# DISTINCTION BETWEEN PRIMARY AND SECONDARY CHROMOSOMAL MUTANTS IN DATURA

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Normal diploid ($2n$) plants of Datura Stramonium have 12 pairs of chromosomes in their somatic cells. It has been shown[1] that certain recurrent mutants in this species are due to the presence of a single extra chromosome in one of the 12 sets. In several of these ($2n + 1$) types, the evidence[2] is conclusive, we believe, that the extra chromosome is a specific one which brings about the peculiarities in the mutant by means of the unbalance which its factors produce over the normal $2n$ condition. Since there are in all only 12 chromosomal sets, one would expect consequently to find not over 12 mutants of the ($2n + 1$) type, each corresponding to the presence of a different extra chromosome. For some time, however, we have had more than 12 such mutants and at present there are over 20 which have been under cultivation for at least two generations.

The occurrence of so large a number of mutants which could be represented by the same chromosomal formula has naturally constituted our main problem with the Daturas for some time past. Evidence, however, has been accumulating from different sources which indicates that these 25-chromosome mutants are not all unrelated types; that, on the contrary, they may be arranged in not over 12 groups consisting of a single mutant or of a Primary mutant and one or more Secondary mutants. The present paper aims to give, in brief, evidence for the grouping of ($2n + 1$) mutants; evidence as to which are Primary and which Secondary mutants; and evidence as to the cause of the difference between the two.

*I. Evidence for Groups of* ($2n + 1$) *Mutants.*—That the ($2n + 1$) mutants arrange themselves into not over 12 groups is indicated by the facts in the following numbered paragraphs:

1. The presence of only 12 sets of chromosomes in the species is presumptive evidence for not over 12 groups to contain the 20 and more ($2n + 1$) types now known.

2. Similarities in external appearance alone would suffice in most cases in placing mutants in their respective groups. The similarities may affect habit, flower and leaf characters or apparently any other part of the plant. Capsules of members of three groups are figured in an earlier publication.[3]

3. A study of the internal anatomy made by Dr. Sinnott[4] has resulted in an arrangement of the mutants into groups which agree very closely with those made on the basis of other evidence.

4. In one group (Echinus-Mutilated), the pollen is dimorphic, half

the grains being supplied with starch and half being practically devoid of starch.

5. In one group (Poinsettia-Wiry) the two members give the same trisomic ratios[2] for the inheritance of a pair of Mendelian characters (cf. table 5).

6. The breeding behavior of the mutants indicates the grouping in that one member may throw another member of a group in its offspring (cf. II., 4 below & table 3).

7. The sizes of the extra chromosomes, which have been measured by Dr. Belling and which are discussed in the succeeding paper, offer additional evidence in regard to the grouping.

From the above evidence we have established 12 groups (if we include the mutant Spinach whose position is as yet doubtful), half of them with Secondaries and half in which no Secondaries have so far been found.

*II. Evidence as to which Are Primary and which Are Secondary Mutants.*—The grouping of mutants was established before it was possible to say with certainty which was to be considered the Primary and which the Secondary in a given group. The data, however, from special breeding tests between members of a single group as well as data collected for other purposes have afforded a series of tests for Primaries and Secondaries which leave little doubt in most cases as to the rank of the mutants within a group. These tests will be considered in numbered paragraphs.

TABLE I

PRIMARY AND SECONDARY ($2n + 1$) TYPES IN OFFSPRING FROM TRIPLOIDS

(Primaries are printed in capitals, secondaries in lower-case type)

| | $3n \times$ SELF | $3n \times 2n$ | TOTAL |
|---|---|---|---|
| 1. GLOBE | 5 | 46 | 51 |
| 2. POIN-SETTIA | 5 | 34 | 39 |
| Wiry | . | .. | .. |
| 3. COCKLE- | | 32 | 38 |
| BUR | 6 | 1 | 1 |
| Wedge | . | 33 | 37 |
| 4. ILEX | 4 | | |
| 5. ECHINUS | 3 | 15 | 18 |
| Mutilated | . | (2?) | (?) |
| Nubbin(?) | . | .. | .. |
| 6. ROLLED | . | 24 | 24 |
| Sugarloaf | . | .. | .. |
| Polycarpic | . | .. | .. |
| 7. REDUCED | 3 | 38 | 41 |

| | $3n \times$ SELF | $3n \times 2n$ | TOTAL |
|---|---|---|---|
| 8. BUCKLING | 9 | 48 | 57 |
| Strawberry | .. | ... | ... |
| Maple | | | |
| 9. GLOSSY | .. | ... | ... |
| 10. MICRO- | 2 | 30 | 32 |
| CARPIC | 4 | 46 | 50 |
| 11. ELONGATE | 2 | 30 | 32 |
| Undulate | .. | ... | ... |
| 12. SPINACH(?) | .. | 2 | 2 |
| Totals ($2n + 1$) | 43 | 381 | 424 |
| ($2n + 1 + 1$) | 11 | 101 | 112 |
| $2n$ | 30 | 215 | 248 |
| $4n$ | 3 | ... | 3 |
| Grand Totals | 87 | 697 | 784 |

1. Offspring from Triploids.—As has been shown,[5] diploids, simple ($2n + 1$) and double ($2n + 1 + 1$) mutants are to be expected in the offspring from triploid parents. Since $3n$ parents have an extra chromosome in each of the 12 sets, their offspring should show the 12 unmodified mutant types, each with a different extra chromosome, provided all such types were viable. A classification into their respective groups of 424 such mutant offspring is shown in table 1. Primaries are represented in capitals and Secondaries in lower case type as also in other tables throughout this paper. The position of Spinach from this and other tests is somewhat uncertain. It may possibly be a Secondary for which the Primary has not yet been distinguished from normals. The table seems to delimit with certainty, however, 11 of the possible 12 Primaries. It will be observed that there is only a single certain exception to the rule that triploids throw Primaries and not Secondaries.

TABLE II

SPONTANEOUS OCCURRENCE OF PRIMARY AND SECONDARY ($2n + 1$) MUTANTS

(Primaries are printed in capitals, secondaries in lower-case type)

| | FROM $2n$ PARENTS | FROM UNRELATED ($2n + 1$) PARENTS | TOTALS |
|---|---|---|---|
| 1. GLOBE | 41 | 107 | 148 |
| 2. POINSETTIA | 28 | 47 | 75 |
| Wiry | . | 1 | 1 |
| 3. COCKLEBUR | 7 | 17 | 24 |
| Wedge | . | .. | .. |
| 4. ILEX | 19 | 27 | 46 |
| 5. ECHINUS | 10 | 11 | 21 |
| Mutilated | 2 | 4 | 6 |
| Nubbin(?) | 1 | .. | 1 |
| 6. ROLLED | 24 | 47 | 71 |
| Sugarloaf | 3 | 9 | 12 |
| Polycarpic | 3 | .. | 3 |
| 7. REDUCED | 25 | 44 | 69 |
| 8. BUCKLING | 27 | 71 | 98 |
| Strawberry | 1 | 1 | 2 |
| Maple | .. | 2 | 2 |
| 9. GLOSSY | 8 | 11 | 19 |
| 10. MICROCARPIC | 64 | 100 | 164 |
| 11. ELONGATE | .. | 2 | 2 |
| Undulate | .. | 1 | 1 |
| 12. SPINACH(?) | 6 | 4 | 10 |
| Totals ($2n + 1$) | 269 | 506 | 775 |
| Related ($2n + 1$) types | .... | 22,123 | 22,123 |
| $2n$ | 32,523 | 70,281 | 102,804 |
| Grand totals | 32,792 | 92,910 | 125,027 |

2. Spontaneous Occurrence of Primaries and Secondaries.—In table 2 are shown the spontaneous occurrences of mutants among over 125,000 offspring from $2n$ and from unrelated ($2n + 1$) parents. By unrelated is meant not belonging to the same group as the new mutant. The tabulations for the $2n$ parents include the years 1918 to 1923; those for the

TABLE III

OFFSPRING OF PRIMARY AND SECONDARY ($2n + 1$) TYPES

(Primaries are printed in capitals, Secondaries in lower-case type)

| | GL | PN | WY | CK | WD | IX | EC | MT | NB | RL | SG | PY | RD | BK | ST | MP | GS | MC | EL | UN | SP | TOTALS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| POINSETTIA | 14 | 8617 | 70 | 5 | | 1 | | | | 5 | 4 | | 3 | 1 | | | | 2 | 1 | | 1 | 30933 |
| Wiry | | 22 | 546 | | | | | | | 2 | | | | | | | | | | | | 2998 |
| COCKLEBUR | 3 | 4 | | 2034 | 1 | 1 | | | | 4 | 1 | | 2 | 4 | | | | 3 | | | | 8860 |
| Wedge | 6 | 6 | | 36 | 472 | 1 | 2 | | | 3 | | | 1 | 3 | | | | 5 | | | | 2477 |
| ECHINUS | | | | | | 1 | 182 | 0 | 0 | | | | 1 | 1 | | | | | | | | 718 |
| Mutilated | | | | | | | 37 | 376 | 0 | 1 | | | 1 | 3 | | | | 4 | | | | 2688 |
| Nubbin(?) | 2 | 2 | | | | | 13 | 0 | 122 | | 1 | | 3 | 6 | | | | 6 | | | | 400 |
| ROLLED | 22 | 16 | | 3 | | 4 | 3 | | | 2023 | 16 | 0 | 9 | 13 | 1 | 1 | 3 | 8 | | | 1 | 11140 |
| Sugarloaf | 6 | 1 | | 2 | | 2 | 1 | 1 | | 155 | 1093 | 0 | 4 | 10 | | | 2 | 13 | | | 1 | 5230 |
| Polycarpic | | | | | | | | | | 1 | 0 | 3 | | | | | 1 | | | | | 123 |
| BUCKLING | 4 | 7 | | 1 | | 4 | | | | 6 | | | 4 | 1041 | 1 | 0 | 3 | 9 | | | | 3101 |
| Strawberry | 3 | | | | | 3 | 1 | | | 2 | | | 2 | 36 | 287 | 0 | | 4 | | | | 947 |
| Maple | 1 | | | | | | | | | | | | | 4 | 0 | 422 | | 1 | | | | 1984 |
| ELONGATE | 1 | 1 | | 1 | | | | | | | | | | | | | | 3 | 66 | 0 | | 1206 |
| Undulate | | | | | | | | | | | | | | | | | | | 4 | 140 | | 592 |

($2n + 1$) parents the years 1920 to 1923. Many different lines are represented in the table and the data for other reasons also are not completely comparable. They are probably sufficiently so, however, for our present purpose. It will be noted that in all cases the Primaries occur spontaneously much more frequently than their Secondaries which are relatively rare. Elongate occurs infrequently for a Primary, but its viability is known to be poor.

3. Percentage of Bad Pollen Grains.—In a preliminary study of pollen sterility undertaken with Mr. Cartledge it was shown that in all cases the Secondaries had a higher percentage of bad grains than their respective Primaries.

4. Breeding Behavior.—An inspection of table 3 will show that, whereas Primaries may occasionally throw their Secondaries, the Secondaries regularly throw their Primaries more frequently than they throw new mutants belonging to other groups. Thus, as shown in table 4, of the 31,000 offspring from Poinsettias about 28% were Poinsettia and about 0.25% were the Secondary Wiry. Conversely when Wirys were the parents, about 0.75% of the offspring were the Primary Poinsettia. In more than half the cases, Secondaries have arisen independently of their own group and have not yet been found among the offspring of their own Primaries. In all cases, however, the Secondaries have thrown their respective Primaries. The percentage of occurrences of Primaries in the offspring of Secondaries has varied from .20% in the case of Buckling from Maple to 3.8% in the case of Buckling from Strawberry.

TABLE IV (CF. TABLE III)

OFFSPRING OF PRIMARY AND SECONDARY ($2n + 1$) TYPES

| | % PRIMARIES | % SECONDARIES | TOTAL |
|---|---|---|---|
| POINSETTIA | 27.86 | .23 | 30,933 |
| Wiry | .73 | 18.31 | 2,998 |
| COCKLEBUR | 22.95 | .01 | 8,860 |
| Wedge | 1.45 | 19.06 | 2,477 |
| ECHINUS | 25.35 | 0.0 | 718 |
| Mutilated | 1.38 | 13.99 | 2,688 |
| Nubbin(?) | 3.25 | 30.50 | 400 |
| ROLLED | 18.16 | .14 | 11,140 |
| Sugarloaf | 2.96 | 20.90 | 5,230 |
| Polycarpic | .81 | 2.44 | 123 |
| BUCKLING | 33.57 | .03 | 3,101 |
| Strawberry | 3.80 | 30.31 | 947 |
| Maple | .20 | 21.27 | 1,984 |
| ELONGATE | 5.50 | 0.0 | 1,206 |
| Undulate | .68 | 23.65 | 592 |

5. Cytological Distinctions.—As is shown in the succeeding paper, Primaries and Secondaries, among those mutants for which adequate

cytological information has been secured, may be distinguished by differences in the configurations of their chromosomes.

*III. Basis of Differences between Primaries and Secondaries.*—The foregoing data point to the Secondary being a modified Primary. Rather extensive breeding experiments, which need not be given here, have proven untenable the provisional hypothesis that the modification might be brought about by a Mendelian factor. As a positive contribution, however, they seem to indicate that the modification is not carried by diploids nor by $1n$ gametes.

TABLE V

SEGREGATION OF MENDELIAN CHARACTERS IN $2n$ OFFSPRING IN TWO GROUPS OF PRIMARY AND SECONDARY ($2n + 1$) TYPES

| | POINSETTIA ($P_2pAa$) | | | | Wiry ($PP'pAa$) | | | |
|---|---|---|---|---|---|---|---|---|
| | *P* | *p* | *A* | *a* | *P* | *p* | *A* | *a* |
| Selfed | 1826 | 220 | 2155 | 738 | 98 | 14 | 116 | 38 |
| Calculated | (1819) | (227) | (2170) | (723) | (100) | (12) | (116) | (39) |
| Ratio | 8 : | 1 | 3 : | 1 | 8 : | 1 | 3 : | 1 |
| Back-crossed | 645 | 281 | 1103 | 940 | | | | |
| Calculated | ( 617) | (309) | (1022) | (1022) | | | | |
| Ratio | 2 : | 1 | 1 : | 1 | | | | |
| | COCKLEBUR ($PpA_2a$) | | | | Wedge ($PpAA'a$) | | | |
| | *P* | *p* | *A* | *a* | *P* | *p* | *A* | *a* |
| Selfed | 835 | 249 | 972 | 112 | 241 | 65 | 249 | 57 |
| Calculated | ( 813) | (271) | (963) | (120) | (229) | (77) | (229) | (77) |
| Ratio | 3 : | 1 | 8 : | 1 | 3 : | 1 | 3 : | 1 |
| Back-crossed | 271 | 238 | 1262 | 501 | 233 | 204 | 219 | 218 |
| Calculated | (255) | (255) | (1175) | (588) | (219) | (219) | (219) | (219) |
| Ratio | 1 : | 1 | 2 : | 1 | 1 : | 1 | 1 : | 1 |

More positive evidence as to the basis of the differences under consideration was secured from the breeding behavior of Wedge, which is a Secondary in the Cocklebur group. From table 5, in which for convenience only diploid offspring are shown, it will be seen that both Poinsettia and its Secondary Wiry give trisomic ratios for the color factors $P$, $p$, but give disomic ratios for spine factors $A$, $a$, indicating that both Poinsettia and Wiry have their extra chromosomes in the set carrying the factors $P$, $p$ but not in the set with the factors $A$, $a$. Similarly the ratios for Cocklebur indicate that this Primary has its extra chromosome in the set carrying the factors $A$, $a$, but not in the set with factors $P$, $p$. Its Secondary Wedge, however, fails to give trisomic ratios for $A$, $a$. The ratios actually found resemble those in disomic rather than in trisomic inheritance and seem to indicate a deficiency in the extra chromosome of Wedge for the locus $A$, $a$, since the evidence strongly indicates that it is a Secondary of Cocklebur. If $A'$ indicates the modified chromosome and $A$ & $a$ go to opposite poles at reduction division in a Wedge plant with the formula

$AA'a$, the gametes would be $A + a + AA' + aA'$. Such behavior would account for the ratios in table 5. If $A'$ is deficient for the factor $A$, the gamete $aA'$ would carry no factor for $A$; hence the disomic ratios between armed and *inermis* Wedges found but not shown in the table. If $A$ & $a$ occasionally should go to the same pole, the gametes would be $A'$ (which would probably die) and $Aa$ which would go to form a Primary Cocklebur occasionally thrown by Wedges.

The hypothesis of a deficiency in the extra chromosome of Secondaries has been strengthened by Dr. Belling's cytological findings. His hypothesis of reversed crossing-over, however, completes the picture by indicating a doubling of a part of the chromosome along with a deficiency of the remaining portion. The matter is considered in the paper which follows, but it should be pointed out that this new conception clears up many of the peculiarities in structure and breeding behavior of Primaries and Secondaries. Especially should be mentioned in this connection the complementary Secondaries in the Rolled and Buckling groups in which each Secondary shows certain characters manifested by the Primary but not shown by the other Secondary.

While we believe the problem of Primary and Secondary mutants is in way of being solved, we do not consider that all Secondaries are necessarily of the same type. Nubbin, for example, has been marked with a question in the tables because certain structural and breeding peculiarities seem to set it apart from the other Secondaries.

*Summary.*—I. The existence of not over 12 groups of $(2n + 1)$ mutants is indicated by the following more or less independent lines of evidence: 1. The occurrence of over 20 such mutants whereas there are only 12 sets of chromosomes in the species. 2. Similarities in external appearance. 3. Similarities in internal anatomy. 4. Pollen dimorphism in one group. 5. Trisomic ratios in one group. 6. Breeding behavior (cf. II, 4). 7. Chromosomal sizes (cf. next paper).

II. Evidence as to which are Primaries and which Secondaries is indicated by following evidence: 1. Primaries and not Secondaries occur in high proportions in offspring from triploids. 2. Primaries appear more frequently spontaneously. 3. Secondaries have a higher average proportion of bad grains in their pollen than their respective Primaries. 4. Secondaries occasionally come from Primaries, but Primaries regularly come from their Secondaries. 5. Differences between Primaries and Secondaries in configuration of their chromosomes (cf. next paper).

III. In seeking for a basis of the differences between Primaries and Secondaries, it has been shown, 1. that the difference is not due to a Mendelian factor, 2. that in one case the Secondary is modified apparently by deficiency of a portion of the extra chromosome, 3. that the cytological findings described in the succeeding paper give a chromosomal basis for observed facts and are confirmed by the occurrence of complementary Secondaries.

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[1] Blakeslee, Belling and Farnham, *Science*, *N. S.*, **52**, 388–90 (1920).

[2] Blakeslee and Farnham, *Amer. Nat.*, **57**, 481–95 (1923).

[3] Blakeslee, *Amer. Nat.*, **56**, 16–31 (1922).

[4] Sinnott and Blakeslee, These PROCEEDINGS, **8**, 17–19 (1922).

[5] Belling and Blakeslee, *Amer. Nat.*, **56**, 339–46(1922).

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# *THE CONFIGURATIONS AND SIZES OF THE CHROMOSOMES IN THE TRIVALENTS OF 25-CHROMOSOME DATURAS*

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Among those progenies of triploids by normals[1] in which the chromosomes have been counted in all or nearly all of the sibs, eleven forms of 25-chromosome plants have occurred in not far from equal numbers. These same primary 25-chromosome forms occurred rarely in the progeny of diploid plants, doubtless arising wholly (or in part) from non-disjunction. Thus out of 1137 pollen-mother-cells of normal diploids (which comprise all plants whose chromosomes were counted, and may be reckoned a random sample) , there were found 8 cases of non-disjunction. In these cases, 11 and 13 chromosomes were present in the second metaphases, instead of 12 and 12. These would produce about 0.4 per cent of 13-chromosome pollen-grains. Non-disjunction occurs also, as might be expected, in the primaries themselves; so that other forms of 25-chromosome plants may spring from any one primary. In 521 pollen-mother-cells of the 11 primaries (and these were all the cells whose chromosomes were counted, and may be regarded as a random sample) there were 4 certain cases of non-disjunction in which two groups of 11 and 14 chromosomes were formed. Since non-disjunction (as distinguished from random distribution of the odd chromosome) changing 12 and 13 into 13 and 12 cannot be ascertained by mere counting, and should be equally frequent with the 11 and 14 distribution; there were probably nearly as many cases of new groups of 13 as the number of groups of 14 seen. Hence, in the pollen of any primary, there might be about 0.4 per cent of 13-chromosome grains having an extra chromosome different from the normal extra chromosome of that primary. Doubtless many of these 13-chromosome grains do not function. But if the same amount of non-disjunction occurs in the egg-cells, we might expect