termediate between tryptophane and nicotinic acid in the mammal remains to be determined.

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*ON THE EVOLUTION OF THE GENUS NICOTIANA**

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Taxonomic Status.—The relatively advanced family *Solanaceae* is allied to such families as the *Labiatae, Hydrophyllaceae, Boraginaceae* and *Polemoniaceae* but most closely related to the *Nolanaceae* and *Scrophulariaceae*. The approximately seventy-five genera have been organized in five to nine tribes or subtribes. Don, Endlicher, Miers and Dunal placed *Nicotiana* in the tribe *Nicotianae*, Bentham and Hooker and von Wettstein, in the *Cestr(in)eae*.

In taxonomic organizations of the *Solanaceae* the genera most frequently associated with *Nicotiana* are *Petunia*, *Cestrum*, *Sessia*, *Vestia*, *Metternichea*, *Retzia*, *Fabiana* and *Nierembergia*. From my point of view the morphological evidence more intimately relates the following genera to *Nicotiana*: *Petunia*, *Cestrum*, *Vestia* and *Fabiana*.

A recent taxonomic reorganization of the genus (Goodspeed[1]) adopts the treatment of Don and recognizes three subgenera and eleven sections with a total of fifty-eight species involved. In subgenus *Rustica* there are sections *Paniculatae* (seven species), *Thyrsiflorae* (one species) and *Rusticae* (one species); in subgenus *Tabacum*, sections *Tomentosae* (five species) and *Genuinae* (one species); in subgenus *Petunioides*, sections *Undulatae* (three species), *Trigonophyllae* (two species), *Alatae* (nine species), *Noctiflorae* (four species), *Acuminatae* (ten species) and *Suaveolentes* (fifteen species).

The significance with regard to evolutionary mechanisms in *Nicotiana* of certain of the inter- and intrageneric relationships defined in the taxonomic status of the genus briefly summarized above will appear in what follows.

Morphology.—*Nicotiana* is a conservative genus in the sense that it does not exhibit characteristic morphological specialization of particular floral or vegetative organs. On the other hand, no other genus of the family, save possibly *Solanum*, shows a greater range of variation in habit, inflorescence and flower than does *Nicotiana*, and the degree of physiological specialization attained by many of its species indicates the extent to which they have evolved.

Between the extremes represented, on the one hand, by desert ephemerals a few inches high and, on the other, by shrubby to subarborescent xerophytic or subtropical perennials to twenty-five feet in height are robust annuals some of which become limited perennials in favorable environments, and also root perennials spontaneously propagating from underground parts regardless of the fate of aerial ones. Inflorescence types include a thyrse panicle and a flat spray, between which complex extremes lie such intermediates and specializations as mono-, di- and pleiochasial cymes, solitary flowers and inconstant, diffuse paniculate-cymose mixtures.

Variability of the flower is chiefly expressed in the form and color of the corolla, stamen insertion and aestivation. In some species the flower is vespertine. The corolla is essentially salverform to trumpetform or tubular, the limb entire to rather deeply lobed. Red, green and yellow in various intensities and combinations occur. Pure white is found in only one species while a corolla predominantly white with a green to purplish flush exteriorly is common. The stamens may be included or exserted, equal or unequal, long or short and inserted at any point from near the base of the corolla to immediately below the limb.

Consideration of major and minor details of habit, inflorescence and flower of *Nicotiana* species reveals correlations suggesting five definite but interrelated morphological nuclei or clusters of species. The *rusticoid* and *tomentosoid* nuclei can be related by shrubby habit, thyrse inflorescence, non-vespertine corolla with tendency for the limb to pass slowly through an upright, horizontal and recurved condition, and low insertion of stamens. The other three—the *alatoid*, *noctifloroid* and *corymbosoid*—also show interrelation. They are herbaceous nuclei; the inflorescence is variable but not a thyrse; the flower is usually whitish, vespertine and salverform; the limb seldom passes through a series of orientations; stamen insertion is higher, sometimes apical.

The *rusticoid* nucleus (subgenus *Rustica* in general, and a group of its member species in particular) shows greatest regularity in leaf form, size and distribution; evenness in distribution of branches; an inflorescence derivable from a leafy shoot and capable of producing other known inflorescence types in the genus by reduction; pronounced regularity of all four whorls of the flower; relatively little adnation of filaments; a conservative condition of corolla throat, stamens, pistil and hypogynous disc. On morphological grounds it is, therefore, taken to represent that portion of the genus in which the most primitive characters have been preserved. By contrast with various species of the *tomentosoid* and *corymbosoid* nuclei, the more strictly *rusticoid* members of subgenus *Rustica* exhibit relatively little polymorphy; the same is true of most members of the *alatoid* nucleus (including species of section *Alatae*) and less true of the *noctifloroid* (including species of section *Noctiflorae*).

The morphological evidence points to the rôle of hybridization in the origin of the five modern nuclei. Thus, one may assume that on a primitive ancestral level a series of progenitors of genera including pre*Nicotiana*, pre*Petunia*, pre*Cestrum* and the ultimate ancestors of other genera now related to *Nicotiana* began to vary each about a distinct morphological mean—differentiation referable to genic and structural alterations accompanied by combination and recombination, selection and genetic and geographic isolation. Two ancestral complexes, the *glaucoid* and the *petunioid*, appeared as a result of hybridization of pre*Nicotiana* with, in the first case, pre*Cestrum* and, in the second, pre*Petunia*, followed by differentiation within two diverging modes of morphological variation thus established. Most of the members of the modern *rusticoid* and *tomentosoid* nuclei arose from the *glaucoid* complex, while, to the same degree, the *alatoid*, *noctifloroid* and *corymbosoid* nuclei were derived from the *petunioid* complex. To this point and to this extent a dendroid diagram of the evolution of

Nicotiana might be constructed. However, certain species and species groups within and without the five nuclei cut sharply across such a diagram and transform it into a reticulum. Thus, there are modern species which owe their origin to hybridization between derivatives of the *glaucoid* and *petunioid* complexes, and others, to hybridization between components of those ancestral complexes themselves.

Distribution.—Today the distribution of the genus is essentially tripartite: South America, North America, Australia and the South Pacific. In South America *Nicotiana* occurs in approximately the entire "tail" of the continent, and also in southern and western Peru, the drier southwest of Ecuador, parts of Bolivia and the southern portion of western Brazil, but is absent from the drainage systems of the Amazon and Oronoco and from the northern countries of the continental "shoulder." In North America it is found native in central and northern Mexico and in the western United States. Scattered stations are known in Guatemala and the West Indies. In Australia it has been collected in all but the tropical north and northwest. In the South Pacific it extends in isolated localities from New Caledonia to the mid-Pacific Marquesas. This distributional picture is a product of the fact that, in general, modern species of *Nicotiana* demand light, a well-drained soil and dry rather than humid heat. Only a few species have set aside one or another of these requirements and occur on the margin of the moist tropics.

Presumably in South America existed the original reservoir from which the present-day assemblage of *Nicotiana* species arose. Certainly there is striking numerical preponderance of South American species and the extent of their differentiation into morphologically and cytogenetically distinct subdivisions has no parallel in the other two distributional areas. More specifically the center of distribution is taken to be the region now represented by the general area of junction of Peru, Bolivia, Argentina and Chile which corresponds to the current distribution of the *rusticoid* nucleus shown above to represent, on morphological grounds, the most primitive of the five clusters of modern species.

The tripartite, geographically discontinuous distribution of the genus is explicable on the assumption that ecologically suitable paths for migration connected South America with each of the other two continents in which species of *Nicotiana* are today native. The obvious suggestion that such a pathway to Australia included Antarctica and Tasmania is supported by the tracing of an extension of the Andean system into the Antarctic continent, the indication that a temperate flora existed there at a relatively recent time and that migration from Antarctica to Australia via Tasmania occurred. The problem of a route permitting passage from South to North America involves the semixerophytic nature of most modern *Nicotiana* species and the tropical zone which now separates the two continents. There may have been a Caribbean path followed by certain progenitors more tolerant than current species of semitropical environments. Along it they could have passed from the northern "shoulder" of South America through the arc of the West Indies to Mexico, a route to which the distribution of other plants points more clearly. On the other hand, if the considerable decrease in temperature postulated for Central America during the glacial epoch induced a somewhat dry subtropical climate, migration might largely have followed the path of today's land connection between the two Americas. From central California to Mexico and from Chile to southern Peru a previously greater westerly extension of land has been postulated. Had a similar extension once existed between Mexico and Ecuador another pathway between central western South America and southwestern North America might have become continuous. In these various connections it is to be noted that both the western United States and Australia have undergone considerable desiccation since the presumptive advent of *Nicotiana*. In the former case this has encouraged the northerly spread of the genus; in the latter it has stranded, segregated and specialized forms already present. The South Pacific localities are considered (Wheeler[2]) to represent eastern migration from the Australian coast probably involving in the extreme instance—the Marquesas—introduction by man.

The distributional evidence indicates that the genus *Nicotiana* has maintained itself from at least a period prior to the formation of an effective Andean barrier, was present in Antarctica when that continent supported a temperate, herbaceous angiosperm flora, and passed from South to North America. It has survived land mass alterations of great magnitude which played a rôle in transforming a restricted, continuous area of distribution into a large, tricontinental and discontinuous one, and were responsible for forcing colonization of new areas and adaptation to new environments upon individual species, as well as for the extinction of others.

Chromosome Number.—In the *Solanaceae* haploid numbers from 7 to 36 have been reported; in the *Scrophulariaceae* the range is from 6 to 30. In the *Solanaceae* over fifty-five per cent of the species investigated show 12 pairs while a multiple of 12 pairs occurs in an additional twenty per cent. In the *Nolanaceae* all the five species counted have 12 pairs and in the *Scrophulariaceae* species with 12 as well as species with 6 pairs occur.

Of the fifty-eight species of *Nicotiana* the chromosome numbers of fifty-five are known. Twenty-eight species show 12 pairs; eleven, 24; four, 16; three, 9; two, 10; two, 19; two, 20; in one species each the haploid numbers are 18, 21 and 22. In related genera the known haploid numbers are: 7 (*Petunia*), 8 (*Cestrum*, *Vestia*), 9 (*Petunia*, *Fabiana*, *Nierembergia*), 22 (*Salpiglossis*).

Subgenus *Rustica* and subgenus *Tabacum* contain only species with 12 or 24 pairs. In the third subgenus (*Petunioides*) four of the six sections contain species showing 24 pairs and all but one section species showing 12 pairs. Two aneuploid series occur, each in a separate section of subgenus *Petunioides:* 9 to 12 pairs in section *Alatae* and 16 to 24 pairs in section *Suaveolentes.*

The rôle of hybridization in *Nicotiana* phylesis suggested by the morphological evidence offers a partial explanation of the origin and evolution of the present-day chromosome number situation just summarized. The primitive ancestral reservoir above referred to which included at least pre-*Nicotiana,* pre*Cestrum* and pre*Petunia,* and which via hybridization on the pregeneric level gave rise to the basic *Nicotiana* complexes, the *glaucoid* and the *petunioid,* is assumed to have been 6-paired. From the two 6-paired complexes a 12-paired *Nicotiana* level arose by interspecific hybridization followed by chromosome doubling and, to a lesser extent, by autoploidy. During the establishment of the 12-paired level a residue of 6-paired species, now extinct, was also in existence. Finally, from the 12-paired level, an almost exclusively alloploid 24-paired level was developed, thus contributing largely to the transformation of a basically dendroid into a reticulate diagram of evolution of *Nicotiana.*

Two aneuploid sequences arose, one between the 6- and 12-paired levels, the other below the 24-paired level. The former is today represented only by 9- and 10-paired species, all of them in section *Alatae,* although earlier existence of 8-paired species may be reflected, as will be indicated below, in the occurrence of the other (16- to 24-paired) aneuploid series. In this connection it is to be recalled that 7-, 8-, 9- and 22- ($x = 11$) paired species are found in modern genera related to *Nicotiana.*

The postulated lower numbers in the 6- to 12-paired aneuploid sequence presumably had a sesquidiploid origin. The higher numbers were doubtless derived by reduction from the 12-paired condition, perhaps in part involving reciprocal translocation (cf. Babcock[3]). There are a number of explanations of the origin of the 16- to 24-paired aneuploid series, all species of which are members of the section *Suaveolentes.* In my opinion two or more 24-paired ancestors of section *Suaveolentes* arose, as did the nine modern 24-paired species, by amphiploidy on the early 12-paired level. During a corresponding period, the same or related 12-paired species, crossing with certain of the not yet extinct 6-paired species, produced by chromosome doubling the 18-paired condition which by reduction initiated the 16-paired group of species. Such establishment of the 16-, 18- and 24-paired progenitors of section *Suaveolentes* was followed by the production of 19-, 20-, 21- and 22-paired species as a result of hybridization, sesquidiploidy, addition through fragmentation or reduction by loss or fusion. Other suggested explanations (Kostoff[4]) involve now extinct 8-, 9- and 10-

paired species in the amphiploid production of 16-, 18- and 20-paired species or take 16- and 20-paired species to be secondary derivatives from an 18-paired one so derived. Yet another (Wheeler[5]) assumes the former existence of a series of 8-paired species which by successive amphiploidy gave rise to the 16- and then the 24-paired condition, with subsequent hybridization between the products resulting in the 18- to 22-paired sequence. In any case, it appears that this modern aneuploid sequence represents a series of secondary derivatives with amphiploidy the fundamental mechanism responsible for their origin.

Chromosome Morphology.—For the genus as a whole a combination of median (*m*) and submedian (*sm*) chromosomes predominates over subterminal (*st*) ones in the ratio of five to three. In each of the three subgenera there is a distinctive ratio of *m* plus *sm* to *st:* in subgenus *Rustica* it is 9:1, in subgenus *Tabacum* 5:2 and in subgenus *Petunioides* 4:3. In addition, there is a basic karyotype characteristic of those species which represent the morphological core of each section. In other words, chromosome morphology in *Nicotiana* exhibits sufficiently distinctive features to have phylogenetic significance (Goodspeed[6]).

In the species of genera related to *Nicotiana* for which chromosome morphology is known, the genoms consist almost exclusively of *m* or *sm* chromosomes. Thus, in species investigated of both *Cestrum* and *Vestia* all of the chromosomes are *m* or approximately *m*, and in 7-paired species of *Petunia* 5 are *m*, 1 is *sm* and 1 is *st*.

The contention that primitive groups tend to possess predominantly *m* chromosomes while those of more advanced status show an increasing proportion of heterobrachial ones is apparently borne out in *Nicotiana*. Based upon this contention, the above postulated aggregation of 6-paired ancestral species probably possessed all *m* chromosomes. Subsequent evolutionary levels, progressive in terms of chromosome multiplication and with aneuploidy as their by-products, were in most instances characterized by differentiation in chromosome morphology, particularly at the 12-paired level. Thus, although the *rusticoid* derivatives (species of subgenus *Rustica*) of the *glaucoid* complex have preserved almost exclusively the primitive *m* type of chromosome, its *tomentosoid* derivatives (species of subgenus *Tabacum*), derivatives of the *petunioid* complex (species of subgenus *Petunioides*) and species derived by hybridization between the two complexes themselves today combine *m*, *sm* and *st* chromosomes in varying proportions. In the *rusticoid* nucleus the tendency toward preservation of the postulated ancestral *m* type of chromosome may be a product of the initial setting apart of a relatively more uniform, and thus more stable, type of internal chromosomal structure, perhaps genically conditioned, together with the persistence of relatively uniform or favorable environmental conditions.

There is evidence suggesting the direction which possible future alteration of the typical *m* karyotype of subgenus *Rustica* may follow. In all 12-paired species of *Nicotiana* at least four nucleoli are seen, and apart from those of subgenus *Rustica* the majority of the species correspondingly show four satellites. In seven of the eight 12-paired species of subgenus *Rustica* only two satellites appear. However, in each of them at least one *sm* chromosome consistently shows a distinct tertiary constriction which is presumably associated with a nucleolus organizer. Such chromosomes, by fragmentation at the distal portion of the less stable heterochromatic regions doubtless associated with these tertiary constrictions, might be expected to become transformed into *st* satellited chromosomes. As a product of an equivalent internal structural differentiation followed by fragmentation the full complement of satellited chromosomes characteristic of the majority of the species of subgenus *Tabacum* and subgenus *Petunioides* could have been derived. The transitional position in terms of satellite evolution which subgenus *Rustica* apparently occupies would argue in favor of its primitive status, one which is indicated on other grounds.

Meiotic Chromosome Behavior.—Complete pairing between structurally homologous chromosomes is shown at *MI* in the majority of species. In certain 12-paired species, however, bridges and fragments at *AI*, and in others frequent occurrence of non-conjunction or non-disjunction followed by irregular distribution of chromosomes, give evidence of the structural hybridity of their genoms. In only one species, an amphiploid on the 24-paired level, has the presence of multivalents been observed. Study of meiotic behavior in races of at least one polymorphic species demonstrates that certain of these races differ from one another by one to several reciprocal translocations and suggests the rôle of structural alterations in initiating species differentiation in *Nicotiana*.

All of the twenty-four monosomics of *N. tabacum* (Clausen and Cameron[7]) and all of the twelve primary trisomics of *N. sylvestris* (Goodspeed and Avery[8]), as well as numerous other chromosomal variants involving loss or addition of one to several chromosomes have been studied in respect to meiotic chromosome behavior. These and similar investigation of variants involving reduction or multiplication of entire genoms—haploids, triploids and tetraploids—have yielded evidence of evolutionary significance. For example, the conspicuous morphological distinctions characterizing each of the primary trisomics of a single species demonstrate the extent to which such chromosome unbalance may be reflected in differences in morphological expression. For example, again, the occurrence at *MI* in tetraploid forms of a number of 12-paired species of valencies higher than four (Fardy and Hitier[9]) suggests the compound character of the diploid representatives of those races.

Meiotic chromosome behavior at *MI* has been studied in a total of two hundred and thirteen F_1 interspecific hybrids of *Nicotiana*. While this number of hybrids represents but a small percentage of the possible combinations of the fifty-eight species of the genus, they include representative combinations pertinent for interpretation of relationships: fifty-three species are involved in the eighty-three intrasectional, forty-nine intersectional and eighty-one intersubgeneric hybrids analyzed.

On the basis of the results of these *MI* analyses the two hundred and thirteen F_1 interspecific hybrids may be classified into five categories: lack of pairing, complete, low variable, high variable and "Drosera Scheme" pairing (cf. Goodspeed[10]). In the high and low variable categories where an extremely wide range in number of pairs may occur the high point in the range is considered significant as indicating the maximum number of appreciable homologous segments in the two genoms involved, while the mode may be an expression of normal expectancy on the basis of the maximum number of homologous segments present or may indicate the number of chromosome pairs possessing relatively large pairing blocks. Similarly, in the lack of pairing hybrids with a range of zero to 4 (frequently 0 to 1, 2 or 3), mode zero, the occurrence of up to 4 pairs presumably represents the maximum, though not consistent, expression of small homologous segments residual in the genoms involved, while the characteristic mode of zero is largely a reflection of the lack of pairing blocks of appreciable size and effectiveness. The presence of a limited number of non-homologous segments in the genoms of the two parental species may explain the occasional occurrence of univalents in a hybrid in which the mode is complete pairing. Therefore, on the basic assumption of the significance of pairing (cf. Stebbins[11]), the maximum extent of pairing in an F_1 interspecific hybrid may be considered as an index of the relationship of the species involved in terms of their residua of ancestral homology. Such application of extent of pairing as a criterion of relationship in *Nicotiana* largely confirms the morphological evidence expressed in the taxonomic organization of the genus. Thus, in approximately ninety per cent of the F_1 hybrids investigated amount of pairing parallels extent of taxonomic relationship of the parental species. In the ten per cent in which extent of pairing does not correspond to degree of taxonomic relationship some few show higher, while the majority show lower, than the amount of pairing to be anticipated. Exceptions of the former type furnish an illustration of the value of a pairing check on a taxonomic organization. Although genetic alterations, reflected in morphological distinctions setting apart two species, have occurred, genically they have more in common than other species of a similar degree of morphological differentiation which show negligible pairing in the F_1 hybrid between them. This means that, in the one case as contrasted with the other, there has been less fundamental divergence from a common (or related) ancestor. On the other hand, lower pairing in F_1 than corresponds to degree of taxonomic relationship between the two parental species may be attributed to one or more of the factors recognized as contributing to secondary disturbance of conjugation.

In terms of hybrids which reach maturity and thus are available for cytological analysis *Nicotiana* is apparently exceptional, at least among genera extensively investigated, in degree of compatibility between its species. Thus, relative remoteness in relationship is not a bar to compatibility, as indicated, first by the fact that approximately sixty-five per cent of F_1 interspecific hybrids show meiotic irregularities, and second, as noted above, that the same proportion involve as parents members of different subgenera or sections. In other words, it appears that factors restricting compatibility have evolved less rapidly than those inhibiting pairing and those responsible for specific morphological distinctions. Certainly in *Nicotiana* high degree of compatibility is characteristic of many present-day species which have diverged considerably in respect both to external morphology and to extent of pairing in F_1 hybrids between them.

In some cases both, and in all cases one, of the species related to the 12-paired ancestry of each of the nine modern American species on the 24-paired level can be identified by meiotic chromosome behavior in appropriate F_1 interspecific hybrids. For three of the nine an amphiploid origin is recognized, with identification of the present-day 12-paired descendants of both the original parental races provided in large part by the "Drosera Scheme" pairing relations exhibited by the hybrids between these descendants and the 24-paired species in question. Of these three, *N. arentsii* furnishes a demonstration of an evolutionary sequence initiated by amphiploidy which is today in progress. Thus, the descendants—*N. undulata* and *N. wigandioides*—of the two 12-paired races which entered into its amphiploid origin possess in their genoms sufficient homologous segments to produce a considerable number of bivalents at *MI* of the F_1 hybrid between them. Therefore, the original parents represented only certain extents of differentiation, both genic and structural, from a common ancestor or closely related ancestors on the 12-paired level. Consistent with this proposition is the occurrence in *N. arentsii* of multivalents which also appear frequently at *MI* in F_1 hybrids between that species and *N. undulata* and *N. wigandioides*. By contrast, *N. tabacum*, another 24-paired species of known amphiploid origin, combines in its ancestry progenitors of two 12-paired species—*N. sylvestris* and *N. otophora* or another member of the same section—which are today taxonomically remote. Correspondingly, the F_1 hybrid between these two 12-paired species shows almost complete lack of pairing and no multivalents appear at *MI* in *N. tabacum*. This evidence indicates that the considerable degree of multivalency exhibited by F_1 hybrids between *N. tabacum* and both *N. sylvestris* and *N. otophora* (or its relatives) is referable to translocations within the genoms of *N. sylvestris* and of *N. otophora* or within the genom of *N. tabacum* during the evolution of these three species.

For the remaining six 24-paired American species an amphiploid origin is indicated by "Drosera Scheme" pairing or approximation thereof at *MI* in F_1 hybrids between each of these species and a specific 12-paired species. In each case the modern descendants of the other original parent on the 12-paired level have presumably become extinct. However, in some instances morphological and in others cytogenetic evidence points to the position in the author's taxonomic organization of the genus which those species would have occupied. Both types of evidence suggest for certain pairs of modern 24-paired species one common 12-paired ancestor, in some instances represented by descendants today, in others not so represented.

The group of related species including *N. repanda, N. nesophila* and *N. stocktonii* provides a present-day illustration of species differentiation from a common ancestor on the 24-paired level. Morphologically the distinctions within the group pattern are considerable but complete pairing in F_1 intragroup hybrids occurs. The case of *N. arentsii,* discussed above, provides a possible picture of the origin of such species groups since the products and by-products of multivalent formation, including substitution between members of subgenoms, to be anticipated in the evolution of *N. arentsii* could initiate species differentiation.

Processes similar to those known to be responsible for origin of the 24-paired level from progenitors of known 12-paired species were presumably operative in initiating the 12-paired level from postulated 6-paired ancestral species (cf. Kostoff[4]). Parallel origin from a primitive 6-paired ancestral level not only of pre*Nicotiana* but also of, at least, pre*Cestrum* and pre*Petunia* permitted inter-pregeneric hybridization. In part the product of such hybridization on the next level of differentiation, when those various genera were establishing their distinct modes of morphological variation, was, as already noted, the definition of the 6-paired *glaucoid* and *petunioid* complexes in *Nicotiana.* A subsequent period of expansion of these two complexes gave a series of species whose ranges of morphological variation in some instances did and in others did not appreciably overlap. Correspondingly, their genoms became to different extents mutually exclusive in terms of genic content and structural organization.

Crossing between 6-paired races of various degrees of differentiation from the pre*Nicotiana* level onward, followed by chromosome doubling, was doubtless a determining factor in the origin of the 12-paired level, just as such amphiploidy has been largely responsible for the origin of the modern 24-paired level. On this assumption, the extent of fundamental relationship between 6-paired ancestors plus accumulation of genic and structural distinctions since establishment of the 12-paired level are reflected in the amount and character of pairing at *MI* of F_1 hybrids between present-day 12-paired species. Thus, complete pairing in such hybrids may be interpreted as the expression of an originally maximum degree of relationship together with minimum later accumulation of genic and structural alterations. The opposite extreme, almost complete lack of pairing, would then be referable to minimum initial relationship plus maximum incidence of distinctions subsequently produced, while low and high variable pairing indicate intermediate expressions of the two factors.

Obviously, the possibility of an auto- as contrasted with an essentially alloploid origin of certain members of the original 12-paired from the 6-paired level cannot be excluded. The occurrence of multivalency in current 12-paired species or evidence of autosyndesis in F_1 hybrids between them should follow such origin. The first of these consequences has not, in my experience, been demonstrated and evidence concerning the second would be difficult to obtain because of lack of large morphological distinctions at *MI* between members of the genoms of *Nicotiana* species. On the other hand, it is possible that autosyndesis, a product of autoploid origin of one or both parents, may explain in certain instances high variable pairing in F_1 hybrids between modern 12-paired species. A 6-paired ancestor in common between two such species, each originating by alloploidy, could be reflected in low variable pairing. In any case, pairing variability is doubtless a product largely of differentiation after attainment of the 12-paired level which has served to reduce the size and alter the arrangement of originally extensive homologous segments.

Conclusion.—The point of view presented here concerning the evolution of *Nicotiana* emphasizes the rôle which hybridization has played in establishing the modern expression of the genus, assuming the existence of a now extinct reservoir of 6-paired pre*Nicotiana* races from which the progenitors of modern 12-paired and 24-paired species levels were derived, largely by amphiploidy. Differentiation on the 6-paired level produced two basic ancestral complexes in the development of which hybridization between pre*Nicotiana* and at least pre*Cestrum* and pre*Petunia* was concerned. From these complexes was produced a series of 12-paired derivatives certain of which are united in the subdivisions of the author's taxonomic treatment of the genus while others trace their origin more directly to both the ancestral complexes via hybridization between their components or immediate derivatives. The present-day 24-paired species level arose largely by amphiploidy involving members of most of the 12-paired clusters of species into which the ancestral complexes became segregated. Throughout the evolutionary sequence genic and structural chromosomal alterations played a part at first more, and later less, definitive than amphiploidy in producing differentiation at successive levels, culminating in the range of morphological variation exhibited by the current *Nicotiana* species assemblage. Documentation of these propositions is afforded by the combined taxonomic, morphological, distributional and cytological evidence, the most pertinent aspects of which have been briefly outlined above.

In evaluating the present and predicting the future evolutionary status of *Nicotiana* it is to be borne in mind that evidence indicating antiquity does not necessarily indicate senescence from the genetic point of view. Although the extent of morphological, and especially physiological, specialization attained by many species of *Nicotiana* suggests a point in the generic cycle past maturity, the degree of polymorphy indicates a plasticity and capacity for expansion characteristic of a relatively aggressive stage of the cycle (Clausen, Keck and Hiesey[12]). The degree of polyploidy already attained by the genus, a product of the continuing and definitive rôle of hybridization in its evolutionary history, might argue for its advanced or even senescent status. On the other hand, it appears that hybridization with the genom or the gene as the unit of differentiation remains a potentially effective mode of future evolution because apparently genetic barriers are accumulating less rapidly than genic alterations determining species distinctions, with the result that there is a high degree of intersubgeneric as well as intersectional and intrasectional crossability. While the present-day range of distribution of the genus in terms of species is, in general, locally restricted and apparently not increasing, the tripartite nature of its global spread argues against the possibility of extinction since, obviously, future climatic or other alterations would be unlikely to be operative over a sufficient geographic area to eliminate all its species. Indeed, on the assumption that the early part of an interglacial period now obtains; the future extension of a genus, most species of which are arid or semiarid types, would be favored by increasingly warmer, drier climates. In other words, despite the fact that numbers of species of *Nicotiana* have doubtless become extinct, that others have become extremely restricted in extent, and that many are today polyploids exhibiting considerable specialization, survival of *Nicotiana* as a genus is favored by its relatively wide range in distribution and morphological character together with its lack of barriers to hybridization.

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*ANTIBIOTIC SUBSTANCES FROM BASIDIOMYCETES I. Pleurotus griseus**

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In a survey[1] conducted in this laboratory it was noted that 213 of the 332 species of Basidiomycetes studied produced substances that inhibited the growth of *Staphylococcus aureus.* Among the species that seemed worthy of further investigation was *Pleurotus griseus.*[2]

P. griseus grew with medium rapidity on a thiamine-peptone agar[1] and a modified Czapek-Dox agar to which corn steep solids had been added. It formed a pink pigment which diffused into the agar. After the fungus had grown for one to several weeks, discs 5.5 mm. in diameter were cut on a radius extending from the center of the colony into the agar adjacent to the colony. These discs were placed on a yeast-peptone agar seeded with *Staph. aureus* and the plates incubated overnight at 37 ± 2°C. Clear zones of inhibition were found around the discs indicating that some substance or substances had diffused from the agar discs into the surrounding seeded medium in sufficient concentration to inhibit the growth of the staphylococci in the agar. A disc cut from the agar at as great a distance as 20 mm. from the edge of the colony frequently produced a zone of inhibition. The zones, while small, were clear, indicating that bacteria resistant to the antibiotic substance or substances were absent. Disc tests, with strains of *Staph. aureus* resistant to other antibiotic substances, indicated that *P. griseus* produced an antibiotic substance which was different from penicillin and different from the active substances produced by several other Basidiomycetes. The antibiotic substance or substances from *P. griseus* did not inhibit the growth of *Escherichia coli.*

P. griseus was grown on several types of nutrient media, differing from one another in the source of nitrogen and carbon, to determine the effect of the medium on the production of the antibacterial substances by the fungus. Cane sugar, dark brown sugar, galactose, lactose, mannitol and corn steep solids were ineffective carbon sources; maltose and soluble starch were poor; dextrose and levulose were the best and nearly equal so far as the