The development rhythm of the flowerbud in some Papilionaceae species

Part I. Gametogenesis in Lupinus elegans (H.B.K.) and Lupinus mutabilis (Sweet.) in reference to flowerbud development

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(Received: February 23, 1976)

Abstract

The developmental rhythm of the bud in the species investigated was found to remain the same within each species, independently of the year and environmental conditions, (glasshouse, field) in which they were fixed.

INTRODUCTION

The present paper is a part of studies undertaken to find an answer to the question whether, in the development of the flowerbud of angiospermous dioecious plants in definite groups such as species, genera, families, autogamous or allogamous plants or others plant groups specific regularities different than in other groups exist. As mentioned in the preceding paper (W. Wojciechowska, 1975a) in most publications concerning gametogenesis in angiospermous dioecious plants, the reader finds with few exceptions, (Davis, 1968a; Charechko-Sawicka, 1940; Rodkiewicz 1961; B. Wojciechowska 1972a, b; W. Wojciechowska, 1975a, b) a separate scheme of investigation of macrosporogenesis and development of the embryo sac and a separate one for studying microsporogenesis and formation of pollen grain. Consequently, the problem is put in the same way in all manuals of plant embryology. Still more scarce is the literature showing the course of gametogenesis in reference to development of the entire flowerbud (B. Wojciechowska, 1972a, b; W. Wojciechowska, 1975a, b). The present paper describe in detail the development of the bud and flower in two further species of the family Papilionaceae — Lupinus elegans (H. B. K.) of the Hartwegi section and Lupinus mutabilis (Sweet.) of the same section.
Lupinus elegans seeds were received from the University Botanical Garden in Uppsala, Sweden and from professor Kazimierski's collection at the Department of Plant Genetics of the Academy of Sciences at Poznań and Lupinus mutabilis seeds from the Agrobotanical Institute at Tapioszelle, Hungary. The seeds were sown in a glasshouse and in the field in the years 1974 and 1975 and the flowerbuds were fixed for 1—2 min in AA and next in FAA. The buds for comparison of the particular stages of their growth were classified according to 8 stages established by B. Wojciechowska (1972a, Figs 1 and 2). In view, however, of the fact that

Fig. 1. Lupinus elegans — development stages of bud and flower I—VIII

in the plants investigated up till now the whole process of microsporogenesis, and in some species the entire process of macromsporogenesis occurs in a completely closed green bud, it proved necessary to follow the initial step of flower development in more detail and divide the stage I of B. Wojciechowska into several substages. As criterion for singling out the substages of stage I, in which the petals of the corolla are inside the closed bud, was assumed the relation between the position of petals and stamens. The successive stages and substages are as follows:

I — Green bud, corolla petals not visible through sepalas (Plate I, Photos 1—7 and Figs 1 I and 2 I)
   I/1 — higher petal of corolla below or at mid height of second whorl of anthers (Plate I. Photos 1 and 2)
   I/2 — higher corolla petal ± equal with tip of second anther whorl (Plate I. Photos 3 and 4)
   I/3 — petals of corolla emerge distinctly above stamens of second whorl (Plate I, Photos 5—7).

II — Corolla petals slightly visible through sepals (Plate I, Photo 8 and Figs 1 II and 2 II)

III — Corolla petals equal with sepals (Plate I Photo 9 and Figs 1 III and 2 III)

IV — Corolla petals slightly excrescent above sepals (Plate I. Photo 10 and Figs 1 IV and 2 IV)

V — Corolla petals distinctly standing out above sepals (Fig 1 V)
Gametogenesis in *L. elegans* and *L. mutabilis*

Fig. 2. *Lupinus elegans* — development stages of bud I—IV, enlarged

VI — Corolla petals fully grown but not opened (Fig. 1 VI)
VII — Flower fully developed and opened (Fig. 1 VII)
VIII — Flower overblown (Fig. 1 VIII)

After embedding the buds in paraffin they were cut longitudinally into 4-15-μ sections. The thickness of the section depended on the size of the bud. The preparations were stained with iron hematoxylin and counterstained with fast green. To facilitate classification into substage each longitudinally sectioned bud was photographed and the photographs were ordered in groups.

RESULTS

In both species macrosporogenesis occurs in a similar way. Simultaneously with the formation in the ovule of one megasporocyte, a single integument is formed. After division a tetrad or triad arises from the me-
Table 1

Comparison of early stages of gametogenesis in *Lupinus elegans* and *Lupinus mutabilis* in reference to flowerbud development
(material fixed in glasshouse and field in 1974 and 1975)

<table>
<thead>
<tr>
<th>Developmental stages</th>
<th><em>Lupinus elegans</em></th>
<th><em>Lupinus mutabilis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Development of ovule and macrosporogenesis</td>
<td>Microsporogenesis</td>
</tr>
<tr>
<td>Stage I/1</td>
<td>from pistils with ovules in the form of undifferentiated eminences to pistils with ovules containing megaspores</td>
<td>in lower whorl</td>
</tr>
<tr>
<td></td>
<td>from undifferentiated anthers to anthers with archesporial cells</td>
<td>in upper whorl</td>
</tr>
<tr>
<td>Stage I/2</td>
<td>from ovules with not yet dividing megaspore to ovules in prophase I</td>
<td>from prophase I to very young microspores</td>
</tr>
<tr>
<td></td>
<td>from prophase I to not yet vacuolated microspores</td>
<td>from prophase I to not yet vacuolated microspores</td>
</tr>
</tbody>
</table>
Lupinus elegans — longitudinal section of buds in stages I—IV, × 10; 1 and 2. stage I/1; 3 and 4. stage I/2; 5—7. stage I/3; 8. stage II; 9. stage III; 10. stage IV
Lupinus mutabilis — gametogenesis in reference to bud development in stage I/3;
1. longitudinal section of bud in stage I/3, × 23; 2. enlarged fragment of bud section shown in photo 1, × 90; 3. macrosporocyte in prophase — enlarged fragment of middle ovule shown in Photo 2, × 1200; 4. cross section of vacuolized microspores from anther of lower whorl shown in Photo 2, × 1200; 5. cross section of vacuolized microspores from upper anther whorl shown in photo 2. × 1200
Gametogenesis in *L. elegans* and *L. mutabilis*

**Plate III**

*Lupinus elegans* — gametogenesis in reference to bud development in stage II. 1. longitudinal section of bud in stage II, × 10; 2. uninucleate embryo sac — cross section of ovule from bud shown in Photo 1, × 1100; 3. cross section of two-celled pollen grain from anther in lower whorl of bud shown in Photo 1, × 1100; 4. cross section of two-celled pollen grain from anther of upper whorl in bud shown in Photo 1, × 1100

Gasporocyte. The tetrads are linear or T-shaped. A triad forms if the second meiotic division does not end in the cell of the micropylar diad remaining generally at the metaphase or anaphase stage. The chalazal cell of the diad always divides normally. From the chalazal cell of the tetrad of triad arises a monospore sac of *Polygonum* type (Plate III, Photo 2).

Microsporogenesis has in both species a course typical for the genus *Lupinus* (Atabekova and Lin Tszyan-Sin, 1962; Kazimierski, 1963).

There is a slight difference between *L. elegans* and *L. mutabilis* in the rate of growth of the petals of the corolla. In *L. elegans*, namely, the petals grow a little slower in relation to the processes of micro- and macrosporogenesis than in *L. mutabilis*. The developmental rhythms of both these species are, therefore, not identical, but very similar. The resemblance is yet so great, that in stage I three substages could be singled out with application of the same criteria of classification (Plate I, Photos 1—7). The course of gametogenesis in reference to bud growth in its early
Plate IV
*Ornithopus pinnatus* and *Lupinus elegans*; moment of formation in both stamen whorls of two-celled pollen grains. 1. *O. pinnatus*, × 65; 2. *L. elegans*, × 13

**Table 2**

*Lupinus elegans*

Comparison of gametogenesis in two collections at stage I/3 in glass-house material fixed in years 1974 and 1975

<table>
<thead>
<tr>
<th>Course of gametogenesis</th>
<th>Collection</th>
<th>Material from Kazimierski’s collection Polish Academy of Sciences, Poznań, Poland</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L. elegans from Botanical Garden in Uppsala (Sweden) sown in Poland</td>
<td>1974</td>
</tr>
<tr>
<td></td>
<td>1974</td>
<td>1975</td>
</tr>
<tr>
<td>Ovule</td>
<td>Most frequent prophase I, in buds with most developed petals further stages of meiotic division</td>
<td>Most frequent prophase I, in buds with most developed petals further stages of meiotic division</td>
</tr>
<tr>
<td>- macrosporogenesis</td>
<td></td>
<td>Most frequent prophase I, in buds with most developed petals further stages of meiotic division</td>
</tr>
<tr>
<td>Microsporogenesis</td>
<td>from tetrads to vacuolated microspores</td>
<td>from tetrads to two-celled pollen grains</td>
</tr>
<tr>
<td>in lower whorl</td>
<td></td>
<td>from very young microspores to two-celled pollen grains</td>
</tr>
<tr>
<td>in upper whorl</td>
<td>from very young microspores to two-celled pollen grains</td>
<td>from very young microspores to two-celled pollen grains</td>
</tr>
</tbody>
</table>
stages of development in *L. elegans* and *L. mutabilis* is shown in Table 1. Photographs 1—5 in Plate II illustrate the course of macro- and microsporogenesis and perianth development in the bud of *L. mutabilis* (Sweet.) in stage I/3 and the photographs 1—4 in Plate III the course of macro- and microsporogenesis in *L. elegans* in reference to bud development in stage II.

It was checked whether the developmental rhythm of the bud in both lupin species is always independent of the origin of the material from one or another collection, of the year in which it was fixed and of the kind of inflorescences on which the buds grew and also of the environment in which the plants were grown.

**Table 3**

*Lupinus elegans*

Comparison of course of gametogenesis in buds of main and lateral shoots at stage I/3
(material fixed in glasshouse in 1974 and 1975)

<table>
<thead>
<tr>
<th>Course of gametogenesis</th>
<th>Main shoots</th>
<th>Lateral shoots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovule</td>
<td></td>
<td></td>
</tr>
<tr>
<td>— macrosporogenesis</td>
<td>prophases I most frequent, in buds with most developed petals further stages of meiotic division</td>
<td>most frequent prophases I, in buds with most developed petals further stages of meiotic division</td>
</tr>
<tr>
<td>Microsporogenesis</td>
<td>from tetrads to two-celled pollen grains from very young microspores to two-celled pollen grains</td>
<td>from tetrads to two-celled pollen grains from very young microspores to two-celled pollen grains</td>
</tr>
<tr>
<td>in lower whorl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>in upper whorl</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Identical developmental phases of the bud for the given substages I were noted in two collections of *Lupinus elegans*: the Swedish one from the Botanical Garden in Uppsala and the Polish one of Kazimierski. Neither were there any significant differences in the *L. elegans* material from the years 1974 and 1975 or in buds collected separately from the main shoot and lateral ones (Tables 2, 3, 4). The development of the bud in *L. mutabilis* is similarly independent of the year and environment (Table 5).

It can be seen from tables 2—5 that in both lupin species the entire process of microsporogenesis, and also quite frequently, the process of formation of two-celled pollen grains, particularly in the upper whorl, occurs in the closed still green bud. The process of macrosporogenesis also takes place in the almost completely closed green bud in *L. mutabilis* and completely closed in *L. elegans*. In stage II of *L. elegans* mostly 1- 2- and 4-nucleate embryo sacs, in the ovules and two-celled pollen grains in both stamen whorls are found. In *L. mutabilis* in stage II tetrads and
Table 4

*Lupinus elegans*

Comparison of gametogenesis course under glasshouse and field conditions in stage 1/3

<table>
<thead>
<tr>
<th>Cultivation condition and years</th>
<th>Glasshouse 1974 and 1975</th>
<th>Field 1974</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Course of gametogenesis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovule</td>
<td>pro phases I most frequent; in buds with most developed petals further stages of meiotic division</td>
<td>pro phases I most frequent; in buds with most developed petals further stages of meiotic division</td>
</tr>
<tr>
<td>— macrosporogenesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microsporogenesis in lower whorl</td>
<td>from tetrads to two-celled pollen grains</td>
<td>from very young microspores to two-celled pollen grains</td>
</tr>
<tr>
<td>in upper whorl</td>
<td>from very young microspores to two-celled pollen grains</td>
<td>from slightly vacuolated microspores to two-celled pollen grains</td>
</tr>
</tbody>
</table>

Table 5

*Lupinus mutabilis*

Comparison of gametogenesis course in stage 1/3 in various years and conditions

<table>
<thead>
<tr>
<th>Years and growth conditions</th>
<th>Glasshouse 1974</th>
<th>Field 1974</th>
<th>Glasshouse 1975</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Course of gametogenesis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovule</td>
<td>Sporadically meiocytes do not divide yet, pro phases I most frequent; in buds with most developed petals sometimes further stages of meiotic division may be found</td>
<td>pro phases I most frequent, in buds with most developed petals sometimes further stages of meiotic division may be found</td>
<td>pro phases I most frequent, in buds with most developed petals sometimes further stages of meiotic division to un-nucleate embryo sacs inclusively may be found</td>
</tr>
<tr>
<td>— macrosporogenesis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microsporogenesis in lower whorl</td>
<td>from sporadically occurring pro phases I to two-celled pollen grains</td>
<td>from sporadically occurring metaphases I to two-celled pollen grains</td>
<td>from metaphases I to two-celled pollen grains</td>
</tr>
<tr>
<td>in upper whorl</td>
<td>from anaphases and telophases to two-celled pollen grains</td>
<td>from tetrads to two-celled pollen grains</td>
<td>from very young microspores to two-celled pollen grains</td>
</tr>
</tbody>
</table>
uninucleate embryo sacs and in both stamen whorls two-celled pollen grains are most frequent.

At the bud stage I/3 in both lupin species the style elongates distinctly and begins to rise above the androecium (Plate I, Photos 5—7 and 9—10). In L. elegans before the opening of the bud (stage IV) the stamens of the second whorl “catch up” with the stigma. In this species selfpollination is frequent. It occurs seldom in L. mutabilis, most frequently in the stage of open flower the style rises above the androecium.

**DISCUSSION**

It may be affirmed on the basis of the investigations performed that the developmental rhythm of the bud in the examined lupin species has an identical course within each species, notwithstanding the year and conditions (glasshouse, field) in which the material was fixed. The minimal difference between L. elegans and L. mutabilis in the rate of growth of the corolla petals as compared to the processes of gametogenesis indicates that these plants are closely related. According to Kazimierski (1963) both these species are classified to the same Hartwegi section. Kazimierski classified all the species of American lupins examined by him to the Hartwegi section which directly or indirectly cross with L. hartwegi giving first generation hybrids with dominating traits of L. hartwegi.

The course of macrosporogenesis in L. elegans and L. mutabilis is typical for the family Papilionaceae (Davis, 1966), with the exception of the occurrence in some ovules of megaspore triads, and agrees with the description given in the work “Embriologija lupina” by Atabekova and Lin-Tsizyan-Sin (1962). In the genus Lupinus megaspore triads were observed in haploids of L. luteus (Kazimierski and Kazimierska, 1970).

Comparison of the developmental rhythm of the bud in the examined species of the genus Ornithopus (B. Wojciechowska, 1972a and W. Wojciechowska, 1977a) with the same rhythm in L. elegans and L. mutabilis revealed the following differences:

1. In the examined serradella species macrosporogenesis starts when in both stamen whorls microsporogenesis is ending, where as in the studied lupin species the first prophasizes appear in the anther almost at the same time as prophase I in the ovules. In further stages however, microsporogenesis precedes macrosporogenesis in the lupin species as well.

2. In the examined serradella species the petals of the corolla grow slower than in the lupin species studied. For instance at the stage when in the green bud the petals are equal with the top of the androecium of the second whorl, in serradella two-celled pollen grains are already present in the anthers of the second whorl, whereas in the lupin species at this phase all stages of meiotic division and nonvacuolized microspores may
be found. Plate IV shows these stages of bud development in *Ornithopus pinnatus* and *Lupinus elegans*, beginning with which two-celled pollen grains are certain to be found in both whorls of stamens. It is seen distinctly that at the moment of two-celled pollen grains formation in both whorls of stamens the corolla petals in *L. elegans* are much more advanced in development than in *O. pinnatus*.

The examined species of the genus *Ornithopus* are doubtlessly autogamous, whereas *L. elegans* can be autogamous and allogamous, and in *L. mutabilis* allogamy markedly prevails. As already mentioned the corolla petals in *L. elegans* grow, as compared with the macro- and microsporogenesis processes, somewhat slower than in *L. mutabilis*. It seems probable, though it still requires confirmation on more extensive material, that autogamy in plants may be associated with a low rate of growth of the corolla petals. Logically, in autogamous plants the bud needs not hurry to open, thus the petals may grow slowly. On the contrary, in allogamous plants pollinated by insects the petals have to grow quickly, though due to the factors responsible for self-incompatibility such a regularity may be effaced. If further investigation confirm this supposition, one could with a good probability distinguish preliminarily autogamous and allogamous plants by analysing fresh buds.

The differences in the developmental rhythm of the flower bud between the studied *Ornithopus* and *Lupinus* species indicate that within one family these rhythms are not identical. In the examined species of the family *Papilionaceae*, however, both those mentioned above and such as *Melilotus officinalis*, *M. albus*, *Robinia pseudo-acacia* investigation of which is under way a common denominator was found. It was, namely, noted that to uninucleate embryo sacs in the ovules either vacuolated microspores or/most frequently, two-celled pollen grains always correspond in the anthers.

The finding of a regularity in the succession of gametogenesis phases and development of the perianth in definite groups of plants may be valuable information for plant physiologists. The differences in the developmental rhythm of the bud between species or varietas seem to support the hypothesis that "florigen is a complex compound present both in stimulators and inhibitors regulating generative plant development". This hypothesis suggests that florigen in such a sense may be different in various plants (Kopcewicz, 1976).

The above given description of growth of the style in successive development stages of the bud illustrated the strict coordination of the development of various flower organs. An interesting experiment pointing not only to a coordination but to an interrelation in the development of generative organs of the flower was carried out by Linskens on the buds of petunia. He cut off the stamens in the petunia bud and found that this inhibited the growth of the style (Rodkiewicz, 1974).
CONCLUSIONS

In two species of American lupins from Hartwegi’s section, *L. elegans* and *L. mutabilis*, the course of gametogenesis was investigated (micro- and macrosporogenesis) in reference to bud development.

It was found that the developmental rhythm of the bud is the same within one species, independently of the year and conditions (glasshouse, field) in which the material was fixed. As compared with the processes of macro- and microsporogenesis the petals of the corolla grow somewhat faster in *L. mutabilis* than in *L. elegans*. Thus, the developmental rhythms of both these species are not quite identical but very similar.

The author wishes to thank mgr Elżbieta Bielińska for making a drawing of the buds and Mrs Eugenia Juja for technical assistance.

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Gametogeneza u Lupinus elegans (H. B. K.) i Lupinus mutabilis (Sweet) na tle rozwoju pąka kwiatowego

Streszczenie

U dwu gatunków lubinów amerykańskich z sekcji Hartwegi, (Lupinus elegans i Lupinus mutabilis) zbadano przebieg gametogenezy (mikrosporogenezy i makrosporogenezy) na tle rozwoju pąka.

Stwierdzono, że rytm rozwojowy pąka przebiega w obrębie każdego gatunku jednakowo, niezależnie od lat i warunków (szklarnia, pole), w jakich utrwalano materiał. W stosunku do procesów makrosporogenezy i mikrosporogenezy płatki korony nieco szybciej rosną u Lupinus mutabilis niż u Lupinus elegans. Rytmy rozwojowe obu tych gatunków nie są więc całkowicie identyczne, lecz bardzo podobne.