CYTOLOGY OF THE FEROX-QUERCIFOLIA-STRAMONIUM TRIANGLE IN DATURA

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The best-known group of the genus *Datura* includes the species *D. stramonium*, *D. quercifolia* and *D. ferox*. For each of the three species an inbred line was used in making the crosses. The *stramonium* tester was our standard Line 1, which was originally obtained from Washington, D. C. The *quercifolia* tester race came from Fort Davis, Texas and the *ferox* tester from near Buenos Aires, Argentina. As has been shown earlier,[1,2] different cryptic chromosomal types occur in *D. stramonium*. In consequence different results would have been obtained if we had used certain other races of this species as a tester. In the present report we are confining ourselves to the results obtained from the use of these testers mentioned.

In the three tester races used, a cytological examination showed that the 24 chromosomes are arranged as 12 bivalents at first metaphase in pollen-mother-cells.

A similar examination of the pollen-mother-cells of the F_1 inter se hybrids, however, showed configurations involving two or more pairs of chromosomes as shown in figure 1. The *stramonium-quercifolia* hybrid has three configurations involving four chromosomes each plus 6 closable bivalents. Each configuration of four is a circle, or when the circle breaks, a chain. The *stramonium-ferox* hybrid also has three configurations: two circles involving four chromosomes each and a circle of four to which a bivalent is attached (configuration of six) plus five bivalents. All the circles show a tendency to break at a particular, fixed point indicated by dotted lines in the formulae. As a result they are more frequently found as chains rather than as circles. The *ferox-quercifolia* hybrid has a "figure-of-eight" configuration which involves four chromosomes plus ten bivalents. Appropriate crosses have shown that, so far as the ends of the chromosomes are concerned, the chromosomes in the two circles of four chromosomes each in the F_1 between Line 1 and Peruvian races of *stramonium* are the same as those found in the Line 1-*ferox* and Line 1-*quercifolia* hybrids. Four of the chromosomes of *D. ferox* and also of *D. quercifolia*, therefore, are the same, so far as their terminal attachments are concerned, as the four modified chromosomes in Peruvian races of *D. stramonium*. Throughout our discussion we shall speak of modified chromosomes in terms of the chromosomes of our standard Line 1 in *D. stramonium*.

Belling's hypothesis of segmental interchange between non-homologous chromosomes[3] will explain the occurrence of circles of four chromosomes found in intra se *stramonium* crosses between our standard Line 1 and certain races found in nature.

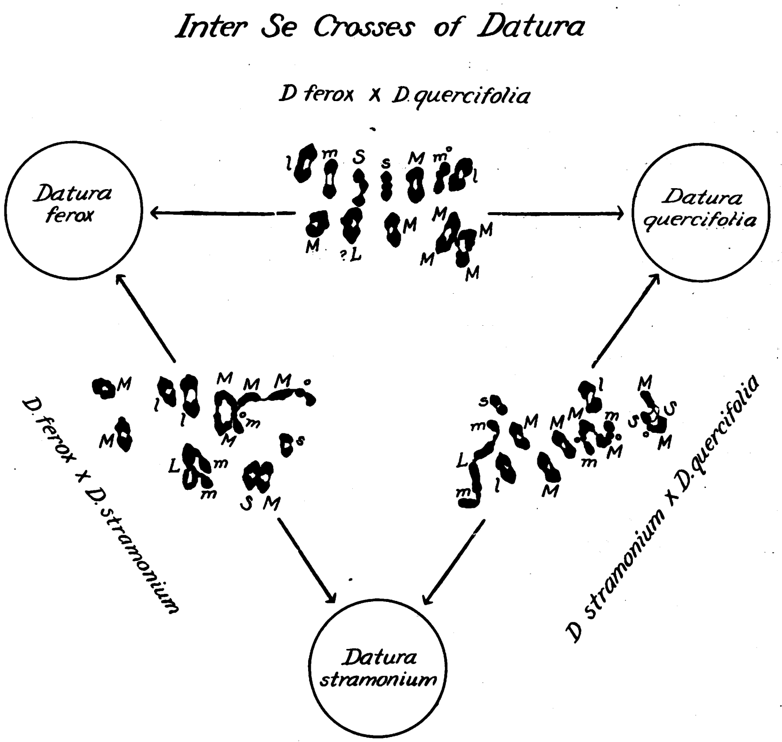


FIGURE 1

The best-known of these configurations is the B circle which is found in crosses between the *stramonium* tester and B races. Segmental interchange (reciprocal translocation) between the 1·2 and 17·18 chromosomes[4] is thought of as having taken place in the origin of the B races from Line 1 to produce the chromosomes 2·17 and 1·18. Since like ends of chromosomes attract each other, a hybrid between these two races shows a circle of four chromosomes which may be represented as follows:

...
1·2——**2·17**
| |
1·18—18·17
...

In this and other diagrams in the text the numbers representing chromosomes other than the Line 1 type are shown in boldface type. Peculiarities of size and shape enable one to identify the four members of the B circle. Appropriate tests have shown that both *ferox* and *quercifolia* are B races.

The other type of segmental interchange typical of *stramonium* races found in Peru involves an interchange between the $11\cdot12^{12}$ and ${}^{21}21\cdot22$ chromosomes.[5] The hybrid between our standard Line 1 tester and these races from Peru shows the following circle of four chromosomes represented thus:

$$\begin{array}{ccc} {}^{12}12\cdot11 & — & \mathbf{11\cdot21}^{21} \\ | & & | \\ {}^{12}\mathbf{12\cdot22} & — & 22.21^{21} \end{array}$$

Appropriate tests have shown that both *ferox* and *quercifolia* have this type of interchanged chromosomes.

The third configurations found in the *stramonium-quercifolia* and *stramonium-ferox* hybrids involve interchanges which have not been found within the species *stramonium* but which are related to one another and are partly identical. In the simpler form, as shown in the *stramonium-quercifolia* hybrid, this configuration is a circle of four chromosomes. In the origin of *quercifolia* we may assume that an interchange took place between the 7.8^{8} and the $19{:}20^{20}$ chromosomes of *D. stramonium* to produce the chromosomes 7.20^{20} and 19.8^{8}. The *stramonium-quercifolia* hybrid then will show a circle of four chromosomes as follows:

$$\begin{array}{ccc} {}^{8}8\cdot7 & —— & \mathbf{7\cdot20}^{20} \\ | & & | \\ {}^{8}\mathbf{8\cdot19} & — & 19\cdot20^{20}. \end{array}$$

The *ferox-quercifolia* hybrid showed ten bivalents plus a "figure-of-eight" configuration which involved four chromosomes. That is, insofar as the ends of the chromosomes are concerned, ten of the twelve pairs are identical in *ferox* and *quercifolia*.

The "figure-of-eight" configuration is of rather infrequent occurrence within the species *D. stramonium*. It may be interpreted as the result of mutual attraction between like humps on two bivalents; these bivalents are united in the hump region only. The explanation offered to account for this type of configuration may be thought of as a limiting case of segmental interchange between non-homologous chromosomes, namely, a reciprocal interchange involving *only* the humps which are attached to ends of the non-homologous chromosomes involved. In thus accounting for the cytological observations, we are merely extending to similar humps the theory that like ends of chromosomes are mutually attracted. Apparently like ends of two homologous chromosomes attract one another, resulting in the formation of a closed bivalent, *regardless* of whether the humps which are attached to the two chromosomes are alike or not. If there has been an interchange of humps between two pairs of chromosomes in the origin of a particular race (or species), then in a hybrid with this race there will be two bivalents, each of which will consist of chromosomes homologous except for their attached humps. The mutual attraction between the two pairs of identical humps which are now located on different bivalents results in the joining together of these bivalents by the humps only.

Thus we can account for the "figure-of-eight" configuration which is found in the *ferox-quercifolia* hybrid. By an interchange of humps between the $7{\cdot}20^{20}$ and $15{\cdot}16^{16}$ chromosomes which we may assume to have taken place in the origin of *D. ferox* from *D. quercifolia*, there resulted the $7{\cdot}20^{16}$ and $15{\cdot}16^{20}$ chromosomes. The *ferox-quercifolia* hybrid then would show a "figure-of-eight" configuration of four chromosomes with the following composition:

$$\begin{array}{c} \mathbf{7{\cdot}20^{20}}\text{—}{}^{20}\mathbf{16{\cdot}15} \\ \langle \quad \rangle \quad \langle \quad \rangle \\ \mathbf{7{\cdot}20^{16}}\text{—}{}^{16}16{\cdot}15. \end{array}$$

The mutual attraction between like humps results in the attachment between these bivalents at their humps only.

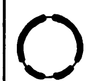
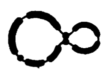
The third configuration in the *stramonium-ferox* hybrid differs from the third configuration in the *stramonium-quercifolia* hybrid in that it involves six chromosomes. It is a circle of four to which a bivalent is attached by its humps. In analyzing the "figure-of-eight" configuration in the *ferox-quercifolia* hybrid above, we have already designated two of the *ferox* chromosomes as $7{\cdot}20^{16}$ and $15{\cdot}16^{20}$. Another *ferox* chromosome involved in this third configuration must be $19{\cdot}8^{8}$, as will be seen from the following consideration. The sizes and connections of chromosomes in the *stramonium-quercifolia* third configuration showed that one of the *quercifolia* chromosomes must be designated as $19{\cdot}8^{8}$. This chromosome in the *ferox-quercifolia* hybrid forms a closed bivalent and therefore *D. ferox* must have the same chromosome ($19{\cdot}8^{8}$) so far as its ends are concerned. In the *stramonium-ferox* hybrid, then, the configuration of 6 chromosomes (a circle to which a bivalent is attached) may be explained as follows:

$$\begin{array}{lllll} {}^{8}8{\cdot}7 & \text{——} & \mathbf{7{\cdot}20^{16}} & \text{—} & {}^{16}16{\cdot}15 \\ | & & | & & \langle \quad \rangle \\ {}^{8}\mathbf{8{\cdot}19} & \text{—} & 19.20^{20} & \text{—} & {}^{20}\mathbf{16{\cdot}15}. \\ \quad \cdots & & \boxdot & & \end{array}$$

This third configuration in the *stramonium-ferox* hybrid has proved interesting because of its instability in back-crosses to *stramonium*. A few plants of two back-crosses were examined cytologically with the results shown in table 1. The configuration of six chromosomes was recovered only once in thirty plants. Eight plants, however, showed a circle of four chromosomes which appears to be identical with the third

TABLE 1

FEROX-STRAMONIUM F_1 BACK-CROSS

No. plants examined	Pedigree number	B	Peruvian	Ferox 3rd	Extracted from 3rd		12 bivalents	Open L bv.
					3a	3b		
								
		(L+m)	(M^o+S)	(M^o+m^o+M^o)	(M^o+m^o)	(M^o+m^o)		
20	290382	15	5	0	6	2	1	1
10	3001200	5	3	1	2	2	1	0
30		20	8	1	8	4	2	1

configuration found in the *stramonium-quercifolia* hybrid and its back-crosses. In addition four other plants showed a "figure-of-eight" configuration which involved four chromosomes. This could not be identical with the "figure-of-eight" configuration that was found in the *ferox-quercifolia* hybrid because of a difference in the sizes of the chromosomes involved. In the *ferox-quercifolia* hybrid the two connected bivalents were M° and M°; in the *stramonium-ferox* back-cross they were M° and m°. Both of these types have been recovered in the second and third back-crosses. The resolution of the configuration of six chromosomes into two separate configurations of four each needs explanation. From cytological evidence it appears that crossing-over between homologous parts of chromosomes will account for this change in configurations. Our hypothesis of how this has taken place will be made clearer from the diagram in figure 2. We assume that crossing-over in the *stramonium-ferox* hybrid took place between the $7\cdot20^{16}$ and the $19\cdot20^{20}$ chromosomes somewhere in the homologous region below the humps. Such crossing-over between homologous regions of otherwise non-homologous chromosomes has been demonstrated by a number of investigators[6] in such diverse forms as *Drosophila, Zea mays, Pisum* and *Oenothera*. The newly formed chromosomes then may be designated as $7\cdot20^{20}$ and $19\cdot20^{16}$. No evidence is available as yet regarding the exact place at which the crossing-over has taken place. The cytological results observed would be satisfied if the crossing-over involved only an interchange of the humps.

Ordinarily the result of such crossing-over is not detectable cytologically because the interchanged chromosomes consist of regions derived from only two chromosomes. In the *stramonium-ferox* hybrid the situation is different. In terms of the *stramonium* tester, the $7{\cdot}20^{16}$ chromosome consists of parts of three chromosomes: its 7 region is homologous with the 7 region of the $7{\cdot}8^{8}$ chromosome, the 20 region with the 20 region of the $19{\cdot}20^{20}$ chromosome, and the 16 hump with the 16 hump of the $15{\cdot}16^{16}$ chromosome. Therefore, if crossing-over takes place in the 20 region of the $7{\cdot}20^{16}$ and $19{\cdot}20^{20}$ chromosomes, the resulting chromosomes will be $7{\cdot}20^{20}$ and $19{\cdot}20^{16}$. Such crossing-over is possible *only* when the organism is heterozygous for the interchanged chromosomes. This is the situation in the *stramonium-ferox* hybrid.

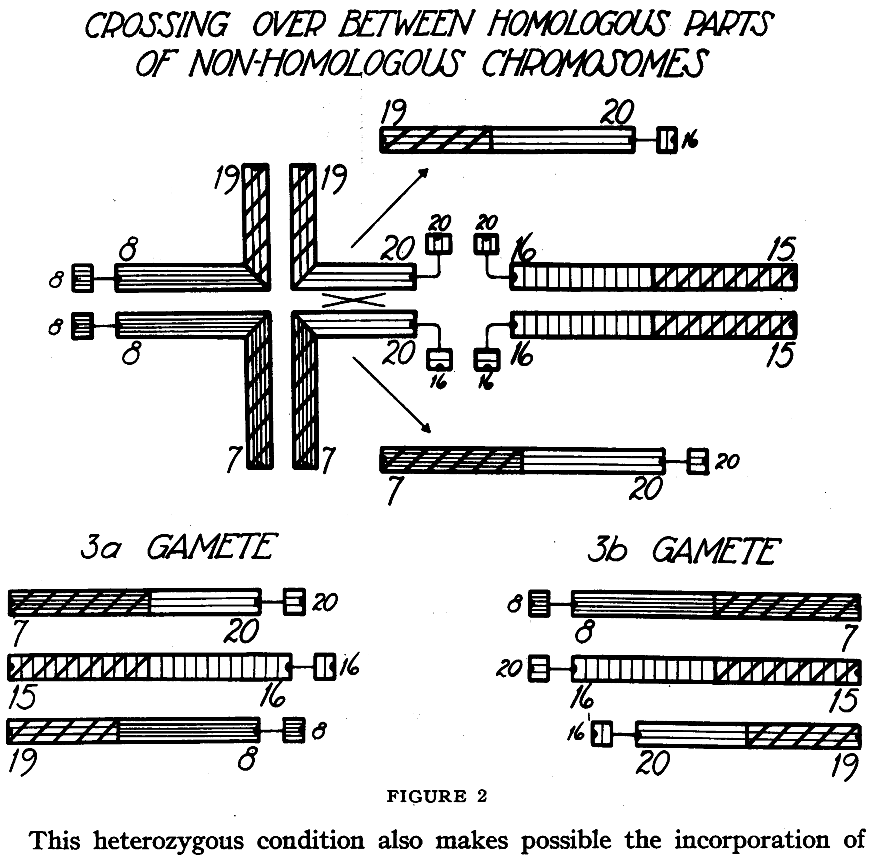


FIGURE 2

This heterozygous condition also makes possible the incorporation of the newly formed chromosomes into viable gametes. The $7{\cdot}20^{20}$ chromosome if combined with the $19{\cdot}8^{8}$ and $15{\cdot}16^{16}$ chromosomes will form a viable gamete; likewise the $19{\cdot}20^{16}$ chromosome if combined with the $15{\cdot}16^{20}$ and $7{\cdot}8^{8}$ chromosomes will form a viable gamete. If each of these gametes is united with a gamete of the *stramonium* tester, as is the case in back-crosses to the *stramonium* tester, then two kinds of configurations will result. The circle of four will be:

$$\begin{array}{ccc} {}^{8}8{\cdot}7 & \text{——} & 7{\cdot}20^{20} \\ | & & | \\ {}^{8}8{\cdot}19 & \text{—} & 19{\cdot}20^{20} \end{array}$$

The "figure-of-eight" configuration will be:

$$\begin{array}{ccc} 15{\cdot}16^{16} & \text{—} & {}^{16}20{\cdot}19 \\ \langle \quad \rangle & & \langle \quad \rangle \\ 15{\cdot}16^{20} & \text{—} & {}^{20}20{\cdot}19 \end{array}$$

Cytologically the circle of four is indistinguishable from the third configuration found in the *stramonium-quercifolium* hybrid. Certainly as far as the ends of the involved chromosomes are concerned it is identical with it. The "figure-of-eight" configuration is new and promises to be a useful tester.

It is now possible to summarize the analysis of the chromosomes involved in the configurations found in these interspecific hybrids. *D. ferox* has five chromosomes, out of a possible twelve, whose ends are identical with the homologous chromosomes in the *stramonium* tester; *D. quercifolia* has six. The numerical representation of the seven *ferox* and six *quercifolia* chromosomes which differ from those in the *stramonium* tester are listed in table 2. In this table also are listed the two new chromosomes extracted from the third configuration in the *stramonium-ferox* hybrid.

TABLE 2

CHROMOSOMES INVOLVED IN THE CONFIGURATIONS FOUND IN STRAMONIUM-QUERCIFOLIA-FEROX HYBRIDS

DATURA STRAMONIUM		D. QUERCIFOLIA	D. FEROX	EXTRACTED FEROX TYPES	
				3*a*	3*b*
Rolled (L)	1.2	2.17	2.17		
Poinsettia (m)	17.18	1.18	1.18		
Cocklebur ($\mathring{M}$)	11.12^{12}	11.21^{21}	11.21^{21}		
Globe ($\mathring{S}$)	$^{21}21.22$	$^{12}12.22$	$^{12}12.22$		
Elongate ($\mathring{M}$)	7.8^{8}	7.20^{20}	7.20^{16}	7.20^{20}	7.8^{8}
Spinach ($\mathring{m}$)	19.20^{20}	19.8^{8}	19.8^{8}	19.8^{8}	19.20^{16}
Reduced ($\mathring{M}$)	15.16^{16}	15.16^{16}	15.16^{20}	15.16^{16}	15.16^{20}

The letters in connection with the chromosomes indicate their relative sizes: L signifies very large, M, medium, m, small medium and S, small. The circle above some of the letters as well as the raised numbers represent humps on these chromosomes.

The isolation of each of these separate "Prime Types" in the homozygous condition is in the process of being made. It is planned ultimately to recombine these "Prime Types" with otherwise Line 1 *stramonium* tester chromosomes to form the resynthesized species *ferox* and *quercifolia*.

As far as the ends of the chromosomes are concerned, *D. ferox* and *D. quercifolia* are closely related.

It should be pointed out again that in our discussions we have classified chromosomal evolution in terms of the chromosomes of our *stramonium* tester Line 1. This is merely a matter of convenience since we are most familiar with the chromosomes of this particular race. It is possible if not probable, that our tester race is a derived rather than a primitive type as may be seen from the following consideration: An examination of more than 500 natural races of *D. stramonium* indicates that the B type is the predominant one except in the United States and Brazil. Our *stramonium* tester Line 1 therefore may equally well represent a segmental interchange that has occurred fairly recently in the evolution of the *stramonium* species. Furthermore, it is known that the *ferox* and *quercifolia* as well as the *leichardtii* tester races are B types. But the third types of interchange which are common to *ferox* and *quercifolia* have not been found in any *stramonium* races.

It should be emphasized that the chromosomal constitutions that have been described refer only to the particular testers used as representatives of *D. stramonium, D. ferox* and *D. quercifolia.* It is known that certain other races of *D. stramonium* would have given different results because of segmental interchanges which apparently took place in their origin. Such cryptic types have been demonstrated by intra se crosses. Although *D. ferox* and *D. quercifolia* have not been studied as yet from this standpoint, these species presumably also contain cryptic types. In an examination of ten races of *D. meteloides* crossed to a *meteloides* tester, two configurations due to segmental interchange were identified in the F_1 with one race. Likewise of three races of *D. innoxia* which were crossed to an *innoxia* tester, one induced a configuration due to interchange. In still another species, *D. metel,* one of the two races which were crossed to a *metel* tester induced a single and the other induced two separate configurations. Therefore the presence of cryptic types has been demonstrated in four species of Datura; in fact, in all the species of this genus that so far have been investigated from this standpoint. It is probable, therefore, that cryptic chromosomal types due to segmental interchange are of wide-spread occurrence.

So far we have found chromosomal configurations in eight different inter se hybrids which involved seven species of *Datura.* In all of these we have found configurations interpreted as due to segmental interchange. Segmental interchange, therefore, may be assumed to have taken place in the differentiation of these species. Just what rôle, however, this process has played in speciation is not yet clear.

Summary.—Three inbred tester races were used in making inter specific hybrids involving *Datura stramonium, D. quercifolia* and *D. ferox.* The results which have been obtained apply to these testers only.

Both *D. ferox* and *D. quercifolia* are "B" and "Peruvian" types. They

show, in common with races of *stramonium* from Peru, two types of segmental interchange between non-homologous chromosomes. The B race has the *stramonium* tester chromosomes 1·2 and 17·18 modified to 2·17 and 1·8. The Peruvian type has the *stramonium* tester chromosomes $11{\cdot}12^{12}$ and $^{21}21{\cdot}22$ modified to $11{\cdot}21^{21}$ and $^{12}12{\cdot}22$.

A previously unknown type of interchange is partly identical in *ferox* and *quercifolia*. In *quercifolia*, the *stramonium* tester chromosomes $7{\cdot}8^{8}$ and $19{\cdot}20^{20}$ are modified to $7{\cdot}20^{20}$ and $19{\cdot}8^{8}$. In *ferox* a further interchange between the $7{\cdot}20^{20}$ and $15{\cdot}16^{16}$ chromosomes has formed the $7{\cdot}20^{16}$ and $15{\cdot}16^{20}$ chromosomes.

In back-crosses of the *stramonium-ferox* hybrid this configuration has given rise to two new configurations, both of which are explainable on the assumption of crossing-over between the homologous regions of the $7{\cdot}20^{16}$ and $19{\cdot}20^{20}$ chromosomes. Such crossing over is detectable in the hybrid because in terms of the *stramonium* tester the $7{\cdot}20^{16}$ chromosome is composed of parts of these three chromosomes $7{\cdot}8^{8}$, $15{\cdot}16^{16}$ and $19{\cdot}20^{20}$. The heterozygous condition of the hybrid makes possible the incorporation of the newly formed chromosomes into viable gametes.

So far as investigated, these species of *Datura* show differences in the architecture of their chromosomes which has been interpreted as due to segmental interchange.

BIBLIOGRAPHY AND NOTES

[1] Blakeslee, A. F., *J. Hered.*, **20**, 177–190 (1929).

[2] Bergner, A. D., and Blakeslee, A. F., *5th Intern. Bot. Cong., Report of Proceedings* (*Sect. Abst.*), **126** (1930).

[3] Belling, J., and Blakeslee, A. F., these PROCEEDINGS, **12**, 7–11 (1926).

[4] The names and relative sizes of the chromosomes, which are here given by their numbered ends, are shown in table 2. The use of numbered ends to designate particular chromosomes has been employed previously in joint papers by A. F. Blakeslee and R. E. Cleland, these PROCEEDINGS, **16**, 177–189 (1930).

[5] The superscripts to the ends 12 and 21 represent terminal humps characteristic of these Line 1 chromosomes. G. A. Lewitsky has demonstrated satellites or knobs on several of the chromosomes in root-tips of *D. stramonium*, *Proc. U. S. S. R. Cong. Genet.*, **2**, 87–105 (1929). They are probably the equivalents of those humps found on meiotic chromosomes. Such humps are convenient markers to identify chromosomes and particular ends of chromosomes.

[6] Brink, R. A., and Cooper, D. C., *Genet.*, **16**, 595–628 (1931). Burnham, C. R., these PROCEEDINGS, **16**, 269–277 (1930). Creighton, H. B., and McClintock, B., these PROCEEDINGS, **17**, 485–497 (1931). Dobzhansky, Th., *Amer. Nat.*, **65**, 214–232 (1931). Emerson, S., and Sturtevant, A. H., *Zeitschr. indukt. Abst.-u. Vererb.*, **59**, 395–419 (1931). Håkansson, A., *Hereditas*, **15**, 2–61 (1931). Muller, H. J., *J. Genet.*, **22**, 335–358 (1930). Rhoades, M. M., *Genet.*, **16**, 490–504 (1931).