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.

MATERIAL AND METHODS

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 Tápíoszelle, 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 macrosporogenesis 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 sepals (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 $\pm$ 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)

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 substages 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)

Developmental stages	<i>Lupinus elegans</i>		<i>Lupinus mutabilis</i>	
	Development of ovule and macrosporogenesis	Microsporogenesis in lower whorl in upper whorl	Development of ovule and macrosporogenesis	Microsporogenesis in lower whorl in upper whorl
Stage 1/1 Higher corolla petal below or at mid height of second whorl of anthers	from pistils with ovules in the form of undifferentiated eminences to pistils with ovules containing megasporocytes	from undifferentiated anthers to archesporial cells from anthers with archesporial cells to anthers with microsporocytes in prophase I	from pistils with not yet differentiated ovules to pistils with ovules with archesporial cell	from undifferentiated anthers to anthers with archesporial cells from not yet differentiated anthers to anthers with microsporocytes in prophase I
Stage 1/2 Higher corolla petal $\pm$ equal with apex of second anther whorl	from ovules with not yet dividing megasporocyte to ovules in prophase I	from prophase I to very young microspores	from still undifferentiated ovules to ovules in prophase I	from prophase I to very young microspores from prophase I to not yet vacuolized microspores

Stage I — closed green bud

Plate I

Lupinus elegans — longitudinal section of buds in stages I—IV, $\times 10$; 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, $\times 23$; 2. enlarged fragment of bud section shown in photo 1, $\times 90$; 3. macrosporocyte in prophase — enlarged fragment of middle ovule shown in Photo 2, $\times 1200$; 4. cross section of vacuolized microspores from anther of lower whorl shown in Photo 2, $\times 1200$; 5. cross section of vacuolized microspores from upper anther whorl shown in photo 2. $\times 1200$

Plate III

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

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*, $\times 65$; 2. *L. elegans*, $\times 13$

Table 2

Lupinus elegans

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

Collection Course of gametogenesis	L. elegans from Botanical Garden in Uppsala (Sweden) sown in Poland	Material from Kazimierski's collection Polish Academy of Sciences, Poznań, Poland	
	1974	1974	1975
Ovule — macrosporangesis	Most frequent prophase I, in buds with most developed petals further stages of meiotic division	Most frequent prophase I, in buds with most developed petals further stages of meiotic division	Most frequent prophase I, in buds with most developed petals further stages of meiotic division
Microsporogenesis in lower whorl	from tetrads to vacuolized microspores	from tetrads to two-celled pollen grains	from very young microspores to two-celled pollen grains
in upper whorl	from very young microspores to two-celled pollen grains	from very young microspores to two-celled pollen grains	from microspores to two-celled pollen grains

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)

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

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 I/3

Cultivation condition and years Course of gametogenesis	Glasshouse 1974 and 1975	Field 1974
Ovule — macrosporogenesis	prophases I most frequent; in buds with most developed petals further stages of meiotic division	prophases I most frequent; in buds with most developed petals further stages of meiotic division
Microsporogenesis in lower whorl	from tetrads to two-celled pollen grains	from very young microspores to two-celled pollen grains
in upper whorl	from very young microspores to two-celled pollen grains	from slightly vacuolized microspores to two-celled pollen grains.

Table 5

Lupinus mutabilis

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

Years and growth conditions Course of gametogenesis	Glasshouse 1974	Field 1974	Glasshouse 1975
Ovule — macrosporo- genesis	Sporadically meiocytes do not divide yet, prophases I most frequent; in buds with most developed petals sometimes further stages of meiotic division may be found	prophases I most frequent, in buds with most developed petals sometimes further stages of meiotic division may be found	prophases I most frequent, in buds with most developed petals sometimes further stages of meiotic division to uninucleate embryo sacs inclusively may be found.
Microsporogenesis in lower whorl	from sporadically occurring prophases I to two-celled pollen grains	from sporadically occurring metaphases I to two-celled pollen grains	from metaphases I to two-celled pollen grains
in upper whorl	from anaphases and telophases to two-celled pollen grains	from tetrads to two-celled pollen grains	from very young microspores to two-celled pollen grains

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-Tszyan-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, whereas in the studied lupin species the first prophase appears 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-incompability 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 vacuolized 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 varieties 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 (K o p c e w i c z, 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 (R o d k i e w i c z, 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 łubinów 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 korney nieco szybciej rosną u *Lupinus mutabilis* niż u *Lupinus elegans*. Rytm rozwojowy obu tych gatunków nie są więc całkowicie identyczne, lecz bardzo podobne.