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The Hunt Institute for Botanical Documentation, a research division of Carnegie Mellon University, specializes in the history of botany and all aspects of plant science and serves the international scientific community through research and documentation. To this end, the Institute acquires and maintains authoritative collections of books, plant images, manuscripts, portraits and data files, and provides publications and other modes of information service. The Institute meets the reference needs of botanists, biologists, historians, conservationists, librarians, bibliographers and the public at large, especially those concerned with any aspect of the North American flora.

Hunt Institute was dedicated in 1961 as the Rachel McMasters Miller Hunt Botanical Library, an international center for bibliographical research and service in the interests of botany and horticulture, as well as a center for the study of all aspects of the history of the plant sciences. By 1971 the Library's activities had so diversified that the name was changed to Hunt Institute for Botanical Documentation. Growth in collections and research projects led to the establishment of four programmatic departments: Archives, Art, Bibliography and the Library.

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+ ch. #5

Stanford - Biology Seminar 10/17/45 (1)

I shall review briefly some of the information acquired in the course of an investigation which has been under way for over forty years in the University of California Botanical Garden at Berkeley. The late W. A. Satchell, for many years Chairman of the Department of Botany at Berkeley, was as some of you may remember, considerably addicted to the use of tobacco. When I arrived in Berkeley in 1909 the first issue of the student newspaper I saw contained a reference to Professor Satchell's loss of his pipe and an admonition that finder bring it or burn a rag -- a commentary on the relatively unsophisticated humor of the undergraduate student of thirty-five years ago.

Professor Satchell's personal interest in tobacco led to the accumulation of pipes and later to the collection of early literature on the history and use of tobacco, and, finally, to growing in the Botanical Garden of seed of as many varieties of tobacco and of other species of *Nicotiana* as he could obtain from Departments of Agriculture, Botanical Gardens, etc. Throughout the world. His first interest he found that descriptions of many of the species as well as of the numerous varieties of *N. tabacum* were inaccurate and that each of the major agricultural varieties was grown under different names in the various parts of the world. Therefore

his interest was first in cataloging the morphological distinctions between these species and varieties, and second in genetic investigation of relationships of what he considered key varieties. He was also concerned with pre-Columbian use and culture, particularly of the species native to Western North America, and in 1912 published *Studies in Nicotiana I*, the first paper on the Nicotiana investigations in Berkeley. I, and later Dr. R.E. Clausen, became associated with these investigations. For the last fifteen years Dr. Clausen has carried on independently his work on the cytogenetics of *N. tabacum*. The series of published studies on Nicotiana begun in 1912 by Professor Satchell, now exceeds 125 papers, most of them under my authorship.

From the beginning I have been concerned with attempts to construct as <sup>nearly</sup> complete a picture ~~as possible~~ of the origins and evolution of the modern species of the genus Nicotiana - a relatively typical genus of flowering plants. I therefore became involved in 1) assembling as complete as possible a living collection of Nicotiana species in the University of California Botanical Garden, 2) determination of their distribution of <sup>in the wild state</sup> the modern species, 3) studies of comparative morphology of these species and 4) <sup>of the modern</sup> cytogenetic

<sup>investigate</sup>  
~~investigation of these species and of hybrids,~~

particularly of ♀, interspecific hybrids. I will now summarize briefly the results obtained along these four avenues of approach to the major objectives -- results obtained in part through the effective cooperation of a number of research assistants and graduate students.

① From my point of view the genus *Nicotiana* consists of fifty-eight valid species of which all but three have been grown in the Botanical Garden at Berkeley for some years. Assembling this collection has taken me twice to Europe and three times to South America (where the majority of the species are found) in the last fifteen years for herbarium studies and collecting, and has involved the assistance of collaborators in Australia and the South Pacific. The problem South American collecting yielded six previously unknown species and many new varieties -- and evidence of *Nicotiana* distribution from the equator to South Patagonia on both the east and the west flanks of the Andes. The problem of successfully growing in Berkeley subtropical to high alpine species is not always easy but has been largely successful.

② As I have already said the majority of the species of *Nicotiana* are native to South America -- the genus is largely American (North and South). In addition a group of species is native in

Australia and a few South Pacific islands. Through man's intervention a few species are rather widely distributed and in cultivation the species of commerce (*N. tabacum* and *N. rustica*) are grown far beyond the limits of their natural distribution. In general the distribution of *Nicotiana* species is temperate with only a few subtropical, from sea level to 13,000 ft.

③ Past morphological and taxonomic work on the genus has been imperfect because a living assemblage of practically all species has never before been available for comparative studies. It has been necessary, therefore, to start from the beginning and build up a body of morphological evidence bearing directly upon fundamental morphological relationships. This has involved studies of species morphology and redescription of all species, and has led to the formulation of a new taxonomic organization of the genus. This taxonomic arrangement (based to a degree on that of Don, 1838) recognizes three subgenera and a total of eleven sections.

④ The cytogenetic investigations of *Nicotiana* have involved many aspects, with emphasis on the cytological.  
a) <sup>details</sup> Studies have been made of the inheritance of character contrasts in intervarietal crosses of *N. tabacum* and of *N. rustica*.

b) Chromosome number and particularly chromosome morphology has been determined in all of the fifty-five species available and in an equal number of varieties. Investigation of chromosome morphology on a comparative basis has been made from metaphase of first division in pollen grains — and it is no mean feat to secure superior preparations of pollen grain divisions in nearly sixty different species.

The following chromosome numbers are characteristic of *Trisetaria* species.

American: 9 (3 species), 10 (2), 12 (28), 24 (9)

Australian ~~and~~ and South Pacific: 16 (4), 18 (1), 19 (2), 20 (2), 21 (1), 22 (1), 24 (2)

Total: 9 (3), 10 (2), 12 (28), 16 (4), 18 (1), 19 (2), 20 (2), 21 (1), 22 (1), 24 (11)

c) Meiotic chromosome behavior has been studied in many of the species and, <sup>particularly</sup> in a large number of  $F_1$  interspecific hybrids.

1) In the case of species the relation of chromosome size and morphology to chiasma frequency and terminalization, and the resulting MI bivalent configurations have been ~~studied~~ determined.

2) We have studied extent and quality of pairing in 133  $F_1$  interspecific hybrids and the results of other ~~investigators~~ have added data on 78 additional  $F_1$  hybrids. In the case of <sup>most of</sup> the 133  $F_1$  hybrids our study has involved analysis of pairing in from 50 to 100 PMC by two ~~investigators~~ working independently. The total of 211

includes a series of 7, interspecific hybrids within sections, between sections of the same subgenus and between sections of all three subgenera. Although <sup>obviously</sup> only a small proportion of the possible hybrid combinations between the fifty-eight species have been made and studied, cytological information is available for practically all hybrids essential for demonstrating degree of relationship, and the total evidence is considerably in excess of that available for any other plant genus.

I want along four such rather broad avenues of approach to a biological objective is of course certain to reveal a number of bypaths which show promise of leading to significant conclusions not necessarily, however, related to the solution of the major problem. Many such bypaths have appeared ~~in~~ during the long course of the *Nicotiana* investigations at Berkeley and a number of them have been followed for different distances. For example: the inheritance of quantitative and qualitative gene alterations induced, primarily in *N. tabacum*, by high frequency radiation (labidity of *N. tabacum*); and again, - the trisomic inheritance in *N. sylvestris* where the full set of twelve primary trisomic types and a variety of derivatives therefrom were studied and certain characters identified in terms of particular chromosomes. Another interesting bypath has been the study of a rather remarkable race of *N. tomentos*,

III

D.

(A) Meiotic chr. behavior

- (a) spp. - rel. ch. size + morph - chiasma fr. + term.
- (b) extent + qual. pairing at MI - 1337, intersp. hybrids (others = 78 add. hybrids) - total of 211 only small prop. of poss. hybrid combi. among 58 spp. but rep. all combi. essential + prob. consid. in excess of any other pl. genus.

E. Travel along 4 such broad avenues → hypothesis - some =

- (1) Prod. qual. + quan. gene alts. by h.f.x. (esp.  $n$ . tab)
- (2) Trisomic inherit.  $n$ . sylvestris (12 primary - many derivatives)
- (3) chr. mosaic  $n$  tom. =  $n$ ,  $2n$ ,  $4n$  PMC + veg. tissues  $2n$ ,  $4n$  +  $8n$ ? competition.

IV. Amplify + document refs. to 4 axes by slides

A. Distribution

- (1) World wide - Amer., Aust., S. Pac. (Lord Howe, New Cal., Isle Pines Seyelles, Tongatabu)
- (2) not tropical - well drained, low rainfall, insul.
- (3) rigorous environ - acidity, low temp, intense insul. (high alt.)
- (4) Obs. suggest - dispersal Antarctica to Amer. + Austr. or SA to Austr. via Antart.
- (5) Trap. route Sd Exodus
- (6) Trap. spp. distrib.

B. Morphol. - sharp morph. distinct. most spp.

- (1) habit + general char.
- (2) flwr. types
- (3) inflo. types
- (4) leaf types
- (5) Anthers (3 views each) - ovaries + gland discs - spp. 3 subgenera
- (6) destination - Top row subg Rustica, 2nd Tab 3 + 4 Pat.

C. cytogenetics

- (1) karyotypes - Rust - pract. ident. = morph. eoid; Tab - more diverse, but karyo. dimorph. of a, b, + c identical = most closely rel. spp. morph.
- (2) chr. pairing MI → complete - upper  $n$  = intrasect, below less complete = intraspec.
  - a) lower left - lack = interubiq; upper left - low variable = intraspec.
  - lower middle - drosera (explan drosera).
- b) extent pairing = reflect. extent genes + their arrang. common or simi. in con. chr. = reflect. of fund. rels. of spp. involved
- c) Tab  $\Delta$  - amphidip.

d) Summary evidence pairing.

- 1) intraspecific = 90% ± complete
- 2) interspecific = 90% ± lacking
- 3) intersubgeneric = 100% ± lacking
- 4) *Angitia* spp x parents = 100% ± presence
- 5) " " x other spp = 85% ± lacking

(3) Prophase - meiosis (pack-diplo-disk)

- 1) Top row -  $\pi$  longiflora
- 2) lower "  $4, \pi$ . plumbea x  $\pi$  glauca (lack of pairing at MI)  
Evidence consistent with MI evid.
- 3) Prophase - good mat. for intensity of meiotic phen.  
(condensation, synapsis, turning etc.)

By paths

?

- 1 - X ray
- 2 - trisomic
- 3 - colch - polyploidy - sylvestris

a 12-gained species of the Peruvian Andes, the PNC of which are  $n$ ,  $2n$  and  $4n$ , and the vegetative tissues of which are apparently mosaic of  $2n$  and at least  $4n$  cells. On the basis of general experience it is a little difficult to understand how  $4n$  and possibly  $8n$  cell lines continue to compete successfully with  $2n$  cell lines to culminate in  $2n+4n$  as well as  $n$  pollen.

I want now to document and amplify the brief references made to the four avenues of approach to the problem of species origins and relationships in the genus *Nicotiana* by showing a few lantern slides.

① Distribution

1-a) World wide. As already said the distribution of the modern species of *Nicotiana* in nature is confined to the Americas, Australia and a few South Pacific islands (New Caledonia, Isle of Pines, Lord Howe Island, Loyalty Islands and Tonga-Talua). *Nicotiana* is not found in the tropical portions of the Americas. Relatively well drained soil, relatively low rainfall and considerable insolation are essential - indeed *Nicotiana* will endure rigorous climates, considerable aridity, low temperature and intense insolation. The distribution as shown on this map suggests dispersal from a South American origin to Australia via Antarctica.

2 b) Map showing routes of the three U.C.B.G. Expeditions to the Andes.

3- c) map - Peru, Chile, Bolivia and Argentina showing distribution of species of section ~~paniculata~~ Subgenus Rustica. (primary)

4. map of same area - showing distribution of species of section Tomentosa.

(2) Morphology - A series of slides showing how sharply most species of Nicotiana are distinguished on the basis of combination of flower and leaf patterns and habit characters.

a) Habit - limited perennial and woody as contrasted with the herbaceous.

limited per. woody & herbaceous

- (5) 1. N. tomentosa (Subgenus Tabacum)
- (6) 2. N. bigelovii and N. nudicaulis (Subgenus Petunioides)
- (7) 3. N. glutinosa (Subgenus Tabacum)
- (8) 4. N. repanda (Subgenus Petunioides)
- (9) 5. N. suaveolens "
- (10) 6. N. Myrsiflora (Subgenus Rustica)  
a new species.

b) Flower types

- (11) 7. Three flower types: N. tomentosiformis, alata & brunneii

c) Inflorescence types

- (12) 8. Three types of inflorescence

d) Leaf types

- (13) 9.

2) The next two slides show special morphological features not previously investigated but significant in determining relationships.

(N) 10. Distinctions in anthers of six species: *N. ramondii* (side, back and front views) *N. glutinosa*, *N. repanda*, *N. selchellii*, *N. saevolepis*, *N. laugsdorffii*.  
and in ovaries and glandular discs in *N. glauca*, *N. tomentosa* and *N. sylvestris* - (from 3 different subgenera)

(b) 11. Studies in aestivation:  
Top row, 2 species of subgenus *Rustica*  
Second row, 2 species of subgenus *Lobocum*  
Third and Fourth rows, 4 species of subgenus *Petunioides*.

(3) Cytogenetics - The following slides illustrate the various types of cytogenetic information secured.

(a) Karyotypes  
12. There are illustrated the karyotypes of the species of two of the three subgenera. On the left are karyotypes of ~~six~~ ~~of~~ ~~the~~ ~~same~~ species of subgenus *Rustica*. Corresponding to the close morphological resemblances and the common distribution of these species (*N. glauca* excepted) the karyotype formula as illustrated here is practically identical for all species but *N. glauca* (a). ~~In the remaining seven species~~ <sup>There are</sup> Only slight size distinctions between the groups of the remaining seven species; except for two <sup>subterminal</sup> chromosomes in *N. benavidesii* (b) only median and submedian chromosomes occur; and one submedian chromosome in each species is subtended.

1  
By contrast with the  
striking uniformity of  
the karyotype pattern  
in subgenus *Rustica*,  
that of the 20 species  
of subg. *Fabrum*  
shows considerable  
diversity — a  
diversity corresponding to  
that expressed in the  
morphology & distribution  
of this subgenus.  
However

2  
Similarly (four of the six  
sections of subgenus  
*Pet.* <sup>(not demonstrated)</sup> show a kary.  
~~formula~~ pattern  
common to the nucleus  
species in each case

① On the right are the karyotypes of the six species comprising subgenus *Tubocum*. Morphologically and distributionally this subgenus is somewhat more diversified than is subgenus *Rustica* and correspondingly the karyotypes of the species comprising it show less uniformity in pattern. However, the cluster or nucleus of three closely related species, *N. tomentosiformis* (a), *N. stophorea* (b) and *N. tomentosa* (c), is characterized by a practically identical karyotype of seven median (or submedian in *N. tomentosa*) and five subterminal chromosomes, one of each morphological type satellited in each species. Thus, each of these species possesses a dimorphic genome,

② particularly striking in the case of *N. stophorea* (b).

(b) Pairing at MI

①7 13- Pairing at MI in the 211 F<sub>1</sub> interspecific hybrids for which data are available can in general be referred to as complete, high variable, low variable, lacking or of the "crosses scheme" type. In intraspecific hybrids generally show pairing as complete or nearly as complete as would occur in the parental species. Such an instance is F<sub>1</sub> *N. beavridaei* x *N. ramondii* (upper right). Also noted here also are the characteristic ring bivalents, each possessing 2 chiasmata, one in each arm of the relatively large median or submedian chromosomes of which there are four of the 10 species are comprised.

Just beneath is illustrated another ~~the~~ intra-sectional hybrid, ♀, *N. benevidensii* x *N. subumbellata* in which pairing is, for some reason, not so nearly complete as is usual in an  $F_1$  hybrid of two species so closely related. (same section)

The two MI plates represented at the upper left show the limits of range of pairing in a hybrid in the low variable category, ♀, *N. tabacum* x *N. glauca*. This is an intersubgeneric hybrid bringing together gametes of 2 species possessing little in common morphologically. That up to

limit?

8 pairs may occur although the mode is very considerably lower, is considered to indicate the <sup>probable</sup> occurrence of a number of small homeologous segments in the two gametes.

Lower left is  $F_1$  *N. otophora* x *N. sylvestris* showing 0 pairs. Although an occasional PNE may show one, two or even three or four pairs the mode in this hybrid and in some six other intersubgeneric hybrids is 0 pairs. Next, and illustrating the final category of <sup>extent of</sup> pairing, the "crosses scheme" are  $F_1$  *N. tabacum* x *N. otophora*, and ♀, *N. tabacum* x *N. sylvestris*. Here the number of pairs is the same as the haploid number of ~~the~~ chromosomes of the parental species with the lower chromosome number. The pairing relations in these three  $F_1$  hybrids will later be referred to in connection with the discussion of the origin of *N. tabacum*.

Approx. 90% of the  $F_1$  intrasectional hybrids indicate close rel. of the parent spp by exhibiting complete or almost complete pairing.

By contrast in only 10% of the hybrids bet. spp of dif. sections of same subgenus and in none of the  $F_1$  hybrids involving spp of dif. subgenera does appreciable pairing occur.

In all  $F_1$  hybrids between any of the 9 amphid. spp and the descendants of their putative ~~ancestral~~ progenitors "Drosarobium" pairing or an approx. thereof occurs. On the other hand, the  $F_1$  hybrids combining these amphid. spp with spp other than those related to their parentage ~~in 50%~~ exhibit little or no pairing in practically all instances.

## (c) Prophase studies)

18 #4 - Here are illustrated prophases of meiosis, pachytene, diplotem and diakinesis, in a species, *N. longiflora* (10 pairs) and in <sup>an intersubgeneric</sup> ~~a~~ ~~lack of pairing~~ hybrid,  $\bar{F}$ , *N. plumbaginifolia* x *N. glauca*, which at MI shows lack of pairing; i.e. a range of 0 to 3 pairs with the mode at 0 pairs.

~~Here~~ The prophase evidence ~~of~~ is obviously consistent with that of MI. Incidentally hybrids of this pairing category provide excellent material for investigation of such meiotic prophase phenomena as speralization, condensation, and the effect of the timing relation of the two species involved, as well as of the nucleolar chromosome relationship.

## (d) High frequency radiation)

19 #5 - Mentioned earlier as an example of the interesting bypaths ~~that~~ which have appeared in the course of the *Nicotiana* investigation was the inheritance of quantitative and qualitative gene alterations induced in *N. tabacum* by high frequency radiation. A few slides now illustrate this.

19 #6 - MI description and

20 #6 - Quartet description following high frequency radiation of P.M.C.

21 #6 - *N. tabacum* (control)

22 -  $X_1$

23 -  $X_1$  flowers

24 - Abnormal mitoses  $X_1$

Trisomic series

- 26- *n. sylvestris* (control)
- 27- Trisomic - whole plant #1
- 28- " " " #2
- 29- Peas - normal and two trisomic
- 30- ~~1/2~~ normal  $2n+1$  and  $2n+2$  (tetrasomic)
- 31- Double trisomic plant

Artificial chromosome doubling via colchicine.

(another bypath)

- 32- *Sylvestris* hexaploid and diploid.
- 33- "  $3n$
- 34-  $4n$

Conclusions

I have been able to present only fragmentary descriptions and illustrations of the principal methods of attack on the problem of species origins and relationships in the genus *Nicotiana*. I hope that they indicate that ~~the~~ <sup>our</sup> search for pertinent information has been intensive and relatively successful and that therefore there may be justification for drawing the following ~~tentative~~ conclusions without much further discussion of the evidence:

- ① A taxonomic organization of present day species of *Nicotiana* based ~~largely~~ upon morphological-distributional evidence is equally expressive of those fundamental relationships inherent in the ~~genetic~~ <sup>cytogenetic</sup> evidence.
- ② More specifically, with reference to karyotypes, a common pattern obtains for <sup>at least</sup> the nucleus species of each ~~section~~ subgenus and section. In other words, the chromosome morphology of the various taxonomic divisions is in general sufficiently definitive to have significance for interpretation of species ~~origins~~ <sup>origins</sup> and relationships. That structural chromosomal reorganizations and alterations in genic constitution have proceeded independently in the evolution of the genus is indicated by the fact that the products of these two processes as reflected (the one in distinctions in karyotypes and the other in distinctions in external morphology) are in many instances not proportional. <sup>manifestly, as already emphasized,</sup> However, evidence from chromosome morphology ~~is~~ <sup>is</sup> large, substantiated <sup>and the taxonomic organization adopted.</sup>

(2) Chromosome behavior at MI in 211  $F_1$  interspecific hybrids has been investigated. These hybrids represent all pertinent combinations, intrasectional, intersectional and intersubgeneric.

(3) From the point of view that chromosome homology reflected in the formation of bivalents at MI is indicative of the extent to which genes and their arrangement in the conjugating chromosomes are common or similar, the amount of pairing in  $F_1$  interspecific hybrids has been employed to provide evidence of the relationships of the <sup>parental</sup> species concerned in each case. I am not overlooking the fact that there are undoubtedly in some cases gene blocks to pairing and that <sup>therefore</sup> our results for any given hybrid might be open to question. However, when 211 hybrids ~~representing all pertinent combinations, consistently~~ <sup>almost invariably</sup> give consistent evidence the conclusion is inescapable that in *Nicotiana* at least (though perhaps not in other genera) pairing <sup>is the</sup> result of <sup>intrinsic</sup> relationships. Significance as a criterion of relationships.

<sup>From this point of view</sup>  
 The evidence based upon pairing in the 211  $F_1$  hybrids investigated, representing all pertinent combinations, intrasectional, intersectional and intersubgeneric, like the evidence from karyotypes, is practically a complete confirmation of the taxonomic arrangement.

④ The modern genus *Nicotiana* is a relatively limited complex of species relatively sharply distinguished morphologically from one another. Intergeneric and interspecific hybridization appears to have played a considerable role in the origin of these modern species. A marked petunioid influence is apparent in the morphology of at least one of the three subgenera of *Nicotiana*. Other related genera such as *Salpiglossis*, *Tobiana* and *Micranthergia* may also have entered into the origin and evolution of modern *Nicotiana* species or have had an origin in common with them.

Within the present framework of the genus, evidence of ~~the~~ the significance of amphidiploidy in species origins is conclusive. Thus the nine American 24 paired species are all of <sup>such</sup> polyploid origin. For *N. tabacum* and *N. rustica* quite complete cytogenetic evidence of amphidiploid origin is available. <sup>In the case of *N. latrosum* the hybrid</sup> This evidence <sup>is</sup> already illustrated <sup>and</sup> involves "Drosera scheme" relations in MI pairing of <sup>7</sup> interspecific hybrids of *N. tabacum* with 12 paired members of section *Tomentosae*, subgenus *Tabacum*, on the one hand, and with *N. sylvestris* of subgenus *Petunioides* on the other; whereas in  $I_4$  hybrids between members of the *Tomentosae* and *N. sylvestris* pairing is lacking. In other words the genome of *N. tabacum* contains two subgenomes, one descended from an ancestor of modern *Tomentosae* and the other from a progenitor of modern *N. sylvestris*. Thus *N. tabacum* is a

hybrid whose amphidiploid origin was the product of interspecific hybridization, in past time, followed by chromosome doubling. Similar cytogenetic information relates *N. undulata*, subgenus *Petunioides*, with members of section *Paniculatae*, subgenus *Pesticia*, in the origin of *N. rustica*. *N. tabacum* and *N. rustica* are species which have been long in cultivation. <sup>that</sup> *N. arentzii*, a recently discovered wild species of the Peruvian-Bolivian frontier, has similarly been shown to be an amphidiploid involving ancestors of two present day, 12-paired species. In the case of some of the other 24-paired species also the modern descendants of <sup>both</sup> their ancestors have now been determined via pairing evidence, whereas in other cases <sup>modern descendants of</sup> only one of the putative parents can be found within existing species. In addition to pairing evidence (which indicates the original amphidiploid ~~parentage~~ ancestry of the 24-paired species) the <sup>of amphidiploid origin</sup> karyotypes of these <sup>24</sup> species present confirming evidence in that <sup>the chromoph of the</sup> two subgenous in each case are often definite reflections of the <sup>chromoph of the</sup> genous of their putative parents. This is particularly striking in the case of *N. arentzii* whose amphidiploid origin is probably more recent than that of *N. tabacum* for instance.

- ⑤ Such evidence of the role of hybridization in the evolution of the genus *Nicotiana* involves the assumption that a considerably larger number of species existed in past time. The fact already referred to that modern descendants of only one of the putative ancestors of certain of the 24-paired amphidiploid species is known is evidence in this connection. From this point of view the genus *Nicotiana* <sup>marginata</sup> is a relic genus.

and finally

- ⑥ There is <sup>some</sup> ~~considerable~~ evidence in hand to suggest, ~~at least~~, that 6 may have been the basic chromosome number for *Nicotiana*, and perhaps for such closely related genera as *Petunia* as well. Part of this evidence is the present day occurrence of 7- and 9-paired species of *Petunia* and of 9- and 10-paired species of *Nicotiana*. On the assumption that 6 is the basic number for *Nicotiana*, 12- as well as 24-paired species represent polyploid derivatives, and from this point of view the few 9- and 10-paired American species are products of hybridization between 6-paired species at present unknown. Eight paired species, again unknown today, <sup>probably</sup> ~~may have~~ had a similar origin and have become responsible through polyploidy for the 16- and 24-paired Australian-South Pacific species. Through hybridization imposed upon such polyploidy the aneuploid series in that species group could