# POLLEN SIZE AND POLYPLOIDY: A REVIEW WITH STUDIES IN *DICHELOSTEMMA* AND *TRITELEIA* (LILIACEAE)[1]

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ABSTRACT: A review of work on the correlation of chromosome numbers and pollen size has shown that attempts to use pollen as a taxonomic characteristic to separate closely related polyploid taxa have generally failed because of the overlap in size between the different levels. Results with *Dichelostemma pulchellum* and *Triteleia laxa* show that pollen size is for the most part correlated with chromosome numbers up to the tetraploid level, but that no definite and trustworthy discontinuities occur between any two successive levels. The range of means for diploids of *D. pulchellum* is 32 μ to 40 μ; for tetraploids, 40 μ to 46 μ; for hexaploids, 40 μ to 44 μ; and for octoploids, 42 μ to 44 μ. The pentaploid pollen is 43 μ. Size ranges for *T. laxa* are as follows: diploid, 40 μ to 50 μ; tetraploid, 42 μ to 48 μ; and hexaploid, 53 μ to 55 μ. The diploid with $2n = 14 + 1$ B had pollen with an index of 44 μ. A comparison of sizes of re-expanded, eight-month old pollen with fresh pollen indicates that dried material is capable of assuming its regular proportions, at least in lactophenol. No particularly large differences were found in the sizes of pollen taken from different anther types—that is, external, short anthers opposite the outer perianth lobes; and internal, long ones opposite the inner perianth lobes. Plants with irregular meiosis often produce high amounts of well-formed and stainable pollen, but in some cases this was not at all true. Production of poor pollen is correlated with the presence of lagging chromosomes at anaphase.

## INTRODUCTION

Discovery that one of De Vries' original mutations of *Oenothera lamarckiana* De Vries (= *O. erythrosepala* Borbas), mutant "gigas," was a tetraploid derivative from that diploid species and subsequent artificial production of numerous other tetraploid forms led naturally to morphological and anatomical, as well as cytological, investigations. Winkler's (1916) thorough study of the "gigas" forms of *Solanum nigrum* L. and *Lycopersicon esculentum* L. was perhaps the first example of the direct production in a laboratory experiment of a new constant genetic type. He grafted the two species together and found that tetraploid adventitious shoots sometimes arose from the scar tissue at the point of grafting. Palisade cells, guard cells, pith cells, trichomes, and pollen grains all were larger in these tetraploid shoots than in the diploids.

Renner (1919) gives extensive pollen measurements for nine species and 41 hybrids of *Oenothera*. In almost all cases the data are based on measurement of 100 or more grains stained with IKI and mounted in glycerin. *Oenothera biennis*

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L., *O. suaveolens* Pers., and *O. parviflora* L. produce two classes of pollen: large, active grains and smaller, inactive ones. This can be adequately explained as the result of gametic or gametophytic lethals that prevent complete development of the pollen or inhibit pollen tube growth. A number of the hybrids show a similar dimorphism, but it is sometimes not as pronounced. *O. hookeri* Torr. & Gray and *O. erythrosepala* show a unimodal size distribution because the four genomes involved carry no gametophytic lethals. The hybrid *(O. hookeri* X *erythrosepala)* X *velutina* is unimodal for apparently the same reason; but *(O. erythrosepala* X *parviflora)* X *gracilis* is bimodal and produces no inactive grains. It may be that pollen size is under genetic control here, regardless of the presence or absence of a lethal. Small-flowered species had the smallest pollen, large-flowered species had the largest, and middle-sized flowers had intermediate pollen. The size differences in Renner's study are obviously not the result of polyploidy because he used only diploids. Perhaps just as interesting is his conclusion that starch grain morphology alone is enough to distinguish most of the species and hybrids in the experiment.

Much of the subsequent work on the actual relationship of polyploidy to cell size was more cursory. Belling and Blakeslee (1923) showed that there was an increase in volume of pollen mother cells from haploid through tetraploid *Datura,* and in addition, that pollen from triploids was characterized by a large number of empty grains and a great diversity in size. A diploid *Oenothera* "gigantea" differed from its tetraploid parents and its sister plants in having smaller nuclei and cells, and three-cornered instead of four-cornered pollen (Håkansson 1925). Comparison of "univalens" and "bivalens" races of the mosses *Amblystegium serpens* (L.) B. & S., *Bryum caespiticium* L., and *Bryum corrensii* revealed a similar connection between chromosome number and cell size (Wettstein 1924, 1937). In some species a positive correlation was found through the whole range of polyploidy from haploid to octoploid and higher, but in others a maximum cell size was reached beyond which an increase in chromosomes could even result in dwarfism.

In an experiment that confirmed Winge's hypothesis, Clausen and Goodspeed (1925) found a two to one pollen volume ratio of hexaploid *Nicotiana* "digluta" (= *N. glutinosa* L. X *N. tabacum* L.) ($n=36$) to tetraploid *N. tabacum* ($n=24$). Additional cases demonstrating the relationship of pollen size to polyploidy were found in *Crepis* (Navashin 1925), *Draba* (Heilborn 1927), and *Rumex* (Kihara and Ono 1926). In their paper on *Rumex,* Kihara and Ono introduce the terms "autopolyploidy" and "allopolyploidy."

Müntzing (1928) published the first comprehensive statistical treatment of the relationship between chromosome number, nuclear volume, and pollen grain size, using eight species of *Galeopsis.* The mean sizes based on a rather small sample of 20 to 30 grains, for the six diploid and two tetraploid species were 31.50 ± 0.14 and 34.01 ± 0.12 μ respectively, with $D/m_D=13.1$. Pollen from ten plants of a single biotype of a *G. tetrahit* line ranged from 32.8 ± 0.31 μ to 36.1 ± 0.36 μ. The danger of drawing conclusions from insufficient data, as Wettstein and most of the others had done, is clearly indicated here. Pollen mother cell and nuclear

volume also show significant differences between diploid and tetraploid species. In all instances, however, *G. speciosa* Miller, a robust diploid, more closely approached the tetraploids than it did any of the remaining eight-chromosomed species. Müntzing also compared measurements on two lines, one diploid and one tetraploid, in water, in glycerin, and in dry air:

in water, $\frac{34.61\ u}{31.45\ u}=1.10$; in glycerin, $\frac{30.25\ u}{27.45\ u}=1.10$; in air, $\frac{30.96\ u}{28.54\ u}=1.09$.

The similarity of the indices appears to indicate that consistency is not especially important in the choice of a mounting medium; however, this is surely a very unsafe generalization.

Dermen (1931) found some correlation between chromosome number and pollen size in *Petunia*. Pollen from diploids was between 32.04 ± 0.13 μ and 37.65 ±0.18 μ in diameter, but that of tetraploids ranged from 39.78±0.21 μ to 43.62±0.19 μ. The small mean sizes of two triploids fell within the diploid range, and the large mean of another fell just short of the tetraploid range. Diploids produced two to 14 percent large, defective grains; tetraploids, six to 19 percent defective grains and one to 20 percent microcytes; and triploids, not unexpectedly, 19 to 37 percent defective grains, 11 to 28 percent microcytes, and three percent giant grains, apparently containing 3*n* (21) chromosomes. There was a close correlation between percentage of abnormal divisions and percentage of defective grains, and it may be that the microspores lack at least one of the basic seven chromosomes.

The relationship is present but not particularly outstanding in several other plants, such as *Allium* (Levan 1932, 1933), *Dianthus* (Howard 1968), and the apple variety Hibernal (Vaarama 1948). In the last-named case the size of mature pollen fluctuates little despite chromosome numbers varying from $n=26$ to $n=35$. Pollen size in *Magnolia* is not at all well correlated with the chromosome number (Canright 1953). In the diploids size latitude of the ellipsoidal pollen runs from 46 X 39 μ to 80 X 58 μ; in the tetraploids, from 51 X 37 μ to 58 X 43 μ; and in the two hexaploids, from 60 X 48 μ to 99 X 65 μ. The tetraploid actually has smaller pollen than the diploid.

Only a few of the multitude of plants showing a correlation that have been investigated in the last 40 years can be given here: *Campanula* (Hubac 1972); *Cannabis, Cosmos,* and *Portulaca* (Blakeslee 1941); *Digitaria* (Gould 1963); *Dactylis* (Müntzing 1937); Lycopersicon (Lindstrom and Koos 1931; Lindstrom and Humphrey 1933); *Medicago* (Julen 1944); *Nasturtium* and *Cakile* (Green 1955); *Petunia* (Ferguson and Coolidge 1932); *Phleum* (Müntzing 1935); *Poa* (Akerberg 1942; Love 1952; Müntzing 1940; Nissen 1950); *Populus* (Johnsson 1945); *Ribes* (Vaarama 1948); *Secale* (Müntzing 1951); and *Valeriana* (Skaluska 1947). Schwanitz (1952a) demonstrated polyploid size correlations for a large number of plants, including *Althaea rosea, Campanula medium, Helianthus annus, Achillea ptarmica, Tagetes patula,* and many others.

Members of the *Sanicula crassicaulis* complex show a smooth, even increase in pollen size (Bell 1954). The diploids and octoploids of *S. crassicaulis* Poepp.

could probably be distinguished by pollen size alone, but the hexaploid has a range nearly as great as their ranges combined; furthermore, the largest pollen is found in the diploid *S. bipinnatifida* Dougl., although its mean is lower. Other diploids have different means and extremes, apparently due to specific differentiation. Total samples show considerable variation, but samples from individual plants are rather uniform.

Niehaus (1971) included data on pollen sizes in the genus *Brodiaea* sens. str. In this case pollen size bears the same direct relationship to chromosome number discussed above, at least in most of the polyploid pairs and series studied. The differences in sizes at the different levels are pronounced, except in *B. elegans* in which one of the tetraploids has pollen 64.1 μ in length and the other, 71.2 μ, and the diploid pollen is 61.2 μ. Pollen of *B. purdyi* decreases from the diploid (61.3 μ) to the tetraploid (55.6 μ).

If discontinuities between polyploid levels are great enough, pollen size may be used as a taxonomic characteristic to separate closely related species. In *Andropogon* mean pollen sizes for taxa with $2n=60$ are 28.9 μ, 33.4 μ, and 35.1 μ; for taxa with $2n=120$, 37.1 μ, 37.2 μ, 38.3 μ, 40.6 μ, and 42.3 μ; and for the taxon with $2n=180$, 48.5 μ (Gould 1957). The two distinct size classes at the $2n=120$ level supposedly reflect differences in mean pollen size of presumed ancestral taxa. Variation in pollen size is due to genetic differences between populations with the same chromosome number and to secondary environmental changes. These are small compared to alterations resulting from polyploidy.

In a paper of unusual interest Hotchkiss (1955) found that tetrads of *Drimys* section *Tasmannia* are conspicuously smaller than those of section *Drimys*. Bailey and Nast (1943) pointed this out earlier and indicated that they found difficulty in distinguishing species and varieties within the section *Drimys* by pollen analysis, but that they could easily separate species of the section *Tasmannia*, as well as the genera *Belliolum*, *Bubbia*, and *Pseudowintera*, by size and form of tetrads, diameter of the germ pore, and other characteristics. Hotchkiss' measurements confirmed this and showed that there is indeed considerable variation in tetrad size in section *Drimys*, contrasted with the more stable sizes in section *Tasmannia*.

Using species rather than polyploid series, Sokolowskaja (1955) compared pollen grain size and chromosome number in some Arctic grasses. The correlation was not exactly glaring in some cases, for instance in *Festuca* and *Poa*, where there was only a gradual increase in species with greater and greater chromosome numbers; but the relationship was considerably more pronounced in *Elymus*, *Puccinellia*, *Aneurolepidium*, and *Anthoxanthum*. In another paper (Sokolowskaja 1958) a similar study was presented comparing the pollen of some Saxifragraceae and Ranunculaceae. Within the various subgeneric groupings studies of *Saxifraga* and *Ranunculus*, the increasing size relationship was present, although it was relatively feeble in several cases: in the section *Stellares* of *Saxifraga*, increasing the chromosome count from $2n=20$ to $2n=28$ and $2n=56$ resulted in pollen 15 u, 17.5 μ, and 18.5 μ, respectively. In the section *Punctatae*, *S. punctata* L. with $2n=28$ has pollen 13 μ in diameter, and *S. nelsoniana* D. Don with $2n=\pm 70$ has pollen only 3 μ larger. The individual species examined in several of the other sec-

tions show no correlation at all. Species with similar pollen sizes may have chromosome numbers differing by a factor of five. In the polyploid pair of *Parnassia palustris*, the correlation was as usual. Species within the various sections of *Ranunculus* showed a much better correlation than was seen in *Saxifraga*. This was true also in the two species each of *Delphinium* and *Caltha* examined. Obviously we need to know more about the polyploid origins of the taxa mentioned above before a clear statement can be made about the pollen size relationships encountered.

In addition to measuring pollen size another popular method of detecting polyploids has been to measure stomata. Positive correlations have been found in *Carya* (Stone 1961), *Cymbidium* (Wimber 1954), and *Secale* (Speckmann, Post and Dijkstra 1965). Sax and Sax (1937) demonstrated that in *Tradescantia* chromosome number and stomatal distribution are more clearly associated than are chromosome number and stomatal length. Similarly, Mochizuki and Sueoka (1955) found no difference in guard-cell chloroplast size between diploid, triploid, and tetraploid sugar beets, but instead a geometric increase in their number. A similar effect was noticed in the mesophyll cells of *Solanum nigrum* L. (Winkler 1916) and in the mosses *Amblystegium serpens* (L.) B. & S. and *Bryum cirratum* Hoppe & Hornsch. (Wettstein 1924). This is to be expected if the volume of the cells increases.

B-chromosomes have a negligible effect on cell size when present in small numbers (Peterson and Munson 1962). Larger numbers can have pronounced effects, however, including increase in nucleus and cell size, variation in pollen size, and increase in the number of aborted grains. The larger grains of a plant with many B-chromosomes, maize in both cases here, may exceed the dimensions of pollen produced by triploids and tetraploids (Randolph 1941 b). There is probably a kindred effect on stomata (Müntzing and Akdik 1948).

Trombetta (1942) has reviewed the matter of the cytonuclear ratio. She mentions two examples outside the higher plants in which cell and nucleus size is dependent on chromosome number, namely in *Spirogyra* and tetraploid echinodern larvae. In the latter case the surface area of the nucleus, rather than its volume, is proportional to the number of chromosomes. The size of the cell is in turn proportional to the nuclear surface area.

Thus, an increase in cell size, whether subtle or obvious, seems to be a general result of an increase in chromosome numbers in plants and animals. Selection may act quickly or slowly to obscure size relations that exist in a series of polyploids, however. Wettstein (1937) found that after 11 years of inbreeding, the size of spores in tetraploid capsules of his moss species had returned to characteristric diploid size. On the other hand two strains of *Datura stramonium* L. were inbred for 13 and 14 years and still held tenaciously to the original polyploid size (Blakeslee 1941). Shetty (1959) found that several allopolyploid species of the Vitaceae showed no correlation at all, and Earnshaw (1942) found a similar situation in maritime plantagos. Once specific differentiation has been attained beyond the mere doubling of the chromosomes, the relationship may or may not hold true. Method of pollination, pollinator, and even location of pollen

on individual insects all could theoretically be affected by the size of pollen grains. In this way size of the pollen could reinforce breeding barriers between polyploids and their diploid parents. In such cases one would expect selection for maintenance of increased pollen size, whereas in anemophilous plants (e.g. the Arctic grasses and *Plantago)* the size of the pollen might be inconsequential in this respect. Size relations resulting from selection must be regarded as relative, in any event, for within a single season pollen size may be affected by any number of modifying conditions.

Nutrition and the position of the flowers may be very important. Testing clones in wet and dry soil, Schoch-Bodmer (1940) found the smallest pollen in *Lythrum salicaria* L. to be associated with lateral flowers from dry soil plants and the largest pollen to be associated with terminal flowers from wet soil plants. In *Oenothera* (Krumbholz, see Schoch-Bodmer 1940) and in *Verbascum* (Schwanitz 1952b) pollen size decreased toward the end of the season, apparently due to decrease in available water. Pollen from the early flowers of *Verbascum* was 24.7 ± 0.61 μ in diameter (based on 100 grains), but that from later flowers was 18.5 ± 0.06 μ. Bell (1954) found no relationship between flower position and pollen size in *Sanicula* but did find a distinct relationship between pollen size and environmental conditions in natural populations. Root crowding in greenhouse pots was an insignificant factor, however. In later study (Bell 1959), found that mineral nutrition affected the amount of variability, but no pattern of pollen size variation related to blooming date or to type of mineral deficiency could be detected in the seven species he studied.

Mineral deficiencies and excesses have also been shown to affect directly nuclear volume and size of chromosomes (Pierce 1937).

Pollen commonly shows more intraindividual than intraspecific variability, for instance, in *Nothofagus, Lycopersicon, Solanum, Allium,* and *Avena* (references in Bell 1959); yet others show considerable uniformity, as in *Betula.* In this last case the diameter of pollen from the top of the tree was not significantly different from that of the lower half, and size differences among the different parts of a catkin were negligible (Clausen 1960).

Another source of size differences within a species in heterostyly. Rather striking examples of pollen dimorphism can be seen in *Psychotria* and *Uragoga* (Baker 1958); in *Rudgea* (Baker 1956); in the majority of species in the tribe Staticeae of the Plumbaginaceae (Baker 1953); in *Amsinckia* (Ray and Chisaki 1957); in *Pontederia* (Ornduff 1966); and in *Lythrum* (Schoch-Bodmer 1940). Bodmer (1927) found in *Lythrum salicaria,* in addition to the usual pollen size differences related to heterostyly, that within the same flower green pollen was somewhat larger than the yellow. This comparison was not valid between different flowers of the same individual.

In *Primula* heterostyly is combined with heteranthery, and the size of the pollen and the number of apparently viable grains are not dependent on the anther position. As is the case with other dimorphic plants, however, full fertility is reached only when the correct pollen type falls on the corresponding stigma: large

pollen grains on the stigmas of the long-styled plants, smaller pollen on the stigmas of the short-styled plants (Ernst 1953).

Pollen from the innermost (long) stamens of *Bombax* was found to be longer than that from the other two kinds. The outer ones have the smallest pollen, whereas the inner, short ones have intermediate-sized pollen; furthermore, pollen from peripheral stamens has a higher expansion capacity than the rest (Davis 1965). A comparison of pollen size in levo- and dextro-rotary flowers of several species of Bombacaceae revealed no differences (Davis 1967).

A number of mechanical problems affecting the results of pollen studies should be mentioned here. The first is squashing and deformation of pollen after mounting. Cushing (1961) found that compression by the cover slip caused a gradual increase in the size of *Corylus* pollen, apparently resulting from the effects of glycerin and glycerin jelly on the elastic properties of the exine. According to Cain (1944) 95 percent alcohol and boiling water treatments of pollen gave similar sized grains in a given species of *Abies*, but acetolysis and KOH resulted in an 8.5 percent enlargement. Buell (1946) showed that treatment of *Pinus palustris* and *P. serotina* pollen with 10 percent KOH did not swell the grains as much as the acetolysis method. He also found that pollen in peat bogs apparently "fossilizes" within a few years in such a way that it will not swell to the same size as herbarium pollen of the same species after acetolysis or the KOH treatment. Ferguson and Coolidge (1932) strongly recommended an oily medium after discovering that grains do not swell uniformly in water. Water mounts are thus of dubious value for correct size determinations, but they may still show the gaps between polyploid levels in some circumstances. Kihara and Ono (1926) made their study of *Rumex* pollen in water and recorded the following: *R. alpina* L. ($n=10$), 185 μ; *R. obtusifolius* L. ($n=20$), 203 μ; *R. crispus* L. ($n=30$), 222 μ. These values are obviously erroneous, expecially for wind-pollinated species: only a few angiosperms are known to have pollen grains this large. Wodehouse (1935) gives 26.4 μ to 31 μ for *R. obtusifolius* and 28.5 μ to 32.0 μ for *R. crispus*, presumably with glycerin jelly as the mounting medium. If his sizes are representative, those of Kihara and Ono are seven times too large; but the gaps between the polyploid levels still show up. The source of this error is more likely an incorrect conversion factor than enormous swelling in water. Kihara and Ono also report no pollen size differences in the subgenus *Lapathum*. Their graph shows a bimodal distribution of pollen size for *R. hydrolapathum* Hudson that falls mostly well within the ranges of the other three species. Because the haploid number for this species is 100, one might expect its pollen to be larger than the rest. That it is not is perhaps an artifact caused by expansion of all grains up to a certain maximum size.

Obviously, however, pollen size does not increase indefinitely with an increasing number of chromosomes, as hinted earlier. In the *Bouteloua curtipendula* complex the relationship holds true up to the hexaploid level ($2n=60$), but above this ($2n=69$ to 103) mean pollen size and coefficient of variation show no increase (Kapadia and Gould 1964).

Löve (1944) added a new dimension to the study of pollen size in *Rumex*. He

found very distinct size differences here (size ratios from the diploid through the octoploid level were 100: 112: 121: 137), and he pointed out that pollen of dioecious plants does not show a bimodal curve resulting from differences in the mass of chromatin in male- and female-determining pollen grains.

Any work on pollen grain size must take these factors into consideration. Care must be taken to record pertinent ecological data and microhabitat conditions that might influence the nutrition and growth, and therefore the pollen size, of the species under investigation. Sampling techniques must be thorough and possible intrafloral and interfloral differences should be checked. In greenhouse material an attempt must be made to keep conditions uniform. Even with all the limitations mentioned above, pollen data can be used to check herbarium material for presumptive polyploids that can then be verified in field studies. Correlation of increasing pollen size with increasing polyploid level has in some cases been easily recognizable, despite the use of pollen from different sources (e.g., in *Sanicula*); but in other cases one can expect that even the most meticulous methods will give disappointing results. As mentioned before, varying selective pressure on pollen size in different groups of plants will affect the usefulness of pollen data. One can surmise that the increasing size of pollen grains in the species of *Sanicula* studied by Bell (1954) did not adversely affect their ability to adhere to pollinating insects. Selection might also act to increase the size of already enlarged tetraploid grains to diminish introgression with the diploid parents, as long as concomitant overlap with some higher polyploid level did not result. The conditions of this statement will obviously depend on the fertility relationships between polyploids in different taxa. Conceivably pollen size could give a rough estimate of the comparative ages of different polyploid populations within a species. The variability of pollen, however, will seldom allow pollen measurements to be used in place of actual chromosome counts.

## MATERIALS AND METHODS

Ideal plants for the type of study undertaken here are those species that form reasonably long polyploid series. Chromosome numbers in *Dichelostemma pulchellum* Heller extend from the diploid ($2n=18$) to at least the octoploid level ($2n=72$). *Triteleia laxa* Benth. has no detected octoploids, but does form two types of tetraploid, one with the base number eight ($2n=32$), the other with the base seven ($2n=28$). All representatives of these different levels were collected by L. W. Lenz from natural populations in California and propagated in the screenhouse at Rancho Santa Ana Botanic Garden. Dr. Lenz also generously supplied the chromosome counts.

Pollen slides were made by dusting fresh pollen from recently dehisced anthers directly into a drop of lactophenol. In most cases this rather viscous medium supported the cover slip well and no squashing occurred. If too little is used, grain deformation occurs; no such slides were used in this study. Bursting was rarely a serious problem.

Measurements were made with an ocular micrometer at a magnification of

900 X. Those made at 450 X were found to be more variable and less accurate. Because the pollen is elliptical, both the polar and the equatorial axes were determined in side view, an easily recognizable and frequently occurring orientation. An average of the two values multiplied by the conversion factor 1.14 gave the pollen area in microns. Fifty grains from each slide were measured to the nearest micron using an ocular micrometer. Other than this, strict attention was given to significant figures, and in statistical work the last figure is often considered "questionable" anyway. Pollen was selected for measurement systematically over at least three fourths of the area under the cover slip in order to avoid bias that might occur from uneven distribution of different sized grains.

Buds for study of meiosis in pollen mother cells were collected between noon and 2 PM (1400 hrs) and fixed overnight in acetic-alcohol. The anthers were then macerated in aceto-carmine and allowed to sit for maximum staining.

Pollen fertility and sterility percentages are based on counts of 1000 to 1500 or more grains. Grains with vacuolate contents were labelled intermediate, and those well-stained were considered viable, though this is well known to give only an estimate of the true viability.

Each plant is identified by a five digit propagation number followed by a dash and the number of the individual, except in two cases in which no propagation number has been assigned by the botanic garden.

## RESULTS AND DISCUSSION

The following discussion concerns data summarized for *Dichelostemma pulchellum* Heller (Fig. 1) and for *Triteleia laxa* Benth. (Fig. 2). Mean pollen size for all diploids of *D. pulchellum* measured was 35 μ, with a range of 32 μ to 40 μ (Fig. 5-10). The largest pollen from a diploid was 1 μ larger than that from the smallest tetraploid and hexaploid, hence the break between polyploid levels is not as great as one might wish for purposes of taxonomic identification. A pollen population with a mean size of less than 36 μ can most probably be regarded as having come from a diploid plant, but above this level, one must be cautious because of the rather high standard deviation.

The tetraploids would be well separated from the diploids were it not for the collections of R. F. Thorne 35775 from Santa Catalina Island and H. J. Thompson 3399 (propagation no. 12526; Fig. 9) from Todos Santos Island near Ensenada, Baja California. These two had essentially the same mean. The overall mean for the tetraploids was 43 μ, well above that of the diploids (Fig. 8). Volumes of diploid and tetraploid grains were roughly in a ratio of two to one. For example, a comparison of smallest to smallest and largest to largest grains from each category gave the ratios 14,800: 27,900 (1:1.87) and 23,700: 44,900 (1:1.89), respectively. The other tetraploid with rather small pollen (Bann-6) is designated as *D. pulchellum* var. *pauciflorum* and was collected near Banning in Riverside County.

Mean size for pollen of all the hexaploids measured was 42 μ, and the range of the means was entirely within that of the tetraploids (Fig. 6). The octoploids

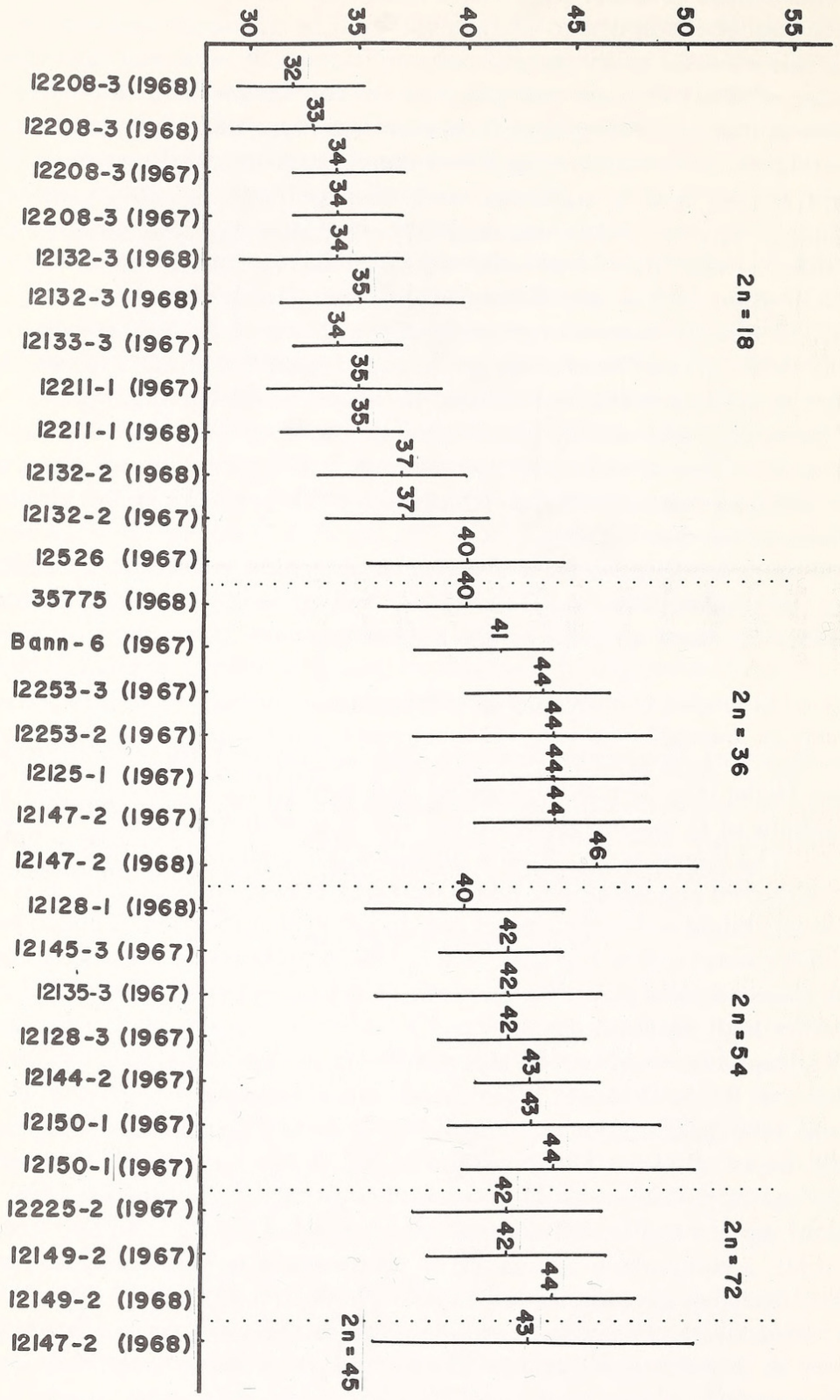



FIGURE 1. Range and mean pollen size in *Dichelostemma pulchellum*. Pollen size in μ is given on the ordinate axis, propagation number and year of cultivation on the abscissa.

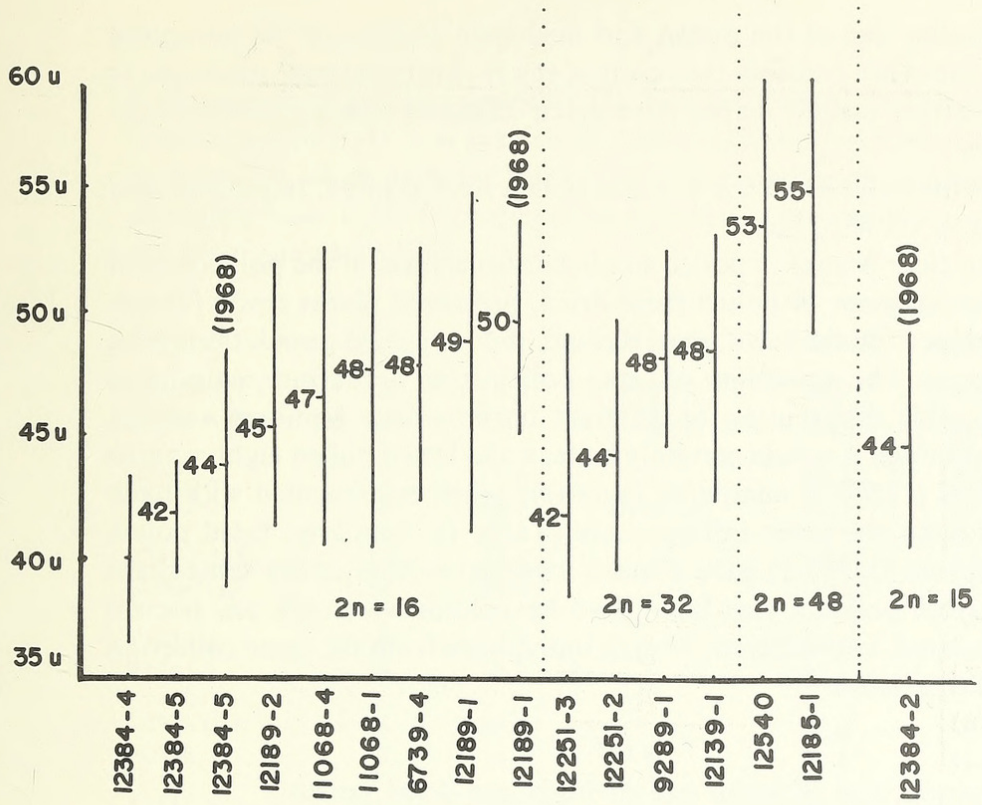



FIGURE 2. Range and mean pollen size in *Triteleia laxa*. Year of sampling is 1967 except where otherwise noted.

had a mean of 42 μ (Fig. 5); and the pentaploid, 43 μ (Fig. 7). Thus, increasing the chromosome number beyond 36 does not necessarily result in a proportional increase in pollen size. The single pentaploid came from a population of tetraploids near Santa Rosa, Sonoma County, and had a mean less than theirs. Standard deviation, and hence variability, increased slightly with increase in chromosome number, but the correlation was not particularly good.

Diploid and tetraploid levels in *T. laxa* are reminiscent of the situation in *Sanicula* (Bell 1954) in that they are also poorly defined by pollen size (Figs. 12 and 14). There is a smooth transition from the smallest pollen of a disploid to the largest, and again this range entirely overlaps that of the two different types of tetraploid. Diploids are 40 μ to 50 μ, with an average of 46 μ; tetraploids range from 42 μ to 48 μ. The one tetraploid (12251) has $2n=28$ and has apparently arisen from a base of seven, whereas the common base in *T. laxa* is eight (Fig. 13). It has misshapen anthers and conspicuously smaller pollen than the other tetraploids.

The hexaploids are well-setoff, but future discoveries could easily abolish the discontinuity (Fig. 15). Octoploids, unknown at present, might also fall within this range. The mean for the two hexaploids is 54 μ.

A diploid with $2n=14+1B$ and a mean of 44 μ was detected in a population of normal diploids with $2n=16$ (Fig. 11). This mean is near the mean for all

diploids. The smaller size of the pollen and mishapen anthers of the tetraploid based on this number are possibly the result of the B-chromosomes, although, as mentioned before, they usually do not have much influence unless present in fairly large numbers.

The ratio of the volumes of the smallest pollen from diploid, tetraploid, and hexaploid plants is 1:1.63: 231.

Although no clear breaks in pollen size occur for certain in the polyploids of either genus, measurement of pollen from dried, preserved plants could furnish presumptive evidence for the location of diploid and polyploid populations in at least some instances. The possibility of using pollen from herbarium material to determine geographic distribution of different chromosome numbers was not tested directly, although it would certainly be feasible. Dried pollen eight months old from a diploid (12208-3) compared favorably when re-expanded with fresh pollen mounted from the same inflorescence (Table 1). Similarly, dried pollen from another diploid (12211-1) gave a mean identical to that of the same plant measured from fresh pollen a year later. Two hexaploids were only one micron apart. Thus compared, two different *T. laxa* individuals from the same collection site were also rather close.

TABLE 1

Comparison of mean size of fresh and dried pollen

| 2*n* | Population | Mean | Age | Species |
|---|---|---|---|---|
| 18 | 12208-3 | 34 ± 1.0 | Fresh | *D. pulchellum* |
| | 12208-3 | 34 ± 0.99 | 8 months | |
| 18 | 12211-1 | 35 ± 1.0 | Fresh | *D. pulchellum* |
| | 12211-1 | 35 ± 1.7 | 9 months | |
| 54 | 12150-1 | 44 ± 2.4 | Fresh | *D. pulchellum* |
| | 12150-1 | 43 ± 1.9 | 8 months | |
| 18 | 12384-4 | 40 ± 1.3 | Fresh | *T. laxa* |
| | 12384-5 | 42 ± 0.95 | 8 months | |

Bell's (1954) measurements of *Sanicula* pollen are about one half from herbarium material and one half from greenhouse plants. Gould (1957) used pollen from both herbarium specimens and dried inflorescences of *Andropogon* and employed herbarium sheets exclusively in his study of *Digitaria* (1963). Kapadia and Gould (1964) combined measurements from wild plants, greenhouse clones, and herbarium sheets in presenting their data on *Bouteloua curtipendula.*

Because Davis (1965) found differences in pollen size among the three anther types in *Bombox,* a similar check was undertaken in *D. pulchellum.*

| | |
|---|---|
| 12208-3 long anthers | 32 ± 1.6 μ |
| 12208-3 short anthers | 33 ± 1.3 μ |
| 12132-3 long anthers | 35 ± 1.8 μ |
| 12132-3 short anthers | 34 ± 1.3 μ |

TABLE 2

Comparison of pollen variability (s), percent normal and abnormal grains, and appearance of PMC's at meiosis in *Dichelostemma pulchellum*. (L) indicates long anthers; (S), short anthers. Data from first member of each pair are from 1967, the other from 1968. If propagation number is given only once, all data under it are from the same year.

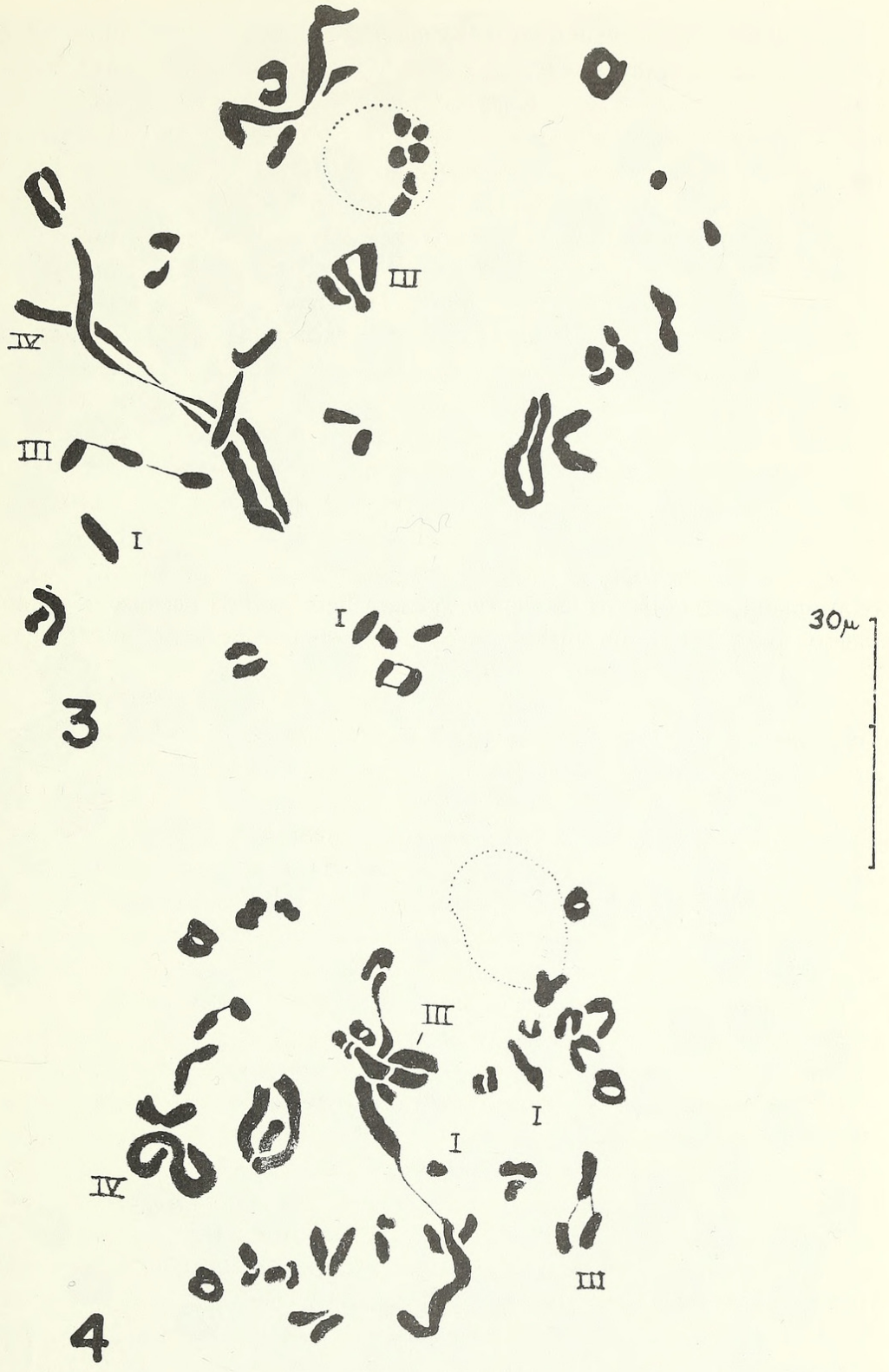
| Prop. No. | 2*n* | s[1] | Meiosis | Pollen % good | Pollen % empty | Pollen % intermediate |
|---|---|---|---|---|---|---|
| 12208-3 (L) | 18 | 16.8 | Regular | 96.7 | 3.30 | — |
| 12208-3 (S) | 18 | 13.9 | Regular | 97.3 | 2.70 | — |
| 12132-2 | 18 | 16.6 | — | 69.2 | 30.3 | 0.500 |
| 12132-2 | 18 | 16.7 | Irregular | 83.7 | 15.8 | 0.502 |
| 12132-3 (S) | 18 | 14.4 | — | 63.0 | 37.0 | — |
| (1968) (L) | 18 | 19.4 | — | 69.9 | 30.1 | — |
| 12211-1 | 18 | 17.5 | — | 99.2 | 0.800 | — |
| 12211-1 | 18 | 10.7 | — | 98.5 | 1.50 | — |
| 12526 (L) | 18 | 24.7 | — | 42.8 | 56.8 | 0.400 |
| 12526 | 18 | — | — | 52.6 | 47.4 | — |
| 12253-3 | 36 | 16.7 | — | 97.1 | 2.30 | 0.600 |
| 12253-2 | 36 | 22.9 | — | 80.3 | 18.5 | 1.20 |
| 12147-2 | 36 | 19.6 | — | 44.5 | 54.4 | 1.10 |
| 12147-2 | 36 | 18.9 | Irregular | 60.0 | 39.2 | 0.840 |
| 12125-1 | 36 | 19.4 | Regular | 99.7 | 0.300 | — |
| 12147-1 | 45 | 23.1 | Irregular | 95.7 | 3.73 | 0.574 |
| | | | | 89.4 | 6.61 | 4.02 |
| 12145-3 | 54 | 16.3 | Irregular | 94.6 | 5.21 | 0.226 |
| 12128-1 | 54 | 20.4 | Irregular | 99.2 | 0.800 | — |
| 12128-3 | 54 | 16.8 | — | 95.6 | 4.39 | — |
| 12150-1 | 54 | 19.5 | — | 98.9 | 0.900 | 0.200 |
| (1967) | 54 | 25.1 | — | 93.5 | 6.54 | — |
| 12149-1 | 72 | 24.9 | Irregular | 63.4 | 35.4 | 1.16 |
| 12149-2 | 72 | 14.2 | Irregular | 100 | — | — |
| 12225-2 | 72 | 15.7 | Irregular | 91.9 | 7.91 | 0.091 |

[1]standard deviation.

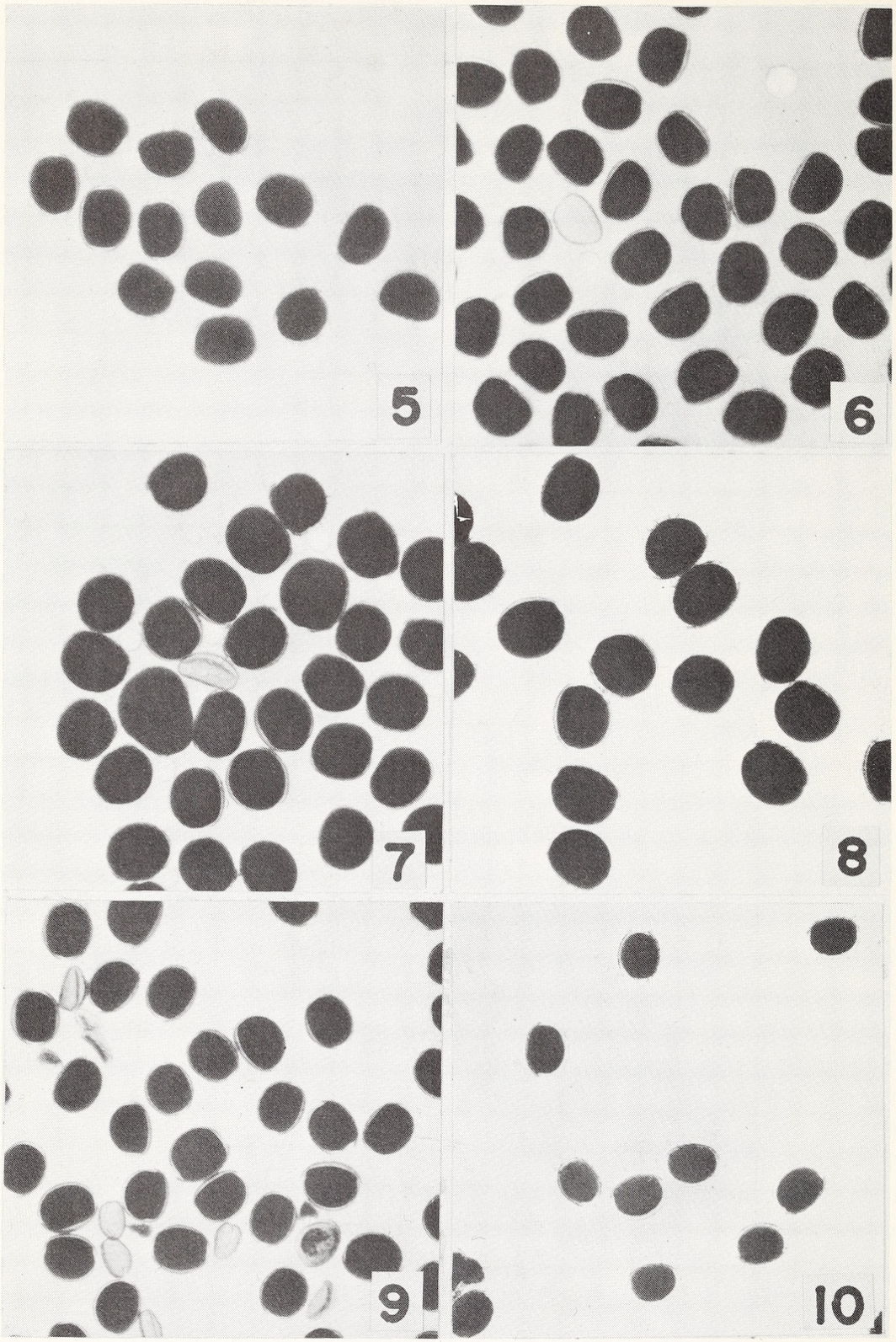
Long anthers are associated with the staminodia and are sometimes wholly or partly sterile; the short anthers are ordinarily plump and well-formed. In the first diploid shown above, however, no difference between the two types was noticed, and the pollen sizes are almost the same. In the second case the long anthers were pale yellow and very thin, and the short ones were orange and plump. The pollen size difference is greater than in the other plant but still not exceptionally pronounced. In the tetraploid (12526) from Todos Santos Island, Mexico, a much larger discrepancy was found: 37 ± 2.4 μ for the long anthers, compared to 41 ± 1.8 μ for pollen taken from both kinds. The majority of mixed pollen samples showed unimodal size distributions, notable exceptions being this latter named plant and the second diploid mentioned above. Whether or not the smaller-sized pollen results from incomplete development of vascular tissue and hence a lowered mineral supply in substandard anthers is unknown.

An attempt was made to correlate variability of pollen, percent normal and abnormal grains, and appearance of PMC's at metaphase I and anaphase I of meiosis (Table 2). Müntzing (1937) found that in *Dactylis* deviations from the normal $2n=28$ (tetraploid) resulted in a decrease in pollen fertility. In general, relationships are obscure in the two genera here studied because of incomplete meiotic data, but some interesting observations can be made nonetheless. The diploid 12208-3 had regular meiosis and a high percentage of good pollen in both long and short anthers. Plants from Kern Mesa (12132-2 and -3) differ widely in the amount of good pollen produced if the individuals studied here are representative. In 1967 the individual 12132-2 produced only 69.2 percent good pollen in one flower and in 1968, 87.7 percent. This indicates most likely a difference within an inflorescence rather than one from year to year. Mean pollen sizes of the two collections are very close, as are their standard deviations. Examination of meiosis showed metaphase to be normal with bivalents only; however, in a long anther 10 out of 72 cells, or 13.8 percent, showed lagging chromosomes. Short anthers showed only a few laggards. Because this plant produced 15.8 percent empty pollen grains, one might be tempted to conclude they nearly all came from the long anthers. This proves to be an unwarranted deduction, for both kinds of anthers from individual 12132-3 produced comparatively low amounts of good pollen. In fact the short anthers actually contained a few more empty grains than the long ones. Long anthers of the Todos Santos plant (12526) yielded 19.3 percent more empty grains than the sample taken from both anther types. There is probably considerable variation in this respect, but non-viable pollen appears to come more or less equally from all anthers. Occasionally, long and rarely short anthers were seen to be pale and shrivelled even before anthesis, in which case they contained less than the usual amount or no pollen at all.

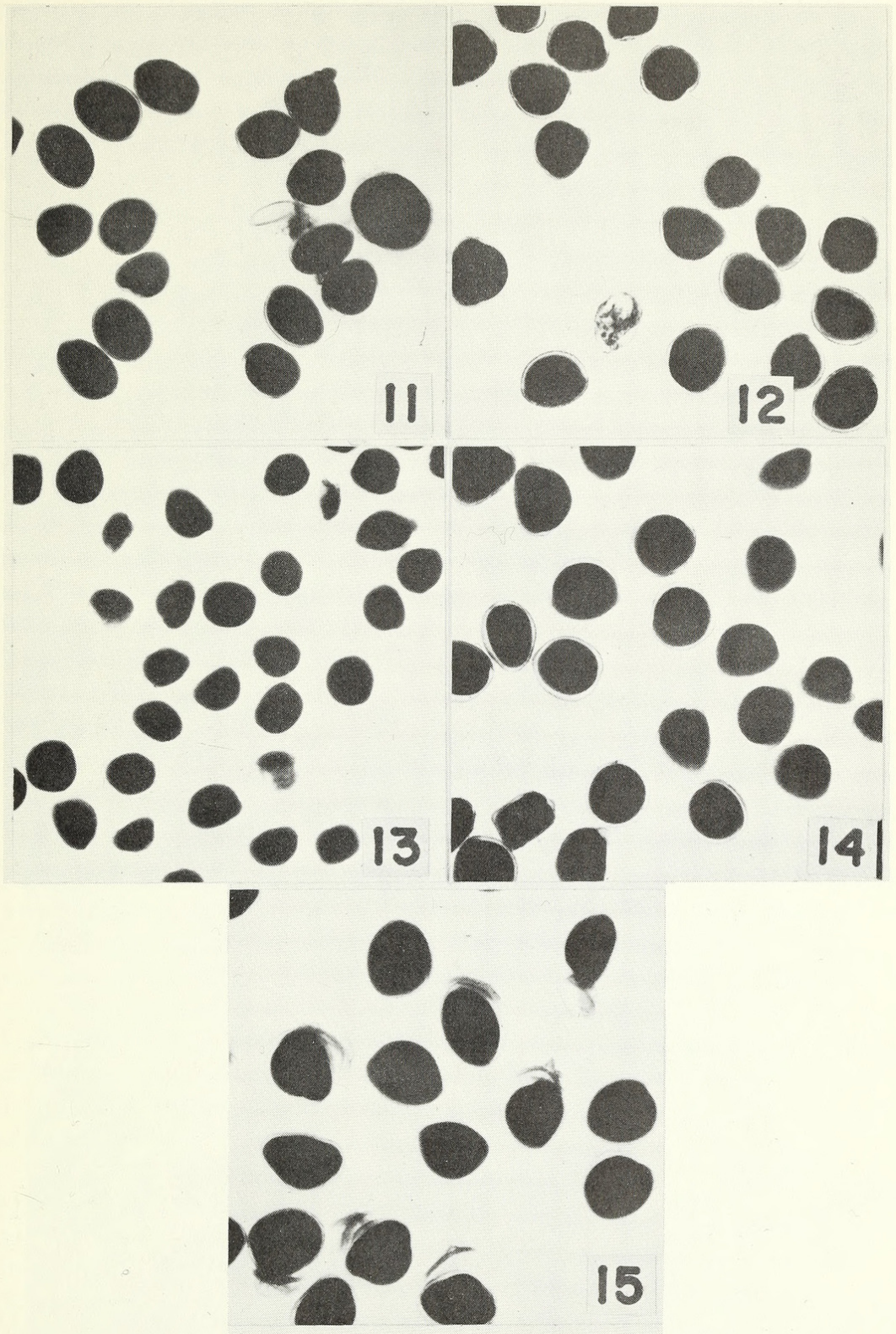
The tetraploid 12147-2 also bore relatively low quantities of good pollen. Irregularities at meiosis included multivalents, bridges (no fragments seen), and lagging chromosomes, in addition to a small number of B-chromosomes. B-chromosomes are deeply stained and are distinct rather than fuzzy; hence, they can usually be recognized without difficulty. One anther had 53 percent lagging chromosomes in the PMC's, a close approximation to the percentage of empty



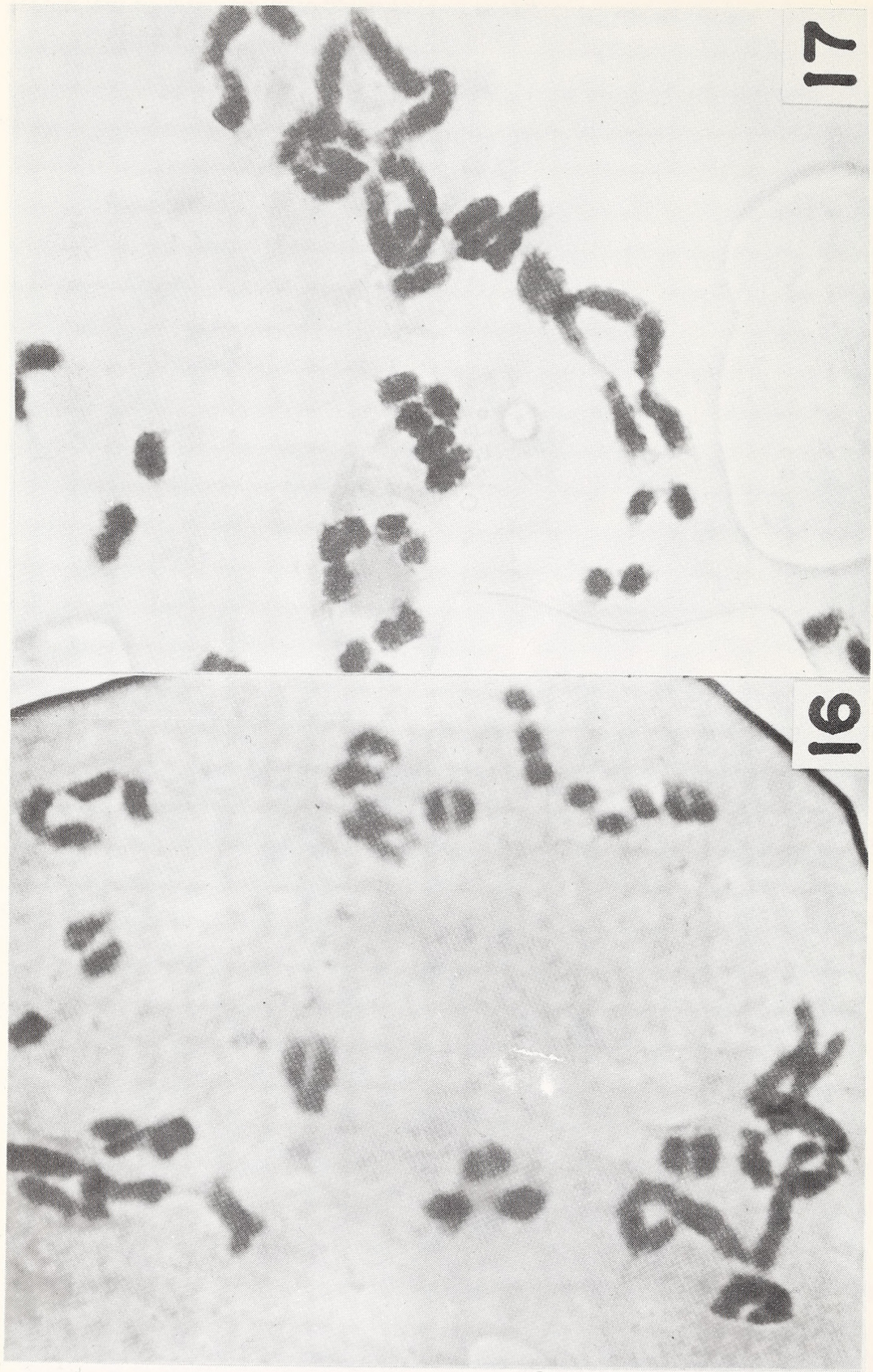

FIGURES 3-4. Chromosome spreads in PMC's of *Dichelostemma pulchellum*. Stained with aceto-carmine. *3.* $2n=54$. No. 12128-3. Note two trivalents, two univalents, and a quadrivalent. *4.* $2n=72$. Two trivalents, two univalents, and a possible quadrivalent either in a ring or with terminalized chiasmata.




FIGURES 5-10. Comparison of pollen from different polyploid levels in *Dichelostemma pulchellum*. **5.** $2n=72$, 12149-2. **6.** $2n=54$, 12144-2. **7.** $2n=45$, 12147-1. **8.** $2n=36$, 12253-3. **9.** $2n=18$, 12526. **10.** $2n=18$, 12208-3. Second number is Rancho Santa Ana propagation number.




FIGURES 11-15. Comparison of pollen from different polyploid levels in *Triteleia laxa*. ***11.*** $2n=15$, 12384-2. ***12.*** $2n=16$, 6739-4. ***13.*** $2n=28$, 12251-3. ***14.*** $2n=32$, 12139-1. ***15.*** $2n=48$, 12185-1. Second number as in Fig. 5-10.




FIGURES 16-17. Chromosome spreads in PMC's of *Dichelostemma pulchellum*. $2n=54$. ***16.*** A quadrivalent and two trivalents. Three bivalents to left of center are associated with the nucleolus (out of focus). ***17.*** A quadrivalent formed by the long metacentrics and a trivalent in upper right. A second quadrivalent of medium long chromosomes is possibly associated at one end into a hexavalent.

pollen grains. Another tetraploid (12125-1) showed only bivalents at metaphase and no lagging chromosomes at anaphase. Pollen mother cells of the pentaploid contained univalents, multivalents, and one to four B-chromosomes; but no chromosomes from the regular complement were ever seen outside the reforming "nucleus" by late anaphase I. Accordingly, pollen was 90 to 96 percent well-formed. All the hexaploids showed a high percentage of well-stained pollen; on the other hand, they all showed irregularities at meiosis (Fig. 16 and 17). Figure 3 shows a very clear picture of hexaploidy in the individual 12128-3 with three bivalents associated with the nucleolus, a pair of trivalents, a pair of univalents, and a quadrivalent of long metacentrics. Each of the octoploids studied had univalents, bivalents, trivalents, and quadrivalents at metaphase (Fig. 4). A few bridges without fragments were seen in one, and a surprisingly large number of cells (11 out of 14) contained a single B-chromosome. No chromosomes from the regular set were ever left behind at anaphase I. Percentage of good pollen was very high, and in one case only five empty grains were seen in 2000 counted.

A logical question to ask is why are so many pollen grains empty in the diploids. Grant (1952) found that in an interspecific hybrid in which chromosome homology already is reduced, changes in the state of nutrition may decrease pairing and associated phenomena even more. The presence of multivalents indicates that the *D. pulchellum* series consists of autopolyploids and probably has not resulted from interspecific hybridization; however, a few of the diploids behave rather oddly in that they do show reduced pairing and may be sensitive to nutritional deficiencies. Fortunately the influence of nutrition is not likely to be important here because both pairing and non-pairing diploids were grown in the same kind of soil under uniform conditions. Type of stamen may have something to do with the amount of good pollen formed, but the relationship is evidently not constant. Long anthers in the Santa Catalina Island population had the smallest pollen and short anthers, the largest; whereas, the reverse was true in the Kern Mesa plant. Although no counts were made, both long and short anthers sometimes produced much less pollen than normal, although it is essentially of the same quality as that from plump anthers. Just what factors might affect one class of anther and not the other at different stages of pollen development is unknown, but again there may be some difference in vasculature. Nutritional deficiencies and unknown effects notwithstanding, the data indicate a clear relationship between percentage of good pollen and presence of lagging chromosomes.

Results from other genera parallel this finding. Sparrow, Ruttle and Nebel (1942) in *Antirrhinum majus* L., Myers (1943) in *Dactylis glomerata* L. and Myers (1945) in *Lolium perenne* L. all found that differences in fertility of autotetraploids (or plants that act like them in the case of *Dactylis*) were not correlated with differences in the frequency of multivalents at metaphase, but instead were dependent on the frequency of lagging chromosomes. As the above data indicate, neither are the accessory chromosomes implicated in a reduction of fertility. Frost (1958) found that fertility was only weakly affected by them unless they were present in high numbers, something over six per cell. Randolph (1941b)

TABLE 3

Comparison of pollen viability, percent normal and abnormal grains, irregularities at meiosis, and gross appearance of pollen in *Triteleia laxa*. Second set of figures for No. 12384-5 and No. 12189-1 is from 1968; otherwise, all data are from 1967.

| Prop. No. | 2*n* | s[1] | Pollen/Meiosis | % good | % empty | % intermediate |
|---|---|---|---|---|---|---|
| 123848-4 | 16 | 13.4 | Pollen normal; all buds sterile, 1968 | 86.4 | 12.0 | 1.60 |
| 12384-5 | 16 | 10.0 | Pollen normal | 90.7 | 9.20 | 0.100 |
| | | 21.4 | Pollen normal | 90.0 | 6.94 | 3.06 |
| 12189-2 | 16 | 21.3 | Pollen normal | 98.6 | 1.40 | — |
| 12189-1 | 16 | 30.0 | Pollen normal | 89.0 | 10.8 | 0.200 |
| | | 19.5 | Pollen normal | 89.3 | 10.7 | — |
| 11068-4 | 16 | 26.3 | Pollen normal; bridges and laggards | 81.2 | 14.6 | 4.20 |
| 11068-1 | 16 | 22.4 | Pollen normal | 85.0 | 14.3 | 0.700 |
| 6739-4 | 16 | 21.6 | Pollen normal | 96.0 | 2.50 | 1.50 |
| 12251-2 | 28 | 30.8 | Pollen very small for a tetraploid | 73.3 | 20.6 | 6.10 |
| 12251-3 | 28 | 27.9 | Pollen very small | 60.0 | 34.9 | 9.10 |
| 9289-1 | 32 | 27.2 | Pollen normal; all buds sterile, 1968 | 96.6 | 2.20 | 1.20 |
| 12139-1 | 32 | 22.8 | Pollen normal; all buds sterile, 1968 | 92.3 | 4.00 | 3.70 |
| 12540 | 48 | 24.6 | Pollen normal | 96.9 | 2.40 | 0.680 |
| 12185-1 | 48 | 31.4 | Pollen normal; a few giant grains | 60.5 | 38.4 | 1.10 |
| 12384-2 | 14 + 1B | 25.3 | 4.73% giant grains | 87.7 | 11.4 | 0.877 |

[1]standard deviation

noticed an increase in the number of aborted grains resulting from the presence of large numbers of accessory chromosomes in maize, and he concluded in another paper (Randolph 1941a) that sterility in autotetraploid maize is largely controlled by specific genes or gene combinations.

A few giant pollen grains were seen in each of the polyploids but none in the diploids (Fig. 7). These have been seen in many other polyploids and result from failure of wall formation at some stage before the pollen tetrads have been completed.

Many of the plants of *T. laxa* selected for pollen analysis in 1967 were unac-

countably perverse in 1968 and proved to be completely or partially sterile. One diploid, however, presented a few useful buds during the season. Pollen mother cells in one flower showed a considerable number of meiotic irregularities, three bridges without fragments, and one to two lagging chromosomes per cell in 10 out of 40 anaphase stages seen. A bud taken several weeks later showed no such problems at all and had only bivalents at metaphase. Table 3 summarizes pollen data for *T. laxa,* including the gross appearance of grains in the lacto-phenol mounts.

Among the diploids two produce from 96 to 98 percent good pollen and two produce around 90 percent. The lowest percentage is obviously associated with lagging chromosomes. It may be inferred that the majority of plants in the population 12384 are somewhat irregular at meiosis both from the lowered amount of good pollen and from the presence of the diploid with x=7 among them (Fig. 11). Except for the tetraploid with $2n=28$ and one other instance the polyploids bear only a small number of empty grains. The other exception has pure white flowers, whereas normal flowers are blue or violet. Albino-flowered forms apparently often show lowered fertility, an estimate of which is given in this case by the mere 60.5 percent stained pollen grains.

## ACKNOWLEDGMENTS

I am indebted to L. W. Lenz, Director of the Rancho Santa Ana Botanic Garden, for guidance and advice throughout the course of this study. All plants from which this study obtained were collected by Dr. Lenz in California. Also, he generously made the chromosome counts available to me. I would also like to thank Sherwin Carlquist, who read the manuscript and pointed out a number of ambiguities. J. S. Henrickson, California State University at Los Angeles, provided assistance with the illustrations.

Finally, I am grateful to the Board of Trustees of the Rancho Santa Ana Botanic Garden for provision of the laboratory facilities with which this work was done.

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