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A WHITE-FLOWERED RACE OF *DATURA* WHICH IS
GENETICALLY DISTINCT FROM SIMILAR WHITE RACES IN
NATURE

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There are two color forms of *Datura stramonium* found growing in the wild throughout most parts of the temperate regions of the world. One form has purple stems and purple flowers, the other has green stems and white flowers. In all other respects the two forms are identical. In most of the current botanical manuals they are classified as distinct species, the name *D. tatula* being given to the purple-flowered form and *D. stramonium* to the white form. In the herbaceous forms of *Datura* there are only these two flower colors, and in all instances plants with purple stems have purple flowers and those with green stems have white flowers. An exception to this last was observed this summer in an F_2 culture from x-ray treated seed, which segregated for a type with purple stems and white flowers. No breeding work has, as yet, been done on this new type. A white-flowered type has also been extracted from hybrids with *D. ferox*. This type differs from the white forms discussed here, in that the cotyledons and the stem below the cotyledons are purple. This type is therefore indistinguishable in the young seedling stage from the purple-stemmed and purple-flowered forms. The recessive gene responsible for this white-flowered condition has been located in the 4 half of the 3·4 chromosome.

In 1915 a lot of *Datura* seed was obtained from the Department of Agriculture in Washington. This seed gave both purple-flowered and white-flowered plants. One of the purple-flowered plants became the origin of our standard Line 1, and one of the white-flowered plants was taken as the origin of Line 2. These lines have been continued by selfing for thirty and thirty-one generations, respectively.

Very early in *Datura* investigations it was demonstrated that the two wild forms, purple and white, differ only in a single pair of genes, and that the gene responsible for the purple-flowered (P) form is dominant over that for white flowers (p).² We have therefore accepted the two color forms as a

single species and have used the name *stramonium* (Linnaeus, 1753) since it preceded *tatula* (Linnaeus, 1762). It was later shown by the use of $2n + 1$ primaries that the pair of genes responsible for the purple- or white-flowered type is located in the 17·18 (Poinsettia) chromosome.³ In table 1 are shown the trisomic ratios obtained in male backcrosses of the $2n + 17·18$ primary when simplex and when duplex for this gene for white flowers (white-1).

TABLE 1
OFFSPRING OF $2n + 17·18$ HETEROZYGOUS FOR WHITE-1

		OFFSPRING	
		PURPLE	WHITE
white-1 $\times 2n + 17·18$ heterozygous (<i>PPp</i>)		1958	1040
	Ratio	(2)	(1)
white-1 $\times 2n + 17·18$ heterozygous (<i>Ppp</i>)		1166	2392
	Ratio	(1)	(2)

Similarly, female backcrosses of the $2n + 17·18$ type heterozygous for purple and white gave the expected trisomic ratios as published earlier. All the other eleven $2n + 1$ primary types gave 1:1, or disomic, ratios in backcrosses. Later records from the $2n + 1·18$ (Wiry) type when heterozygous for purple and white, and linkage records with other genes in the 17·18 chromosome show that this gene for white flowers is in the ·18 half of the 17·18 chromosome.

The increase in the rate of mutation, both gene and chromosomal, observed in plants grown from aged seeds has been previously reported.^{4, 5, 6} Progenies from plants grown from seed which had been stored under ordinary laboratory conditions for more than four years gave a marked increase in the number of pollen abortion mutations. It was pointed out that these mutations usually occurred as sectors, rarely involving an entire plant, and that one plant might have unmutated sectors as well as one or more different mutated sectors. This is taken to indicate that the mutant sectors originate from initial cells of the embryonic stem which have undergone mutation within the resting (aging) seed.

In the progeny of 315 plants grown from seeds which had been aged from $7\frac{1}{2}$ to $9\frac{1}{2}$ years a total of eleven new morphological types due to mutation have been secured. This is a mutation rate of 3.5 per cent. During the twenty-five years of *Datura* breeding work only nine new morphological or "visible" types have been secured in the progenies of untreated normal parents. It would be very difficult to accurately calculate the rate of mutation in these controls, but it is extremely low. It is also likely that some of these nine mutants may themselves have been caused by the aging of seed. In the same 315 plants from old seed, a total of 30 pollen abortion types (new mutations) had been observed by Dr. Cartledge in earlier tests. This would be a rate of 9.5 per cent. In these tests the mutations which

show as visible morphological effects are thus about one-third as frequent as those which cause pollen abortion.

Among the progenies from plants grown from the seeds which were $7\frac{1}{2}$ years old at the time of planting there was one culture which immediately drew our attention. This culture segregated in the seed pan for a green-stemmed type which in all appearances was like Line 2. When these green-stemmed plants matured they bore white flowers and were indistinguishable from Line 2 or from any of the other white-flowered races which had been collected from the wild.

As it seemed likely that these white-flowered plants were the result of some admixture with Line 2 or some other laboratory accident all other selfed capsules which had been harvested from this plant (320546(3)) were immediately planted. Table 2 gives the tabulation of these plantings.

TABLE 2
OFFSPRING OF SELFED CAPSULES OF 320546(3)

CAPSULE	PURPLE	WHITE
A	160	34
B*	111	25
C	50	..
D	57	..

* Capsule B also segregated for "peach" independently of white.

These results indicated that a mutation for white had actually occurred and that it had become established as a sector involving capsules A and B. This new white-flowered race (white-2) was then crossed with white-1, using Line 2 as the standard white-flowered race. The resulting F_1 plants had purple stems and when mature bore purple flowers. One of these F_1 plants was selfed and among 143 individuals in the resulting F_2 generation there were 84 purple and 59 white seedlings. These figures approximate a 9:7 ratio (80.5:62.4) expected in the F_2 from a cross of two independent factors which produce a similar effect. It was therefore evident that we had obtained, by a mutation presumably caused by some aspect of aging, a new white which was different genetically, but which was not distinguishable in appearance from white-1.

The appropriate steps were taken to locate this new gene for white flowers in a particular chromosome. Table 3 shows the results of male backcrosses of four different $2n + 1$ primaries which were heterozygous for white-2. It will be noted that all but the $2n + 15 \cdot 16$ type give a close approximation to a 1:1 ratio. The $2n + 17 \cdot 18$ type which in table 1 gave 1958P to 1040p (2:1) when heterozygous for white-1 now gives a perfect 1:1 ratio (129P to 129p) with white-2. The $2n + 15 \cdot 16$ type is here the primary which gives a 2:1, or trisomic ratio, when simplex for white-2 or a 1:2 ratio when duplex for the gene.

Having located the gene for white-2 in the 15·16 chromosome we undertook to determine which half of the chromosome contained the locus for the gene. As there is only one of the two possible secondaries of the $2n + 15·16$ type available it was impossible to test for both halves by the use of secondaries. The secondary $2n + 15·15$ when heterozygous for white-2 and used as the female in backcrosses gave $92P$ to $71p$ diploid offspring and $29P$ to $20p$ $2n + 15·15$ offspring. The nearness of these ratios to a 1:1 ratio, especially in the case of the $2n + 15·15$ offspring, is evidence that the locus for the gene for white-2 is not in the ·15 half. It was therefore assumed that the gene must be located in the ·16 half of the 15·16 chromosome. Further breeding tests with tertiaries, or compensating types, which contained the ·16 half as extra material gave positive evidence that the gene is located in the ·16 half. Although the results of tests of linkages with other genes in the 15·16 chromosome are still incomplete the results

TABLE 3
MALE BACKCROSSES OF $2n + 1$ TYPES HETEROZYGOUS FOR WHITE-2

Disomic Ratios			PURPLE	WHITE
white-2 $\times 2n + 5·6$ heterozygous	(<i>Pp</i>)		17	16
“ $\times 2n + 17·18$ “	(<i>Pp</i>)		129	129
“ $\times 2n + 21·22$ “	(<i>Pp</i>)		416	398
	Ratio		(1)	(1)
Trisomic Ratios				
white-2 $\times 2n + 15·16$ heterozygous	(<i>PPp</i>)		829	451
	Ratio		(2)	(1)
“ $\times 2n + 15·16$ “	(<i>Ppp</i>)		1022	2050
	Ratio		(1)	(2)

which we have obtained confirm the supposition that the gene for white-2 is in the ·16 half.

Since we now had two genetically different white-flowered types which were indistinguishable from each other in appearance, it seemed desirable to determine whether among the many collections of similar white races from the wild there might not also be more than one gene responsible for the white-flowered condition.

Seed of 1350 races of *Datura* have been assembled since the beginning of our investigations with *Datura*. This assemblage of races has been secured from as widely scattered parts of the world as possible, through our own collections and through the kindness of many correspondents who have sent in seed. The methods used in collecting these samples have been described in an earlier paper.⁷ Of these 1350 races, 323 have been classified as belonging to some one of the other nine herbaceous species of *Datura* or to the *Brugmansia* (Tree *Datura*) group. Of the remaining 1027 collections of

D. stramonium 496 have been of the green-stem and white-flowered type; the rest have had purple flowers.

The possibility that there might be more than one gene responsible for white flowers among the many wild races was tested by crossing as many as possible of these white races with standard white testers. If the white flowers of any of these races had been determined by a gene different from that responsible for the white flowers of the standard, the F_1 plants resulting from the cross would have purple flowers. The majority of these crosses were made directly to Line 2 which had been taken as the standard for

TABLE 4

GEOGRAPHICAL DISTRIBUTION OF 409 WHITE RACES OF *D. stramonium* WHICH WERE FOUND TO BE EQUIVALENT TO WHITE-1

ORIGIN	NO. OF RACES TESTED	ORIGIN	NO. OF RACES TESTED
North America		Asia	
United States		China	6
(17 states)	70	Siberia	1
Mexico	4	India	4
West Indies	11	East Indies	8
South America		Iraq	1
Chile	10	Africa	
Brazil	21	Algeria	4
Europe		Belgian Congo	3
England	1	East Africa	2
France	6	West Africa	4
Spain	22	South Africa	6
Switzerland	6	Australia	4
Portugal	7	Botanical Gardens	37
Italy	42	Commercial Sources	5
Hungary	83		
Russia	38		
Germany	3		

white-1. A few of the crosses were made to other white races which in turn had previously been crossed to Line 2 and had been found to be of the white-1 type.

Of the 496 white races available, it was possible to obtain crosses with a standard and the resulting F_1 in 409 cases. All of these F_1 plants had green stems indicating that the white-flowered condition of these races is determined by the same gene as that for white-1. It was not necessary to grow to maturity the F_1 plants of these tests since seedlings of white-1 are entirely green. If any new gene for white had entered into these crosses the resulting seedlings would have been purple-stemmed as were those from the cross of white-1 with white-2.

Table 4 shows from what widely separated parts of the world these 409

white races were secured. In Europe and Asia *D. stramonium* is usually found in the white-flowered form; the reverse of this is true in the United States. This accounts for the larger number of white races tested from Europe. Forty-two races are listed in the table as having been obtained from Botanical Gardens or from commercial sources. The true origin of these from the wild cannot be determined.

In 1937⁷ a study was made of the geographical distribution of the chromosomal prime types (*PT*'s) in *D. stramonium*. It was shown that there were only five recurrent prime types among 583 races tested. With the exception of *PT*3 which occurred only in the purple form, all five *PT*'s were found in both purple and white races. Since the summary of 1937 was compiled three white-flowered *PT*3 races from Mexico have been procured. White-flowered races from all five recurrent *PT*'s are included in the present tests with our standard white-1, and in all the gene for white flowers was found to be equivalent to that present in white-1. This is additional evidence that our sample of white races truly represents the wild white-flowered *Daturas*, and is suggestive that the species existed in the white-flowered form before the differentiation into the various *PT*'s occurred.

No other mutation to the green-stemmed or white-flowered condition has ever appeared in all our experiments with *Datura*, which have included x-ray and radium treatments of pollen and seed, heat and cold treatments, injection of chloral hydrate, wounding, and aging of pollen and seed. These treatments have produced many other mutations as evidenced by over 300 new morphological types which have segregated in the F_2 generations. A mutation giving rise to a white type would not have been overlooked as might be possible in the case of a less striking form.

It is not known what the fundamental mechanism may be that brings about genetic changes (mutations) in aged seed. This seed was aged in the laboratory under conditions very different from what would have taken place naturally. It has, indeed, been reported in an earlier paper⁸ that *Datura* seed buried in the ground, in a dry condition, for 22 years gave no appreciable increase in the rate of pollen abortion mutations over that found in controls. It may be that the laboratory aging of seed while not directly causing mutation may in some manner make the embryo, or even young seedling, susceptible to some other active mutation-causing agency. That the particular mutation under discussion here must have occurred relatively early is suggested by table 2. Capsules *C* and *D* of the plant within which the mutation to white-1 occurred yielded only purple normal seedlings, indicating that the branches on which they were formed came from initial cells in the seed which had undergone no mutation. Capsule *A* produced purple normal and white normal offspring and so came from an initial cell which had undergone a single mutation. Capsule *B* besides giving purple and white offspring also gave plants of a new type called "peach"

(located in the 13·14 chromosome) on account of the slender and rather pale leaves suggestive of peach leaves. From this capsule there were 88 purple normal, 20 white normal, 23 purple peach and 5 white peach offspring, which is a close approximation of a 9:3:3:1 ratio. It appears in this case that the mutation for white took place fairly early in the embryonic shoot and that somewhere in a succeeding cell carrying the white mutation a second mutation for peach occurred.

Since so many white-flowered races obtained from many widely separate regions of the world, and representing all the recurrent prime types, have been tested by crosses with the standard white-1 and have, in all cases, been determined to be genetically equal to the standard, it is probable that no new gene for the white-flowered condition has become established in the wild. If a mutation to a white-flowered type had occurred in nature there is no evident reason why it should not have survived the processes of natural selection. The white races are in all respects as vigorous and as productive as the purple races. However, we know that natural mutations, or spontaneous mutations from untreated material, are rare in *Datura*. Of the 1350 races of *D. stramonium* which have been assembled eight morphological types differing by a single gene from the standard Line-1 have been recorded. All these have been found to be due to recessive genes, and all have been located in particular chromosomes. As stated above, the rate of mutations from untreated material in our own cultures is also extremely low.

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