reaches a 7-cell size. At this period endothelium starts to form. The growth of the integuments is not strictly synchronized with the development of the embryo sac: sometimes, at the 4-nucleate stage of the embryo sac both integuments grow over it forming the micropyle canal, and sometimes they reach only midway up the nucellus (Plate II, photo 5, and Table 1). Development of the ovules in one ovary is not synchronous. For instance in one ovary undifferentiated ovules may be seen simultaneously beside ovules with megasporocytes in prophase I; in another ovary ovules in different stages of the first meiotic division; and in still another ovary, ovules in the stages one- two- and 4-nucleate embryo sacs, lie next to each other etc. (Fig. 5).

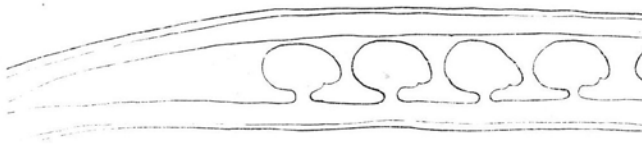


Fig. 4. *O. sativus* ovules pointing by micropylar part to ovary base (apotropic)

The steps of development of male and female generative organs in reference to bud development are shown in Table 1.

For determination of pollen germination capacity *in vitro* pollen grains were collected from various bud development stages. Serious difficulties were encountered when sowing pollen grains taken from buds in stages II and III, although at stage II the beginning of bursting

of the endothecium was observed (Table 1). On the few successful preparations from these stages the pollen grains germinated on 2 per cent agar medium with 10 per cent sugar added. Beginning with stage IV of bud development pollen grains were easily sown and *in vitro* investi-

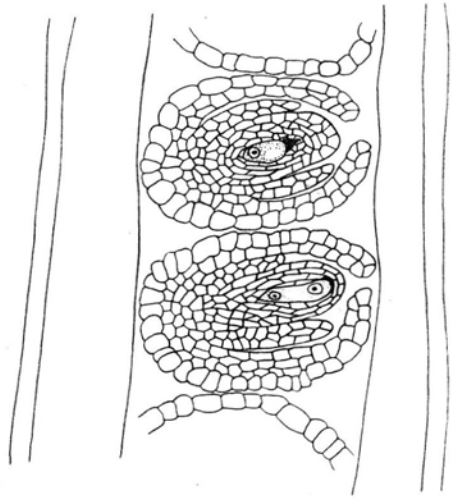


Fig. 5. Asynchrony in development of ovules from one ovary

gations were performed in 3 replications. Pollen grains from open flowers (stage VII) were sown 2 per cent agar medium with various sucrose concentrations (10, 20, 30, 40%) and without its addition. The longest tubes were found to grow at 20 and 10 per cent sugar concentrations, therefore studies of pollen grains germination at stages IV—VII

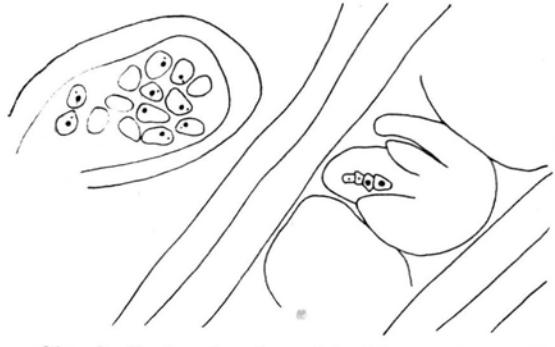


Fig. 6. Fragment of longitudinal section through bud in stage Ie, see Plate II, photo 4

of flower development were performed only at these optimal sugar concentrations. It was found that pollen grains from closed flowers (stages IV—V) germinated better, less well just before flower opening (stage VI) and from open flowers (stage VII, Table 2). Germination of pollen grains from young buds in stage IV was most frequent (86%)

Table 1

Developmental stage	Microsporangium structure	Microsporogenesis, pollen, formation, pollination — Lower stamina	Microsporogenesis, pollen, formation, pollination — Higher stamina	Ovule — position — integuments	Macrosporogenesis and embryo sac development — fertilization	Bud length	Bud width (μ)
I a	one epidermis layer; endothecium, are seen; intermediate layer poorly or not at all visible; one layer of mononucleate tepetum	microsporocytes do not divide yet	microsporocytes in prophase II	orthotropic eminences	no differentiation	718	334
I b	epidermis; spreading endothecium; intermediate layer poorly or not at all visible; one layer of mononucleate tapetum	from microsporocytes at various stages of meiosis (prophase I — telophase II) to vacuolized microspores	from sporadically occurring tetrads to vacuolized microspores; sporadic dividing microspores and two--cell pollen grains;	from orthotrophic ovules to distinctly bending ones; intergument absent or beginning to form	from undifferentiated ovules to those with macrosporocytes in prophase I	1081	473
		most frequent: tetrads or very young microspores	young microspores are most frequent*		not dividing macrosporocytes are still most frequent		
I c	epidermis, spreading endothecium; intermediate layer still sometimes visible; disappearing tapetum	from tetrads to strongly vacuolized microspores; rare two-cell pollen grains;	from young microspores to two-cell pollen grains;	ovules mostly slightly bent; integument absent or beginning to form	from undifferentiated ovules to differentiated ones with late stages of 1st meiosis division	1476	495
		vacuolized microspores most frequent	vacuolized microspores are most freqeunt		most frequent macrosporocytes in prophase I		
I d	epidermis sometimes already cracking, extended endothecium; intermediate layer lacking; remains of tapetum or none	from vacuolized microspores to two-cell pollen grains;	two-cell pollen grains, sporadically vacuolized microspores	ovules from bent to campylotropus; integument at base of nucellus or reaching higher	from prophase I stages in macrosporocytes to mononucleate sporadically binucleate embryo sacs	1659	546
		two- cell pollen grains are most frequent			macrosporocytes in prophase I are most frequent		
I e	epidermis cracked; extended endothecium, intermediate layer lacking; remains of tapetum or none	two-cell pollen grains		ovules from bent to campylotropous; integuments at base of nucellus or reaching half way up it	from diads to mononucleate embryo sacs	1715	611
					tetrads and mononucleate embryo sacs most frequent		
II	epidermis cracked or lacking; extended endothecium, sometimes already cracked; intermediate layer and tapetum lacking	two-cell pollen grains		campylotropous ovules; from integuments reaching half way up the nucellus to those surrounding the nucellus and forming the micropylar canal	sporadically mononucleate, more frequently 2—4 nucleate embryo sacs	2126	700
					most frequent are 4-nucleate ones		
III	remains of epidermis still visible or lacking; endothecium greatly extended, in a number of anthers cracked; intermediate layer and tapetum lacking	two-cell pollen grains		campylotropous ovule; integuments from not completely surrounding the nucleus to coalesced forming the micropylar canal	most frequent are newly formed 7-cell embryo sacs, sporadically 1-, 2-, 4-, and 8-nucleate free ones; small egg cell; synergides well visible; polar nuclei not coalesced; antipodes well visible or already degenerated	3000	732
IV	epidermis lacking, endothecium cracked; pollen grains mostly falling out	two-cell pollen grains firts cases of pollination		campylotropous ovule; integuments most frequently form micropylar canal--sometimes inside the integument it is not yet coalesced	most frequent are 7-cell embryo sacs, sporadically 8-cell free ones; egg cell and synergides well visible; polar nuclei not coalesced or beginning to coalesce; antipodes degerated or not	3663	839
V	burst endothecium; pollen grains falling out	two-cell pollen grains more frequent pollination		campylotropous ovule; integuments form micropylar canal	7-cell embryo sacs, large egg cell synergides well visible, polar nuclei not coalesced, coalescing or already coalesced; antipodes degenerated or not	4512	925
VI	burst endothecium; pollen grains have fallen out	two-cell pollen grains numerous pollination		campylotropous ovule; integuments form micropylar canal	mature embryo sacs; first fertilization	5381	1010
VII	burst endothecium; pollen grains have fallen out	two-cell pollen grains mass pollination		campylotropous ovule; integuments form micropylar canal	mature embryo sacs; numerous fertilizations or several-or some dozen-cell embryos		

* Young microspores — microspores with centrally located nucleus without vacuoles or with nucleus already slightly pushed aside.

but the pollen tubes growing from them were significantly shorter than those from older flowers. Beginning with stage V and up to stage VII there were no significantly differences in pollen tube length (Table 3). It results from those investigations that *in vitro* pollen grains from

Table 2

In vitro germination of pollen grains collected from flowers at various stages of development (IV—VII)
Medium 10 and 20% sucrose in 2% agar
Comparison of means by Duncan's test at $\alpha=0.05$ level

	Bud development stages			
	IV	V	VI	VII
Bliss degrees	68.6	66.5	49.9	48.3
Per cent	86.0	83.6	58.0	55.6

	2	3	4
Sd × tm=	5.85	6.13	6.32

buds in stages V—VI are most visible. This is expressed by a high percentage germination and by the growth of the pollen tubes, which are as length as those of the pollen grains in open flowers. Observation of germinating pollen grains in situ both in permanent preparations and on stigmata stained with lactophenol confirm the results obtained in vitro. Pollen grains germinating in situ were found on stigmata from

Table 3

Length of pollen tubes (μ) 24 h after sowing *in vitro* pollen grains collected at various stages of bud development (IV—VII)
Medium 10 and 20% sucrose in 2% agar
Comparison of means by Duncan's test at the $\alpha=0.05$ level

Bud development stages			
IV	V	VI	VII
26.58	38.32	41.68	39.03

	2	3	4
Sd × tm=	7.50	7.86	8.11

stage IV. At this stage the per cent of stigmata with germinating pollen grains was higher in plants growing in glasshouse conditions than in those in the field. Immediately before anthesis (stage VI) the per cent of stigmata with germinating pollen grains exceeded 60 per cent (Table 4) both in the field and glasshouse plants. In newly opened

flowers the per cent of pollinated stigmata varied within the limits of 70—80 per cent, thus 20—30 per cent were not selfpollinated to the moment of anthesis, this giving a chance of pollination from outside. As already mentioned, in some nontypical buds the style was longer by 0.5—1.5 mm and stood above the petals (lPate III, photo 1). In order

Table 4

Per cent of stigmata with germinating pollen grains at various stage of flower development (IV — VII)

Development stages	IV	V	VI	VII
Glasshouse	19.5	24.3	61.9	83.0
Field	6.8	19.4	66.7	71.7

to establish whether the standing out stigmata can be selfpollinated, the buds were isolated. It was established that 80 per cent of with standing out stigmata — after flower opening got dried after certain period of time; these buds when artificially pollinated with their own pollen or that from neighbouring flowers or other plants produced normal pods. Hence it may be concluded that a certain per cent of the flowers is not selfpollinated solely because of a mechanical obstruction.

DISCUSSION

Considerations on problems of the developmental rhythm of male and female gametophytes and of the perianth in bisexual flowers of angiosperms plants lead to the supposition that there exist specific correlations within definite plant groups (e.g. within plants of the same species, genus, family, order, class, or within auto- and allogamous plants, or within plant groups with a similar type of early organogenesis etc.).

In the serradella species — *O. sativus* and *O. compressus* (2x and 4x), investigated in this aspect (B. Wojciechowska, 1972) and in the presently studied species *O. pinnatus* anatomically and morphologically differing from the other two, the developmental rhythm of the buds is similar. For instance in all these species the development of the embryo sac takes place when binucleate pollen is already formed in the anthers. What is more, binucleate pollen was found in all these species even in green buds before the complete formation of the corolla (stage I e). On the other hand in red clover (*T. pratense*) in buds with completely formed petals, microspores were found (W. Wojciechowska and Strzyżewska — unpublished). As it follow's from the above description, in spite of autogamy, the development cycles of

pollen and the embryo sac in the species of *Ornithopus* studied do not coincide, although as shown by pollen viability tests, the highest germination capacity is reached in the closed flower and is synchronized with the maturity of the embryo sac.

It is worth to emphasize that both the species examined by B. Wojciechowska exhibited the same development rhythm of buds both in glasshouse and in the garden, the only exception was the earlier germination of pollen grains on the stigmata of *O. compressus* and *O. sativus* 2x in the glasshouse. This indicates that fairly big differences in temperature and moisture between the glasshouse and the garden did not change the developmental rhythm of the bud in these species.

Beside the work jointly describing the development of both gametophytes of *Ornithopus* sp. (B. Wojciechowska, 1972) only a few papers were found in the available literature dealing with this problem in other species (G. Davis, 1968a and 1969; Rodkiewicz, 1961).

The investigations of Rodkiewicz on the interdependence of the developmental phases of the embryo sac with the phases of pollen development in *Lilium candidum* are in stricking agreement with the results of the present study. In *O. pinnatus*, the same as in *Lilium candidum*, pollen grains are already mature when the mother megaspore cell is at the end of prophase of the first meiotic division. It should be noted that in *L. candidum* macrosporogenesis occurs according to the *Fritillaria* type and in the genus *Ornithopus* according to the *Polygonum* type. Nevertheless such complete agreement was noted. Could this be accidental? Davis, comparing the processes in anthers and ovules of *Eucalyptus meliodora* (1968a) and *Eucalyptus stellulata* (1969) found other correlations than in *L. candidum* and *Ornithopus* sp., and other in each of the two species of the genus *Eucalyptus*. In both *Eucalyptus* species, however, similarly as in *Ornithopus* sp. and *L. candidum*, in the initial phase of bud development the differentiation of archespore tissue in the anthers occurs earlier than in the ovules which form at later stages. The further development of the microsporocytes and microspores, however, in both the studied *Eucaliptus* species does not precede the development of megaspores and embryo sacs as is the case in the investigated *Ornithopus* species and in *L. candidum*.

In *O. pinnatus*, like in *O. sativus* and *O. compressus* there is definitely some correspondence between the different phases of mega- and microspores, but no strict parallelism was found; for instance, when in *O. pinnatus* anther tetrads are found, then the ovules in the pistil may be either not yet differentiated or with macrosporocytes already in various stages of prophase I, but it is quite certain, that mononucleate embryo sacs are not yet to be found. (Table 1). In *O. pinnatus* these variations are among other things the consequence of the fact

that both the ovules in one ovary and the stamina in one androecium do not develop simultaneously.

Descriptions of stages of development of all or at least of the majority of ovules in one ovary with numerous ovules are seldom found in the literature. It may be concluded, however, from these descriptions that in some plants there are no differences in the development of the particular ovules in one ovary, as for instance in *Eucalyptus stellulata* Sieb. (Davis, 1969), whereas in others there are distinct differences dependent on the position of the ovules in the ovary, as for instance in *L. candidum* (Rodkiewicz, 1961), where the ovules in the central part of the ovary are most advanced in the development. Lately the same author (1973a) found asynchrony of divisions in the ovules of one multi-ovular *Epilobium* ovary. He investigated in particular ovaries the development of megaspore tetrads observing the callose walls with the use of the fluorescence method in anilin blue.

As far as development of anther in one androecium is concerned according to Rodkiewicz (1973b), "The beginning of meiosis and further its succesive phases up to the moment of tetrad formation occur more or less synchronously in the whole anther and in all the anthers of the stamina in one whorl" (underscored by W.W.). This is not contradicted by the wide differences observed in the stage of microsporocyte and microspore development in the stamina the anthers of two different whorls of one androecium in *O. pinnatus* (Table 1 and Plate II, Photo 1). As already mentioned, in *O. pinnatus* rather frequently slight deviations were found within one anther (Plate I, Photo 4).

In the end phase of pollen grain formation (in the period of mitosis I), as mentioned above, simultaneous nucleus divisions were never observed even within one loculus. Beside pollen grains in various phases of first mitosis, in the same loculus microspores were found, not dividing yet as well as two cell pollen grains. In red clover (*Trifolium pratense* L. — W. Wojciechowska and Strzyżewska, unpublished) similarly and in grasses, for instance in the genera *Festuca* and *Lolium* (Sulinowski-personal communication) in the end phase of pollen grain formation there was no synchronization. Pollen grains in this phase are already covered with sporoderm, and the tapetum is usually completely degenerated; each pollen grain constituting by now a distinct unit. Rodkiewicz (1973b) also mentions the lack of synchronization in divisions of microspore and pollen grains after the degeneration of the tapetum.

It is probable that the development rhythm of the bud may be modified by the action of external factors as for instance temperature, light, auxin application etc. The morphogenetic action of these factors on the early development of the gynoecium and androecium was demonstrated by Heslop-Harrisson (1956—1959). It is not excluded

that those factors affect also the developmental rhythm at later phases of bud formation. This problem is connected with the method of physical or chemical castration. It was for instance found that irradiation of buds with X-rays at a suitable moment (Schneider, 1965; Helting, 1968; Poszwińska, 1966) impairs the pollen forming tissue, whereas the female organs develop after a certain time intact. It is believed that this occurs because in many plant species as in *Ornithopus* sp. microsporogenesis is more advanced than megasporogenesis. X-rays destroying the already differentiating anthers do not impair the undifferentiated ovules. Eventual damage to the forming ovules is more easily repaired than that to the androecium at a more advanced stage.

It should be mentioned that the developmental rhythm of the bud may be affected by its position in the inflorescence. In the heterogamous species *Epaltes australis* Less (*Compositae*) Davis (1968b) found that "microsporogenesis in florets of the capitulum conforms to the normal acropetal succession in that youngest anthers are in florets at the centre of the capitulum, but development of the ovules follows the reverse procedure".

A characteristic phenomenon in *O. pinnatus* is the occurrence of a certain, not very large, number of buds with standing out stigmata. The same was observed in other plants such as *Medicago* sp. by Filatov, Petrova, (1972); Marcus and Wilsie (1957) describe a from of *Medicago* in which stigmata were standing out in all the buds of the plant.

It is noteworthy that the pollen tubes of the species *O. pinnatus* like these of the species investigated by B. Wojciechowska (1969, 1972), *O. sativus* and *O. compressus*, when growing *in vitro* reached a maximal length beginning with stage V when the ovules already contain full-grown 7-cell embryo sacs.

CONCLUSIONS

In the autogamous plant *O. pinnatus* the development of microspores is not simultaneous in the two stamen whorls and it precedes the development of the embryo sacs. Two-cell pollen forms in green buds before the full formation of the corolla, but its germination capacity is highest much later when the embryo sacs are mature but still enclosed in the flower.

A characteristic phenomenon in *O. pinnatus* is the occurrence of a small number of buds with stamina sticking out beyond the perianth so that allogamy is possible.

It was found that the developmental rhythm of the bud in the species *O. pinnatus* which is morphologically and anatomically different

from other serradella species, is analogous to the same rhythm in the autogamous species *O. sativus* and *O. compressus* investigated by B. Wojciechowska. The developmental rhythm of the bud in these three species varies within certain definite limits and was found to be the same in investigations performed in different years and under different conditions (glasshouse, garden). Further investigation from this aspect of a greater number of bisexual flowers of angiosperms plants might give a basis for more general conclusions.

The author is indebted to mgr E. Bielińska for preparing the drawings. The technical assistance of Ms E. Juja is acknowledged.

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Gametogeneza i procesy zapylenia u Ornithopus pinnatus (Mill.) Druce na tle rozwoju pąka kwiatowego

Streszczenie

U *Ornithopus pinnatus* badano rytm rozwojowy pąka. Gatunek *O. pinnatus* jest odrębny morfologicznie i anatomicznie od gatunków *O. sativus* i *O. compressus* zbadanych przez B. Wojciechowską (1969, 1972). Pomimo samopylności cykle rozwoju pyłku i woreczka zalążkowego u przebadanych gatunków nie przebiegają równocześnie, chociaż, jak wynika z badań żywotności pyłku, maksimum zostaje osiągnięte w zamkniętym kwiecie i jest zsynchronizowane z dojrzałością woreczka zalążkowego.

Charakterystycznym zjawiskiem u *O. pinnatus* jest występowanie pewnej nieznacznej liczby pąków z wystającymi ponad okwiat znamionami, co ułatwia obcozapylenie.

Stwierdzono, że rytm rozwojowy u tych trzech gatunków waha się w pewnych określonych granicach i jest jednakowy w badaniach prowadzonych w różnych latach i różnych warunkach (szklarnia, ogród). Wydaje się celowe przebadanie pod tym kątem większej liczby roślin okrytozalążkowych obupłciowych, ażeby stwierdzić ewentualne prawidłowości. Dotychczas, prace badające odpowiedniość między fazami mikrosporogenezy, megasporogenezy i rozwoju okwiatu, są bardzo nieliczne. Dalsze badania, być może, dałyby podstawy do ogólniejszych wniosków.