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Pollenfertility and Seed Formation in the *Ornithogalum umbellatum/angustifolium* Complex (*Liliaceae/Scilloideae*)[1]

By

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Key Words: Angiosperms, *Liliaceae, Hyacinthaceae, Scilloideae, Ornithogalum, O. umbellatum, O. angustifolium.* – Polyploid complex, pollen shape, pollen fertility, seed formation, seedlings, weather circumstances.

Abstract: The pollen fertility and seed formation of six species of the *Ornithogalum umbellatum/angustifolium* complex and of seven related species were studied. Four types of pollen grains could be recognized. The pollen fertility varied greatly in this complex and is not related to the ploidy level. The seed formation of *O. umbellatum* showed an adaptation to a subcontinental-Mediterranean climate, that of *O. angustifolium* to an Atlantic climate. In both cases raindrops seem to be important for pollination, in view of the absence of insect pollinators. After open pollination 113 seedlings were obtained in four species. Their chromosome numbers were determined. Nearly all the cultivated seedlings were aneuploid, which points to a positive selection of euploids in nature, because aneuploid individuals are rare in the wild.

The complex of *Ornithogalum umbellatum* L. and related species like *O. angustifolium* (briefly called "*O. umbellatum/angustifolium* complex") (x = 9) in Europe comprises a polyploid series ranging from diploid (2n = 18) to hexaploid (2n = 54). The mode of reproduction of these taxa varies. Some species reproduce by vegetative propagules (bulbils), e.g. *O. umbellatum* L. (2n = 36, 45, 54), *O. angustifolium* Bor. (2n = 18, 27, 36) and *O. refractum* Kit. ex Schlecht. (2n = 54). Other species of the complex reproduce sexually by seeds, e.g. *O. monticolum* Jord. & Fourr. (2n = 18, 20), *O. baeticum* Boiss. (2n = 18) and *O. algeriense* Jord. & Fourr. (2n = 50). Some related taxa, such as *O. exscapum* (2n = 18, 20), *O. nivale* Boiss. (2n = 16), *O. comosum* L. (2n = 18), *O. collinum* Guss.

[1] Biosystematic Studies on the *Ornithogalum umbellatum/angustifolium* Complex III. – Previous parts of this series are Part I: Taxonomy. Proceeding Kon. Ned. Acad. Wet. series C, **85** (4), 563–574 (1982) and van Raamsdonk (1984).

(2n = 36), *O. gussonei* TEN. *p.p.* (2n = 72, 90) and *O. woronowii* KRASCH. (2n = 16) also reproduce sexually. Plants which produce bulbils usually show a reduced seed formation. This phenomenon can be observed frequently in plant groups which reproduce in both of these ways. In *Polygonum viviparum* L. an increase in the production of bulbils is combined with a decrease in the number of flowers (LAW & al. 1983). In *Ficaria verna* HUDSON some races reproduce almost solely by axillary tubercles and produce hardly any seed (GADELLA 1977). *Gagea spathacea* (HAYNE) SALISB. is completely or partially seed sterile and multiplies by means of bulbils produced under the tunic of the bulb (GUSTAFFSON 1946). The way in which reproduction is organized in a species largely determines the variation within and between populations. For that reason a detailed consideration of the mode of reproduction of the mentioned taxa is necessary.

In this paper the results of a study on seed formation after open pollination of eight cytotypes will be presented. Some of these seeds were germinated. The chromosome numbers of the seedlings are analyzed. A description of the types of pollen grains and their fertility is provided.

Material and Methods

The observations on pollen fertility are based on a study of 180 plants. The fertility of the pollen grains and their shape were studied by squashing anthers in a solution of acetocarmine 1% with ferriacetate, after gentle heating. The seed formation of plants belonging to eight cytotypes was observed for eight years (1976–1983). The number of seeds are listed in Table 2. In the month of May during these consecutive years the following eight meteorological factors were recorded (data kindly provided by the Royal Dutch Meteorological Institute, KNMI, De Bilt): 1) mean temperature, 2) average amount of rainfall per hour, 3) total amount of rainfall per month, 4) total number of hours of rainfall per month, 5) the mean relative moisture per month, 6) the number of 3-hour periods of cloudy weather without sunshine per day, 7) total amount of hours sunshine per month, 8) total amount of radiation per month. The influence of these factors on the formation of seeds was studied. Seedlings obtained after pollination were analyzed cytologically as well as morphologically (number and shape of the bulbils, fertility of the pollen grains). The female parents belonged to different cytotypes. – All of the plants were grown in the experimental garden of the State University of Utrecht, voucher specimens are deposited in herbarium U. The plants were separated into groups according to their geographical origin, cytotype and chromosome banding pattern (VAN RAAMSDONK 1984).

Results

1. Pollen Shape. There are four different types:

A: The pollen grains are stuck together in a mass (pollinium) in each locule. The individual grains are more or less dissolved and completely amorphous (Fig. 1*a*). This type did not occur frequently: only in the

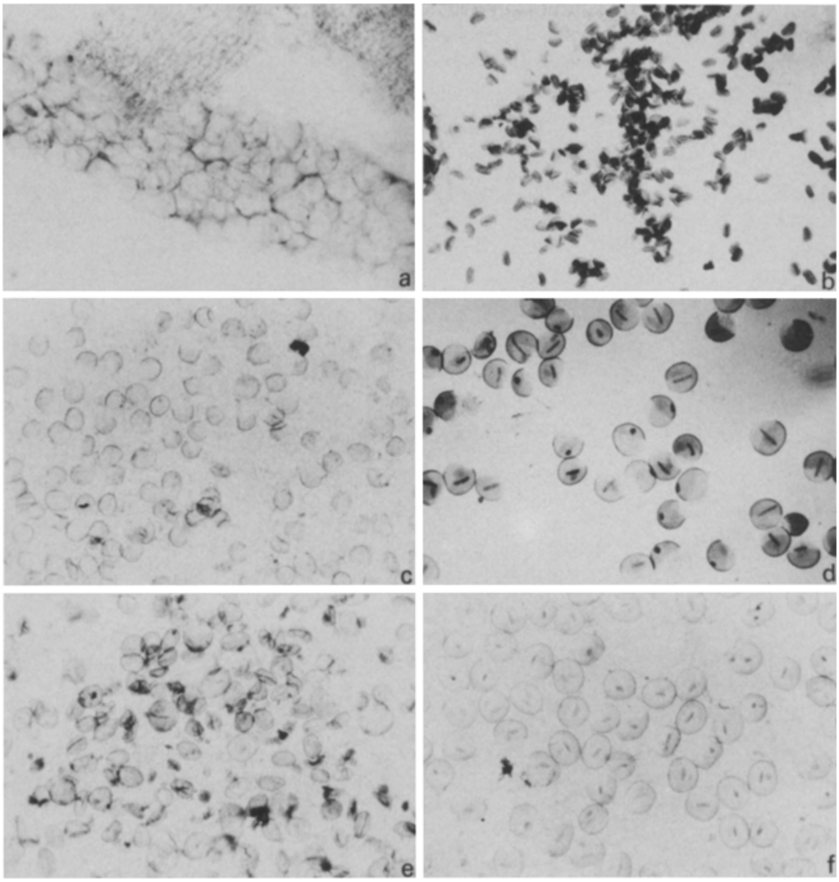

Fig. 1. Photographs showing: *a* pollen grains amorphous and stuck together [type A, *O. monticolum* (2 n = 18), French Alps); *b* pollen grains small and dented [type B, *O. monticolum* (2 n = 18), French Alps]; *c* pollen grains sterile, spherical [type C, *O. umbellatum* (2 n = 45), French Alps]; *d* pollen grains fertile [type D, *O. angustifolium* (2 n = 27), The Netherlands]; *e* a mixture of types B, C, and D [*O. umbellatum* (2 n = 54), The Netherlands]; *f* pollen grains of type D [*O. algeriense* (2 n = 50), Spain]

Dutch pentaploids, some tetraploid populations and two populations of *O. monticolum*.

B: The pollen grains are small and dented with a very regular shape (Fig. 1 *b*). This type occurs in *O. monticolum* with a very regular shape. It is usually combined with the following two types (Fig. 1 *e*).

C: Pollen grains are spherical and sometimes contained a granular

Table 1. The relative amount (in percentages) of pollen type A, of pollen type B and of the pollen fertility for each cytotype. The cytotypes are divided into geographical groups. The Standard Deviation is given in brackets

Name	2n	Origin	Type 'A'	Type 'B'	Fertility (%)
O. monticolum	18/20	France: Haut-Alpes	100 (0)	0 (0)	15 (33)
	18	France: Alpes-Mar.	0 (0)	99 (1)	1 (1)
	18	Alps	0 (0)	33 (30)	66 (32)
O. baeticum	18	Spain	0 (0)	52 (40)	47 (40)
O. angustifolium	18	Poland	0 (0)	50 (14)	45 (14)
	27	N.W.-Europe	0 (0)	25 (18)	73 (19)
	36 a	Netherlands	100 (0)	0 (0)	0 (0)
	36 b	Poland	0 (0)	21 (16)	77 (17)
O. refractum	54	Balkan	0 (0)	40 (49)	60 (49)
O. umbellatum	36	France: Yveline	0 (0)	25 (7)	72 (3)
	36	France: Var	23 (11)	57 (12)	26 (5)
	36	West-Germany	100 (0)	0 (0)	100 (0)
	36	Netherlands	100 (0)	0 (0)	0 (0)
	45	Netherlands	95 (6)	3 (4)	8 (7)
	45	W. and S. Europe	0 (0)	38 (33)	53 (37)
	45	E. Europe	0	5	95
	54	Provence/Texel	0 (0)	76 (30)	16 (30)
	54	W. and S. Europe	0 (0)	34 (31)	57 (33)
	54	Denmark	0	90	5
	54	Netherlands	0 (0)	81 (14)	15 (13)
O. algeriense	50	Spain	0 (0)	6 (11)	91 (14)
O. exscapum	18	Italy	0 (0)	5 (5)	95 (5)
O. nivale	16	Crete	0 (0)	40 (56)	60 (56)
O. comosum	18	Balkan	0 (0)	1 (0)	99 (0)
O. collinum	36	Balkan	0 (0)	15 (10)	86 (11)
O. gussonei p.p.	72	Italy	0 (0)	3 (4)	96 (4)
	90	Italy	0	30	70
O. woronowii	16	Caucasus	0 (0)	31 (41)	67 (38)
O. platyphyllum	18	Turkey	0	2	98

substance. These grains lack a nucleus (Fig. 1 *c*). This type is of regular but limited occurrence in most cytotypes and taxa.

D: The pollen grains are spherical and contained a nucleus (Fig. 1 *d* and *f*).

2. Pollen Fertility. Pollen grains of type A can be fertile, those of type B and C are always sterile and those of type D are always fertile. In most plants either pollen type A or D is present. Plants with pollen grains of the fertile type D usually lack pollinia. In Table 1 a survey is given of the pollen fertility of the different cytotypes. A considerable difference in fertility occurs in *O. monticolum*. Both a sterile and a semi-fertile group occur. It appears that in *O. umbellatum* the Dutch pentaploids, which all have pollinia, are sterile while the other pentaploids are fertile and do not have

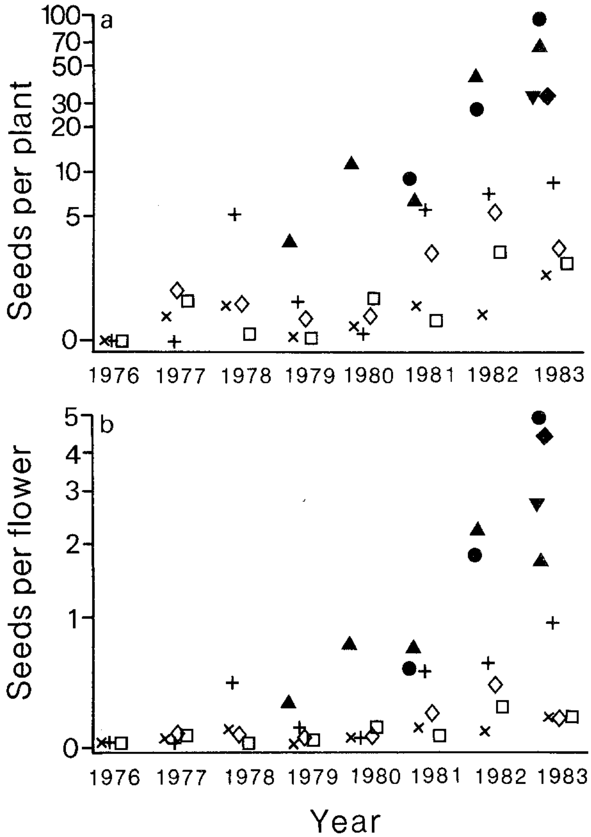

Fig. 2. The seed formation per plant (*a*) and per flower (*b*) of eight cytotypes for 8 successive years. The years are shown on the ordinate and a logarithmic scale of the number of seeds is shown on the abscissa; the symbols indicate the following cytotypes: ×: *O. angustifolium* 2n = 27, +: *O. angustifolium* 2n = 36, ◇: *O. umbellatum* 2n = 45, □: *O. umbellatum* 2n = 54, ▲: *O. monticolum* 2n = 18, ▼: *O. baeticum* 2n = 18, ●: *O. collinum* 2n = 36, ◆: *O. algeriense*

sticky pollen grains. Fertile pollen grains of type A were found in one tetraploid population of *O. umbellatum*. Differences between the ploidy levels of *O. angustifolium* and *O. umbellatum* are not very conspicuous.

The diploid species *O. exscapum*, *O. nivale*, *O. comosum*, *O. woronowii* and *O. platyphyllum*, and the polyploid species *O. algeriense*, *O. collinum*, and *O. gussonei* show a high fertility.

3. Seed formation. The total amount of seeds per plant (Fig. 2*a*) and per flower (Fig. 2*b*) is shown for eight cytotypes. From both figures it is

Table 2. The number of seeds per flower of eight cytotypes (upper part) and the value of eight weather parameters (lower part) for seven successive years

	1977	1978	1979	1980	1981	1982	1983
O. angustifolium 2n = 27	0.03	0.07	0.01	0.03	0.08	0.06	0.15
O. angustifolium 2n = 36, Netherlands	0.0	0.41	0.07	0.02	0.47	0.53	0.93
O. umbellatum 2n = 45, Netherlands	0.04	0.04	0.03	0.03	0.17	0.37	0.15
O. umbellatum 2n = 54	0.05	0.01	0.01	0.07	0.03	0.22	0.15
O. monticolum 2n = 18	–	–	0.23	0.70	0.65	2.14	1.68
O. collinum 2n = 36	–	–	–	–	0.15	1.80	5.02
mean temperature °C	11.9	12.1	11.5	12.3	13.3	12.8	*10.6*
Average amount of rainfall mm/hr	1.86	1.80	2.24	0.97	2.04	*0.89*	1.02
Total amount of rainfall/month mm	60.0	24.3	133.1	*12.8*	95.6	30.3	123.1
Hours of rainfall/month	32.2	13.5	59.4	*13.1*	46.7	33.8	119.9
Mean relative moisture %	73	78	76	*62*	76	71	84
3 hour periods of cloudy weather per day	5	5	5	*4*	4	4.3	6
Hours of sunshine/ month hr	225.1	167.8	191.7	278.6	190.5	239.4	*103.8*
Total amount of radiation/ month J/cm^2	54317	47257	50016	61067	49854	62231	*36930*

clear that the non-bulbiliferous plants (for which the closed symbols are used) produce more seeds than the bulbiliferous plants do (indicated by open symbols). An increase in seed formation could be observed from 1976 to 1983, and a more sudden change after 1980. The collection was transferred to another experimental plot in the autumn of 1980 and placed in frames facing South instead of West. It seems likely that this change in exposure is responsible for the increase in seed formation. The correlation between seed formation and weather conditions in the month of May have been calculated for 1976 to 1983. The data on the seed formation per flower and the weather conditions are presented in Table 2. The correlation coefficients for the four bulbiliferous cytotypes are presented in Fig. 3, because only these cytotypes were studied throughout the period 1977–1983. The seed formation of the two cytotypes of *O. angustifolium* show a positive correlation with the amount of rainfall. Their seed formation seems to be negatively correlated with the amount of sunshine. Hardly any correlation could be found between the seed formation of the cytotypes of *O. umbellatum* and the weather type. The only factor which seems to influence the seed formation in *O. umbellatum* is the average

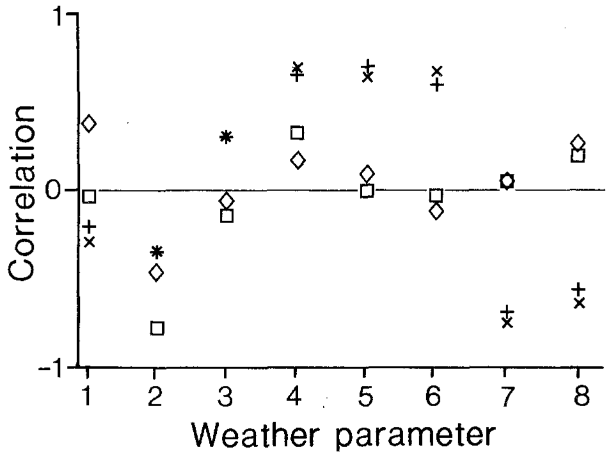

Fig. 3. The correlation coefficients of the seed formation of four cytotypes and eight weather parameters. The symbols indicate the same cytotypes as in Figs. 2 and 4. For explanation see text

amount of rainfall. Mean temperature and average amount of rainfall influences the seed formation of the two species studied almost to the same extent.

None of the flowers of the species cultivated were visited by insects. Ultra-violet light was used to locate nectar guides, but none were found.

4. Seedlings. The data on the average chromosome number and the pollen fertility for a number of seedlings are presented in Fig. 3. The female parent, the number and the shape of the bulbils are also indicated. Most of the seedlings have aneuploid chromosome numbers which may vary even within the same individual. Usually the seedlings resemble the plant from which the seeds have been derived. One seedling, grown from the seed of *O. angustifolium,* closely resembles *O. umbellatum* and has a higher chromosome number than the plant from which it arose. As a rule seedlings with $2n = 27$–36 had bulbils which were long and tapering, as in *O. angustifolium.* Seedlings with the chromosome number $2n = 36$–54

were characterized by 9–76 short and round bulbils, which were like the bulbils of the *O. umbellatum* female parent. Two seedlings with 2n = 35–36 formed both types of bulbils. One seedling of *O. umbellatum* is an exception to this rule: It combined the chromosome number 2n = 30–33 with 10 small spherical bulbils. The seedlings raised from *O. algeriense* and *O. collinum* turned out to be aneuploid, too, and in some cases formed very few bulbils. The chromosome numbers ranged from 42 to 50 in seedlings obtained from *O. algeriense* parents and ranged from 34 to 37 in seedlings obtained from *O. collinum* plants. The latter group of seedlings showed a much smaller range of aneuploidy than those of *O. umbellatum* and *O. angustifolium*. The pollen fertility of the seedlings varied to a considerable extent. The pollen type A was almost absent in seedlings obtained from the Dutch pentaploids of *O. umbellatum*. It is especially remarkable that the pollen sterile Dutch tetraploids of *O. angustifolium* produced seeds that developed into plants with a high pollen fertility.

Discussion

The occurrence of fertile odd-numbered polyploids (at the triploid and pentaploid level) and of sterile even-numbered polyploids (at the diploid and the tetraploid level) is remarkable. Odd polyploids are often sterile in many groups of plants. In *Hieracium* the triploid hybrids between sexual strains of *H. pilosella* (2n = 36) and *H. hoppeanum* or *H. peleterianum* (2n = 18) are completely sterile. Most pentaploid hybrids between sexual strains of *H. pilosella* with 2n = 36 and 2n = 54 are also sterile (GADELLA 1982). CZAPIK (1966) analyzed the meiosis of *O. angustifolium* (but published her results under the name of *O. umbellatum*). She found various numbers of trivalents, bivalents and univalents in the first metaphase stage of female meiosis. In the second meiosis of the microsporogenesis chromosome numbers between 5 and 22 occur (average number 13 or 14). 93–100% of the pollen grains proved to be germinable (CZAPIK 1966). These data correspond with the findings presented here. The pollen fertility is not disturbed by the occurrence of trivalents and univalents, or by varying and deviating chromosome numbers.

The seed formation of *O. angustifolium* is positively correlated with the amount of rainfall in the month of May. In years when May was relatively dry (1980, 1982) fewer seeds were formed. *O. angustifolium* is clearly adapted to an Atlantic climate, which is more humid than the climate which *O. umbellatum* prefers. *O. umbellatum* is a subcontinental species which formed more seeds in the drier springs of 1980 and 1982. These results agree with their geographical distributions (VAN RAAMSDONK 1984).

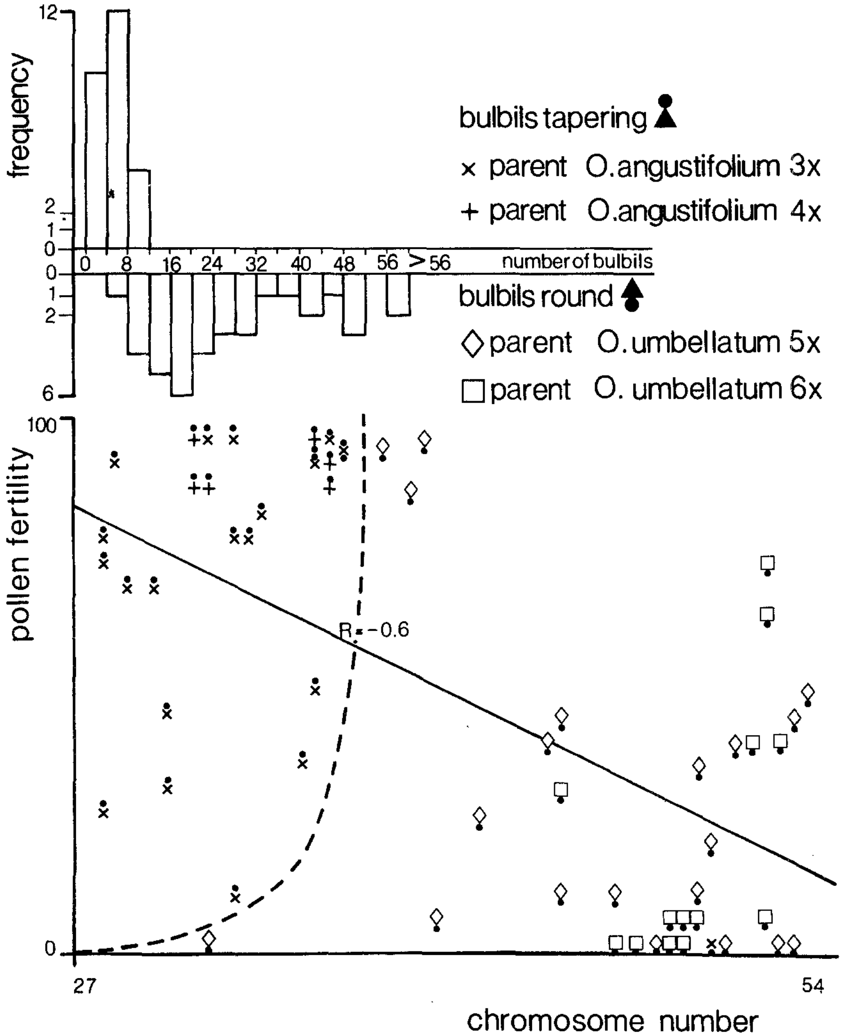

Fig. 4. On top a frequency diagram of the number of bulbils is given for each of the two shapes of bulbils. The scatterdiagram below shows the average chromosome numbers on the ordinate and the pollen fertility percentages on the abscissa for a number of seedlings. The position of the point indicates the shape of the bulbils. The symbols indicate the cytotype to which the female parent belongs. A broken line separates *O. angustifolium* from *O. umbellatum*

The seed formation of both species is correlated to a certain extent with the amount of rainfall in spring. A possible explanation is that rainfall is an important vector for pollination to the stigma of the same flower or to that of other flowers.

The chromosome numbers of seedlings of *O. angustifolium* 2n = 27 analyzed by CZAPIK (1966) ranged from 18 to 30. In my own collection they ranged from 26 to 36 (in all 40 seedlings investigated). CZAPIK, however, harvested after controlled pollination between triploid parents and not after uncontrolled or open pollination as in the present study. Therefore, the possibility of hybridization should not be excluded. This may also be deduced from the morphology of the seedlings. The offspring of *O. algeriense* form very few bulbils, whereas wild plants produce none at all. This points to the hybrid origin of the seedlings. The progeny of *O. angustifolium* (2n = 36) are highly fertile and have chromosome numbers deviating from that of the female parent. For that reason it is unlikely that this cytotype reproduces pseudogamously. Pseudogamous reproduction was taken into consideration in view of the complete pollen sterility of *O. angustifolium* (2n = 36), but the same plants did not produce seeds when they were kept apart in order to prevent pollination with foreign pollen. Apparently, these plants produce seeds after being pollinated with pollen of other plants with fertile pollen grains.

In view of the fact that aneuploid plants are hardly ever found in nature (CZAPIK 1965, 1966, GADELLA & VAN RAAMSDONK 1981, NEVES 1952, TORNADORE and GARBARI 1979) positive selection for euploid seedlings seems likely. Aneuploid chromosome numbers are the rule in seedlings which are the product of open or controlled pollination of triploid plants (CZAPIK 1966; present study). Apparently natural selection operates against such aneuploid types. From these data it is clear that triploid plants reproduce virtually only in a vegetative manner. In all cases the amount of seed formation is negatively correlated with the production of bulbils.

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