are not comparable with measures of the fainter and more remote systems. The surprisingly small dispersion about the curve again emphasizes the general similarity in linear dimensions of the globular clusters of the galactic system. The plotted crosses refer to clusters whose computed distances involve only the earlier measures of diameter and integrated magnitudes; the distances for the others are based chiefly on magnitudes of bright stars and variables. The open circle represents N. G. C. 4147.

A study of the deviations from the mean curve of figure 1 shows no systematic relation of deviation to class of cluster or to galactic latitude. This lack of correlation with latitude is unexpected, in view of the effect on the measurement of distances that space absorption in low latitudes should produce. It may indicate that the angular diameters and the apparent total brightness are about equally undermeasured wherever the absorption is serious.

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INCREASED MUTATION RATE FROM AGED DATURA POLLEN

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Since recent tests of aged seeds showed increased mutation rate, as indicated by pollen abortion,[2] together with parallel increase in the rate of visible recessive gene mutations in the same material,[3] it was thought possible that aging pollen might have similar effects. A rather limited series of experiments, using very simple methods of handling the material and making the tests, was accordingly undertaken. The results, however, were so positive, and the possible significance and applicability of the method so general, that it seemed best to describe the methods used and the results thus far obtained.

The plants used were grown from fresh seed of the Standard Line 1 of Datura. This line has been highly inbred, and has been passed through a haploid. It has been used in other mutation rate experiments, and as the standard in the general breeding work with Datura. Anthers were picked

just before they opened, and were placed on separate numbered slips of paper in a Syracuse dish. The pollen remained in the anther or on the paper beneath until used. A pollen sample from each flower was examined microscopically to see that it was normal in appearance. The uncovered dishes containing the pollen were stored in a cupboard which was heated by electric light bulbs connected to a thermostat. The thermometer in the cupboard registered 29 to 31°C. during the experiment, except that on one night the thermostat stuck and the temperature of the cupboard went down to about 18°C. Only two of the eight series of flowers were subjected to this change. The pollen was stored at about 30°C. in order to keep it somewhat above room temperature, and hence drier than it might otherwise have been. This temperature (about 85°F.) falls within the range of air temperatures to which flowers of Datura are normally subjected in the greenhouse and field. The pollen was shielded from the direct rays of the electric lamps. When pollinations were made, the dish with the pollen was carried to the greenhouse and the pollen there applied, either directly from the anther or from the paper slip, to the stigma of a previously emasculated flower. The flowers used as females were somewhat varied in age, but all were in good condition and neither extremely young nor extremely old. Crosses were attempted at intervals from two to twenty days after the pollen was stored. The distribution of the pollinations made and capsules set, in relation to the age of the pollen, is shown in table 1.

TABLE 1

Age of pollen, days	2–3	4–5	6–7	8–9	10–11	12–13	14–15	16–17	18–20	Total
Pollinations made	4	6	5	8	42	25	26	22	20	158
Large capsules set	2	2	0	3	1	4	0	0	0	12
Small capsules set	1	2	2	0	7	2	0	0	0	14

The use of pollen abortion as an index of the mutation rate has been discussed in an earlier paper[2] where the technique employed is described. Two types of abortion, due respectively to gene and to grosser chromosomal mutations, can be distinguished with a fair degree of accuracy by differences in appearance of the aborted grains. The classification of mutations in table 2 is based on this appearance of the grains and not on cytological study or breeding behavior. The highest rate of pollen abortion mutations found in our aged seed experiments was 8.7% for 21 mutations in 242 plants grown from seven- to eight-year old seed.[2] Experiments with heat and other factors (excepting strong radiation) have given lower rates from treated seeds. It seems clear, however, that higher rates have been induced by the use of aged pollen. A total of 29 pollen abortion mutations have been recorded in 193 plants from pollen aged for four to thirteen days, giving a mutation rate of 15.0%. The 191 control plants, grown from

the fresh seeds of a self (made with fresh pollen) on a sib of the plants used for the tests, showed no mutations. The data are shown in table 2. The mutation rates from progenies of fewer than five plants average very high, but the number of plants is extremely small. Percentages for these cases have been put in parentheses in table 2. From these small progenies there are 18 plants recorded with nine, or 50.0%, showing pollen abortion. The conditions of the storage of the pollen, especially in respect to moisture content, were not accurately controlled. It would appear that the conditions which adversely affected the size of the progenies tended to raise the mutation rate. Among the larger progenies, those from eight- to ten-day old pollen show higher rates of mutation than do those from five- to six-day old pollen. The rates are, respectively, 13.9% from 122 plants with 17 mutations, and 5.7% from 53 plants with three mutations.

TABLE 2

POLLEN ABORTION MUTATIONS FROM AGED POLLEN

POLLEN AGE, IN DAYS	PARENT (341) NOS.	PROGENY NO.	SEEDS SOWN	PLANTS GROWN	PLANTS RECORDED	NORMALS	MUTATIONS: CHROMOSOMAL	MUTATIONS: GENE TYPE	MUTATIONS: TOTAL	PERCENTAGE
4.00	93 × 37*A**	3401821	8	3	2	1	1	0	1	(50.0)
5.00	60 × 71*A*	3401822	1	1	1	1	0	0	0	(0.0)
5.25	73 × 37*B*	3401815	42	35	34	32	1	1	2	5.9
5.75	71 × 77*B*	3401816	29	19	19	18	1	0	1	5.3
6.75	54 × 77*A*	3401823	5	3	2	1	0	1	1	(50.0)
7.00	93 × 77*B*	3401824	5	4	4	2	1	1	2	(50.0)
8.00	73 × 93*A*	3401817	37	19	19	16	2	1	3	15.8
10.00	37 × 77*B*	3401825	5	4	4	3	0	1	1	(25.0)
10.25	71 × 71*B*	3401818	169	114	103	89	9	5	14	13.6
10.75	93 × 73*A*	3401826	2	1	1	0	1	0	1	(100.0)
11.00	53 × 77*A*	3401827	5	2	2	0	2	0	2	(100.0)
13.25	25 × 25*C*	3402081	200	4	2	1	0	1	1	(50.0)
...	Totals	...	...	209	193	164	18	11	29	15.0
Fresh	2 × 2	3401	...	...	191	191	0	0	0	0.0

* Letters *A*, *B*, *C* designate separate flowers used as males.

The mutations from aged pollen involved the whole plant; that is, they were of the sort to be expected if the mutations were brought in by one of the gametes, and were not the sectorial type of mutation found when seeds are aged or otherwise treated.[2] Two sectorial mutations, one among the test plants and one among the controls, were also found. Since it is most likely that these originated in the seeds after fertilization the plants concerned are not recorded. Spontaneous sectorial mutations may be expected, in this material, to occur in about 0.3% of the plants. The data in table 2 are based upon one-flower samples, but two or more flowers have been recorded for all except two of the plants which showed mutations. It is, of course, in the case of a given mutation, not possible to say whether the

mutation occurred in the male or in the female gamete; or whether the mutation was induced by the treatment, or would have occurred without treatment. However, in Line 1 Datura material, spontaneous non-sectorial pollen abortion mutations occur about one per 500 plants, so that the chances that more than one or two of the 29 mutations found have this origin are very remote. The present investigation furnishes added evidence to that in an earlier publication[4] which indicated that gametic lethals do not occur in plants. The mutative changes responsible for the pollen abortion here found may be supposed to have entered the plants through the sperm cells of the aged pollen grains.

The method of increasing mutation rates by aging of the pollen for a few days seems to be one that might be employed in any material of which the pollen can be stored. A large proportion of dicotyledonous plants will, probably, yield viable pollen after much longer aging than that here reported, judging from the report of Holman and Brubaker.[5] Correns[6] aged pollen of Mercurialis for 120 days, and showed that aged pollen gave progenies with altered sex-ratios. Hiorth[7] kept Antirrhinum pollen alive for 28 days at room temperature, and from it obtained several hundred F_1 plants, but grew only four F_2 progenies which produced no visible gene mutations. He found that heating pollen to near the lethal point gave no increase in the rate of visible gene mutation.

Mutations which occur in the mature pollen grains may be of particular interest because the simplicity, accessibility and durability of this material seem to make it especially useful for studies on the nature and incidence of mutation. It seems possible also that mutations in nature may be dependent, to an unsuspected extent, upon the conditions to which the pollen has been subjected before it functions in fertilization. The considerable number of mutations found in the experiments here described supports the suggestion of Navashin,[8] in regard to the chromosomal mutations induced by aging seeds—that the metabolic conditions within the cell rather than external agents may be the cause of such mutations.

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