Geographical Distribution of Chromosomal Prime Types in *Datura stramonium* *

By

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The geographical distribution of plants and animals has received considerable attention in the study of evolution of species. Since we believe the problem of evolution of species can best be stated in terms of the evolution of their chromosomes, we have for some years been making a study of the chromosomal changes which have taken place in nature within the genus *Datura*. The present paper has to do with Prime Types (PT's), or races with modified chromosomes in terms of a standard race, within the single species *D. stramonium*.

At the present time we have in our collection over 90 prime types of various kinds which have been obtained from radiation and heat treatments, from aged seeds and pollen grains and from races in nature. Prime types have been discussed in an earlier publication (1). Only those obtained from nature need concern us here. That chromosomal races existed in *Datura* was early recognized from breeding behavior (2). By this means we were able to classify 198 of our strains into 73 "A" races which gave normal inheritance of the purple-white factors and 125 "B" races which gave abnormal inheritance of these factors. Belling (3) showed that the peculiarities of the so-called "B" race (now called prime type 2) could be explained by his hypothesis of segmental interchange. Since that time we have been accumulating evidence regarding the occurrence in nature of prime types in *D. stramonium* and other species of this genus.

In 1929, a preliminary report (4) was made regarding the "cryptic" chromosomal types of *D. stramonium* which up to that time had been found in nature. These prime types, as we now term them, were called cryptic because there was nothing in their appearance which could be used to distinguish their chromosomal constitution from that of our standard Line 1. They may show recognizable differences, however, due to genes. In the paper cited we were unable to distinguish all the PT's we now know and had determined the end arrangements of the modified chromosomes in only PT 2. At that

* "Awarded the A. Cressy Morrison prize for 1936 by the New York Academy of Sciences."

time also there was no knowledge available regarding the end arrangements of modified chromosomes in species of the genus *Datura* other than *D. stramonium*. Consequently, no relationship could be suggested between the PT's in different species. The numbers of races in our earlier paper were not sufficient to justify the plotting of their geographical distribution. Further tests enable us now to report on the chromosomal constitution of 583 races and to say something regarding their distribution in the six continents, as well as to suggest relationships between prime types and evolution in the genus *Datura*.

The method employed in testing races for prime types in *D. stramonium* was essentially the same as that used for races in other *Datura* species. An assemblage of races was secured from as widely different parts of the world as possible through our own collections and through the kindness of correspondents who cooperated in obtaining seeds for our tests.¹⁾ Each collection of seeds from a given locality which generally came from a single plant was assigned an S.I. (seed introduction) number. In our own collections it was possible to know with some precision how many plants and how many loci were represented from a given region. Our custom was to sample a patch of plants by gathering seeds from two or three individuals in the group, each receiving a separate S.I. number and becoming the starting point of a new race. Each patch may be considered a

1) The investigation is indebted for seeds of *D. stramonium* to members of the *Datura* group and to the following:—A. H. G. Alston, G. E. Anastasia, W. A. Archer, A. Aslander, B. T. Avery, C. A. Backer, A. M. Banta, P. Baranov, H. D. Barker, H. H. Bartlett, E. Baur, Mathilde Bensaude, D. Bois, R. Bolton, D. N. Borodin, S. Boshnakian, T. Brinkley, N. L. Britton, L. A. Brown, S. C. Bruner, S. Calderon, Rosalie Caples, W. M. Carne, J. de Carvalho e Vasconcellos, E. Cheel, A. Chevalier, R. Chodat, H. H. Chung, J. L. Collins, C. Conzatti, H. F. Crampton, J. N. Couch, C. B. Davenport, Gertrude C. Davenport, G. A. de Mol, P. H. Dorsett, R. A. Emerson, Y. Emoto, N. Esposta, J. M. Fogg Jr., P. Font-Quer, L. Fourton, K. Fujii, Lulu Gaiser, R. R. Gates, Edith Grosvenor, V. Goessens, G. K. Gunjko, J. W. Harshberger, L. J. Heatly, A. H. Henckel, C. M. Holmes, F. J. Hopkins, S. Horowitz, P. G. Howes, J. B. Hurry, M. A. Jabbar, L. I. Kazakevich, D. A. Keck, Anna M. Kornhauser, H. H. Laughlin, I. F. Lewis, D. H. Linder, F. E. Lloyd, A. Lutz, R. Maire, E. Manoilov, O. Mattiolo, J. R. A. McMillan, M. A. Meunissier, N. W. Monteverde, Daisie Morrison, J. Nonidez, J. F. Normand, A. Noronka, I. V. Novopokrovsky, R. T. Palhinha, G. Pallacci, R. N. Parker, Emily Perkins, Orra P. Phelps, E. P. Phillips, A. A. Pristupa, W. Razdorskij, B. E. Read, G. M. Reed, P. H. Rolfs, K. Ryerson, J. G. Salas, R. Salgues, A. Sangiovanni, Virginia Schoonmacher, A. Serck, S. Shantz, P. Shevchenko, D. K. Larinov, R. Stewart, N. Stshibrya, A. H. Sturtevant, Z. Szabo, F. Thone, N. P. Vercholanova, C. T. White, O. E. White, Anna R. Whiting, C. M. Woodworth, Elaine Young, J. L. de Zuazo, A. de Zulueta.

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Without the cooperation of these just mentioned it would not have been possible to obtain records from such widely distributed regions of the world. We should appreciate receiving samples of a few seeds from individual plants of *D. stramonium* from regions not represented in our tables as well as seeds of other species of *Datura* which we hope to study in a similar manner.

separate locus. Sometimes the races from a single patch differed chromosomally. In such cases the patch might be listed as the locus for several different prime types or prime type associations. When collections were sent to us it was not always certain whether the seeds in a packet came from one or from many plants, but it has been our practice to record each packet as a separate S.I. As a preliminary test of the homozygosity of the race, about a dozen plants of each S. I. are grown. Examinations are made for pollen abortion but the chromosomes are not examined in this generation. It has occasionally happened that segregation of pollen abortion types has been discovered in this first test generation. Probably segregation of PT's which are unaccompanied by pollen abortion might similarly have been discovered if it had been feasible to make the necessary cytological examinations. A more profitable procedure was found to be an examination of the chromosomes in hybrids between a selected plant of the given S. I. race and our standard Line 1 (PT 1).¹⁾ If the given plant happened to be heterozygous for a PT it would be an even chance which component appeared in the F₁ hybrid examined, hence the accidental choice of a heterozygous plant as the representative of the race to be tested would in no way affect the random character of the sample. If in the hybrid only twelve bivalents are observed in the first meiotic division in pollen mother cells, the S. I. in question is listed as the same PT as the tester, viz. PT 1. If, however, a characteristic configuration of four or more attached chromosomes is found, the S. I. is listed as belonging to one of the other PT'S. Familiarity with the sizes and arrangements of the chromosomes in the configurations and further tests with certain other of our prime types (1) have made possible an accurate classification of the types which differ from PT 1.

Prime type 1. Throughout this investigation one race has been used as a standard in terms of which the chromosomes of *D. stramonium* and other species have been interpreted. Our Line 1 is a highly inbred race which came originally from Washington, D. C., and has been listed as PT 1. The twelve chromosomes of PT 1 may be arranged in Table 1 according to their relative sizes beginning with the largest (L) as 1·2. Thus far terminal humps have been located on eight of these twelve PT 1 chromosomes. They serve as convenient markers to identify chromosomes and particular ends

1) In the task of determining the chromosomal condition in F₁ hybrids, during a number of summers we have been aided by the following:—Helen Besley, Louise Buck, Margaret B. Chambers, Priscilla Chinn, Caroline A. Denton, O. J. Eigsti, Rachel Haynes, I. Hein, Ruth E. Henderson, Dorothy C. Hewitt, Lucy B. Hodges, Alfhild E. Johanson, R. H. MacKnight, Lois I. Platt, J. S. Potter, Sophia Satina, Jennie L. S. Simpson, H. E. Warmke.

of chromosomes. They are probably the equivalents of satellites or knobs which G. A. Lewitsky (5) demonstrated on several chromosomes in a study of root-tip cells of *Datura*. They are represented by superscripts at the appropriate ends of the chromosomes.

Table 1

L- ¹ ·2	M- 9·10 ¹⁰	m- 17·18
1 - 3·4	M-11·12 ¹²	m- 19·20 ²⁰
1 - 5·6	M-13·14	S- ²¹ 21·22
M- 7·8 ⁸	M-15·16 ¹⁶	s- 23·24 ²⁴

Prime type 2. The "B" type which had been distinguished by breeding behavior is now called prime type 2. Instead of the chromosomes 1·2 and 17·18 of PT 1, it has the modified chromosomes 1·18 and 2·17. The hybrid between PT 2 and PT 1 shows ten bivalents plus a circle of four chromosomes in the following order:—2·17-17·18-18·1-1·2—.

When the hypothesis of segmental interchange was first proposed, it seemed to necessitate the abortion of half of the gametophytes. Hybrids between PT 1 and *stramonium* races of PT 2 have good pollen. The configuration rarely occurs as a flat circle on the spindle of the first meiotic division. Since alternate chromosomes have become attached to fibers leading to the same pole, adjacent ones to fibers leading to opposite poles, one finds a twisted circle of four chromosomes horizontally placed across the equatorial region. This zigzag arrangement is more striking in circles of six or more chromosomes, but is clearly seen in smaller circles. This orderly separation of adjacent chromosomes to opposite poles results in the formation of viable gametophytes. *Intrase stramonium* hybrids rarely show this circle broken.

The question was raised (4) whether all of the PT 2 races were really the same. By our methods only the ends of chromosomes are identified; ordinarily we cannot locate the region in the chromosome at which the interchange took place. In 1930 an examination was made of crosses between the first PT 2 race, which came from a commercial firm in Erfurt, Germany, and fourteen other PT 2 races selected from various parts of the world. One each was selected from North Carolina (U.S.A.); Chile (a purple race); Australia; Madeira Islands, French Somaliland, Pretoria and Johannesburg in South Africa; Kashmir, India; Tashkent, Asia; Portugal; Hungary; two from Italy and one from a Botanical Garden in Brazil. In all these fourteen hybrids the 1·18 and 2·17 chromosomes formed equal bivalents, and these PT 2 races therefore may be considered identical.

Prime type 3. Instead of the chromosomes 11·12 and 21·22 of PT 1, prime type 3 has the modified chromosomes 11·21 and 12·22. The hybrid with PT 1 shows ten bivalents plus a circle of four chromosomes in the following order:—22·21-21·11-11·12-12·22—. The

circle formation is found only rarely. Failure of attachment at the two ·22 ends produces a chain of four; failure of attachment also at the ·11 ends produces two open unequal pairs, 11·21–21·22 and 11·12–12·22. Since the members of each pair separate independently of the other, chromosomes which would be adjacent in a circle formation may go to the same pole, resulting in deficiencies and hence in inviable gametophytes. This chromosomal behavior probably explains in part why these hybrids show a variable amount of aborted pollen. That the environment has some effect on the amount of abortion is indicated by the fact that when the plants are grown in the field they regularly show about 25 per cent of aborted grains whereas this amount varies in greenhouse plants.

Prime type 4. Instead of the chromosomes 3·4 and 21·22 of PT 1, prime type 4 has the modified chromosomes 3·21 and 4·22. The hybrid with PT 1 shows ten bivalents plus a circle of four chromosomes in the following order:—22·21–21·3–3·4–4·22—. Failure of attachment at the ·22 ends produces a chain of four. Unlike the condition found in the PT 3 hybrids, the configuration in this PT 4 hybrid seldom is resolved into open pairs with resultant deficiencies and bad pollen. Prime type 4 hybrids, therefore, have good pollen.

Prime type 7. The modified chromosomes in prime type 7 have been formed by the interchange of humps rather than of larger segments of the chromosomes. Thus instead of the 9·10¹⁰ and 19·20²⁰ chromosomes of PT 1, those of PT 7 are 9·10²⁰ and 19·20¹⁰ in which the superscripts represent the humps. The hybrid with PT 1 shows ten bivalents plus a “necktie” The latter consists of two bivalents which are connected at the hump region only and is represented as follows:

$$9 \cdot 10^{10-10} 20 \cdot 19$$

$$9 \cdot 10^{20-20} 20 \cdot 19$$

The hybrid with PT 1 shows 50 per cent of pollen abortion. This is true also for other PT's which show a “necktie” configuration in hybrids. Since the members of each bivalent may become oriented on the spindle independently of the arrangement in the other bivalent comprising the “necktie”, inviable combinations are to be expected in half of the cases, as explained in a previous paper (1).

In races which were tested several years ago, PT 7 was identified mostly by means of pollen tests. The routine then was as follows: each new race was crossed to both PT 1 and PT 7. If the hybrid with PT 1 showed 50 percent of aborted pollen grains and the hybrid with PT 7 showed good pollen, the race was classified as belonging to the PT 7 group. The “necktie” configuration of four chromosomes is frequently missing and is easily overlooked. This was particularly

true in the earlier examinations. In the more recent tests, PT 7 has been identified both by the pollen and by cytology.

Prime type 87. In addition to the prime types discussed in earlier paragraphs, the following two have been found each from only a single locality. Prime type 87 was obtained from Oaxaca, Mexico, from a plant which was also PT 2. It has the chromosomes 3·12, 4·22 and 11·21 instead of the 3·4, 11·12 and 21·22 chromosomes of PT 1, and in consequence the hybrid with the latter shows a circle of six in the following order: —22·4—4·3—3·12—12·11—11·21—21·22—. The chromosomes in this circle may be only partially arranged in zig-zag fashion on the spindle; or, because of weak attachments at the ·11 and ·22 ends, these six chromosomes may be arranged as a chain of six or a chain of four plus an open unequal pair. Consequently chromosomes which would lie adjacent to one another in a circle formation may go to the same pole and hence result in deficiencies and inviable gametophytes. The hybrid with PT 1 shows abortion of upwards of half of the pollen grains. Because it has the 11·21 chromosome in common with PT 3 and the 4·22 chromosome in common with PT 4, PT 87 may be the result of segmental interchange between the 12·22 chromosome of PT 3 and the 3·21 chromosome of PT 4, although as yet we have not found any PT 4 races in Central America to hybridize with the PT 3 races there.

Prime type 88. A second isolated prime type is PT 88 which was obtained from Entrerios, Argentina. It has the modified chromosomes 15·23 and 16·24 instead of the 15·16 and 23·24 chromosomes of PT 1. The hybrid with PT 1 shows a circle of four chromosomes as follows:—15·16—16·24—24·23—23·15—. Like PT 2, the hybrid with PT 1 shows a circle that rarely is broken. Practically no pollen is aborted.

Among the prime types secured from radiation treatment, a considerable number were produced by simple translocations in which there was a free fragment and a translocated portion of the chromosome. The hybrid of such prime types with PT 1 shows an open chain, never a closed circle. All the prime types so far found in nature were the products of segmental interchange. Except for PT 87 which was found only once, these interchanges involve only two non-homologous chromosomes.¹⁾

1) In Table 1 of our earlier paper (4) two configurations of six were recorded in the hybrid with PT 1. One race was from Concepcion, Chile, and the other from Kashmir, India. In both cases the records show the occurrence of configurations of six in some pollen mother cells and configurations of four in other cells on the same slide. We are not prepared to offer an explanation of these discrepancies in our early records. The two S. I. races were retested and found to react only as normal PT 2.

It might be considered surprising that so few prime types have been obtained from a rather wide sampling in view of their frequency after treatments. There are only five which have been found more than once. Interchanges must have occurred several times in our cultures from untreated parents. Thus the tertiary $2n + 4.6$ has appeared spontaneously twice, the $2n + 13.18$ also twice and the $2n + 2.9$ once. In these five instances the tertiary chromosomes are evidently formed by segmental interchange from normal PT 1 chromosomes. However, attempts in these cases to obtain the complementary tertiary chromosomes in homo- or heterozygous prime types have been unsuccessful. In addition, tests were made to see if, in carrying on our various $2n + 1$ types through selfing for several years, segmental interchanges had not occurred in the sublines which had thus been established. Back-crosses to PT 1 in all of 24 cases showed only 12 bivalents and indicated that these 24 inbred strains were free from prime types induced by segmental interchange. We have no proof therefore that PT's have established themselves in our cultures except through previous treatment.

In Table 2 are listed 583 races with their geographical origin and the prime types which they represent. They are also separated into those with purple (P) flowers and those with white (W) flowers. A summary is given in Table 3. It will be noted that the sum of the number of races under the PT's is considerably more than the number of races in the second column. This is due in part to the fact that a single race may show more than one independent configuration in the hybrid with PT 1 and thus be credited to more than a single PT in the columns to the right. It is due also in part to the fact that some of the races are listed as both P and W. In Table 4 are listed those loci in which more than a single PT was found either in different individuals or associated in the same plant. In 14 loci more than a single PT was found in different individuals in the same locus. In 121 races associations of two PT's in the same plant were observed. All the races of PT 3 in nature are also PT 2, but we have isolated a pure race of PT 3 in our cultures. The maximum number of PT's associated in a single plant is three and such a condition has been observed twice.

Datura stramonium is widely distributed throughout the world in the semitropics and in the warmer portions of temperate regions. The location of 583 races is shown in Table 2 and on maps in figs. 1-6. In the table, races have been grouped first according to the geographical unit to which they belong and secondly under this, to the political subdivisions of this unit. Almost two-fifths of the collection came from the United States; so large a number has not been obtained

Table 2. Distribution of chromosomal prime types among 583 races of *Datura stramonium*. P—purple flower color; W—white

No. Loc	Races			Origin	PT 1			PT 2			PT 3	PT 4			PT 7			Other PT's
	Total	P	W		Total	P	W	Total	P	W	P	To- tal	P	W	To- tal	P	W	
1	1	1	0	CANADA	1	1	0											
155	*216	171	46	UNITED STATES	*162	134	29	24	16	8		32	27	5	15	10	5	
9	21	21	0	Massachusetts	15	15	0	2	2	0					5	5	0	
6	10	10	0	Connecticut	10	10	0											
12	15	6	9	New York	14	6	8								1	0	1	
2	4	2	2	New Jersey											4	2	2	
11	13	12	1	Pennsylvania	11	11	0	2	1	1					1	0	1	
7	9	9	0	Delaware	7	7	0					2	2	0				
15	23	16	7	Maryland	19	15	4	3	0	3		1	1	0				
48	65	55	10	Virginia	36	32	4	14	11	3		23	19	4	3	3	0	
14	17	16	1	North Carolina	11	11	0	3	2	1		6	5	1	1	0	1	
1	2	1	1	District Columbia	2	1	1											
3	3	3	0	West Virginia	3	3	0											
1	2	2	0	Alabama	2	2	0											
1	1	1	0	Florida	1	1	0											
1	1	1	0	Tennessee	1	1	0											
3	3	2	1	Kentucky	3	2	1											
4	7	6	1	Indiana	7	6	1											
2	2	2	0	Illinois	2	2	0											
4	*6	5	2	Missouri	*6	5	2											
2	2	0	2	Arkansas	2	0	2											
3	3	1	2	Kansas	3	1	2											
2	2	0	2	Michigan	2	0	2											
1	1	1	0	Texas	1	1	0											
1	1	1	0	New Mexico	1	1	0											
2	3	1	2	California	3	1	2											
11	16	6	10	WEST INDIES	6	1	5	5	1	4		4	4	0	5	4	1	
1	1	1	0	Puerto Rico	1	1	0											
2	4	0	4	Virgin Islands	4	0	4											
5	8	5	3	Barbados				4	1	3		4	4	0	4	4	0	
1	1	0	1	Montserrat				1	0	1								
1	1	0	1	Haiti	1	0	1											
1	1	0	1	Cuba											1	0	1	
7	10	8	2	CENTRAL AMERICA				10	8	2	3							1 (P)
5	5	3	2	Mexico				5	3	2	2							1 (P)
1	4	4	0	San Salvador				4	4	0								
1	1	1	0	Guatemala				1	1	0	1							
58	81	56	25	SOUTH AMERICA	15	0	15	65	56	9	51							1 (W)
4	4	4	0	Colombia				4	4	0								
1	1	1	0	Venezuela				1	1	0								
14	15	0	15	Brazil	15	0	15											
1	1	0	1	Argentina														1 (W)
18	32	32	0	Peru (purple)				32	32	0	32							
5	9	0	9	Chile (white)				9	0	9								
15	19	19	0	„ (purple)				19	19	0	19							
82	*140	9	132	EUROPE	8	2	6	125	3	122	1	*23	4	20	8	2	6	
1	1	1	0	England				1	1	0								
1	3	0	3	Germany				3	0	3								
2	5	0	5	Switzerland				5	0	5								
4	6	1	5	France	4	1	3	2	0	2		1	0	1				
18	25	4	21	Spain				21	2	19	1	14	2	12	7	2	5	
8	*8	3	6	Portugal	2	1	1	3	0	3		*5	2	4				
22	36	0	36	Italy	1	0	1	35	0	35		3	0	3	1	0	1	
10	37	0	37	Hungary				37	0	37								
16	19	0	19	Russia	1	0	1	18	0	18								
23	33	5	28	ASIA	7	5	2	26	0	26								
5	7	5	2	Japan and Korea	7	5	2											

Table 2 (Continued)

No. Loci	Races			Origin	PT 1			PT 2			PT 3	PT 4			PT 7			Other PT's
	Total	P	W		Total	P	W	Total	P	W		Total	P	W	Total	P	W	
6	7	0	7	Java				7	0	7								
2	7	0	7	Siberia and Turkestan				7	0	7								
5	7	0	7	China				7	0	7								
4	4	0	4	India and Ceylon				4	0	4								
1	1	0	1	Mesopotamia				1	0	1								
18	†26	9	19	AFRICA	3	0	3	12	1	11		†12	9	5	†12	8	6	
2	2	1	1	Madeira Islands				1	0	1		1	1	0	1	1	0	
4	4	0	4	Algeria				4	0	4								
1	1	0	1	Liberia				1	0	1								
3	3	0	3	Portuguese W. Africa	3	0	3											
2	2	0	2	Belgian Congo				2	0	2								
1	1	0	1	French Somaliland				1	0	1								
1	1	0	1	British E. Africa				1	0	1								
4	†12	8	6	South Africa				2	1	1		†11	8	5	†11	7	6	
5	5	2	3	AUSTRALIA	1	0	1	2	0	2		2	2	0	2	2	0	
2	4	4	0	HAWAII								1	1	0	4	4	0	
	†51	23	31	BOTANICAL GARDENS	†14	9	8	29	6	23		6	6	0	8	8	0	
362	583	294	296		217	152	69	298	91	207	55	80	53	30	54	38	18	2

* One race heterozygous for purple and white.

† Two races heterozygous for purple and white.

‡ Three races heterozygous for purple and white.

Table 3. Distribution of chromosomal prime types among 583 races of *Datura stramonium*. P—purple flower color; W—white

No. Loci	Races			Origin	PT 1			PT 2			PT3	PT 4			PT 7			Other PT's
	Total	P	W		Total	P	W	Total	P	W		Total	P	W	Total	P	W	
1	1	1	0	CANADA	1	1	0											
155	*216	171	46	UNITED STATES	*162	134	29	24	16	8		32	27	5	15	10	5	
11	16	6	10	WEST INDIES	6	1	5	5	1	4		4	4	0	5	4	1	
				CENTRAL AMERICA				10	8	2	3							1(P)
7	10	8	2	SOUTH AMERICA														1(W)
58	81	56	25	EUROPE	15	0	15	61	56	9	51							
82	*140	9	132	ASIA	8	2	6	125	3	122	1	*23	4	20	8	2	6	
23	33	5	28	AFRICA	7	5	2	26	0	26								
18	†26	9	19	AUSTRALIA	3	0	3	12	1	11		†12	9	5	†12	8	6	
5	5	2	3	HAWAII	1	0	1	2	0	2		2	2	0	2	2	0	
2	4	4	0	BOTANICAL GARDENS								1	1	0	4	4	0	
	†51	23	31		†14	9	8	29	6	23		6	6	0	8	8	0	
362	583	294	296		217	152	69	298	91	207	55	80	53	30	54	38	18	2

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from any other country. Races which were obtained from Botanical Gardens or Commercial seed houses are listed in the table under Botanical Gardens apart from other races because one cannot be

certain of their origin. For this reason they have not been indicated on the maps.

Prime type 1 is the predominant type in the United States; in fact, it is the only type that has been found west of the Atlantic coast. It is the only type which we have obtained from Brazil and Japan. It occurs also on some of the West Indian islands; in several countries of Europe, particularly France; in Portuguese West Africa and Australia.

If totals only are considered, the widespread distribution of PT 2 is somewhat masked by the fact that PT 1 is the predominant type in the United States from which so large a proportion of our races have come. PT 2 is the predominant type in Central America, Peru and Chile, Europe, Asia and Africa.

Prime type 3 associated with PT 2 is the only type found in Peru where all the plants observed had purple flowers. In Chile, where both purple-flowered and white-flowered races occur, the purples were PT 3 and 2 while the whites were PT 2 only. PT 3 associated with PT 2 was also obtained in Central America and Spain.

Prime type 4 is more widespread in its distribution. It is common in Virginia and North Carolina and has been found also in Maryland and Delaware in the United States; in the Barbados in the West Indies; in southern Europe, especially Spain and Portugal; in South Africa; Australia and the Hawaiian Islands.

Prime type 7 is a predominant type in South Africa and the Hawaiian Islands. It has been found along the Atlantic coast of the United States, in the Barbados, Spain, Italy, the Madeira Islands and Australia.

On the maps are indicated the loci from which races were obtained. Compound symbols made up of two or more single symbols indicate the association of more than one PT in the same plant. Numerals adjacent to a symbol indicate the number of loci in which the type was obtained in the area covered by the symbol. In each of twelve loci, two separate symbols had to be used because in these loci were found separate plants which belonged to two different PT's or PT combinations as shown in Table 4. In two loci three separate symbols were used because in each there were separate plants which belonged to three different PT's or PT associations. Not indicated on the maps are four loci beyond the limits of the part of the map of the United States shown in fig. 1 and two loci in the Hawaiian Islands.

Fig. 1 shows that PT 1 is the predominant type in the United States. Prime type 4 centers in Virginia and North Carolina where it may occur associated with PT 2 or PT 7 or with both. There is

Table 4. Loci in which more than one prime type is represented.
P—purple flower color; W—white

Locality	Plants with more than one PT				Plants with one PT			
	No.	P	W	PT's	No.	P	W	PT's
Massachusetts Tarpaulin Cove	1	1		2,7	3	3		1
Barnstable					1	1		2
Pennsylvania Spring Mills	1		1	2,7	1	1		1
Delaware Georgetown					1	1		4
Virginia Partlow	1	1		4,7	1	1		1
Toano	5	5		2,4	1	1		2
					1	1		4
Petersburg	2	2		2,4	2	2		1
Gardiner's Cross Roads I	1	1		4,7	1		1	7
					1	1		1
Gardiner's Cross Roads II					1		1	2
Gordonsville					1	1		1
					1	1		4
Richmond	1	1		2,4				
Yorktown	1		1	2,4				
North Carolina Jackson	2	2		2,4				
Murfreesboro I	1		1	2,4,7	1	1		1
Murfreesboro II					1	1		4
Barbados Locus I	1	1		4,7				
	1	1		2,7				
Locus II	1	1		4,7				
Locus III	1	1		4,7	1		1	2
Locus IV					1	1		2
					1			4
Mexico Santa Cruz	1	1		2,3				
Oaxaca	1	1		2,87				
Huexotla	1	1		2,3				
Guatemala Guatemala	1	1		2,3				
Peru (all purple)	32			2,3				
Chile (purple only)	19			2,3				
France Perpignan	1		1	2,4				
Spain Barcelona I	1	1		2,3				
Barcelona II	1		1	2,4				
San Adrian I	2			2,4				
San Adrian II	3		3	2,4				
San Adrian III	3		3	2,4				
Montseny I	1		1	2,4				
Montseny II	2		2	2,4				
Palos	3		3	2,7				
Aragon	1	1		4,7				
Madrid	1	1		4,7				
Portugal Lisbon I	1		1	2,4				
Lisbon II	1		1	2,4				
Italy Rome I	1		1	2,4,7	3		3	2
Rome II	1		1	2,4	1		1	2
Piedmont	1		1	2,4				
Madeira Islands	1	1		4,7				
South Africa Pretoria	1		1	2,7				
Johannesburg I	*8	5	4	4,7				
	1	1		2,4				
Johannesburg II	*1	1	1	4,7				
Bulawayo	1	1		4,7				
New South Wales Gunnedal	1	1		4,7				
Queensland Moreton Bay	1	1		4,7				
Hawaii Molakai	1	1		4,7				
Botanical Gardens								
Madrid, Spain	4	4		4,7				
Rio, Brazil	1	1		2,4				
Havana, Cuba	1	1		4,7				

* One race heterozygous for purple and white.

a scattering of PT 2 and of PT 7 to a lesser extent along the Atlantic coast in the United States.

Fig. 1. Distribution of prime types in Central and Eastern United States. In this and following maps, numerals adjacent to a symbol indicate the number of loci of this type. Because of limited space in some states along the Atlantic Coast, the symbols have been drawn to the right with lines indicating the loci. Prime type 1 was obtained also at Gulfport, Florida; Haynes Canyon, New Mexico; Sacramento and Palo Alto, California.

Fi . 2. Distribution of prime types in Mexico, Central America and the West Indies

In contrast, South America, as indicated in fig. 3, shows separation of types. All of the collections from Brazil belong to PT 1 type. In Chile all white plants were PT 2; all purple plants, PT 2 plus PT 3. In Peru only purple flowered plants were found, all being PT 2

Fig. 3. Distribution of prime types in South America.

Fig. 4. Distribution of prime types in Europe.

Fig. 5. Distribution of prime types in Asia, East Indies and Australia.

plus PT 3. In addition a new type (PT 88) was obtained from northern Argentina. A rather careful search for *Daturas* was made by one of us while on a collecting trip through the Argentine. From our own observations in the field, from inspection of specimens in the principal herbaria of the country and from reports of botanists whom we consulted, it is safe to say that although *D. ferox* is common, *D. stramonium* is not known to occur in the central and southern parts of this country. The collection which gave us PT 88 is the only record we have of the occurrence of *D. stramonium* in the Argentine. It is possible that further collections in the same general region might disclose the fact that PT 88 is not a sporadic prime type but is representative of the races in this part of the country.

Fig. 6. Distribution of prime types in Africa and Southwestern Asia Minor.

In fig. 4 one can see that PT 2 is the predominant type in Europe; in fact it is the only type except in Portugal, Spain, southern France and western Italy and one locus in northern Russia.

The predominance of PT 2 types also on the mainland of Asia is shown in fig. 5. The condition is a continuation of that in eastern Europe. Prime type 1 occurs in Japan and Australia. In the latter, PT 4 and PT 7 also have been found.

While it is true, as shown in fig. 6, that PT 2 is widespread in most of Africa, in southern Africa PT's 4 and 7 predominate and on the west coast PT 1 has been found.

Discussion

The preceding paragraphs have presented the data regarding the distribution of prime types in our collection of races of *D. stramonium*. Certain facts seem to stand out rather clearly. There are areas in which certain types appear to occur exclusively so far as their races have been tested. Among these are the Peruvian area which contains purple-flowered PT 3 associated with PT 2, Brazil which contains a white-flowered PT 1, Eastern Europe and Western Asia with a white PT 2, and Japan with PT 1 including both purples and whites. On the other hand there are areas in which a mixture of types is found. As examples may be mentioned the Eastern states of the United States, the Southwestern part of Europe including Southern France, Spain, Portugal and Western Italy, Central America and the West Indies, and Australia.

Datura stramonium is a weed and is likely to be found in disturbed soil wherever the climatic conditions are favorable. Its place of origin is unknown. Gray's *Manual* as well as Britton and Brown's *Flora* describe the two Linnean species, *D. stramonium* and *D. tatula* as distinct species although the two are merely color forms determined by a pair of genes for white and purple respectively. The purple form is listed as naturalized from tropical America and the white form as naturalized from Asia. The latter statement, however, is qualified by a question mark. Let us see if evidence from our prime types may have a bearing on this problem of the origin of the purple and white forms in the United States. Our collection of races from this country is predominantly purple (171 P: 46 W) and is predominantly PT 1 (162 PT 1; 24 PT 2; no PT 3; 32 PT 4; 15 PT 7). In South America the only known source of PT 1 is in Brazil, but all the plants of which we have records from Brazil are whites. Whites are distinctly in the minority in the United States. Among the races from Central America, purples predominate but they are all PT 2's. PT 2 is rare in the United States. The West Indies have all the types which occur

in the United States although our collections from there show a relatively smaller proportion of PT 1's and the latter differ from those in the United States by being mostly whites. If we look outside this country for a place of origin of the predominant purple PT 1's which occur here, the only place which our records suggest is Japan, but the data from that country so far are rather meager.

We have made tabulations of the proportions of purples and whites among the different prime types as shown in Table 5. In states where PT 2 occurs, whites have been found four times as frequently in PT 2 as in PT 1, a fact which suggests that if PT 2 was introduced into this country, it was brought in chiefly in the white-flowered form. If we seek for such a source of supply, it would be natural to look to Europe where white PT 2's predominate or perhaps to Africa whence they might have been brought in with the slave trade. A glance at Table 2 and the map in fig. 1 will show that in the United States, PT 2, PT 4 and PT 7 are confined to the eastern states. The western states which show only PT 1's, though relatively poorly sampled, have twice as many races tested as has North Carolina, for example, which has representatives from all the PT's which occur in North America. This eastern area, therefore, may be considered the area where the different types were introduced or perhaps where they originated. From Table 5 it might appear that

Table 5. Occurrence of purples and whites in prime types in selected states in United States

5 states with PT 2 records			4 states with PT 4 records			6 states with PT 7 records		
Prime type 1			PT 2			PT 1		
Total P W			Total P W			Total P W		
92	84	8	24	16	8	73	65	8
Ratios	10.5	1		2	1		8	1
Expectation from PT 1 records			22			28.5		
						3.5		
						13		

both PT 4 and PT 7 are somewhat more likely to be whites than purples. The tendencies at best are slight and the numbers are not large. It is doubtful, therefore, if conclusions can be drawn safely from these facts which would favor PT's 4 and 7 also being introduced in white plants.

Frequently collections from a single patch of plants (locus) disclosed more than a single PT or color form. Such cases are summarized in Table 4. They include cases in which a single plant had to be recorded as belonging to two or three PT's. In other cases different PT's were represented in separate individuals in the same locus. The association of PT's together in the eastern United States

is summarized in Table 6. Prime type 7 occurs associated with PT 2 about as frequently as one would expect from the proportion of PT 2 races tested for the region in question. Prime type 4, however,

Table 6. Occurrence of prime type 2 with PT 4 and PT 7 in United States

4 states with PT 4 records						6 states with PT 7 records					
Not prime type 4			Prime type 4			Not prime type 7			Prime type 7		
Total	Not PT2	PT2	Total	Not PT2	PT2	Total	Not PT2	PT2	Total	Not PT2	PT2
82	74	8	32	20	12	120	102	18	15	12	3
Ratios	9.25	1		1.66	1		5.6	1		4	1
Expectation from non-PT 4 records				29	3	Expectation from non-PT 7 records				12.7	2.3

appears to be associated with PT 2 about four times as often as one would expect. The same reasoning employed in discussing the association of white flower forms with PT 2 would suggest that PT 4 came into the United States in association with PT 2. This conclusion could not be extended to all other countries since it will be observed from Tables 2 and 3 that PT 4 is predominantly purple in Africa and exclusively purple in Australia and in Botanic Gardens.

Prime type 4 and PT 7 are seen from Table 4 to be frequently associated together. Out of 32 occurrences of PT 4 in the United States, three were associated with PT 7. In other parts of the world, however, they are associated with one another more frequently. Thus out of 80 PT 4's, 28 were associated with PT 7. In terms of PT 7 the frequency of association is still more striking. There are 54 PT 7's in our collection. Of these, 28 or more than half were found associated in the same plants with PT 4. These facts suggest that PT 7 may have originated in a PT 4 race and have been separated through hybridization in cases in which it occurs alone. Similarly, since PT 2 is widespread and PT 3 is restricted in its range but is always associated with PT 2, it may be concluded that PT 3 arose in a PT 2 race.

We have discussed the origin and distribution of types in terms of PT 1 as a standard. A standard we believe is essential, but the choice of PT 1 was accidental, due to its predominance in this country. We could use any standard type without implying thereby that this race was phylogenetically the origin of other races in this and other species. With our present knowledge, we should now choose PT 2 in all probability. Prime type 2 appears to be more widely distributed. It also shows a closer relationship with other species tested. Thus the standard tester races of *D. ferox*, *D. quercifolia*, *D. Leichhardtii* and probably *D. pruinosa* contain the PT 2 modified

chromosomes 1·18 and 2·17. The tester race of *D. discolor* has the PT 2 chromosome 2·17 but the 1·18 chromosome has been modified by further interchange. If one were to use any single PT of *D. stramonium* as the primitive form from which to derive all other species of the genus, it would appear natural, therefore, to choose PT 2.

Another PT which shows relationships with other, though fewer, species is PT 3. The PT 3 chromosomes 11·21 and 12·22 occur in the tester races of *D. ferox* and *D. quercifolia*, which with *D. stramonium* make up the *stramonium* group. Furthermore, the tester race of *D. discolor* is a modified PT 3. If one were to choose a race from Peru as a standard, one would have PT 2 associated with PT 3. In consequence hybrids between it and the two other members of the *stramonium* group would show fewer configurations than if any other known race of *D. stramonium* were used as a standard. It is perhaps significant that the distribution of the two other members of the *stramonium* group overlaps that of PT 3. *D. quercifolia* occurs in Central America where PT 3 and PT 2 also occur associated together, and *D. ferox* is abundant as the only *Datura* species common in central Argentina and spreads over into Chile where the purple *D. stramonium* plants are PT 3 associated with PT 2.

One of us in 1925 discovered hybrids between purple *D. stramonium* and *D. ferox* growing in great abundance in the extensive vineyard of the "Quinta Normal" in Santiago, Chile. We cannot be sure of the direction of the stream of evolution and hence dare not say that *D. ferox* and *D. quercifolia* were derived from the "Peruvian" PT 2-PT 3 type or that the "Peruvian" type of *D. stramonium* was derived from one of these other species. We can say with confidence, however, that their chromosomes are related. The Peruvian type is therefore of especial interest. All the PT 3 races from Peru and Chile are similar in appearance with a characteristic lobing of the leaves and with long spines on the capsules. The one race from Spain is the same in appearance and suggests a migration to or from this mother country. The three races in Central America resemble less closely the plants from Peru.

Prime types 4 and 7 are widely scattered. They differ from PT's 1, 2 and 3 in that in no great area do they appear to be present to the exclusion of other PT's. Their characteristic chromosomes have not been found in any of the other species tested. Prime types 4 and 7 therefore may be listed as probably peculiar to *D. stramonium*. There is no evidence that they have arisen in other species.

We have discussed the distribution of PT's as if the interchange of chromosomal segments to produce each PT had occurred but once

as a mutation in a single plant, and the PT race thus formed had been distributed to other regions. Such an interpretation is supported by the *intrase* crosses between PT 2 races from widely separated regions already mentioned. They indicated that the modified chromosomes in these cases were alike so far as the interchanged segments were concerned. The relatively few PT's in nature would also suggest the extreme rarity of the segmental interchanges necessary to their formation. In our collection of PT's induced by treat-

Table 7. Chromosomes involved in prime types

PT 1 chromo- somes	Times modified to from PT's		
	Follow- ing treat- ment	From nature	
		Recurrent types	Isolated types
1-2	18	1	
3-4	12	1	1
5-6	11		
7-8	14		
9-10	17	1	
11-12	17	1	1
13-14	19		
15-16	18		1
17-18	8	1	
19-20	13	1	
21-22	10	2	1
23-24	12		1

ment, there are 82 which are due to segmental interchanges or simple translocation. As shown in Table 7, the modified chromosomes in these PT's are well scattered among the 12 PT 1 chromosomes. There are several instances in which the PT's involve the same chromosome ends. In only one instance, however, could the two PT's possibly be identical in respect to the place where the interchange occurred. Further study might show that in this case also the two PT's were not

actually the same. We can be sure that duplication of segmental interchanges following treatment at least is extremely rare. There are lines of evidence on the other hand which indicate that breaks in chromosomes may occur repeatedly at the same place. It has already been shown that two of the three different tertiary $2n + 1$ types which have appeared spontaneously in our cultures have appeared again independently. These tertiary forms have an extra chromosome formed by segmental interchange, and in these two repeated types the evidence seems good that the interchanges took place at the same level of the chromosomes. Moreover in our secondaries, such as the $2n + 2:2$ type (in which the extra chromosome appears to be formed by a break in two homologous chromosomes and the reattachment of similar parts to form double half chromosomes) the breaks in the chromosomes appear to be at the same place in all the cases in which the same chromosomes are affected. There is evidence from our experience, therefore, indicating that there are preferred places in chromosomes at which breaks repeatedly take place. These facts offer some presumption in favor of the same segmental interchange having occurred independently in different regions. However, there

is no direct evidence in favor of the polyphyletic origin of prime types in nature.

The modified chromosomes in prime types we have interpreted as resulting from segmental interchange between non-homologous chromosomes of our standard PT 1. Our method of testing, however, detects only the changed ends. It is likely that chromosomal changes have occurred inside the chromosomes of prime types in addition to gene mutations which are known to have occurred in some cases and which may differentiate geographical races. It is not believed that such possible internal chromosomal changes or gene mutations would have any necessary relation to the type of interchange responsible for the prime types in question. This conclusion is reached from a study of tertiary $2n + 1$ types derived from prime types. All the recurrent prime types have repeatedly been backcrossed to PT 1 with retention of the hybrid chromosome configuration until the plants were indistinguishable in appearance from our standard PT 1. Ultimately the prime type is then extracted in homozygous condition free from any genes which may have been peculiar to the race from which the PT was originally obtained. By the proper breeding procedure each of the modified chromosomes may be made the extra in a $2n + 1$ tertiary type in which all the chromosomes except this extra belong to PT 1. Thus from PT 2 we have isolated the two tertiaries $2n + 1 \cdot 18$ and $2n + 2 \cdot 17$. By this means we have isolated the modified chromosomes as extras in $2n + 1$ forms from all the recurrent prime types. We have in these cases apparently replaced all the unmodified chromosomes by PT 1 chromosomes and through crossing over have likely also replaced a large proportion of the modified chromosomes by parts of the PT 1 chromosomes from which they had been derived. Any recessive genes remaining would certainly be suppressed by the dominant allelomorphs in the unmodified chromosomes. The appearance of these purified $2n + 1$ tertiaries derived from the prime types is what would be expected on the supposition that segmental interchange alone were responsible for their formation.

It is noteworthy that no simple translocations have been observed in nature in *Datura*. They have occurred not infrequently, however, following radiation treatment. They would have given a means of adding extra chromosomal material and thus changing the chromosomal balance with a wide range of effects upon the structure of the plant affected. Thus from the radium-induced prime type in which the $\cdot 2$ half of the $1 \cdot 2$ chromosome had been translocated to the $11 \cdot 12$ chromosome with the $\cdot 1$ half left free, we have eliminated the free fragment and synthesized a pure-breeding type (6) homozygous for

the 2·11·12 chromosome which differed in many characteristics from the normal on account of the extra chromosomal material which it contained. We have developed a method (7) whereby it is possible to synthesize pure-breeding types through the addition of internal excess material. This involves the combination of induced prime types in which the segmental interchange occurred between the same chromosomes but at different levels. The fewness of prime types of *Datura* in nature renders this particular method of adding chromosomal material internally unlikely in our genus. We are still of the opinion earlier expressed (8) that the addition of excess chromosomal material would furnish the kind of differences observed between species and that in some way such addition of blocks of genes has been operative in evolution. We must confess, however, that the present study presents no evidence in support of this belief. Our contribution refers to changes in the end arrangements of chromosomes. Though similar changes have occurred within all the species of *Datura* adequately tested and have at least accompanied the formation of new species, we do not yet know their significance in evolution. Our study at least presents a body of facts regarding what has actually taken place in nature in the vital units responsible for variation in the organic world. The relation of these units to the origin of species is a field which has been sadly neglected. We feel it cannot be too strongly emphasized that the clue to the evolution of species lies in a study of the evolution of their chromosomes.

Summary

Data are presented on the distribution of chromosomal prime types (PT's) in 583 races (287 purples, 289 whites and 7 races which were heterozygous for P and W) from 362 loci exclusive of 51 races from Botanic Gardens and commercial firms. Prime types found in nature consist exclusively of races produced by segmental interchange between non-homologous chromosomes in terms of a standard race, PT 1. With the exception of two single races, only five prime types (PT's 1, 2, 3, 4, 7) have appeared in our collections. Some areas are represented by a single PT or PT association. Thus, all the races from Eastern Europe with the exception of one race from Russia and all the races from Asia with the exception of Japan are white-flowered PT 2; all the races from Brazil are PT 1 and also have white flowers; all the races from Peru are PT 3 associated with PT 2. In some areas a mixture of types has been found, for example, in South-western Europe, the West Indies and Central America. In the United States most of the races are purples and predominantly PT 1 with PT's 2, 4 and 7 occurring scattered within the eastern area. Whites

are found more frequently among the PT 2's than among the PT 1's, a fact which suggests that PT 2 was introduced into this region as a white race from Europe or Africa where white PT 2's are predominant. Prime type 3 has always been found associated with PT 2 which latter PT is the one most widely distributed. It is concluded that PT 3 probably arose in a PT 2 race. The two other species of the *stramonium* group, *D. quercifolia* and *D. ferox*, have been found within the range of the "Peruvian" PT 2-PT 3 race. Since the tester races of these two species both have the four chromosomes of PT 2 and PT 3, it is suggested that these two species are phylogenetically related to *D. stramonium* through PT 2 and 3. Prime type 2 chromosomes are found in the majority of the *Datura* species tested. Prime type 4 and PT 7, however, appear to be limited to *D. stramonium*.

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