GENETICAL STUDIES ON THE INTERRELATIONSHIPS OF CERTAIN VIRUSES CAUSING NECROSIS IN THE POTATO.

THESIS

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November, 1940.

C O N T E N T S.

INTRODUCTION

This paper is concerned with a study of the inheritance of a type of reaction in the potato plants following infection by certain viruses.

It has long been suggested that Solanum tuberosum L., with a somatic chromosome number of 48 occupies a place in the polyploid series 24, 36, 48, 72 etc. of the Solanaceae. Nevertheless, potato investigators have repeatedly endeavoured to explain their breeding results on a diploid basis, a procedure which must inevitably lead to erroneous conclusions if, as is likely, the polyploid nature of S. tuberosum is a reality.

In contrast to diploid studies polyploid genetics are rendered more difficult by the duplication of factors and blending of character expressions consequent upon the polyploid state, to which the immense variation in foliage types, habit and tuber colour of the potato bear eloquent witness. In the present paper the analysis of a usually highly complicated situation has been facilitated greatly by the discovery of a lethal necrotic reaction in the potato which is apparently determined by a single gene, completely dominant in a single dose.

Within/

Within recent years it has become ever increasingly evident that no genetical investigation is complete without a full consideration of the cytological possibilities involved. In fact genetics and cytology are truly complementary and no apology is therefore required for the somewhat detailed discussion that certain cytological topics have received in the following pages. Unfortunately no co-ordinated account of the cytology of the potato exists; thus it has been found necessary to sift and compare the various accounts scattered through the literature and to examine critically the data relevant to the problem under consideration. For instance, the question of secondary association as observed in the potato requires to be viewed against a background of wider cytological experience. This phenomenon is widespread among the potatoes and on present theories demands for its satisfactory explanation that 6 shall be the basic number of the genus Solanum, and consequently that the cultivated potato be an octoploid. With this the genetical evidence so far obtained disagrees and a survey of the literature has shown up many discrepancies for which existing theories of secondary association do not adequately account. The whole problem requires a much fuller discussion than I have been able to give it /

it but an exhaustive treatment was not felt to be justified in the absence of original contributions and of familiarity with the material discussed. On the other hand, the significance of secondary association as an indicator of latent homologies, if true, is very great and I have attempted to assess the evidence for and against from a genetical view point. The need is stressed for a thorough re-investigation of potato cytology and the phenomenon of secondary association in particular.

The evidence presented herein seems to offer a sound basis from which further incursions into potato genetics may be made with some measure of confidence. The majority of characters in the cultivated potato such as leaf shape, pubescence, tuber shape, time of maturity are quantitative in their expression and unsuitable for straightforward genetical analysis. With the simplified biochemical methods now available, however, it should be possible to analyse the inheritance of tuber colour and most probably other discontinuous characters are to be looked for among the wild South American potatoes. The possibilities of the present material are by no means exhausted and many aspects remain to be worked out. Additionally it is hoped that an examination of the distribution of the lethal necrotic genes among the South American material may lead to interesting results.

POTATO VIRUSES

The complications of potato virus work began when it was realised that several distinct types of disease were implicated in the degeneration of potatoes. Work in Europe and America was carried on largely independently with the result that the literature became overburdened with descriptive names, such as rugose mosaic, mild mosaic, crinkle mosaic, curl, streak, simple mosaic, etc. of virus diseases, occurring in the two countries, some of which have since proved to be identical in essence. At first no discrimination was made between the disease and its cause, nor was it appreciated that different potato varieties reacted differently to the same virus complex. The situation in America was further complicated by Johnson's (1925) discovery that all stocks of American varieties were permeated by a virus, the "healthy potato" virus. In an attempt to clear up the existing confusion Johnson (1927, 1929) formulated a scheme which would be internationally intelligible by classifying viruses according to their physical and chemical properties in vitro. These methods do not, however, afford any information on the properties of viruses which are of direct interest, their properties as pathogens, nor are they applicable to/

to such viruses as are not readily inoculable by needle or rubbing. At a later date Quanjer (1931) attacked the problem from the aspect of the morbid anatomy of the infected host, by means of which he differentiated potato viruses into six classes viz. anecrotic mosaics, phloem necrosis, acronecrosis, acropetal necrosis, pseudo-netnecrosis and concentric necrosis of the tuber, according to the reactions of certain potato varieties to be used as differential hosts. From the classificatory point of view the scheme is of little value, as the external symptoms of the various classes are far more readily recognisable than are their morbid anatomy. But it is of immediate importance in the fact that the old term "streak" was found to cover two quite distinct types of disease, acronecrosis and acropetal necrosis, differing both in the expression and internal effects produced on the differential hosts. We are concerned with the first of these, namely top necrosis or acronecrosis.

A preliminary account of this disease was published by Quanjer and Botjes (1929) but it is evident that Schultz (1925) observed similar disease symptoms at an earlier date on certain varieties and seedlings grafted with scions from the American Green Mountain. He distinguished these from ordinary "streak"/

"streak" to which Green Mountain is susceptible. According to Quanjer (1931) top necrosis is defined as:- "necrosis radiating from only a rather small percentage of the internal phloem strands, into the surrounding parenchyma, this in turn surrounded by a cork cambium, except in the tender tips, which are soon killed; occurring in foliage, stem and tubers."

In general the first visible symptoms of top necrosis are the development of large numbers of fine necroses or necrotic spots penetrating the tissue of the terminal two or three leaves of a shoot, usually at the junctions or endings of the finer veins, eventually killing the whole shoot apex and spreading down the stem. If the plant is young and growing strongly it may be entirely killed, but in older or maturing plants the spread of necrosis seems to be checked and only one or two shoots may be affected (Figure 2). Necrotic plants usually form few if any tubers, some or all of which are spotted and blotched by black necrotic patches. In storage the necrosis spreads to the tuber buds and by the following season the tubers are frequently reduced to a mass of cork. Partially necrotic tubers produce apparently normal sprouts which soon become smothered with necroses to which the young plants succumb. Unblemished tubers give rise to healthy plants which show/

show no signs of necrosis. In most cases then, the total effect of top necrosis is the elimination of the infected plant, or part, and its vegetative progeny. (Figures 5, 11-16).

Bawden (1932) supports Quanjer's (1931) observations that the necroses arise first in the phloem, later spreading into the neighbouring tissues. Thickening of the cell walls of the primary phloem elements is the first visible pathological symptom, followed by the separation of the adjacent cell walls and the infiltration of a brownish gum-like deposit into the intercellular spaces so formed. Individual cells may lose their cell contents coincident with thickening of the walls or may also become filled with the gum-like deposit. Similar changes precede the development of necrotic areas in the tubers. In any one section of the stem few of the external and internal phloem groups were found to be necrotic by Bawden and the spread of necrosis from these was in the direction of the wood, the xylem parenchyma of which became severely affected. It was found that localisation of spread of necrosis in old plants or in plants grown at high temperatures was due to the production of cork cambiums which presumably checked the spread of the virus. In young plants the spread of necrosis is so rapid as to either prevent the formation of or to over-ride such cork barriers.

Quanjer/

Quanjer and Botjes (1929) appreciated that top necrosis might be caused by more than one virus, since, certain varieties which contained a virus or viruses producing top necrosis when grafted to other varieties were themselves killed when grafted with virus containing scions from a second set of varieties. About this time the first isolations of the so-called potato mosaic viruses were made; the first to be isolated and named were viruses X and Y* from a complex of the two by K. M. Smith (1931). These were followed by virus A (Murphy and McKay 1932) virus B (Clinch and Loughnane 1933, Bawden 1936) and virus C (Bawden 1936)./

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*Virus nomenclature at the present time is in a chaotic state since no one system has received general recognition. Smith's practice of naming viruses alphabetically was followed with other potato viruses and worked quite satisfactorily. Johnson (1935) elaborated a scheme for naming viruses according to the hosts on which they were found with a number, indicating the specific virus. Smith (1937) recently put forward a further modification of Johnson's scheme by substituting the Latin generic name for the vernacular name of the host and classifying viruses according to the taxonomy of their hosts. The fallacies of such a scheme are obvious. Not only is it artificial in the extreme but far less information is conveyed by a designation such as Solanum Virus 14 than by the much maligned symptom names. The situation is not exactly eased by Holmes'(1939) latest revision of classification in which he assigns binomials to all the viruses known. There seems no reason why viruses should not rank with other organisms and, providing that sound criteria, other than those of similarity of expression determine the groupings, this appears to be the most logical scheme proposed as yet. In the present account, however, it has seemed best to use those names by which the viruses are best known.

1936). "Pure cultures" of virus X were easily obtained and Murphy and McKay (1932) found that virus A was carried almost without symptoms in the varieties Irish Chieftain and Golden Wonder. Virus B was found to occur in most plants of the variety Up to Date but always associated with the X virus and the same applies to virus C which was first detected in plants of Di Vernon (Salaman and Bawden 1932, Bawden 1936). These five viruses X, Y, A, B and C, together with the aucuba mosaic viruses F and G isolated by Loughnane and Clinch (1935) and Clinch, Loughnane and Murphy (1936) are the components, as far as is known at present, of all the European mosaic diseases of potatoes. Of these we are directly concerned with four only, namely X, A, B and C.

When individual potato varieties were graft infected with these viruses they were found to react in one of three ways to each of the four viruses, the mode of reaction of any one variety depending upon the infector virus (Murphy and McKay 1932, Bawden 1936, Cockerham 1930). The virus might be carried without symptoms; a mosaic of varying severity might develop; or the plant might show the top necrotic reaction. American varieties were found to behave similarly in some respects (Dykstra 1935, 1939).

The usual technique in such experiments is

to/

to graft a virus containing scion to a shoot of the plant to be tested. Shoots develop from the axillary buds below the graft and symptoms first appear on these 12-14 days from the time of grafting afterwards becoming systemic. Most workers have evolved their own methods of leaf inoculation. Essentially they consist in rubbing or scratching several leaves on a plant with a swab or needle carrying virus infected sap. The symptoms following this type of inoculation are frequently different from those attending graft infection, depending upon the virus and the potato variety. In essential features the top necroses caused by all four viruses are the same, the differences lie principally in the initial symptom picture. The usual type caused by virus X (Figure 4) is that previously described but in some varieties, e.g. Thorn II, the necrosis is rather slow and indeterminate, due in part to the stocky and slow growth of the plant. This same type of reaction was found in a family with Thorn II as the female parent, (Thorn II x Pepo). The scoring of the seedlings was so uncertain that the results had to be discarded as unreliable. In other progenies plants have been noted in which top necrosis was preceded by the appearance of relatively few large black lesions on the terminal leaves, in place of the more usual network of/

Plate I.

Virus X

Fig. 1. First stages of top necrosis in a plant of Epicure. The apical leaves show the typical necrotic spots.

Fig. 2. Early stages of top necrosis in Edgecote Purple. The apical leaves are beginning to wither and dry up and necrotic streaks can be seen spreading down the stems.

Fig. 3. A leaf from a seedling showing local necrosis following inoculation by rubbing with a sap extract from X infected Arran Victory plus carborundum.

Fig. 4. Systemic top necrosis in Cardinal, the duplex N^X variety.

Plate I.

Fig. 1

Fig. 2

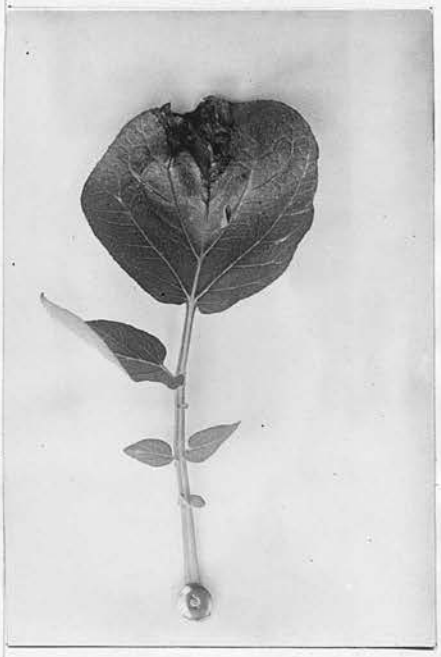


Fig. 3

Fig. 4

Plate II.

Virus X

Fig. 5. Tubers from a seedling (1.55). Upper right and lower left, diseased tubers showing necrotic areas involving the eyes, which have been killed. Upper left, cut surface of a necrotic tuber; lower right, cut surface of a healthy tuber.

Fig. 6. The recessive type; a single leaf of Arran Victory showing a mild mosaic.

Plate II.

Fig. 5

Fig. 6

of fine necroses, though the ultimate effects of infection were the same. In others again, the first observable symptom of reaction was the wilting of the youngest leaves of the shoots, followed immediately by the appearance of fine necroses. The nature of these differences remains to be investigated. Presumably they will be found to be constant and determined by heritable factors.

Virus X is known to exist in several strains, some more virulent than others (Salaman 1933, 1938, Köhler 1937, 1938). The strain used in this investigation seems to be of medium strength since on Datura it produces a bright yellow mottle without any necrosis such as the severe X strains induce on this plant (Cockerham unpub.). With the exception of the anomalous Virus D (Bawden 1934) now considered to be an aberrant strain of X (Bawden 1936), all the X strains behave similarly as far as the top necrotic reaction is concerned (Bawden 1936).

Schultz (1925) in his first experiments induced top necrosis in the variety Duke of York by grafting to it scions from the American Green Mountain. Since Duke of York reacts in this way only to viruses A and C (Cockerham unpub.) and since Green Mountain cannot carry C (Dykstra 1939) this appears to be the first record of top necrosis resulting from virus A infection./

infection. At a later date Salaman (1930) found that Great Scot went down with top necrosis on grafting with crinkle infected scions. Crinkle is a mixture of viruses A and X (Murphy and McKay 1932) and the A component again seems to have been the cause of this reaction since Great Scot is necrotic only to this virus (Cockerham 1939). A similar reaction was recorded in Up to Date and British Queen by the Irish workers (Murphy and McKay 1932, Clinch and Loughnane 1933) and other varieties have since been added to the list. Of the four viruses under discussion virus A probably causes the most distinct types of top necrosis. In most necrotic reacting varieties the appearance of necrosis is preceded by a blotchy yellow mosaic on the terminal leaves accompanied by some curling and waving of the affected leaves. Necrosis is often confined to a few large lesions which later appear over the entire shoot apex. The necrotic network following the veins characteristic of virus X is not always obvious. Spread of necrosis to the petioles and main stem follows as with the other viruses (Figure 9). A peculiar variant of this reaction has been found in several named varieties and seedlings. Here the only evidence of the lethal reaction is the appearance of a small necrotic patch of tissue in the apex of the growing shoot. This necrosis may spread down the stem/

Plate III.

Virus A

Fig. 7. Epicure showing top necrosis. Note the absence of necrotic patches on the larger apical leaves and the necrotic state of the shoot apex.

Fig. 8. Final stages of apical top necrosis in Epicure.

Plate III.

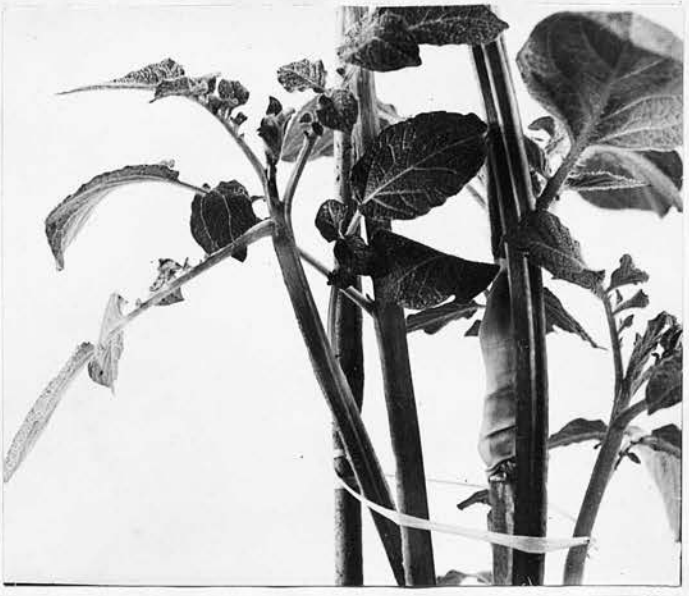


Fig. 7

Fig. 8

Plate IV.

Virus A

Fig. 9. Systemic top necrosis in Great Scot. The large shoot shows the typical blotch necrosis of Virus A as well as apical necrosis.

Fig. 10. Leaflets from normal, right, and top necrotic, left, plants of Up to Date.

Fig. 11. Necrotic tubers from Duke of Perth in surface view, upper and lower left, and cut open, upper right. Cut, healthy tuber on lower right.

Fig. 12. The two surfaces of a halved necrotic tuber of Duke of Perth showing the characteristic mode of spread of Virus A necrosis in tubers, viz. outwards and inwards from the vascular ring.

Plate IV.

Fig. 9

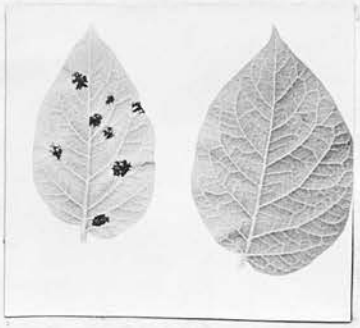


Fig. 10

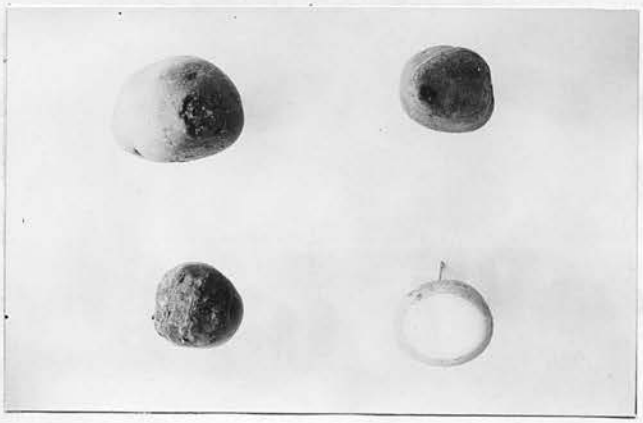


Fig. 11

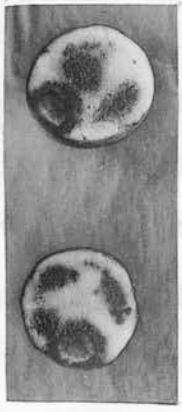


Fig. 12

stem, appearing as translucent brownish patches, the resulting symptoms becoming indistinguishable from ordinary top necrosis, or the apices of the affected shoots may be killed, no other disease symptoms making a subsequent appearance. In the latter case the plant may continue growing from axillary buds further down the stem. In a few cases hypertrophic spots of tissue have been seen below the shoot apices of infected plants and these later became necrotic. Again the necrotic parent seems to determine the behaviour of the dominant carrying seedlings of a progeny. The second type of reaction is shown by the variety Maud Meg and when crossed with Pepo, a carrier of virus A, the necrotic plants in the progeny all showed this same type of reaction. This apical top necrosis seems to have escaped the notice of most observers since varieties such as Epicure, Arran Crest and King Edward have been recorded as carriers of virus A (Bawden 1936) whereas they are really top necrotic to it (Cockerham 1939).

Strangely enough virus B has been studied more, though unwittingly, than any of the other three viruses. This follows in part from Quanjer's (1931) choice of Arran Victory and President as being the best differential hosts for international comparison of viruses, since Arran Victory is necrotic only to virus B and President only to viruses B and C. Thus, Schultz/

Schultz (1925), Quanjer and Botjes (1929, 1930) and Quanjer (1931) all describe graft transfers from American varieties (Green Mountain, Bliss Triumph, Rose 4 and Irish Cobbler) which produced top necrosis in President, among other European varieties. An identical reaction was produced by a virus carried without symptoms by Duke of York (Quanjer 1931). Bliss Triumph and Green Mountain are both unable to carry virus C (Dykstra 1939) so that there is no doubt that the first top necroses described were those produced by virus B infection. Clinch and Loughnane (1933) studied the combination of X and B occurring naturally in most stocks of Up to Date but believed they were dealing with only one virus. The B component escaped identification on account of its easy inoculability by needle to "carrier" potato varieties (Quanjer 1931) and to the test plants employed (Datura and tobacco) and the absence of characteristic symptoms on these test plants (Smith 1931, Clinch and Loughnane 1933, Bawden 1936, Dennis 1939). The presence of B in Up to Date and the inoculated hosts was demonstrated by grafting scions to President and Arran Victory which both developed top necrosis. The compound nature of Up to Date "streak" was finally elucidated by Bawden (1936) where "a position was reached in which, of two Datura plants showing identical symptoms one, when grafted/

grafted to President gave top necrosis whilst the other gave only a mottle, whether grafted or needled". Though plants of Up to Date have been found without virus B (Murphy and McKay 1932) the stocks of Duke of York uncontaminated by X propagated at this Station appear to be the only ones available with which reliable indicator tests on various hosts might be carried out.

By grafting Up to Date scions on to plants of the American seedling U.S.D.A.41956, reputed to be immune to all strains of the X virus, Dykstra (1935, 1939) believed he could obtain pure samples of virus B by filtering out the X component. Dennis (1939) repeating this procedure, carried out an extensive series of tests with plants of U.S.D.A.41956 containing supposedly pure virus B. Grafts from tomatoes previously inoculated with virus from this source produced top necrosis in the varieties Epicure, King Edward and Arran Crest, which repeated tests have shown to be anecrotic to virus B (Cockerham unpub.). The top necroses must therefore have been due to virus X and the whole work is thereby laid open to question.

There is little noteworthy in the external symptoms of pure virus B top necrosis (Figure 13). On young plants the primary necroses are of the network type following the veins and these are usually preceded by an easily visible mosaic. Where very young shoots are/

Plate V.

Virus B

Fig. 13. Arran Victory showing typical top necrosis.

Fig. 14. A plant of President showing an earlier stage of top necrosis.

Fig. 15. Right, a leaflet from a plant of Arran Victory showing local lesions following inoculation by rubbing with a sap extract from B-infected Duke of York plus carborundum; left, leaflet from a control plant inoculated with water plus carborundum.

Fig. 16. Top row, necrotic tubers from Angus Leader in surface view, right, and cut open, left; bottom row, tubers from Arran Chief showing early stages of necrosis on the surface and young sprout, left, and cut open, right.

Fig. 17. Necrotic tubers of Golden Wonder, left, and Thorn 2 right; top row, in surface view, bottom row cut open.

Plate V.

Fig. 13

Fig. 14

Fig. 15

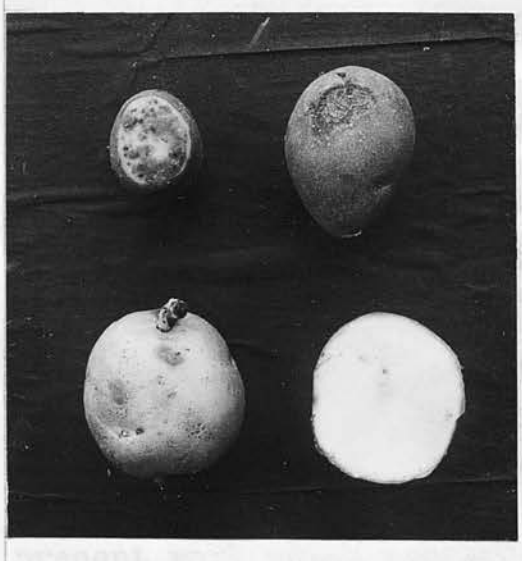


Fig. 16

Fig. 17

are attacked the necrosis seems to cause the rapid collapse of the apical leaves. The extent of the distribution of virus B and its effects on carrier varieties are largely unknown. It seems to be carried without symptoms by a number of American varieties (Dykstra 1935, 1939, Quanjer 1931) but is commonly carried by only a few European sorts, principally Up to Date, Duke of York and Zeeland Blue.

The differential varieties President and Arran Victory behaved similarly as far as their top necrotic reactions were concerned until Salaman (1930b) found plants of Di Vernon containing a virus which caused President to go down with top necrosis but induced only a mild mottle in Arran Victory. Top necrosis followed graft infection of Duke of York, Up to Date, Eclipse and Majestic (Salaman and Bawden 1932) by this virus which Bawden (1936) later termed virus C. The original stock gave no indication of the presence of virus X, no symptoms appearing on 35 Daturas inoculated by needle (Salaman and Bawden 1932) but C seems never to have been found uncontaminated with X. A virus latent in the varieties Monocraat and Roode Star was found by Quanjer (1931) to produce top necrosis in Duke of York. This may possibly have been virus C too. The part of the present work which concerns viruses B and C has only been/

been made possible by the use of the pure stocks of these viruses, carried in Duke of York and Edgecote Purple respectively propagated at this station and kindly placed at my disposal by Dr. Cockerham.

The development of the necrotic network on the terminal leaves of necrotic reactors to virus C is often accompanied by a brilliant yellowing of the interveinal tissue. The black necrotic veins stand out in striking contrast to the yellowing leaf tissue. This fades with the collapse and dropping of the leaves two days or so after the first appearance of symptoms (Figure 18). Practically nothing is known of the distrubution or mode of transmission of this virus, nor does it seem to be of any great economic import. Dykstra (1939) found no corresponding virus in America and virus C induced top necroses in a number of American varieties.

The most peculiar feature of top necrosis in general is that time has not borne out Schultz's (Schultz et al. 1934) contention that as a disease it would become a serious menace in the field. In fact top necrosis has remained typically a laboratory disease. When Quanjer (1931) first investigated this "disease" it was new to Holland though he had observed it "in the 'massif central' of France". Top necrosis has been seen in the field by the present writer on several occasions/

Plate VI.

Virus C

Fig. 18. A seedling showing top necrosis in an advanced stage. Note the characteristic veinal lesions on the yellowing apical leaves and the necrotic areas, both in the infected stem and below the graft.

Fig. 19. A plant of Up to Date showing the early stages of top necrosis.

Fig. 20. Local lesions on a leaf of Majestic following inoculation by rubbing with a sap extract from C-infected Edgecote Purple plus carborundum.

Fig. 21. Extreme left, necrotic tuber from Duke of York in surface view, top, and cut open, bottom. Centre, necrotic tubers from Ballydoon, top, and Pepo, bottom; extreme right, the same cut open. Note in each case that the spread of necrosis appears to be from the surface inwards.

Plate VI.

Fig. 18

Fig. 19

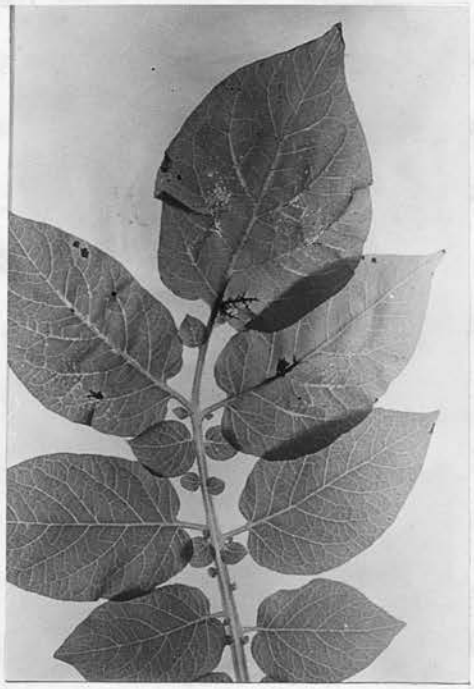


Fig. 20

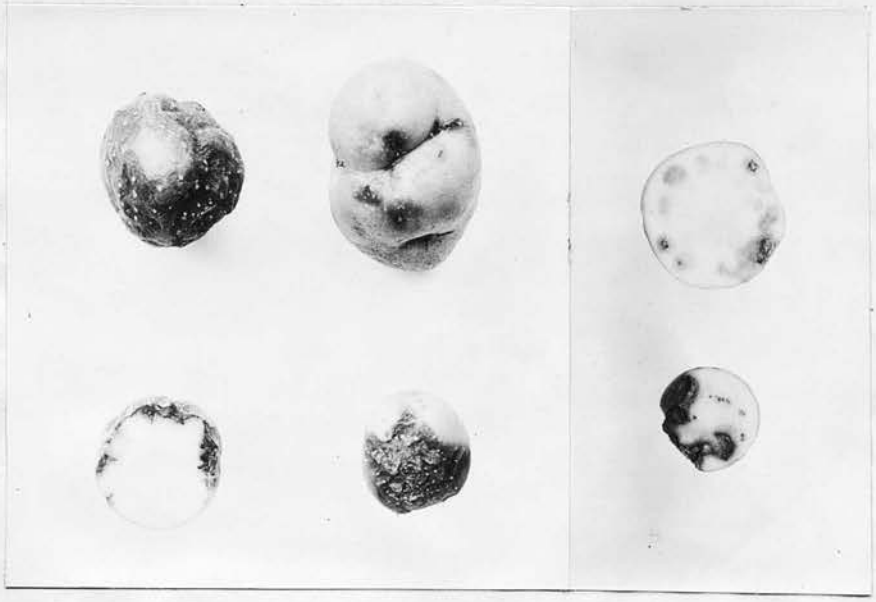


Fig. 21

occasions but there can be no doubt of its extreme rarity. If we go deeper into the matter, a perusal of the literature reveals a probable reason for this anomaly . It has been a matter of common observation that, from the mixed yield of necrotic and unblemished tubers produced by a top necrotic plant only the unblemished tubers give rise to healthy plants in the following year and no trace can be found in these of the virus which produced the lethal reaction in the mother plant (Salaman and Bawden 1932, Murphy and McKay 1932, Murphy and Loughnane 1936, Bawden 1936). Grafting crinkle (A and X) infected scions to Kerr's Pink (to which variety virus A is lethal) Murphy and McKay found that the plants developed necrotic symptoms and that the second generation plants yielded only a mild mosaic on President in place of the full crinkle disease. They found also that Kerr's Pink was similarly able to filter out the streak component (virus B) from a mixture of streak and interveinal mosaic. Necrosis was associated with recovery from the disease, a recovery "due not to the dieing out of diseased plants, but to the actual recovery of plants which were once infected, and in which the disease must have been systemic since it affected the tubers". Earlier, Salaman (1930a) had produced similar necrotic reactions in Great Scot by grafting plants with crinkled scions, remarking/

remarking that "crinkle in Great Scot in the field is very uncommon"; he might as well have said absent. For this phenomenon of recovery coincident with necrosis Murphy and McKay (1932) put forward the suggestive explanation that "if the virus is localised in these cases in the visible lesions, the complete death of the tissue involved is connected with the disappearance of the disease". They quote the work of Johnson (1925) with tobacco mosaic in Nicotiana glutinosa, of Kunkel (1928) with grass mosaic in sugar cane and of Valleau and Johnson (1930) with tobacco mosaic in potato as other instances of recovery and apparent immunity of plants from virus infection associated with necrotic lesions. The practical absence of top necrosis from field crops was found to be the result of elimination of streak infected plants and their progeny by the violence of the reaction. (Murphy and Loughnane 1936). Whence it was concluded that the absence of permanent infection by virus X from some varieties such as Epicure, King Edward and Arran Crest and by virus A from others like Kerr's Pink, British Queen and Up to Date was the direct result of their intolerance to these viruses and that in fact, no variety would be found to contain naturally any virus to which it reacted with top necrosis (Murphy 1936, Cockerham 1937a, Clinch, Loughnane and Murphy/

Murphy 1938). It seems then that the ability of a variety to react in the top necrotic manner upon graft infection with certain viruses in the greenhouse confers on that variety an immunity to infection by the same viruses in the field (Clinch et al. 1938, Cockerham 1937b, 1939 and unpub.). Varieties of this type were dubbed "field immune" by the Irish workers (Clinch et al. 1938).

In a statistical study of the distribution of virus diseases in Scotland in 1937 Cockerham (1939) has shown that the incidence of severe mosaic was least among those varieties "field immune" to both the A and X viruses or to A alone and that such severe mosaic as did occur in those varieties was due to virus Y or to combinations of X and Y respectively.

The evidence, though mostly circumstantial is overwhelmingly in favour of this hypothesis. Information from the literature and from greenhouse experiments also favours the idea that localisation is responsible for the inability of viruses to spread in hosts which are intolerant to them. Bawden (1936) found that attempts to inoculate leaves of President by needling sap from Up to Date were followed by the appearance of black circular local lesions on the inoculated leaves. These remained discrete and did not spread. Whilst the X component produced a systemic mottle, virus B was never recovered from such

President plants. The same author transferred virus A successfully to tolerant hosts of this virus by using Clinch and Loughnane's (1933) carborundum method but failed to do so when intolerant varieties were inoculated. On the other hand Epicure, Arran Crest and King Edward developed top necrosis from the X virus in the Up to Date combination with no formation of local necroses. Clinch and Loughnane (1933) also describe reddish local lesions on leaves of President inoculated with sap from Daturas containing virus B. A particularly clear instance appears in Smith's (1931) paper. Up to Date was used as the source of virus X and when needle inoculated to President and Arran Victory, both of which are intolerant to B, the primary symptoms took the form of dark red necrotic lesions which developed along the needle scratches on the inoculated leaves. These lesions were regarded as characteristic of virus X but it is clear that they must really have been due to virus B. A systemic mottle developed in both hosts, due to X, but it is recorded that Arran Victory often gave "a 'streak' reaction to the X virus in the current season. This takes the form of irregular lesions on the lower leaves which gradually spread; the leaves then turn yellow and drop". No doubt this may have been an incipient top necrosis resulting from virus B infection.

In/

In the present investigation it was decided, primarily as a labour saving device, to inoculate most of the plants of ten seedling progenies which were to be tested for their virus X reactions. Two or three leaves of each plant were rubbed with a sap extract from X infected Arran Victory plants containing a little carborundum powder. A few plants developed typical top necrosis after about 12 or 14 days, but a much larger proportion developed large black necrotic lesions on the inoculated leaves (Figs 3, 15 & 20). These did not spread and the only other symptoms shown by such plants was a somewhat doubtful mottle on the young leaves, but no virus was ever recovered from them. At a later date all the local lesion plants were grafted with X containing Arran Victory scions and, with very few exceptions, they all developed top necrosis. The correlation between the local lesion reaction on inoculation and the top necrotic reaction on grafting seems a significant one. Whether a precisely similar course of events occurs in the field is not known. It would seem that it must do so. The difficulties of distinguishing between significant and incidental virus lesions and between these and insect punctures and such like mechanical lesions in the field are considerable, and it is probable that infected leaves might drop off and easily escape notice. The behaviour of/

of intolerant varieties grown in close contact with infector plants carrying their respective lethal viruses would no doubt repay investigation.

The value of this character of field immunity is very real and there seems no reason to suppose its liability to breakdown pending the possible development of "biologic strains" of viruses such as have complicated the question of disease resistance in cereals. On this point it is instructive to draw a comparison between the field immunity of potato varieties to viruses and that type of resistance to rust fungi in other plants which also depends on the localisation of the pathogen following the extreme violence with which the host reacts to infection. The comparison cannot be pushed too far in view of the dissimilarity of the infecting agents but it is at least suggestive.

One of the most fully investigated cases is that of the Norwegian "Viking" Red Currant which was the only currant found to be immune from attack by Cronartium ribicola, the White Pine blister rust (Hahn 1929, 1936). The only visible symptom of infection was the development of small watery pustules on the undersides of young leaves which later developed necrotic flecks. No uredia were ever formed on this variety. A cytological study of this resistance by

Anderson/

Anderson (1939) proved that spore germination and incubation took place equally well on leaves of resistant or susceptible Ribes and that penetration of the leaf tissues of the resistant Viking was effected. Degeneration of hyphae in the resistant host started 48 hours after infection but all traces of these had disappeared after 96 hours. Though the cells of the invaded mesophyll reacted very definitely before the disappearance of the hyphae, hypertrophy of tissue forming the watery pustules only began 72-96 hours from the time of infection and the first necrotic cells only appeared after the disintegration of the hyphae. While they are functioning the fungal hyphae are apparently always in contact with living cells and the death of the necrotic cells is only indirectly related to the former presence of the hyphae. Nusbaum (1935) has described a precisely similar behaviour in apple varieties resistant to infection by Gymnosporangium juniperi-virginianae and quotes Evans' (1933) work on strains of onion immune to Urocystis cepulae. Here, badly degenerated and dead mycelium was found in host cells which were still apparently healthy. Wheat varieties resistant to Puccinia graminis tritici by virtue of a like reaction were described by Stakman (1915) as hyper-sensitive. One explanation put forward by Stakman and other workers (Newton/

(Newton 1922, Allen 1923) was that the fungus was starved on account of the death of all the cells immediately surrounding the invaded areas, an explanation which the recent studies quoted above do not support. The production of a toxin by the parasitised cells which kills the invading hyphae has been postulated by others. In the case of the Viking red currant, which shows no visible anatomical differences from the highly susceptible Ribes nigrum, R. sativum and Grossula ria hirtella, it seems that the death of the fungus in this host must be associated with an antagonism or physiological incompatibility between parasite and host.

In the case of the virus lesions we have no guide from the visible behaviour of the pathogen in the host. Clinch and Loughnane (1933) found, by discolouring with alcohol the apical leaves of President plants beginning top necrosis that starch concentrations marked the areas which the visible necrotic lesions later occupied. In a study of the local lesions produced on rubbing leaves of Nicotiana glutinosa with tobacco aucuba mosaic extract Sheffield (1936) states that the first microscopic symptom of infection was the appearance of a strip of dark staining material between certain of the cell walls. This band usually formed first between cells of the lower epidermis and the spongy parenchyma, spreading later

to the upper surface and accompanied by mitotic division of the cells immediately within it. Lesions first appeared about 48 hours after infection as small colourless shining patches, but their mature dark-staining appearance was the consequence of the necrotic disc isolating the epidermal cells which die and dry up.

Holmes (1934, 1936, 1937, 1938) has already identified genes in Capsicum, Nicotiana species and Solanum melongena which are responsible for the localising of tobacco mosaic virus on inoculated leaves of these hosts. It is clear that this reaction is similar in kind to that of top necrotic potatoes and depends on the extreme susceptibility or incompatibility of the host to the pathogen.

Whatever the mechanism involved in the localising or top necrotic reactions an obvious means presents itself of combating infection by two of the most important and widespread potato viruses, X and A, and incidentally of combining virtual immunity to all four viruses X, A, B and C.

MATERIAL AND METHODS.

Genetical investigations were therefore undertaken at this station to determine the mode of inheritance of the top necrotic reaction. Crosses and selfs were made of a number of named varieties whose reactions to virus X were known and the progenies tested for their reactions to graft infection with this virus. It was soon found that the acro-necrotic reaction was dominant and the segregations suggested that the inheritance was not simple, though no definite scheme was formulated (Cockerham 1937b, 1939).

When the present work was begun it was surmised from the available figures and the genetical constitution of the potato that the segregations were probably those of a tetraploid. The data accumulated in the two years elapsing have borne out this assumption and provided a most interesting problem.

Only a limited number of cultivated varieties has been dealt with, determined principally by their abundance of flowers and fitness as pollen and seed parents, qualities both fairly rare among cultivated potatoes. The material consists in a number of selfs and crosses between necrotic and anecrotic types. The limitations imposed by pollen fertility have made the obtaining of reciprocal crosses a difficult matter, though/

though some of the gaps have been filled in the present season (1940). Unfortunately, or otherwise, the pollen parent most frequently used, namely Kepplestone Kidney, has proved to be abnormal genetically and tended to obscure the results in the first place. The work was extended to cover an investigation of the inheritance of virus A, B and C top necroses and of linkage between the factors determining these reactions.

The principal methods employed have already been indicated in the foregoing account. The testing and scoring of individual progenies takes a considerable time. The seed of selected crosses is usually sown the year previous to testing, the plants grown in isolation to avoid virus infection from extraneous sources, and the tubers of each plant harvested and bagged separately in the autumn of that year. The tubers are potted up in the following year, beginning in February and grown in an insect-proof greenhouse at a temperature of approximately 60°F. though the maximum is usually considerably higher in summer.

When the plants reach a convenient size for handling, one shoot of each is grafted with a scion containing the virus with which the progeny is to be tested. Pure stocks of each of the viruses X, A, B and C, carried in Arran Victory, Irish Chieftain, Duke of York and Edgecote Purple respectively were used. The saddle graft has been most frequently employed

and/

and the stock and scion unite very readily if the union is bound with raffia and rubber tape and the plant stood under the bench for a couple of days until the graft recovers. The "take" was usually 100% with this method. Shoots spring from the axillary buds immediately below the graft and symptoms appear on these about 14 days from the time of grafting, sometimes sooner if the plants are growing very quickly and the temperature high. From then on the plants are examined weekly and any observable symptoms scored. Failing the production of a satisfactory response after a suitable time, a plant is either regrafted, if the first union is a poor one, or a sap extract is inoculated to a test plant and the presence or absence of virus in the grafted plant judged from the reaction thereon. Hyoscyamus niger has been most extensively used in this connection as it gives distinct reactions with viruses X, B and C and is markedly free from a confusing natural mottle shown by several other Solanaceous plants. The means of testing for virus A was to graft scions from the suspect plant to a reactor variety such as Up to Date or Great Scot; failing satisfactory results from any of these tests a fresh tuber is set up and the plant retested.

The method of raising progenies outlined is almost essential for producing sufficient material for linkage/

linkage work, but the number of losses in the field during the first year and from rots during storage often made replacements impossible and decreased the value of some of the material considerably. Cuttings can be propagated quite easily, however, and both this difficulty and the time required to raise a progeny may be overcome by propagating and testing seedlings raised in the one year.

If the plants grow rapidly there is seldom any difficulty in scoring their reactions but if growth is checked symptom expression is apt to become indeterminate. It is believed that, with the exception of the family G 415 (Maud Meg x Pepo) tested with virus A, there are very few doubtful or wrongly scored plants. The influence of these will of course be greater on linkage data.

The genetical data cannot be adequately discussed without an explanation of the principles on which they depend, necessarily somewhat lengthy by reason of their complexity, and with which the next section is concerned.

CROSSING-OVER AND TETRAPLOID SEGREGATION.

Genetics is primarily concerned with the effects of segregation and recombination of characters. The evidence which affords proof that the factors determining these characters, the genes, are borne in the chromosomes is now too well known to bear repetition here, but it seems well to include a resumé of the evidence leading to the proof that genetical crossing over is the result of cytological crossing over.

Early cytological observations had shown that at a certain stage of meiosis the chromosomes went into close association and later emerged in pairs, the constituent threads of which were also divided, the bivalent at diakinesis being termed a tetrad. At intervals along their length the pairs of half chromosomes or chromatids appeared to change partners and to these changes of partner Janssens (1909) gave the name chiasms (chiasmata, Darlington 1929). To explain their occurrence Janssens put forward a theory which attributed their origin to the breakage and rejoining of the paired chromosomes. In support of this he argued that the two maturation divisions would have no significance in the absence of reconstruction of the chromosomes, and that the existence of four gametes would have no justifiable basis unless they were individually different/

different. Moreover the chiasmata lacked causality unless they were the result of change of partner. All of these points, although stated from a priori viewpoint at the time, have since been strikingly verified.

Morgan (1911) adopted this chiasmatype hypothesis when he formulated his theory of linkage to explain the fact that, whilst recombination between groups of linked genes followed the random proportions demanded by Mendel's second law, recombination within such groups did not. Here it was found that the proportion of new combinations was lower, that of old combinations higher than would be expected. He made in addition two further assumptions, namely that the genes are arranged in linear order in the chromosomes, and that recombinations are the result of exchange of homologous segments between partner chromosomes.

Nevertheless it has taken a considerable time to amass proof of these two theories for the precise reason, as Darlington (1937) has pointed out, that the two methods of study, cytological and genetical, are necessarily complementary. The following lines of evidence have been selected as proving most clearly the fundamentals of crossing over.

In his now classical researches on non-disjunctional females in Drosophila, Bridges (1916) noted/

noted the occurrence of "equational exceptions". These arose as the result of two X chromosomes pairing and crossing over and afterwards passing to the same cell instead of disjoining normally. From such a cell arose a daughter which was homozygous for a recessive character for which the mother was only heterozygous. In all, eighteen such cases were found. These were only explainable on the basis that crossing over had occurred at the "four strand stage", i.e. when the chromosomes had divided into their constituent chromatids. They necessitate that each of the two X chromosomes after crossing over consist of one non-crossover and one crossover chromatid, which is manifestly impossible if crossing over occurs between whole chromosomes. The final proof of four strand crossing over was provided by a case where one strand was a double crossover and the other a single crossover at the same level ($\frac{v+ \quad f}{+sg \quad +} \rightarrow \frac{v \quad sg}{+ \quad +}$).

As a result of X-raying a line of such primary non-disjunctional females as those just described, Anderson (1924, 1925) obtained one individual which had both its X chromosomes attached at one end and gave only equational exceptions in its progeny. By repeating this procedure with a female heterozygous for a number of sex linked genes it was possible to study crossing over between the two component chromosomes/

chromosomes of the attached X individuals, which behaved as if they were independent in this direction. All the various crossover types were found except that in which the two components were identical crossovers. Again showing that crossing over takes place in the four strand stage, but with only two strands crossing over at any one level. Whilst the loci farthest away from the point of attachment of the two chromosomes showed an almost random distribution, the percentages of crossing over decreased as this point was approached showing that assortment of chromosomes is determined by the spindle attachment or centromere. Bridges and Anderson's (1925) work with triploid Drosophila confirmed all these results, showing in addition that where three homologous chromosomes were present, pairing and crossing over took place equally throughout their length. More recent evidence in support of four strand crossing over has been provided by the work of Emerson and Beadle (1933), Beadle and Emerson (1935) and Redfield (1930, 1932) on Drosophila, Rhoades (1933) on Zea, and Whiting and Gilmore (1932) on Habrobracon.

By marking certain portions of a chromosome cytologically and genetically it has been possible to demonstrate that crossing over involves an interchange of homologous segments. This has been done by Creighton/

Creighton and McClintock (1931) in a line of Zea where a reciprocal translocation between chromosomes 8 and 9, with the arm of the ninth chromosome carrying the genes c (colourless aleurone) and wx (waxy endosperm), was distinguished from its mate by the possession of a terminal knob. The locus of c is so near to the knob region as to render separation of the two, by crossing over, unlikely. In the cross knobbed-interchange, with C and Wx in different homologues, by knobless-normal (cWx) a test of the hypothesis is provided by the appearance of CWx individuals with knobbed normal chromosomes (Figure 22). Cytological examination confirmed that crossing over had taken place in the region marked by the genetic factors. Brink and Cooper (1935) have given an even more elegant and convincing proof of Morgan's theory by making use of a strain of maize heterozygous for two translocations involving chromosome 1. Stern's (1931) work with races of Drosophila carrying two translocations affecting the sex chromosomes has given equally valuable evidence.

It was at one time held that chiasmata were the result of alternate reductional and equational separation of identical chromatids. This is now known to be untrue. The most cogent evidence in this direction is provided by the interlocking of non-homologous chromsomes and the pairing of fragments.

Mather/

Mather (1933, 1935) found a critical case of double interlocking in Lilium regale. The pachytene configuration must have been of the type known as false – both homologues of one pair passing between those of the other (Figure 23). With chiasma formation occurring on either side as well as between the interlocked homologues, which are themselves paired by chiasmata, it is obvious that each loop of the interlocked bivalent must separate sister chromatids of the interlocking chromosome (Figure 24). The so-called classical theory (McClung 1927, Sax 1930, 1932) of alternate reductional and equational separation of chromatids would demand the formation and subsequent breakage of a second chiasma in the central loop. This is rendered highly improbable in Lilium since there is scarcely any reduction in chiasma frequency between diplotene and metaphase. This type of configuration is of rare occurrence, having been found in only two additional cases, in Lilium elegans by Beal (1936) and by Upcott (1936) in Eremurus (v. also Darlington 1929a). Sax and Anderson (1934), in a review of the known cases of interlocking prior to 1934, attempted to reconcile these and additional ones in Tradescantia with the classical chiasmatype hypothesis. However, Tradescantia represents a rather special case since, following upon strong polarisation of/

Plate VII.

Fig. 22. Diagram of the chromosomes carried by two Zea plants crossed by Creighton and McClintock (1931) to demonstrate the correlation between cytological and genetic crossing over.

Fig. 23. Diagram showing false interlocking of two bivalents. Both homologues of one bivalent pass between those of the other.

Fig. 24. The genetical interpretation of double interlocking. Left, pachytene and right, diplotene. Crossing over must have occurred at the critical chiasma (after Mather 1933a).

Fig. 25. Diagram showing different types of division of unequal chromosomes owing, on the assumption of chiasmatypy, to the different relationships of the inequality, the centromere and the chiasma, which may or may not be formed between them. (Where several chiasmata are formed the possibilities are more numerous.) The arrows represent the direction of the change undergone by the bivalent between diplotene and metaphase with terminalisation (incomplete in B). The inequalities and the centromeres are shown blank. A, first division regularly reductional owing to the centromeres lying next to the inequality. B, first division regularly equational (second division reductional) owing to the centromeres lying at the opposite end from inequality and one chiasma being formed between them. C, first division reductional or equational according to whether a chiasma is formed on one side of the centromere or the other. C_2 shows a lateral chiasma. A, Trimerotropis, Circotettix, Acridium, Stenobothrus, most sex chromosomes (and autosomes fused with sex chromosomes). B, Phrynotettix, chromosome "B"; Melanoplus (Hearne and Huskins, 1935); C, Phrynottetix, chromosome "C"; Mecostethus gracilis and Trimerotropis citrina (Carothers, 1931); Stauroderus (D., 1936 d); Peziza (Matsuura and Gondo, 1935).
(From Darlington, 1937).

Plate VII.

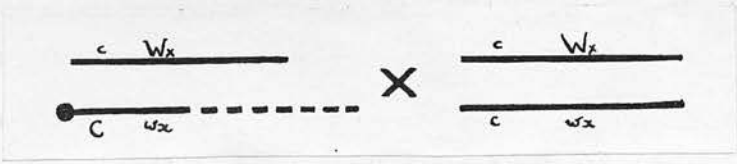


Fig. 22

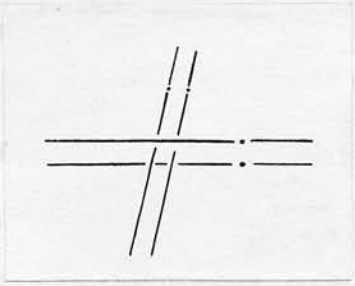


Fig. 23

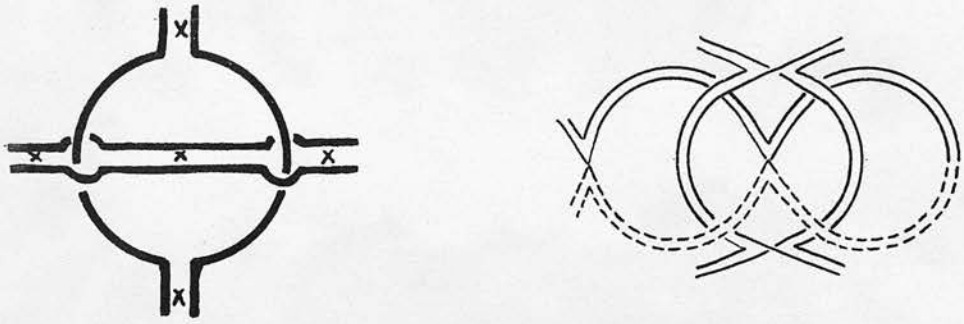


Fig. 24

Obligatory Facultative

Reductional

Equational

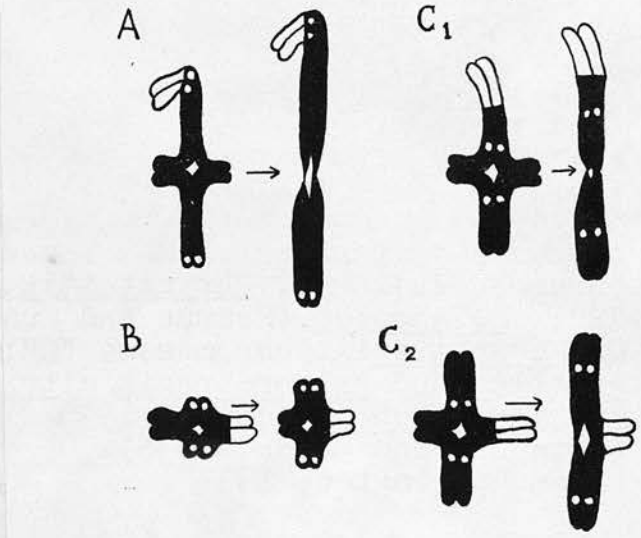


Fig. 25

of the nucleus, pairing begins at the ends of the chromosomes. Thus interlocking of the proximal type, i.e. with the interlocked chromosome in the same loop as the centromere, is likely to be more frequent, as these authors found, than the random proportions of proximal and distal types demanded by the revised chiasmatype hypothesis (Darlington 1930b, 1931).

Janssens (1909, 1924) assumed that, in addition to crossing over between two of the four chromatids all four threads might interchange at one point. The evidence admits of only the first of these two hypotheses and Darlington (1930b, 1931) has put forward a revised partial chiasmatype hypothesis which states:- "(i) that a chiasma is constituted by (genetical) crossing over between two of the four chromatids taking part in it or (ii) that association at diplotene is between chromatids derived from the same chromosome" (1931). Where duplications in the form of fragments pair with their homologues these are often too small to permit of the formation of more than one chiasma. Since their two ends are easily recognisable the chiasma must represent a change of partner on either side of which sister chromatids remain paired. (Mather (1935) in Lilium Henryi and L. japonicum, Darlington (1929b) in Tradescantia). Crossing over in segmental interchanges and inversions provide further direct proof.

Less/

Less direct evidence is afforded by the fact that chiasma frequency and genetical crossing over pursue parallel courses. Thus, crossing over and chiasma formation are only known in the sex chromosomes of Drosophila males (Darlington 1934, Dobzhansky 1932); interference in chiasma formation and crossing over obey the same rules (Haldane 1931); variation in temperature has a similar effect on both processes (White 1934).

In the present account Darlington's partial chiasmatype theory has been adopted.

As yet no entirely satisfactory explanation for the mechanism of crossing over exists. Belling's (1927, 1933) theory is largely observational in nature and leaves several aspects, such as interference, unexplained. Whilst Darlington's torsional hypothesis (1935 et seq.) has many points in its favour it does not appear to the writer to offer a convincing explanation for the exactness of crossing over. The fact that genes are never lost at crossing over whereas they may be at translocation of artificial breakage (e.g. after X-raying) may imply some specific difference between these two processes (Belling 1933) but it would seem that we know too little as yet of the nature of gene reproduction to offer more than tentative explanations of these phenomena.

It/

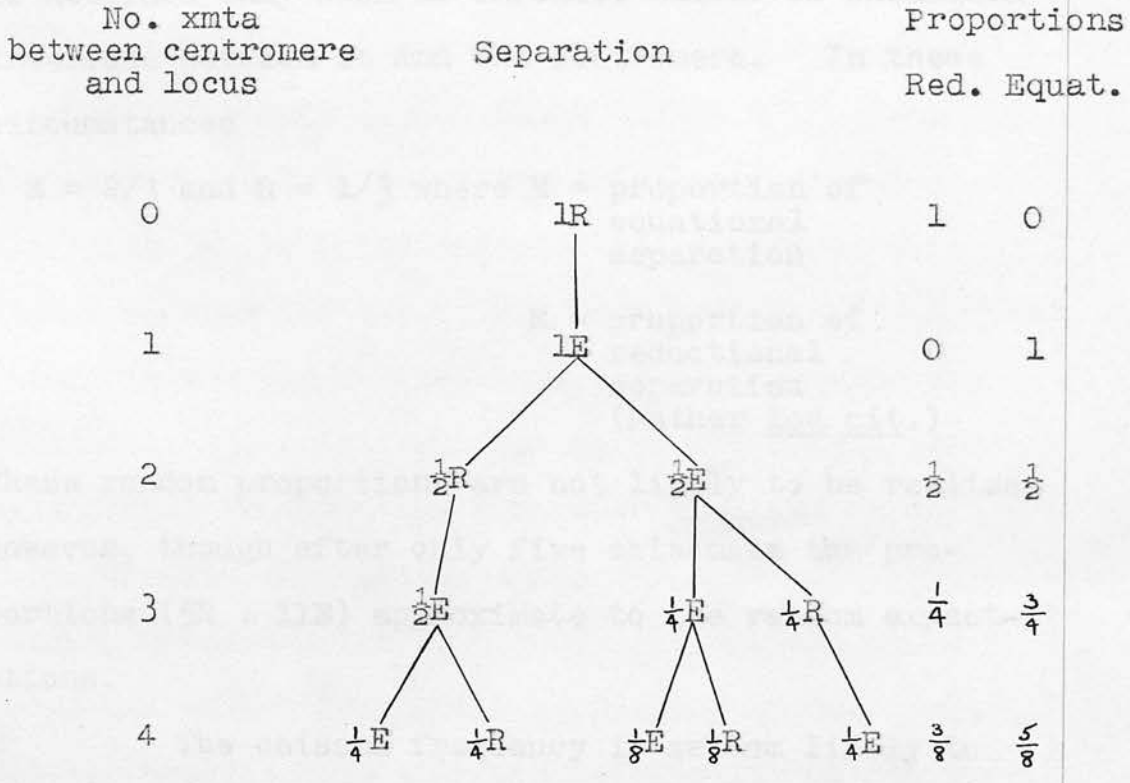
It seems well to discuss some of the implications of crossing over in diploids before introducing the more complex results expected in tetraploids.

At the first stage of meiosis, leptotene, the chromosomes appear as single unpaired threads. Homologous chromosomes begin to pair at zygotene and complete this phase at pachytene. During this time the exchanges of partner occur which become visible as chiasmata at diplotene, when the opposite members, having split longitudinally into their component chromatids, fall apart from one another. Pairing at metaphase is then by virtue of chiasma formation alone (Darlington 1929).

Anderson (1925), and Bridges and Anderson (1925) have shown that not only is separation at anaphase governed by the centromere, but that the percentage of homozygosis in attached X's and triploid female Drosophila decreases as the region of the centromere is approached, i.e. separation at the centromere is reductional. In the case of a locus situated some distance from the centromere however, the type of separation at first anaphase will depend on the number of chiasmata intervening between the locus and the centromere. Since crossing over only takes place between two of the four strands (Anderson (1925)/

(1925), Bridges and Anderson (1925)) exchanges between chromatids passing to the same pole and those between chromatids passing to opposite poles will be expected in equal frequency. Where a locus separates reductionally at first anaphase it is obvious that it must separate equationally at second anaphase. Similarly equational separation at first anaphase must be followed by reductional separation at second anaphase.

From these principles Mather (1935) has deduced the amounts of equational and reductional separation to be expected at a locus situated 1, 2, 3, 4, 5 and n chiasmata from the spindle attachment. A locus distant one chiasma from the centromere will always separate equationally. If two chiasmata intervene, separation will be equational and reductional in equal proportions. With three chiasmata between the locus and the centromere, bivalents separating reductionally after the second chiasma will show equational separation in half the cases and reductional separation in the other half; and so on.



(After Mather 1935).

The general formulae for the proportions of equational and reductional separation to be expected after any number of chiasmata may be derived quite simply. It can be shown that entirely random separation of a locus is attained only when an infinite number of chiasmata intervene between it and the centromere. In these circumstances

E = 2/3 and R = 1/3 where E = proportion of equational separation

R = proportion of reductional separation (Mather loc. cit.)

These random proportions are not likely to be realised however, though after only five chiasmata the proportions (5R : 11E) approximate to the random expectations.

The chiasma frequency is seldom likely to remain constant, as has been assumed in the above theoretical deductions. Where the situation of a locus is such that a mean number of one chiasma is formed between it and the centromere this variability will be expressed by the upper limit of equational separation never being attained. Likewise, where the mean number is greater than one the proportion of reductional separation will tend to increase at the expense of equational separation as a result of double crossing over. Similarly, it has been assumed that crossing/

crossing over occurs between the four threads at random, but, if the two strands crossing over at one chiasma partially determine those crossing over at the next these theoretical proportions will not hold. The occurrence of such chromatid interference does not appear to be widespread however (Anderson (1925), Emerson and Beadle (1933) Mather (1933b)). Some information on this point may be deduced from the frequency of chromatid interlocking at anaphase, a high frequency indicating a preponderance of reciprocal chiasmata (Darlington and Dark 1932). Emerson and Rhoades (1933) point out that chromatid interference will result in more than 50% recombination.

The unequal bivalents found in certain Diptera Carothers (1931) Wenrich (1916), and spore formation in the Ascomycete Neurospora (Dodge 1929, Lindegren 1932) have afforded information on the proportions of equational and reductional separation in these organisms. In the absence of any peculiarity of chromosome behaviour type of segregation is not detectable genetically. Where the two members of a bivalent are unequal in size, the type of separation is readily determinable by observation. Wenrich (loc. cit.) in Phrynotettix and Carothers (loc. cit.) in Trimeropteris found that different bivalents showed characteristic proportions of the two types of separation./

separation. Where the inequality lies next to the centromere separation is always reductional; but where there is a possibility of several chiasmata forming between the inequality and the centromere the proportions of separation types will be determined by the conditions reviewed above (Figure 25). In certain ascomycetes, notably in Neurospora crassa, with which Lindegren's work is concerned the eight ascospores are formed in linear order in the ascus. By germinating each spore and noting the arrangement of spores in the ascus the proportions of reductional and equational separation for different known factors may be ascertained. The variation of these proportions with temperature provides additional proof that chiasma formation and genetical crossing over are similarly determined. The literature on similar determinations has been reviewed by Brieger(1933).

The situation in autotetraploids is akin to that already described for the attached X Drosophilas, in that each gamete may derive two chromatids from the same configuration, in contrast to the simple condition in diploids, where one chromatid passes to each gamete. Each chromosome is here represented by four identical homologues which come together in pairs at random during pachytene (Darlington 1929a). In the absence of polarisation of the nucleus pairing will begin at several/

several points, leading to the association of all four chromosomes in pairs at different levels. Chiasma formation takes place at random as in diploids* and leads to the attachment of portions of different chromatids to the same centromere. With complete terminalisation of chiasmata at diplotene since both pairing and crossing over are at random we should expect to find all the possible combinations of four chromosomes associated terminally if sufficient chiasmata are formed. On these expectations there are ten types of quadrivalents, all of which have been found in tetraploid Primula sinensis (Darlington 1931) and Datura (Belling 1927). Failure of chiasma formation between paired homologues at pachytene leads to the substitution of a trivalent or two bivalents for a quadrivalent (Figure 26). At metaphase the multivalents become arranged on the equator of the cell. Their various types of co-orientation i.e. their arrangements with respect to the spindle axes, and the variables determining these arrangements have been classified by Darlington (1937). The different types depend on the way in which the distances apart of the centromeres and the rigidity of the configurations/

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* Mather (1936, 1937, 1940) has demonstrated that chiasma formation in diploids pursues a regular time sequence determined by the differential and interference distances - the random distribution is only apparent.

configurations are affected by the distribution of the chiasmata. Interstitial chiasmata, inasmuch as they tend to bring the centromeres of paired chromosomes closer together so increasing their strength of mutual repulsion, result in more rigid configurations than terminal chiasmata.

The four types of co-orientation are as follows:-

(1) linear - having the centromeres close together and lying in one spindle axis (Figure 27,L).

(2) parallel - pairs of centromeres lie on parallel and independent axes, a configuration not found in the triploid (Figure 27,P).

(3) convergent - two centromeres lie axially with respect to a third (Figure 27,C).

(4) indifferent - one centromere lies indifferently with respect to the others (Figure 27,I).

Of these various types, non-disjunction is most likely to follow a linear, parallel or indifferent arrangement whilst a convergent one will disjoin regularly, adjacent chromosomes passing to opposite poles. In addition, linear and indifferent configurations are most likely to segregate unequally, the terminal one or two chromosomes disjoining and leaving the central member stranded on the metaphase plate. The proportions of non-disjunction and unequal segregation will naturally be higher where interstitial chiasmata increase the rigidity of the configuration.

Of/

Plate VIII.

Fig. 26. Diagram showing the multivalent and other configurations possible at diakinesis in a tetraploid with complete terminalisation. Certain of the associations of four are possible in diploid structural hybrids (v. Ch. V) xta = chiasmata; the numbers given are those necessary for the formation of the different types of association. (From Darlington 1931)

Fig. 27. Divergent methods of co-orientation in trivalents (linear, L; convergent, C; and independent, I) and quadrivalents (parallel, P, and discordant, D). In D_2 the two loops are at right angles.

Fig. 28. Segregation in a quadrivalent in the general case. Two loci are shown as marked each with four different allelomorphs. The two linked allelomorphs in the interphase nuclei are the two which are joined to the same attachment at first division. They must then separate at second division. Locus a shows reductional separation at the first division (top) and so cannot give double reduction, i.e. two identical allelomorphs in the same gamete. Locus b shows equational separation as a result of crossing over between it and the spindle attachment. In some of the cases (middle) the equationally separating chromosomes reach different interphase nuclei and so double reduction is again impossible. In other cases (bottom), however, the two equationally separating chromosomes reach the same interphase nucleus, and double reduction occurs in half the cases for any allelomorph. The symbols e and a are the mean frequencies of equational separation and non-disjunction at the first division respectively, and the figure shows that double reduction occurs in only ae of cases. (Mather 1936).

Univalents
Bivalents
Trivalents

Quadrivalents

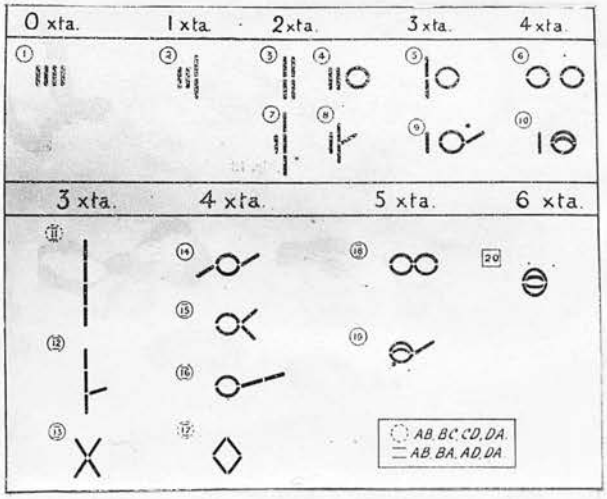


Fig. 26

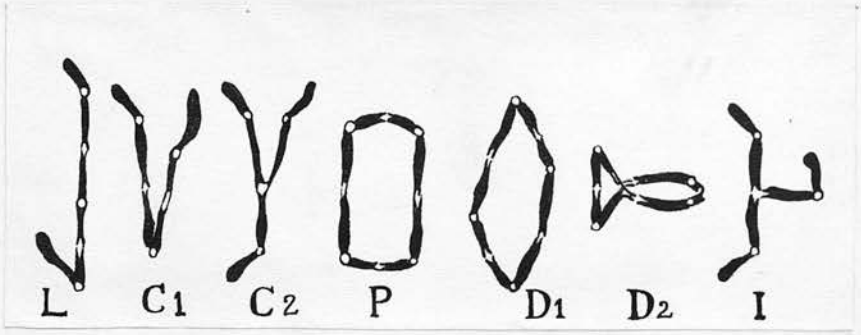


Fig. 27

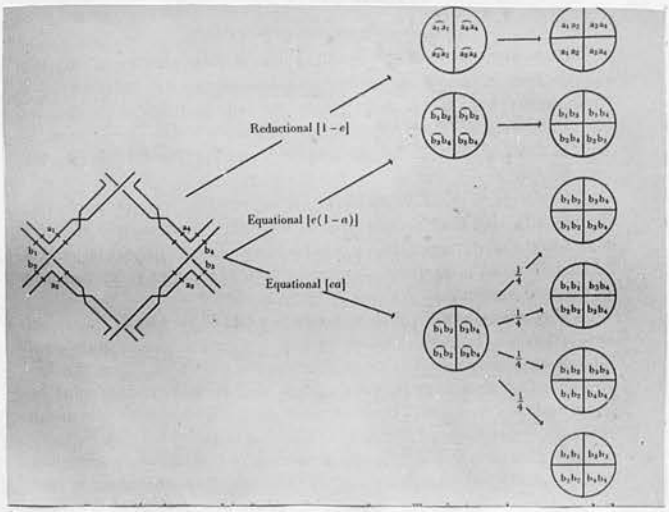


Fig. 28

Of 100 quadrivalents analysed by Upcott (1939a) in Tulipa sylvestris the co-orientation was convergent in 46, parallel in 40, indifferent in 10, and linear in 4. The maximum type of co-orientation, convergent, is here in the majority. Some information of the proportions of disjunct and non-disjunct arrangements at metaphase is available in the case of interchange heterozygotes where precisely similar conditions to those governing multivalents obtain. Håkansson (1931) found a ring of 4 in Pisum to be arranged non-disjunctionally in about half the divisions whilst in Campanula (Gairdner and Darlington 1931) a ring of 4 was found to be arranged in this manner in about 2/3 of the divisions. It is thus obvious that conditions will vary both from one species to another and one quadrivalent to another within the same complement depending upon the frequency and distribution of the chiasmata.

In most tetraploids investigated unequal disjunction of quadrivalents into 3 and 1, in place of the regular 2 and 2 has been found, (Belling and Blakeslee (1924) in Datura, Darlington (1931) in Primula sinensis, Upcott (1935) in Tomato). This does occur in the potato also (Ellison (1936) Meurman and Rancken (1932)) but it is evident that such gametes are inviable since aneuploid types have not so far been found/

found in this species (Ellison 1935). Hence, any consequences of such segregation may be safely ignored in the following discussion.

Depending upon the type of configuration and its subsequent disjunction, chromosomes which have crossed over may pass to the same pole at anaphase, thus providing the requisite conditions for the occurrence of equational exceptions. This possibility was envisaged by Darlington (1929b and 1931) who termed the process "double reduction". In an individual simplex for a given dominant factor, i.e. of constitution Aaaa, double reduction will lead to an excess of recessive types and the occurrence of duplex (AAaa) individuals where none would be expected. The occurrence of exceptional segregates in tetraploid progenies of Datura (Blakeslee, Belling and Farnham 1923) and of Dahlia (Lawrence 1929, 1931a, Lawrence and Scott-Moncrieff 1936) has been so accounted for. We have therefore to find an estimate of the frequency of occurrence of double reduction if breeding results from autotetraploids are to be analysed satisfactorily.

Single/

Single Factor Segregation.

General Case.

In the diagram (Figure 28) the locus "a" occupied by the four allelomorphs a_1, a_2, a_3, a_4 is so situated that a chiasma never intervenes between it and the centromere. Since separation of sister chromatids must be reductional at second anaphase, there can be no double reduction. The three possible types of interphase nuclei ($a_1a_1:a_2a_2::a_3a_3:a_4a_4$), ($a_1a_1:a_3a_3::a_2a_2:a_4a_4$), and ($a_1a_1:a_4a_4::a_2a_2:a_3a_3$) occur with equal frequency and give the gametes (a_1a_2), (a_3a_4), (a_1a_3), (a_2a_4), (a_1a_4) and (a_2a_3) in equal numbers. This corresponds to Muller's predictions (1914) based on the assumption that the chromosomes behave as independent units. It is obvious that a precisely similar result will follow the replacement of a quadrivalent by two bivalents, providing that pairing between the four homologues is entirely at random. On the basis of Muller's segregations, Haldane (1930) worked out the proportions of gametic types to be expected from higher autopolyploids introducing, in addition the proportions to be expected if the eight chromatids behaved entirely independently of one another. This he termed random chromatid segregation. Linkage formulae based on chromosome segregation were put forward in a paper on tetraploid/

tetraploid Primula sinensis by De Winton and Haldane (1931) whilst the corresponding formulae for random chromatid segregation were published by Sansome (1933). These latter papers are further discussed below.

Though approximations to both these types of segregations have been found (Random chromosome - Primula sinensis, De Winton and Haldane (1923, 1930), Sömme (1930), Zea Randolph (1938), Datura Blakeslee et al.(1923) and others; random chromatid - Rubus Crane and Darlington (1927, 1928), tomato Sansome (1933), Lindstrom (1935)) it is generally recognised that they are limiting types and that most tetraploid segregations will be intermediate in nature. By a very successful analysis of their genetic behaviour Mather (1935, 1936) has greatly increased the amount of information to be derived from autotetraploid segregations. Statistics have been developed by means of which an estimation of the excess of recessives in the simplex and duplex cases may be arrived at by the combination of data from back-cross and self progenies. The analysis has been extended to cover cases of close linkage of two factors in the simplex type and estimates of the frequencies of equational separation and non-disjunction of the paired chromosomes at first anaphase may be derived therefrom. Since the results are capable of sound explanation on a cytological basis we are able to arrive/

arrive at a much more satisfactory interpretation of the genetical data than that provided by either the random chromosome or random chromatid analyses.

As this method of estimation is built up on the cytological principles set out above, the theoretical analysis is given in some detail. The argument follows that of Mather's (1936) paper.

In the diagram (Figure 28) the locus "b" is represented as always having one chiasma between it and the centromere. Only one pairing arrangement of the four possible ones is shown. At anaphase therefore, identical daughter allelomorphs will be attached to different centromeres, thus fulfilling the first condition for double reduction. The chromosomes which have crossed over may proceed to opposite poles or to the same pole. In the first case the six different gamete types will be produced in equal numbers as above. In the second case where two equationally separating chromosomes reach the same nucleus each interphase nucleus may divide so that identical allelomorphs pass to the same or different gametes. Since the mode of division of the two interphase nuclei is uncorrelated there are four equally probable forms of behaviour at second anaphase; "the occurrence of these homozygous gametes in the general case marks the occurrence and extent of double reduction" (Mather 1936). Therefore, since the/

the amount of double reduction will be a function of the product of the frequencies of crossing over and genetical non-disjunction it follows that this amount "is a function of the cross-over distance between the locus and the spindle fibre" (Mather loc. cit.). The segregation for any given gene in a tetraploid will therefore differ according to its crossover distance from the centromere and the extent to which the chiasma frequency is affected by the environment.

Simplex and Triplex Cases.

Of the four possible tetraploid constitutions the simplest to deal with are the simplex (B_1b_3) and triplex (B_3b_1) types, since their two segregations are complementary.

The quantities we have to consider are:-

(1) the mean frequency of equational separation at the locus concerned, dependent upon its distance from the centromere and the frequency of chiasma formation between the locus and the spindle attachment. Let this be "e".

(2) the frequency with which adjacent chromosomes, having crossed over, pass to the same pole at first anaphase. Let this be "a".

Let the plant be simplex for the factor B. Then in 1 - e of cases separation at the locus is reductional and the result will be the same whether the/

the two spindle attachments pass to the same pole at anaphase or not. The gametic proportions will be 1 Bb : 1 bb. In e of cases separation at the locus will be equational, but in 1 - a of these cases adjacent chromosomes pass to opposite poles and the result is the same as before. In (ae) of cases both conditions for double reduction will be fulfilled and the gametic segregation will be 1 BB : 2 Bb : 5 bb (for details see Appendix II p. 188).

Combining these gametic segregations in their correct proportions, the expected gametic segregation for the simplex plant will be

	BB	Bb	bb
	ae	4 - 2ae	4 + ae

and the triplex

	4 + ae	4 - 2ae	ae

If the heterozygotes are not distinguishable from the homozygous dominants these become

	B	b
	4 - ae	4 + ae
and	8 - ae	ae

If α represents the produce ae, the results of selfing simplex and triplex plants will be

	B	b
Simplex	$48-8\alpha-\alpha^2$	$16+8\alpha+\alpha^2$
Triplex	$64-\alpha^2$	α^2

if a and e have the same values in male and female gametogenesis.

If/

If a has its random value of 1/3 (Mather 1935) and e equals 0 these reduce to the expressions of Muller (1914), whilst if e has its random value of 6/7 (Mather 1935), to those for random chromatid segregation. Thus if it is found that a given tetraploid segregation merely approximates to the ratios expected on the random chromatid basis we have no knowledge of how the values of a and e are distributed, and hence no information is gained of the cytological conditions obtaining. In species with a low chiasma frequency there will be a greater tendency towards the replacement of quadrivalents by pairs of bivalents and, though more rarely, by trivalents and univalents. This will be expressed by a reduction of the values of both e and a, since in bivalents chromosomes which have crossed over nearly always pass to opposite poles. Even in trivalents the chance of genetical non-disjunction of the required type is small, partners in a chiasma nearly always passing to opposite poles (Rhoades 1933). Again, random chromatid segregation demands that there shall be an infinite number of chiasmata between the locus and the spindle attachment, a condition manifestly impossible in species with a low chiasma frequency or with localised chiasma formation. Secondly, that disjunction of the members of a quadrivalent shall be at random, and this, as we have already seen, is by no means the rule. On the whole therefore, random chromatid/

chromatid segregation may be rejected as an unsatisfactory basis upon which to analyse tetraploid genetic data.

Breeding Results.

The breeding experiments have shown that four genes designated N^X, N^A, N^B and N^C separately determine the top necrotic reactions to viruses X, A, B and C respectively. An inspection of the accompanying tables reveals that the segregations approximate to those expected of a tetrasomic gene present and dominant in a single dose. One variety appears to be duplex for the N^X gene. It is believed that this represents the first unquestionable instance in the potato of a character determined by a single gene. There are deviations from the simple tetraploid expectations in almost every progeny, and it is with an examination of these that we are especially concerned.

It will be seen at once that, with the exception of the crosses involving Kepplestone Kidney, carrying the N^X gene, and a single family, Liddesdale Lads x Pepo, involving the N^B gene, all the backcross data are derived from families of a similar type, namely, N x n. Further, these N x n families agree with the Kepplestone Kidney N^X x n^x crosses in showing an excess of recessives over the expected 1:1 ratio of a simplex tetraploid backcross. On the other hand, they/

they are in marked contrast to those Kepplestone Kidney families of a reciprocal type, namely $n^x \times N^X$, which show a deficiency of recessives on an expected 1:1 ratio. Selfed progenies of Kepplestone Kidney and of two other N^X carrying varieties taken together show no heterogeneity and the segregations approximate to a 3:1 ratio. In the absence of reciprocal crosses two explanations of these deviations in the various families according to whether the dominant carrying parent acts as male or female are open to us. Either the variation in the proportions of n^x individuals may be ascribed to interactions of cytoplasmic factors; or this apparent differentiation is dependent solely on the varieties employed, the excess of recessives in those N x n families in which Kepplestone Kidney is not a parent being due to double reduction, following upon quadrivalent formation between the chromosomes carrying the genes concerned.

The single cross of a reciprocal type concerns the N^B gene (Liddesdale Lads x Pepo, $n^b \times N^B$). There is no doubt of the accuracy of the scoring of this progeny and it agrees with the two other $N^B \times n^b$ families in showing an excess of recessives, but disagrees with the Arran Victory x Kepplestone Kidney ($N^B \times n^b$) family which has a deficiency of recessives. Moreover, although the Kepplestone Kidney x n^x families and/

and other N^X x n^x matings taken together show no heterogeneity it is noteworthy that the former do show a greater number of recessives in proportion to the family totals than the latter.

There seems no doubt that the individual genes studied are showing tetrasomic segregation and good reasons have been put forward in a later section for believing that the cultivated potato is an auto-tetraploid. The second of the above two explanations has therefore been adopted as being both the simplest hypothesis and the one with which the available evidence is most in agreement. In the present season progenies have been raised and crosses made which will either support or negative this interpretation.

To avoid breaking the continuity of the discussion the detailed workings of the results have not been included here but will be found in Appendix II where they may be checked.

The group of families in which Kepplestone Kidney is concerned will be left on one side meanwhile.

The data on the N^X gene are provided by 13 back-cross families of the type N^X x n^x and two selfs. The former may be grouped into 5 concerning the variety Epicure (Table 1), 5 crossed with Pepo (Table 2), and 3 of unlike parentage (Table 3). There is no heterogeneity either within or between these groups and (Tables 1 - 10), with the exception of Liddesdale/

S I M P L E X.

N^X TABLE 1.

N^X x n^X	N	n	T
Epicure x Shamrock	41	51	92
" x Alness	33	35	68
" x Argyle Fav.	14	22	36
" x Alannah (1)	11	21	32
" x " (2)	29	31	60
	128	160	258

* Heterogeneity $\chi^2_4 = 2{\cdot}552$. P = 0·70-0·50

N^X TABLE 2.

N^X x n^X	N	n	T
Epicure x Pepo	10	12	22
White City x Pepo	51	54	105
Liddesdale Lads x Pepo	29	29	58
Maud Meg x Pepo	72	84	156
Southesk x Pepo	62	69	131
	224	248	472

Heterogeneity $\chi^2_4 = 0{\cdot}3208$. P = 0·99-0·98

* The index of χ^2 indicates the number of degrees of freedom.
Values of P, the probability of obtaining a similar result by chance, of 5% or less have been taken as indicating that the segregations differ significantly from one another.

S I M P L E X

N^X

TABLE 3.

N^X x n^x	N	n	T
Benest x Alness	30	41	71
Craigs Defiance x Flourball (1)	91	77	168
Craigs Defiance x Flourball (2)	60	60	120
	181	178	359

Heterogeneity $\chi^2_2 = 2{\cdot}96$. P = 0·30-0·20

TABLE 4.

F_2 N^X selfed	N	n	T
Edgecote Purple	15	8	23
Liddesdale Lads	107	33	140
	122	41	163

Heterogeneity $\chi^2_1 = 1{\cdot}323$. P = 0·30-0·20

Heterogeneity χ^2 between back cross F_2 data = 0·37473. P = 0·70-0·50

S I M P L E X.

ADDITIONAL TESTS.

N^X

TABLE 5.

N^X x n^X	N	n	T
Epicure x Alannah (1)	11	21	32
" x " (2)	29	31	60
	40	52	92

Heterogeneity $\chi^2_1 = 1{\cdot}668$. P = 0·20-0·10

N^X

TABLE 6.

N^X x n^X	N	n	T
Craigs Defiance x Flourball (1)	91	77	168
Craigs Defiance x Flourball (2)	60	60	120
	151	137	288

Heterogeneity $\chi^2_1 = 0{\cdot}5613$. P = 0·50-0·30

S I M P L E X.

ADDITIONAL TESTS.

N^X

TABLE 7.

N^X x n^X	N	n	T
Epicure x Pepo	10	12	22
Others x Pepo	128	160	288
	138	172	310

Heterogeneity $\chi_1^2 = 0{\cdot}1215$. P = 0·80-0·70

N^X

TABLE 8.

N^X x n^X	N	n	T
Epicure x Others	128	160	288
Others x Pepo	224	248	472
	352	408	760

Heterogeneity $\chi_1^2 = 0{\cdot}6837$. P = 0·50-0·30

S I M P L E X.

ADDITIONAL TESTS.

N^X TABLE 9.

N^X x n^X	N	n	T
Epicure x Others	128	160	288
Benest x Alness) Craigs Defiance x Flourball (1) and (2))	181	178	359
	309	338	647

Heterogeneity $\chi^2_1 = 1{\cdot}322$. P = 0·30-0·20

N^X TABLE 10.

N^X x n^X	N	n	T
Others x Pepo	224	248	472
Benest x Alness) Craigs Defiance x Flourball (1) and (2))	181	178	359
	405	426	831

Heterogeneity $\chi^2_1 = 0{\cdot}6003$. P = 0·50-0·30

Liddesdale Lads x Pepo and Craigs Defiance x Flourball (1) and (2) the families all agree in showing an excess of recessives over the expected 1:1 ratio. Both the Craigs Defiance progenies are somewhat anomalous in that a large number of losses occurred in the field during the first year. These caused a more serious discrepancy when the same families were tested for their virus A reactions, there being over a third of the original total of 338 missing. This was unfortunately realised too late for the matter to be rectified, and, since the cross was a valuable one, Craigs Defiance being simplex for all four necrotic genes and Flourball nulliplex for the same genes it was decided to continue testing the available material. Since, in the N^X families 21 plants are missing from family (1) and 29 from family (2) it seems likely that part of the discrepancies may be due to this factor. For the rest of the crosses, the excess of recessives is continent with the expectations of the occurrence of some double reduction.

The two selfs, Edgecote Purple and Liddesdale Lads, together show scarcely any deviation from a simplex selfed ratio of 3:1 and there is no indication of hererogeneity (Table 4).

By means of a combined estimation (see Appendix II, p.197) using these data from back-cross and/

and selfed matings a value for α, the index of separation of the N^X gene may be obtained. This proves to be 0·164±0·1091. Using this value of α, it can be shown that there is no heterogeneity between the back-cross and self data concerning this gene. The agreement in this case is very good (Table 4 and see Appendix II, p. 199). In addition to an excess of recessives double reduction in a simple back cross is expected to give rise to a small number of duplex individuals. In the present case the duplex types are not distinguishable from the simplex ones and the classification of the data is therefore as complete as circumstances permit, though the amount of information obtainable is thereby reduced.

The peculiarities of the Kepplestone Kidney crosses have already been touched upon. The three back crosses in which this variety is the female parent agree with those N^X x n^X families just considered in showing an excess of recessives over the expected 1:1 ratio (Table 11). In each case the proportion of recessives to the family total is greater in the Kepplestone Kidney crosses than in the others. There is no significant heterogeneity between pairs of Kepplestone Kidney and other varietal crosses involving the same recessive carrying male parent (Tables 12 and 13)/

S I M P L E X.

N^X

TABLE 11.

N^X x n^X	N	n	T
Kepplestone Kidney x 70(13)	12	12	24
" x Alness	7	13	20
" x Shamrock	37	65	102
	56	90	146

Heterogeneity $\chi^2_2 = 1{\cdot}649$. P = 0·50-0·30

N^X

TABLE 12.

N^X x n^X	N	n	T
Kepplestone Kidney x Shamrock	37	65	102
Epicure)) x Shamrock Benest)	33	35	68
	70	100	170

Heterogeneity $\chi^2_1 = 2{\cdot}519$. P = 0·20-0·10

S I M P L E X.

N^X TABLE 13.

N^X x n^X	N	n	T
Kepplestone Kidney x Alness	7	13	20
Epicure)) x Alness Benest)	63	76	139
	70	89	159

Heterogeneity $\chi_1^2 = 0{\cdot}7304$. P = 0·50-0·30

N^X TABLE 14.

N^X x n^X	N	n	T
Pepo x Kep. Kidney	41	25	66
Others x Pepo	224	248	472
	265	273	1265

Heterogeneity $\chi_1^2 = 5{\cdot}001$. P = 0·05-0·02

13), but a comparison of the two totals, Kepplestone Kidney x n^x and Other varieties x n^x shows that they do not agree, the deviation being greater than the random sampling error allows (Table 15). The four selfed progenies of Kepplestone Kidney are homogeneous and conform well with a 3:1 ratio. They also agree with the two simplex selfed families described (Tables 16 and 17).

The ten n^x x Kepplestone Kidney families are a very heterogeneous collection, the heterogeneity χ^2 for 9 degrees of freedom being 28·49 (Table 18). The largest proportion of this is contributed by the four progenies with President, Up to Date, Scot and Peachbloom respectively as the female parents (Table 19). The remaining six all agree in showing a considerable deficiency of recessives on a 1:1 basis (Table 20). This cannot be attributed to faulty scoring since the dominants never escape observation and the consistency of the results rules out any other explanation. In fact even among the four aberrant progenies President x Kepplestone Kidney and Peachbloom x Kepplestone Kidney are the only ones in which the, in this case unexpected, excess of recessives may possibly be attributed to this cause. The deviations from equality in the Scot and Up to Date families are very large, being 45 and 56 respectively. As would be expected,

a/

S I M P L E X.

N^X

TABLE 15.

N^X x n^X	N	n	T
Kep. Kidney x n^X	56	90	146
Rest x n^X	533	586	1119
	589	676	1265

Heterogeneity χ^2_1 = 4·7436. P = 0·05-0·02

TABLE 16.

F_2 N^X Selfed	N	n	T
Kepplestone Kidney 1	13	6	19
" " 2	27	7	34
" " 3	11	4	15
" " 4	52	21	73
	103	38	141

Heterogeneity χ^2_3 = 1·031. P = 0·80-0·70

S I M P L E X.

TABLE 17.

F_2 N^X Selfed	N	n	T
Kepplestone Kidney	103	38	141
Rest	122	41	163
	225	79	304

Heterogeneity $\chi^2_1 = 0{\cdot}1043$. P = 0·80-0·70

N^X

TABLE 18.

n^X x N^X	N	n	T
President x Kep. Kidney	50	50	100
Up to Date x "	78	22	100
Scot x "	92	47	139
Claymore x "	93	64	157
Peachbloom x "	58	62	120
Kerr's Pink x "	73	53	126
British Queen x "	45	27	72
Pepo x "	41	25	66
A. Victory x "	84	67	151
Golden Wonder x "	98	73	171
	712	490	1202

Heterogeneity $\chi^2_9 = 28{\cdot}49$. P = very small.

S I M P L E X.

N^X TABLE 19.

n^x x N^X	N	n	T
President x Kep. Kidney	50	50	100
Up to Date x "	78	22	100
Scot x "	92	47	139
Peachbloom x "	58	62	120
	278	181	459

Heterogeneity $\chi^2_3 = 27{\cdot}29$. P = very small

N^X TABLE 20.

n^x x N^X	N	n	T
Claymore x Kep. Kidney	93	64	157
Kerr's Pink x "	73	53	126
British Queen x "	45	27	72
Pepo x "	41	25	66
A. Victory x "	84	67	151
Golden Wonder x "	98	73	171
	434	309	743

Heterogeneity $\chi^2_5 = 1{\cdot}523$. P = 0·30-0·20.

a heterogeneity test between the totals of crosses with Kepplestone Kidney as the male parent and female parent respectively confirms their dissimilar nature. Anticipating the results from other segregations it has been found that the effect of Kepplestone Kidney as a male parent is to cause a deficiency of recessives. Thus, in the cross Arran Victory x Kepplestone Kidney (N^B x n^b) there is a deficiency of recessive n^b plants, not as great as in the n^x x N^X families, but still a deficiency. This seems to lend support to the idea that the behaviour of Kepplestone Kidney is abnormal and that the apparent association of an excess of dominants with its presence as a male parent in crosses with other recessive carrying varieties is due to its carrying a lethal factor which reduces the viability of the recessive (n) types, whatever the gene concerned. Whether the factor is genic or cytoplasmic it is not possible to say and only further work can verify this hypothesis. It is particularly important to know whether the behaviour of the few Kepplestone Kidney x n^x families is characteristic of this type of cross.

The data on the inheritance of the remaining three genes, N^A, N^B and N^C are at present rather scanty. Six progenies have been tested for their reactions to virus A, with somewhat variable results. One of these had Kepplestone Kidney as a male parent and showed the same/

same deficiency of recessives as did the N^X families concerning this variety. The remaining five were all of the $N^A \times n^a$ type. Of these the three Pepo crosses do not agree in their behaviour (Table 21). The families Liddesdale Lads x Pepo and Maud Meg x Pepo both have an excess of recessives over the 1:1 ratio, as we might expect to find if double reduction is occurring here too, but the third one, Southesk x Pepo has a deficiency of recessives. Since the figures shown represent the results from only half of the latter progeny it is perhaps inadvisable to regard them as typical of the whole. The Maud Meg x Pepo progeny was a highly unsatisfactory one to score. The reaction of the female parent to virus A infection has been described already; it is definitely a top necrosis but a very protracted one which fails to appear on some occasions even when the union between stock and infector scion is above suspicion. The same type of reaction was manifested in this particular progeny and it is highly probable that the number of recessives, n^a, is too large. The correct proportions will no doubt be obtained by further testing. The shortcomings of the Craigs Defiance crosses have also been touched upon and in the absence of confirmatory crosses it is difficult to know whether their behaviour is to be regarded as typical or not (Table 22). They both agree/

S I M P L E X.

N^A TABLE 21.

N^A x n^a	N	n	T
Liddesdale Lads x Pepo	21	25	46
Southesk x Pepo	37	27	64
Maud Meg x Pepo	57	92	149
	115	144	259

Heterogeneity $\chi^2_3 = 7{\cdot}008$. P = 0·05-0·02

N^A TABLE 22.

N^A x n^a	N	n	T
Craigs Defiance x Flourball (1)	57	44	101
Craigs Defiance x Flourball (2)	46	39	85
	103	83	186

Heterogeneity $\chi^2_1 = 0{\cdot}0809$. P = 0·80-0·70

agree with the Kepplestone Kidney cross in showing a deficiency of recessives (Tables 23 and 24). The mixed nature of the data is particularly unfortunate but there seems no reason to suppose that further results will invalidate the hypothesis that virus A top necrosis is also determined by a tetrasomic gene N^A, with which, in fact, the present data do not disagree.

The four crosses concerning the N^B gene agree well with expectations. The Liddesdale Lads x Pepo and Craigs Defiance x Flourball families are reciprocal in type, $n^b \times N^B$ and $N^B \times n^b$ respectively, and homogeneous, both showing an excess of recessives over the expected equality (Table 25). This is in conformity with the idea that the inheritance of this gene is also tetrasomic and that double reduction is occurring. The single Arran Victory x Kepplestone Kidney family has been mentioned above and is peculiar in again showing a deficiency of recessive plants. This family and the other three taken together are apparently homogeneous though the value of P, the probability, is low (Table 26).

The virus C data consists of four families only, two of which are selfs in type and the others are back crosses. In each case the families were raised from berries of the same cross. Both Epicure and/

S I M P L E X.

N^A TABLE 23.

Back-cross	N	n	T
Arran Victory x Kep. Kidney	80	63	143
Other N^A x n^a	218	227	445
	298	290	588

Heterogeneity χ_1^2 = 2·121. P = 0·20-0·10

N^A TABLE 24.

N^A x n^a	N	n	T
Craigs Defiance x Flourball (1) and (2)	103	83	186
Others x Pepo	115	144	259
	218	227	445

Heterogeneity χ_1^2 = 5·203. P = 0·05-0.02

S I M P L E X.

N^B TABLE 25.

Back-cross	N	n	T
n^b x N^B Liddesdale Lads x Pepo	21	23	44
N^B x n^b Craigs Defiance x Flourball (1)	62	70	132
Craigs Defiance x Flourball (2)	39	53	92
	122	146	268

Heterogeneity $\chi^2_2 = 0{\cdot}5242$. $P = 0{\cdot}80\text{-}0{\cdot}70$

N^B TABLE 26.

N^B x n^b	N	n	T
Arran Victory x Kep. Kidney	70	64	134
Other N^B x n^b	122	146	268
	192	210	402

Heterogeneity $\chi^2_1 = 1{\cdot}643$. $P = 0{\cdot}20\text{-}0{\cdot}10$

and Alannah carry the gene N^C and the two families are homogeneous, as are also the two Craigs Defiance crosses (Tables 27 and 28). These latter again show an unexpectedly high number of dominants for a 1:1 ratio if the N^C gene is showing double reduction, but as before, it must be borne in mind that quite a large proportion, 70 in this case, of the total plants are missing. A value of λ for the N^C gene may be estimated from these data as was done for the N^X gene; whence $\lambda = 0.0666 \pm 0.2060$. The high standard error is inherent in the data. As with the simplex N^X data, using this value for the index of separation it is possible to test the homogeneity of the back cross and self data. In this case the agreement is quite good the value of P being 0.20-0.10 (Table 28).

The results from the work with the A, B and C viruses must be regarded as preliminary to further investigation. On the whole they follow the more reliable data on the N^X gene and suggest that the four genes determining the top necrotic reactions to their respective viruses are all situated near enough to the ends of their respective chromosome or chromosomes for crossing over to occur between them and the centromere. Whether any grounds exist for postulating linkage between any of these four genes will be examined in an ensuing section.

S I M P L E X.

N^C

TABLE 27.

F_2 N^C x N^C	N	n	T
Epicure x Alannah (1)	21	7	28
" x " (2)	34	18	52
	55	25	80

Heterogeneity χ^2_1 = 0·7891. P = 0·20-0·10

N^C

TABLE 28.

N^C x n^c	N	n	T
Craigs Defiance x Flourball (1)	83	78	161
Craigs Defiance x Flourball (2)	55	53	108
	138	131	269

Heterogeneity χ^2_1 = 0·000. P = 1·0

Heterogeneity χ^2_1 = 1·7261. P = 0·20-0·10
between back-cross F_2 data

Duplex.

So far only one variety has been found which by its segregations shows itself to be duplex for the W^X gene. This is Cardinal.

The duplex (B_2b_2) type presents a more complicated case on account of the fact that here any chromosome may pair either with a like, or an unlike, or both kinds of other chromosome in contrast to the simplex, where the dominant carrying chromosome must be paired with an unlike at any level. We can simplify the case by supposing that the locus is so situated that there is rarely or never a change of partner between it and the centromere. In 1/3 of cases, therefore, like chromosomes will be associated in this region and in 2/3 unlike. Where like chromosomes are paired there can obviously be no equational separation and hence no double reduction. Where unlike chromosomes are paired the amount of equational separation, e, and of non-disjunction, a, will be the same for either pair. If crossing over in the two pairs is assumed to be uncorrelated the gametic segregation will be C $5-\alpha$, c $1+\alpha$ (See Appendix II, p.189).

An important result follows changes of partner between the locus and the spindle attachment in/

in the duplex. A change of partner in the simplex must still leave the dominant carrying chromosome paired with a recessive carrying one. With two chiasmata and no change of partner between them there is 50% equational separation at a locus beyond the second. If there is an exchange between the chiasmata the proportion after the second chiasma is increased to 100% (Mather 1935). This represents the maximum effect in the simplex. In the duplex, however, in the 1/3 of cases where like chromosomes are paired near the centromere, a change of partner will result in unlike chromosomes being paired. With two chiasmata and no change of partner there will be no increase of equational separation. But with such an exchange separation would be 100% equational for a locus beyond the second chiasma. "Hence partner exchanges should result in the duplex showing a greater increase in the number of recessive gametes over 1 in 6 than it does over 1 in 8 in the simplex" (Mather 1936). The three progenies of Cardinal selfed tested for their virus X reactions all agree in showing an approximate 15:1 segregation expected from a duplex tetraploid (Table 29). The two back cross progenies however do not agree. Here the discrepancy is probably due to the excess of recessives in the Abundance cross resulting from faulty scoring (Table 30). An estimation of the duplex index of separation/

D U P L E X.

N^X

TABLE 29.

F_2	N_2^X Selfed	N	n	T
Cardinal	1	44	3	47
"	2	27	2	29
"	3	25	4	29
		96	9	105

Heterogeneity $\chi_2^2 = 1{\cdot}400$. P = 0·50-0·30

N^X

TABLE 30.

n^X x	N_2^X	N	n	T
Golden Wonder x Cardinal		74	12	86
Abundance x Cardinal		70	26	96
		144	38	182

Heterogeneity $\chi_1^2 = 4{\cdot}733$. P = 0·05-0·02

Heterogeneity between back cross F_2 data $\chi_1^2 = 2{\cdot}5434$. P = 0·20-0·10

separation, β , may be obtained by combining the two sets of data (see Appendix II, p.198), giving $\beta = 0.4193 \pm 0.1575$. Substituting this value of β in the maximum likelihood expression it will be seen that the two sets of data, back cross and self show relatively good agreement with one another (Table 30 and Appendix II, p.198).

A particularly interesting feature of the duplex index of separation has been pointed out above, namely that it is likely to be larger than the simplex index since partner exchanges in the duplex lead to a higher proportion of equational separation of the locus concerned. Comparing the values of and for the N^X gene just obtained we see that this is clearly so, though the difference is not significant, t being equal to 2.024 for three degrees of freedom (see Appendix II, p.199). Small values of α and β indicate that the genes concerned are near the centromere, and the difference between them will consequently be small. With increasing distance between the locus and the centromere these values increase, and, since a greater frequency of changes of partner is possible, the differences between them will also increase. In the present case the data suggest that changes of partner are taking place between the locus of the gene N^X and the centromere.

The/

The significance of the small value of α estimated for the N^C gene cannot be profitably discussed in view of the small amount of data aviilable. No selfed progenies of varieties simplex for either of the other two genes, N^A and N^B have yet been tested so that corresponding estimations for the indices of separation of these is not possible at present.

Linkage.

The results from the named variety work have shown that certain combinations of genes occur far more frequently than others. Thus, varieties necrotic to the X virus are nearly always found to be necrotic to virus A alone only one has been found necrotic only to virus X (Cockerham 1939). On the other hand there seems to be little or no evidence of any close association between these two characters and those responsible for necrosis to either of the viruses B or C. These results suggest that at least the N^X and N^A genes are linked. Accordingly two families were tested for their reactions to all four viruses and two were tested with viruses A, X and B. A fifth family, Maud Meg x Pepo has not been included here on account of the unreliability of the scoring. These progenies have, of course already been analysed for their single factor segregations. For the detection of linkage between the /

the various genes the data from the above four families have been regarded as homogeneous and grouped as in the accompanying table (Table 31) according to the pairs of genes concerned. This seems justifiable in spite of the fact that discrepancies have been detected among some of the single factor segregations. The discrepancies are now spread over four classes in place of two and they are far outweighed by aberrations due to linkage the detection of which is the primary object. The data may then be analysed by the χ^2 method (for details of which see Mather 1938, and Appendix II, p. 201). The assumption is made that, since all the families are back crosses, the two genes in each case are showing 1:1 ratios and segregating independently. χ^2 is partitioned into three components, two of which are concerned with the deviation of the single genes from the expected 1:1 ratio and the third with detecting association of the two factors in segregation, the expectation being that they are independent. In the present case the deviations from expectation may be found for each individual family and for the totals and the homogeneity of each component may be tested. The procedure is explained only for the first of the groups in Table 31, being precisely similar in each case. A χ^2 for three degrees of freedom calculated on the totals of all the families segregating/

TABLE 31.

Family	AX	Ax	aX	ax	T	Hetero	geneity χ^2	Degrees of Freedom
Arran Victory x Kep. Kidney	75	5	6	57	143	Gene pair,	A,a 1·96589	
Liddesdale Lads x Pepo	21	0	2	23	46		P = 0·70-0·50	
Craigs Defiance x Flourball (1)	49	7	1	42	99	Gene pair,	X,x 1·1573	3
" x " (2)	38	6	5	34	83		P = 0·70-0·50	
Deviation of totals	183	18	14	156	371	Linkage	1·150	
χ^2_3 = 258·106 P = v. small							P = 0·80-0·70	
	AB	Ab	aB	ab				
Arran Victory x Kep. Kidney	43	30	29	30	132	Gene pair,	A,a 2·2454	
Liddesdale Lads x Pepo	13	6	7	17	43		P = 0·50-0·30	
Craigs Defiance x Flourball (1)	33	22	16	26	84	Gene pair,	B,b 2·7740	3
" x " (2)	20	25	16	23	97		P = 0·50-0·30	
Deviation of totals	109	83	68	96	356	Linkage	5·3012	
χ^2_3 = 10·36 P = 0·02-0·01							P = 0·20-0·10	
	XB	Xb	xB	xb				
Arran Victory x Kep. Kidney	42	32	29	31	134	Gene pair,	X,x 1·2669	
Liddesdale Lads x Pepo	14	8	6	16	44		P = 0·80-0·70	
Craigs Defiance x Flourball (1)	35	31	24	37	127	Gene pair,	B,b 2·6332	
" x " (2)	16	27	22	25	90		P = 0·50-0·30	3
Deviation of totals	107	98	81	109	395	Linkage	6·1542	
χ^2_3 = 5·0 P = 0·20-0·10							P = 0·20-0·10	
	BC	Bc	bC	bc		Gene pair,	B,b 0·4301	
							P = 0·70-0·50	
Craigs Defiance x Flourball (1)	35	27	35	34	131			
" x " (2)	16	23	33	19	91	Gene pair,	C,c 0·0039	1
Deviation of totals	51	50	68	53	222		P = 0·98-0·95	
χ^2_3 = 3·86 P = 0·30-0·20						Linkage	4·5715	
							P = 0·05-0·02	
	AC	Ac	aC	ac		Gene pair,	A,a 0·1495	
							P = 0·70-0·50	
Craigs Defiance x Flourball (1)	27	30	29	15	101	Gene pair,	C,c 0·0645	1
" x " (2)	28	17	17	22	84		P = 0·80-0·70	
Deviation of totals	55	47	46	37	185	Linkage	5·3146	
χ^2_3 = 3·54 P = 0·50-0·30							P = 0·05-0·02	
	XC	Xc	xC	xc		Gene pair,	X,x 0·1245	
							P = 0·80-0·70	
Craigs Defiance x Flourball (1)	37	45	45	30	157	Gene pair,	C,c 0·0187	
" x " (2)	29	23	25	27	94		P = 0·90-0·80	1
Deviation of totals	66	68	70	57	261	Linkage	3·1862	
χ^2_3 = 1·54 P = 0·70-0·50							P = 0·10-0·05	

The subscripts of χ^2 indicate the numbers of degrees of freedom.

segregating for the genes N^X and N^A is very large showing that the individual classes deviate far from the expected equality. Detailed analysis shows that all four families agree in showing that the segregations are not independent. From this it is concluded that the genes N^X and N^A are linked. A similar procedure with the other five combinations of genes enables us to see that in no case do the single gene ratios differ significantly from the 1:1 expectation but that, whilst segregation of the gene pairs N^B-N^C, N^A-N^C, and N^X-N^C is independent, segregation of the two pairs N^A-N^B, and N^X-N^B is not so. The value of P, the probability that segregation is independent is barely significant in the N^X-N^B case but the four families show no heterogeneity of the linkage components. It will be seen from the table that the calculations for the gene pairs N^B-N^C, N^A-N^C and N^X-N^C have been made from but two families, Craigs Defiance x Flourball (1) and (2). Though the single factor ratios all agree with a 1:1 expectation the linkage component in each case shows some heterogeneity. This may be attributed to the somewhat incomplete nature of the progenies, previously discussed. Having detected linkage between the N^X, N^A and N^B genes some means of estimating its extent must be found. This necessitates some further consideration of the theoretical aspects of tetraploid behaviour./

behaviour. In diploids linkage is measured by the frequency of recombination gametes since these are a direct indication of the frequency of recombination chromatids. In tetraploids however, we are faced with a situation where gametes arise which carry recombination strands but are, in fact, indistinguishable from gametes carrying non-recombination strands of certain types. The term recombination gamete is therefore used to describe those types of gametes which give rise to one of the two types of zygote of which the parent zygote was incapable of producing disregarding the distribution of the two genes among the chromosomes concerned.

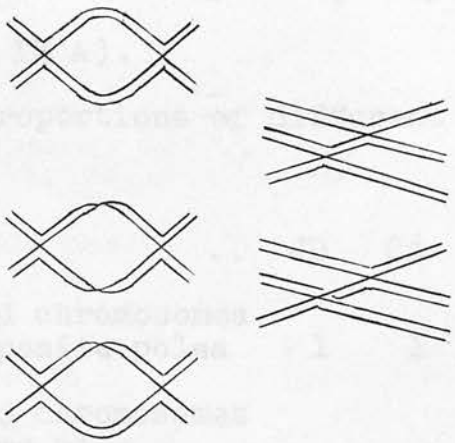
In the absence of chromatid interference the upper limit of recombination in diploids is 50%, even numbers of cross-overs restoring the original combinations. The situation is complicated in tetraploids by the fact that, since more than two homologous chromosomes are present, changes of partner are possible. Such changes of partner will clearly reduce the frequency of double crossovers. In a tetraploid simplex for two factors in single coupling, visible recombination results only from those chiasmata involving the dominant carrying chromosome with a recessive carrying one. Following double crossing over between these with no change of partner between the two chiasmata the situation will be the same as in diploids/

diploids and non-crossover, single crossover and double crossover chromatids will result in the proportions of 1:2:1, giving 50% of recombination. If there is a change of partner, the two chiasmata involving the dominant carrying chromosome but different recessive carrying ones, the proportions of non-, single and double crossovers are 5:6:1, again giving 50% of recombination (Plate IX). This represents the upper limit of recombination between those chromatids taking part in chiasmata with the dominant carrying chromosome. The total amount of recombination depends on the gametic combinations with the other strands not taking part in these particular chiasmata.

From the previous discussion it will be realised that the genetic situation in the duplex type is too complex for linkage determinations. Similarly data from triplex plants are of little use and attention will be confined to a consideration of linkage in the single coupling and single repulsion phases.

Let c and d be the two factors under consideration, the zygote showing single coupling (CD, cd_3). If c is nearer the centromere the possibility of equational separation of this locus may be neglected and the calculation thereby simplified. Let a be the proportion of cases in which paired chromosomes, having crossed over, pass to the pole at anaphase and/

Plate IX.



A B

Diagram illustrating the effect of a change of partner on the proportions of strands with 0, 1 and 2 cross-overs resulting from two chiasmata.

A. No change of partner. There are three possible relations of the strands at the two chiasmata, viz. reciprocal (top) complementary (middle) and diagonal (bottom). The first two occur equally often, in the absence of chromatid interference, and the last twice as often as either of the others. It will be seen that this leads to proportions 1:2:1 of 0, 1 and 2 cross-over strands and hence 50 per cent. recombination in the four strands concerned.

B. With change of partner. There are two possibilities with regard to the strand relationships at the two chiasmata, viz. giving no double cross-overs (top) or one double cross-over (bottom). These occur equally often in the absence of chromatid interference, and lead to proportions of 5:6:1 of 0, 1 and 2 cross-over strands, i.e. again giving 50 per cent. recombination in the strands concerned.

and let two chiasmata be constantly formed with no change of partner occurring between the loci of C and D on the dominant carrying chromosome. The relations of chromatids at the second chiasma depend on whether the two chiasmata are reciprocal, complementary or diagonal (Plate IX A).

The proportions of different gametic types are then,

		CD	Cd	cD	cd
1 - a	paired chromosomes to opposite poles	1	1	1	1
¼a	paired chromosomes to same pole. Chiasmata reciprocal	2	-	-	2
¼a	paired chromosomes to same pole. Chiasmata complementary	2	-	-	2
½a	paired chromosomes to same pole. Chiasmata diagonal	(½ 2 (	-	-	2
		(½ 1	1	-	2

In the ½a of cases where paired chromosomes with diagonal chiasmata pass to the same pole double reduction occurs in ¼a of them, giving the proportions 1CD:1Cd:2cd. Summing these proportions for the different gametic types, we get, (4+3a)CD:(4-3a)Cd: (4-4a)cD:(4+4a)cd. Where a change of partner is occurring between the loci of C and D, other conditions being the same, the dominant carrying chromosome may pass to the same pole as either recessive chromosome with/

with which it crosses over but not both. Double crossing over occurs in half the cases, in the other half it does not. The proportions of gametic types are then,

		CD	Cd	cD	cd
With double crossing over occurring.					
½ - a	chromosomes to opposite poles	1	1	1	1
½a	chromosomes to same pole	1	1	1	1
½a	chromosomes to same pole	(½ 1	1	1	1
		(½ 2	-	1	1
a	chromosomes to same pole	1	1	1	1

Summing these proportions we get (2+5a)CD: (6-5a)Cd:(5-2a)cD:(3-2a)cd. The series shows a higher proportion of recombination gametes than the other one.

In their paper on linkage in the tetraploid Primula sinensis De Winton and Haldane (1931) disregard the possibility of two chromosomes which have crossed over passing to the same pole. Their formulae for single coupling and single repulsion are similar to those of the diploid and p, the recombination value, can never be greater than 50%. For single coupling the gametic series they give is 1-p:p:p:1-p so that with double crossing over and no change of partner, from the above summations, p = 8-7a/16, and where there is a change of partner p = 11-7a/16. From this it/

it is clear that for any value of a in the first case p can never be greater than 50% whereas in the second case for certain values of a it can. Thus it is possible, using this formula for linkage, for single coupling to give more than 50% recombination, "although, as regards the effective chiasmata the strands taking part cannot show more than 50%".(Mather 1936). In this case, then, De Winton and Haldane's p is not a true measure of the amount of recombination occurring and estimates of p from their formulae will accordingly be less than the true value on account of their disregard of the possibility of genetical non-disjunction. In the case of single repulsion, recombination will only occur following crossing over between the two dominant carrying chromosomes, so that the upper limit of recombination will probably be less than 50%. Where the proportion of recombinations is large the additional complications introduced by changes of partner and interference do not allow of any simple analysis of the situation and De Winton and Haldane's formulae then seem the only ones to use. The theoretical possibility of distinguishing between 50% of crossing over and no linkage in the cases of double coupling and repulsion which these authors deduce is also unlikely to be of practical value or to represent the true situation.

Primula sinensis seems rather peculiar in its behaviour. The S, B and G genes with which these authors/

authors deal are situated in one of the longest chromosomes with the locus of G being the farthest away of the three genes from the centromere (De Winton and Haldane 1935) and yet the single factor ratios for these genes show no evidence of double reduction, the only abnormalities of segregation being due to pentasomy. The possibility of genetical non-disjunction is regarded as a relatively remote contingency by these authors yet crossing over does occur between each of the loci and there seems to be no lack of quadrivalent configurations which would lead to such non-disjunction (Darlington 1931).

Estimation of Linkage.

In the present instance only in the case of the gene pair N^A-N^X are the proportions of recombinations small enough to warrant an estimation of the value of p. The data are from single coupling crosses so that this association alone will be considered in detail. We have already seen that the proportions of gametic types provide the only evidence on which a consideration of segregation may be based and these must be expressed in terms of the variables which,

(a) determine the recombination of chromosomes by crossing over and

(b)/

(b) determine the combinations of the chromosomes in the gametes.

Estimates of the recombination value are only reliable if the linkage between two factors is close enough for double crossing over and partner exchanges to be sufficiently rare as to be neglected. The proportions of gametic types are then determined by the frequency of crossing over between the two loci and the occurrence of double reduction, which will be correlated for the two genes.

If c and d are the two genes in single coupling (CD,cd_3) c being nearer to the spindle attachment, let the frequency of equational separation at c be e and the frequency with which the spindle attachments of the two chromosomes pass to the same pole at anaphase be a. Then, let the frequency of chiasma formation, i.e. twice the frequency of crossing over on the chiasma type hypothesis, be p_1 where separation at c is reductional and p_2 where separation at c is equational. The total chiasma frequency p will then be $(1 - e)p_1+ep_2$.

In 1-a of cases chromosomes pass to opposite poles so that equational separation is ineffective. The proportions of gametic types are then

	CD	Cd	cD	cd
$(1 - a)(1 - p)$	4	-	-	4
$(1 - a)p$	2	2	2	2

In/

In a of cases paired chromosomes pass to the same pole but in 1-e of these separation is reductional at c and the proportions are

	CD	Cd	cD	cd
$a(1 - e)(1 - p)$	4	-	-	4
$a(1 - e)p_1$	3	1	-	4

In e of the cases separation at c is reductional and the proportions are

	CD	Cd	cD	cd
$ae(1 - p_2)$	3	-	-	5
$ae\ \frac{1}{2}p_2$	3	-	1	4
$ae\ \frac{1}{2}p_2$	2	1	1	4

By summing these the gametic series

CD $4-ae-2p+ap+\frac{1}{2}aep_2$

Cd $2p-ap-\frac{1}{2}aep_2$

cD $2p-2ap+aep_2$

cd $4+ae-2p+2ap-aep_2$

is obtained. The series expected from two factors in single repulsion is easily derived if it is borne in mind that the dominant carrying chrmosomes are paired in only one third of cases. In the remaining two thirds each marked chromosome is paired with a recessive carrying chromosome and the possibility of independent double reduction at both loci must be considered.

Estimation/

Estimation of a, e and p is possible from single coupling or repulsion data alone if the terms involving aep_2 are neglected. The discrepancies involved will not be very great since these terms will, in general be small as compared with the others.

Of the four families scored for both the N^X and N^A genes we have seen that two of them, Arran Victory x Kepplestone Kidney and Craigs Defiance x Flourball (1) are showing some deviation from the typical N^X segregation and only one of these, Liddesdale Lads x Pepo, is showing a typical N^A segregation, i.e. an excess of recessives over the 1:1 ratio. Hence it is rather difficult to know which data will give the most reliable estimate of the crossover percentage for the two genes. Back cross and F_2 data, from which a correcting factor for these deviations might be derived are only available for the N^X gene. Accordingly a provisional value for p has been calculated from the totals of all four families. This must be regarded as the best possible estimate of the recombination value under the circumstances. In each case the genes are in the coupling phase and the frequencies are N^AN^X 183, N^An^X 18, n^aN^X 14, n^an^X 156. The values of a, e and p are then determined by a combined estimation (see Appendix II, p. 203), giving p = 0·2372, a = 0·3636 and e = -0·9189. This value of p, the frequency of chiasma formation/

formation between the loci of N^A and N^X represents 11·86 of crossing over, since, on the chiasmatype hypothesis one chiasma represents 50% of crossing over. Part of the discrepancies of the data are eliminated in the calculation of a and e and little importance is to be attached to the fact that a has approximately its random value of 0·3333. Similarly the negative value of e is of course absurd and indicates a deficiency of one of the classes probably the recessives in this case. The somewhat unsatisfactory nature of the data is also indicated by the high standard error of the crossover percentage, ±17·485%. As calculated by De Winton and Haldane's formula p has the lower value of 8·624%.

The crossover percentage for the genes N^A and N^B, on De Winton and Haldane's formula calculated from the results from the same four progenies is 42%. This value is too large to be reliable and it is difficult to tell from this figure whether the gene N^B is really located in the same chromosome as the N^A and N^X genes.

At present therefore it is only possible to say that linkage between the N^A and N^X genes is fairly close and that the N^C gene is situated in a different chromosome from either of these and the N^B gene. The location of the latter gene can only be decided by further work.

The/

The accompanying table (Table 32) showing the distribution of these four genes and their allelomorphs among 129 named varieties has been drawn up from data collected by Dr. Cockerham. Disregarding for the moment those varieties carrying combinations of the genes N^B, N^A and N^C or their recessive allelomorphs with either the N^X or n^x genes it will be seen that the frequencies of the remaining classes are approximately equal. Thus, of 70 N^A carrying varieties 36 also carry N^B and 34 n^b, 34 carry N^C and 36 n^c, and so on. Now, an examination of the frequencies of those classes which comprise combinations in which N^X or its recessive allelomorph are concerned shows that, whilst the n^xN^A, n^xn^a, n^xN^B, n^xn^b etc. frequencies are approximately equal there is marked irregularity in distribution of the N^X types. On an expectation of equal numbers in all classes a χ^2 test indicates that, with the exception of the N^Xn^b - N^XN^B pair, the frequencies of all the other pairs of combinations in which N^X or its recessive allelomorph are concerned deviate significantly from equality (Table 33). This is to be traced to the low frequency of the N^X gene, as it will be seen that N^X-carrying varieties form the lowest proportion of the total (Table 32). The reason for this low frequency is at present obscure. The linkage data are as yet too scanty to give reliable indications/

TABLE 32.

Frequencies of different gene combinations among 129 named varieties.

	Total	N^X	n^x	N^A	n^a	N^B	n^b	N^C	n^c
N^X	26	-	-	25	1	9	17	19	7
n^x	103	-	-	45	58	53	50	49	54
N^A	70	25	45	-	-	36	34	34	36
n^a	59	1	58	-	-	26	33	34	25
N^B	62	9	53	36	26	-	-	25	37
n^b	67	17	50	34	33	-	-	43	24
N^C	68	19	49	34	34	25	43	-	-
n^c	61	7	54	36	25	37	24	-	-

TABLE 33.

Deviations of frequencies of N^X,n^x combinations from equality.

Combinations	Obs.	Deviation on 1:1 expectation	χ^2_1	P.
$N^X n^a$: $N^X N^A$	1:25	11·08		v. small
$N^X n^b$: $N^X N^B$	17: 9	1·231		0.30-0.20
$N^X n^c$: $N^X N^C$	7:19	2·77		0·10-0·05
$N^X N^A$: $n^x N^A$	25:45	2·857		0·10-0·05
$N^X n^a$: $n^x n^a$	1:58	27·54		v. small
$N^X N^B$: $n^x N^B$	9:53	15·61		v. small
$N^X n^b$: $n^x n^b$	17:50	8·128		0·01
$N^X N^C$: $n^x N^C$	19:49	6·618		0·02-0·01
$N^X n^c$: $n^x n^c$	7:54	18·11		v. small

indications as to the relative viability of the various genotypes but it seems unlikely that the irregularities are due merely to the limited numbers of varieties tested, particularly in view of the even distribution of the other three genes and their allelomorphs.

In conclusion, an interesting point is the discovery so far of only one duplex type, namely Cardinal, duplex for the N^X gene. Now, on back-crossing a simplex tetraploid we expect to get occasional duplex individuals and it seems plausible to assume that Cardinal has been a chance selection of one of these. Had the duplex condition been associated with any visible economic quality such types might well have been encountered in greater frequency.

CYTOLOGY.

At an earlier stage in this account it was deduced on general grounds that the cultivated potato is a tetraploid. This assumption has been fully borne out by the genetical results which, moreover, require for their satisfactory explanation a degree of homology amongst the four sets of chromosomes such as would be expected in an autotetraploid. The next step therefore, was, obviously, to survey the existing accounts of meiosis in Solanum tuberosum L. to see what evidence they afforded this hypothesis. The results were disappointing. Not only is there neither information on the frequency of occurrence of quadrivalents nor on the chiasma frequency in this species, but there are no figures of complete metaphases from which an estimate might be obtained. On some points the various accounts are conflicting and the basic number of the genus Solanum has been queried on the grounds of observation on secondary association.

In an attempt to fill the most important gaps in the information smear preparations were made of pollen mother cells from most of the pollen parents used in the genetical work, together with a few miscellaneous seedlings. Temporary smears were examined in aceto-carmine, whilst permanent smears were/

were fixed in Belling's fluid and stained by Newton's gentian violet method, using a 0·5% solution of gentian violet. Both methods gave moderately good results. Owing to the large number and small size of the chromosomes and consequent crowding of the metaphase plates, however, the writer did not feel sufficiently competent to interpret all the configurations observed. Although quadrivalents were seen in most of the varieties examined, it was found impossible to determine with what frequency they occurred nor were any readily analysable metaphases found. The unsatisfactory nature of these statements requires no emphasis; nevertheless this work did allow of a better assessment of the results claimed by other authors. Where personal observations are able to substantiate points in the following account, mention will be made of the fact.

In the first recorded accounts of mitosis in the potato the somatic number of chromosomes was variously estimated as 36 (Nemec 1899, Muller 1925, Lutman 1926) 34 (Mano 1904) and 14-16 (Young 1922, 1923). Stow (1927) appears to have been the first to establish 48 as the somatic chromosome number from counts in root tip material, and 24 as the haploid number in pollen grains. These counts were confirmed by Fukuda (1927). Levitski and Benetskaja (1927) also counted/

counted approximately 48 chromosomes in somatic cells but confused the issue by claiming that the variation in the number was due to fragmentation of chromosomes. More recent work has left no doubt as to the constancy of 48 as the diploid number of chromosomes in Solanum tuberosum (Heyn 1930, Meurman and Rancken 1932, Longley and Clark 1930). From an examination of their figures it is evident that the low estimates of earlier workers are the result of poor fixation and difficulties of observation. Young's (1922, 1923) exceptionally low counts may, according to Heyn (1930) be the result of mistaking diakinetic stages in pollen mother cells for premeiotic divisions.

In common with other Solanaceous species the somatic chromosomes of the potato are short, varying from 1-3 μ in length (Ellison 1935), having median, submedian or apparently subterminal spindle attachments (Meurman and Rancken 1932, Ellison 1935, (Upcott) in Crane 1936, Šepeleva 1937). Some chromosomes appear to have secondary constrictions but the most permanent feature of the complement is the possession of a pair of satellited chromosomes (Upcott figures two pairs in the variety Langworthy). These were first noticed by Heyn (1930) and have been described by most workers since then, but owing to their small size they might easily escape recognition, as has been generally appreciated./

appreciated.

Noticing size differences between members of the complement Ellison (1935) attempted to correlate varietal differences with the numbers of chromosomes which could be assigned to six arbitrary length classes. The lengths were obtained by measuring camera lucida drawings (x 5000) and found to vary from 14-16 mms (1 mm = 0·2μ). The classes were arranged so that the first was comprised of chromosome figures 4-6 mms in length, the second 6-8 mms and so on. The distribution of chromosomes among the length classes was found to vary among varieties. Certain male fertile varieties had an equal number of chromosomes in each group, from which the conclusion was drawn that pairing may be facilitated by equality in length, leading to reduction in meiotic abnormalities and attendant male sterility. Apart from any other considerations, it is difficult to see what importance can be attached to these observations. Not only are the classes of an entirely arbitrary nature but, on the author's own statements, "since the variation allowed is 0·5-1 mm. certain chromosomes may equally well be classified in one of the adjacent groups". In one variety (Champion), 18 out of 30 chromosomes measured exactly 8 mms and it was uncertain to which group they should be assigned.

Comparing/

Comparing the complements of Langworthy and Up to Date with their russet skinned mutants Golden Wonder and Field Marshal, it was claimed that mutation was accompanied by an increase in the numbers of the longer chromosomes. The material was re-examined by Crane (and Upcott) 1936, and no confirmation of Ellison's results obtained. "Since Golden Wonder is a periclinal chimaera with an inner core of Langworthy, and particularly if only a single layer is involved in constituting the difference between these varieties, the cytological results of Ellison are indeed remarkable" (Crane 1936).

Nevertheless, Ellison's work has made it clear that the potato is remarkably constant in its somatic number, no variants from 48 being found amongst 100 seedlings examined from each of two selfed progenies of Majestic and Flourball, in addition to the named varieties examined. Levitsky and Benetskaja's claims (q.v.) may probably be attributed to the mistaking of satellites and secondarily constricted parts of chromosomes for whole chromosomes.

Recently Šepeleva (1937) has compared the somatic complements of seven species including the cultivated variety Great Scot of S. tuberosum. She finds that approximately the same types of chromosomes are repeated in the different species though the proportions/

proportions of the various types may differ. It is noteworthy that the single European cultivated variety differed in no essentials from the others and that here again only two satellite bearing chromosomes were seen (Figures 30-35).

An argument in favour of the allotetraploid nature of the potato has been put forward , on the assumption that the presence of only two satellited chromosomes indicates the differentiation of the complement into two homologous sets. But it should be noted that a similar interpretation of morphological differentiation within a complement of Zebrina pendula by Darlington (1929b) had later to be abandoned, since the species proved to be a functional diploid. In the absence of evidence to the contrary we must therefore regard the existence of only two satellited chromosomes where four might be expected, as of doubtful significance.

The widespread occurrence of pollen sterility among cultivated potato varieties has interfered with breeding so much, in the matter of choice of parents, that we find quite a wealth of literature dealing with this phenomenon. But, with the exception of Ellison's (1936) paper, very little of this concerns itself with an intimate study of meiosis. Since, therefore, most of the pollen sterility literature has little bearing on/

Plate X.

The somatic complements of some S. American potato species. (From Sepeleva 1937).

Fig. 30. S. Henryi (2n = 24) Commersoniana.

Fig. 31. S. subtilius all. (2n = 24). Glabrescentia.

Fig. 32. S. Vavilovii (2n = 24) Polyadenia.

Plate X.

Fig. 30

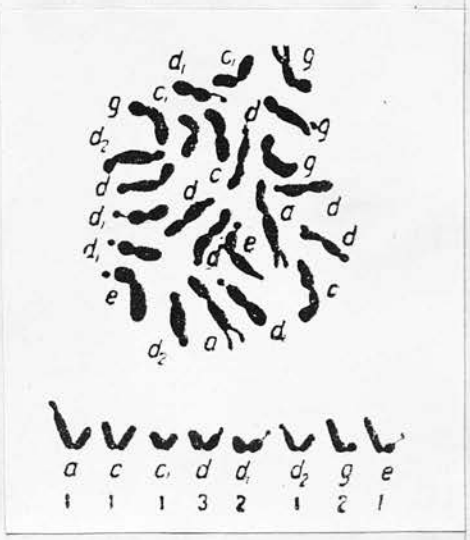


Fig. 31

Fig. 32

Plate XI.

Fig. 33. S. Keselbrenneri (2n = 24) Andigena.

Fig. 34. S. tuberosum L. s. lat. var. Great Scot.

Fig. 35. S. goniocalyx (2n = 24) Andigena.

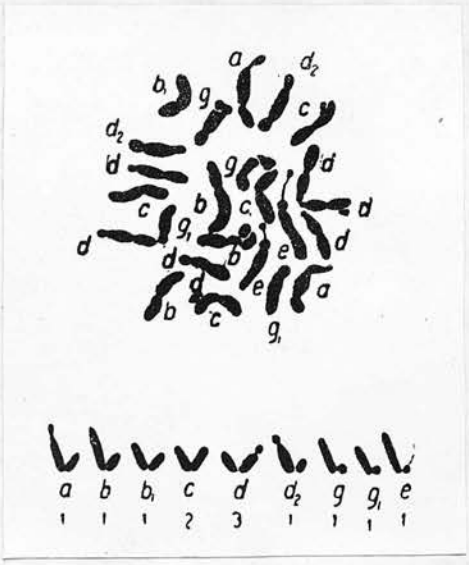


Fig. 33

Fig. 34

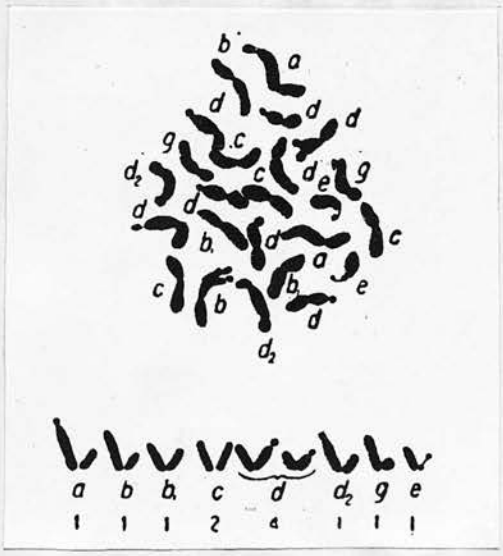


Fig. 35

on the present discussion some of it has been assembled and discussed in a section to be included as an appendix to this paper.

It is well known that the number of potato varieties producing abundant flowers and pollen is surprisingly small and even in the most fertile ones 10-20% of bad pollen is found. Consequently, reduction division in the majority of varieties might be expected to show a greater or lesser degree of irregularity. It has been with the object of correlating any observed irregularities with pollen sterility that most of the cytological investigations have been undertaken.

According to Heyn (1930) serial development of pollen mother cells occurs in an anther; either the youngest stages are at the base, with the oldest at the distal end, or the oldest stages occur in the centre with the youngest at both ends of the anther. All the anthers of one flower do not develop at the same rate. This latter condition was frequently found by the present writer. If one anther of a flower contained pollen mother cells at the right stage it was very seldom that the remaining ones were at the same point of maturation. The contents of any one anther were scarcely ever synchronous in their development.

Little study of prophases has been made owing to the difficulties of observation, and diakinesis appears/

appears to be the first stage at which critical observation is possible. In normal varieties spindle formation takes place quite regularly though multipolar spindles are mentioned by Fukuda (1927) and Heyn (1930).

Earlier workers doubted the authenticity of multivalent associations at diakinesis and first metaphase, any clumping of chromosomes being attributed to fixational effects (Bleier 1931). However, although Meurman and Rancken (1932) state that pairing in most fertile varieties was generally by bivalents they frequently observed multivalents. Examples of tri-, tetra-, penta- and hexa-valents are illustrated by them (Figure 40). Heyn (1930) remarks on the occurrence of trivalents but is unable to reconcile their presence with his assumption that the potato is a diploid. It seems well to mention at this stage that the phenomenon of secondary association, close association of groups of bivalents, assumed to be an expression of homology is widespread among potatoes. Consequently, if observations on multivalents are confined to polar views of metaphase plates it will be extremely difficult to distinguish between true multivalent formation and mere approximation of bivalents. This difficulty is appreciated by Meurman and Rancken (loc. cit.) but they state that, although many of the multivalent groups seen in polar views may be the result of secondary/

secondary association some, at least, are the result of chiasma formation at prophase, as is confirmed by side views of metaphase plates. The following analyses of I metaphase plates are included for what they are worth (Figures 38, 39).

Variety	I	II	III	IV	V	VI
Pepo	5	8	8	6	-	-
Vesijarvi	5	3	3	4	-	2
Deodara	-	-	-	3	-	2

Ellison (1936) assembled the fifty varieties he investigated into maturity groups, describing the peculiarities of male gametogenesis in each group in turn. More reliance can be placed on the statements relating to multivalents in that the observations were made at diakinesis and so are not open to criticism on the ground of confusion with secondary association. He noted a limited occurrence of multivalents among the first early group of varieties, seldom more than twelve chromosomes being so associated. Quadrivalents and hexavalents are claimed to have been frequently seen and trivalents occasionally, though bivalents were the most frequent configurations. Quadrivalents and a single octovalent are recorded from the second early group but the incidence of multivalents was found to be much higher among members of the late maturing group. Two octovalents and a septivalent are illustrated/

illustrated from the variety Sefton Wonder (Figures 41 a-c). In the varieties St. Malo, Field Marshal and Irish Chieftain meiosis was also observed in the ovule. Quadrivalents are recorded from the first of these but multivalents seem to have been absent from Irish Chieftain.

Beyond these somewhat general statements there is no information regarding the exact frequency of multivalents in any one variety. From my own experience I am of the opinion that these descriptions of figures of high valency must be regarded with a certain amount of scepticism. Unless the diakinesis and metaphase plates were exceptionally clear it was often a difficult matter to discern the limits of quadrivalents let alone septi- and octovalents, as a result of the crowding of the plates.

The variability of pairing at metaphase is mentioned by most workers. Thus Meurman and Rancken (1932) found from 8-10 univalents in metaphase plates of some varieties, whilst in others more than half the complement was represented by univalents (Figures 36). Heyn (1930) makes similar mention of up to 10 univalents lying off the equatorial plate. Longley and Clark (1930) go to the extent of classifying the 37 varieties they investigated according to the degree of metaphase pairing. They do not even record the presence/

Plate XII.

Fig. 36. Side views of metaphase I in Great Scot (6), and Deodara (7) and of anaphase I in Vesijärvi (8), Great Scot (9) and Early Rose (10) showing precocious, lagging and dividing (8) univalents.

Fig. 37. Polar views of second metaphase plates with 48 chromosomes from Magnum Bonum, Up to Date, Vesijärvi and Early Rose (19, 20, 21 and 22) showing closely associated and connected groups of chromosomes; 23 shows a dividing restitution nucleus.

Fig. 38. Polar views of I metaphases from Deodara (3) and Great Scot (4) showing multiple chromosome groups. These may be either multivalents or secondarily associated groups of bivalents. Also polar views of second anaphase plates (5a and b) from Pepo showing slight secondary association.

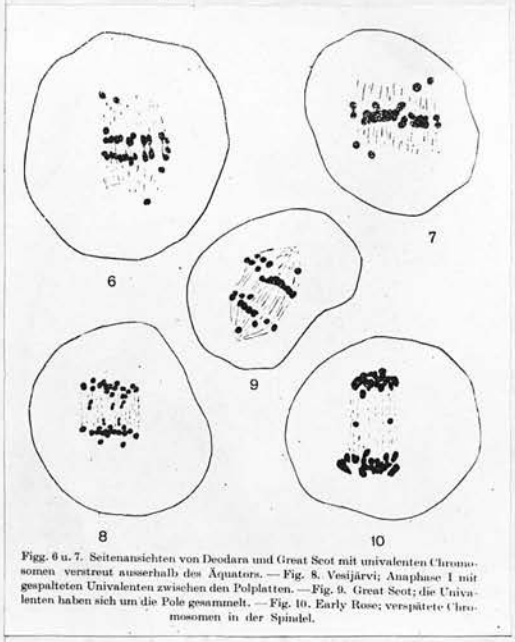
Fig. 39. Polar views of I metaphase plates from Pepo and Vesijärvi showing univalents, bivalents and multivalents.

Fig. 40. Side views of a series of multivalents observed by Meurman and Rancken (1932, Fig. 15).

(Figures 36-40 from Meurman and Rancken 1932).

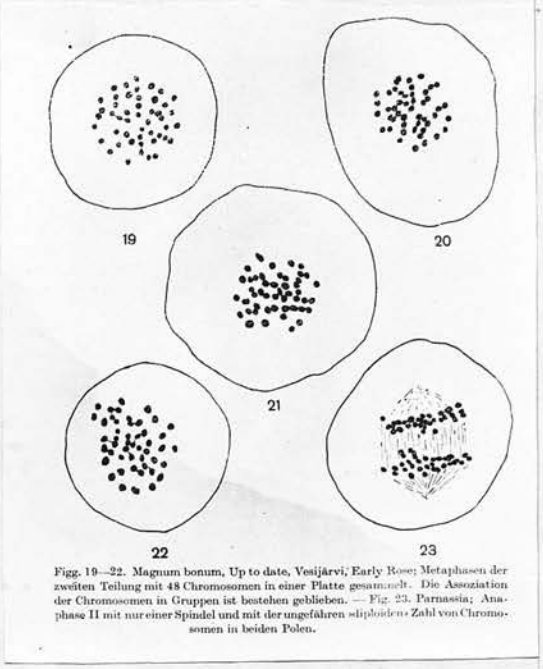
Fig. 41. Diakinetic octovalents, septivalents and sexivalents respectively, observed by Ellison (1936, Figures 17-20) in the varieties Sefton Wonder (russet Great Scot) and Incomer.

Plate XII.



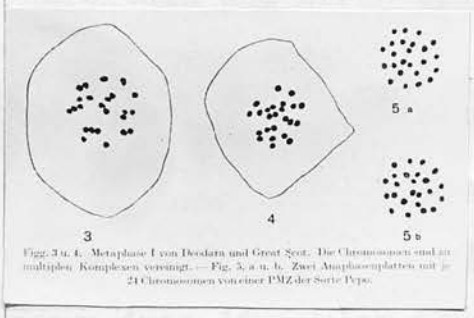
Figg. 6 u. 7. Seitenansichten von Deodara und Great Scot mit univalenten Chromosomen verstreut ausserhalb des Äquators. — Fig. 8. Vesijärvi; Anaphase I mit gespalteten Univalenten zwischen den Polplatten. — Fig. 9. Great Scot; die Univalenten haben sich um die Pole gesammelt. — Fig. 10. Early Rose; verspätete Chromosomen in der Spindel.

Fig. 36



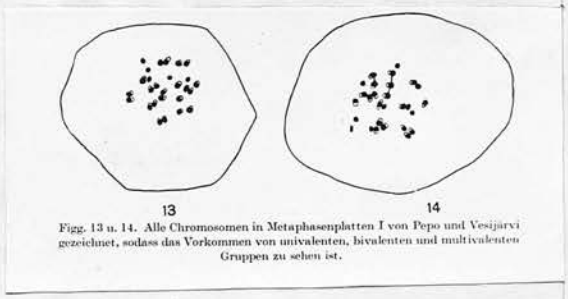
Figg. 19—22. Magnum bonum, Up to date, Vesijärvi, Early Rose; Metaphasen der zweiten Teilung mit 48 Chromosomen in einer Platte gesammelt. Die Assoziation der Chromosomen in Gruppen ist bestehen geblieben. — Fig. 23. Parnassia; Anaphase II mit nur einer Spindel und mit der ungefähren «diploiden» Zahl von Chromosomen in beiden Polen.

Fig. 37



Figg. 3 u. 4. Metaphase I von Deodara und Great Scot. Die Chromosomen sind in multiplen Komplexen vereinigt. — Fig. 5, a u. b. Zwei Anaphasenplatten mit je 24 Chromosomen von einer PMZ der Sorte Pepo.

Fig. 38



Figg. 13 u. 14. Alle Chromosomen in Metaphasenplatten I von Pepo und Vesijärvi gezeichnet, sodass das Vorkommen von univalenten, bivalenten und multivalenten Gruppen zu sehen ist.

Fig. 39

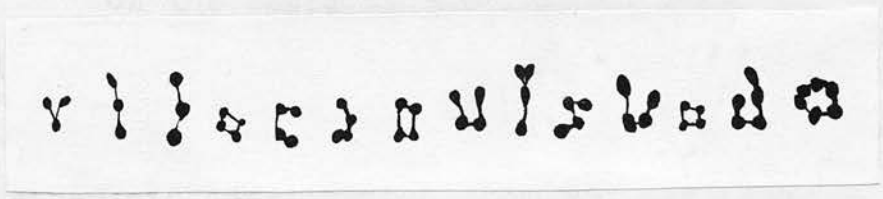


Fig. 40

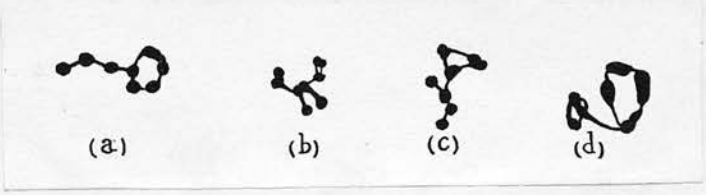


Fig. 41

presence of multivalents.

Consequently the distribution of chromosomes at anaphase would be expected to show some irregularities. Even where a large number of univalents are present, however, they are apparently included in one or other of the interkinesis nuclei, the laggards which become excluded, regarded by Bleier (1931) as being fairly rare, forming micronuclei (Figure 36). Unequal distribution following upon non-disjunction does occur since Ellison (1936) frequently found second metaphase plates showing 23 or 25 chromosomes in Sefton Wonder, Irish Chieftain and Arran Consul. That the gametes formed from such nuclei are non-functional is shown by the absence of aneuploid plants.

Scanty though the foregoing data are, some useful conclusions may perhaps be drawn by bringing them into line with the existing information on polyploid behaviour.

On the basis of the observations of sexi-, septi- and octovalents it has been inferred that the cultivated potato must be an octoploid, since a simple autotetraploid constitution does not permit of such configurations being formed (Ellison 1936). Our problem is therefore to see if the evidence really does support this conclusion.

First/

First of all, one or two general statements may be made regarding multivalent association of chromosomes. Multivalent formation in a polyploid depends on:-

(a) changes of partner taking place among the paired homologues, and

(b) the number of chiasmata formed in proportion to the length paired at pachytene.

In illustration of the second point the case of triploid Hyacinthus (Darlington 1937, Stone and Mather 1932) may be quoted, where there are three length types of chromosomes. The chiasma frequencies are 6·2, 3·6 and 2·0 respectively and each type has a characteristic frequency of univalent formation, viz. 2·8, 0·1 and 11·9% respectively. The high percentage of univalents amongst the short chromosome type is explained by the low chiasma frequency. The unexpectedly high proportion of univalents in the long chromosome class is attributed to the "interference with free assortment resulting from their extreme length" (Darlington 1937). The chromosome length-chiasma frequency relationship will therefore be a critical one for short chromosome species (see Mather 1937, 1940).

Since the chromosomes pair in two's at pachytene the odd chromosome in triploids will often tend to be left out of association. This results in a/

a higher proportion of univalents than in tetraploids where association of two chromosomes along their length leads to the replacement of a quadrivalent by two bivalents (Tables 34 and 35). But, assuming the chiasma frequencies to be the same in each the number of trivalents in the triploid will be greater than the number of quadrivalents in the tetraploid, since fewer chiasmata are required per configuration. Even in autopolyploids then association will never be complete as the following comparisons testify. Comparing the percentage of potential multivalents formed with the chiasma frequency where this is known (Table 36) we find that, as a rule, high chiasma frequency is correlated with a high percentage of multivalents, as condition (b) above would imply.

Lamm (1938) estimated the chiasma frequency in each of a pair of twin seedlings which arose in the progeny of an F_1 hybrid between the triploid Solanum chaucha and a cultivated variety of S. tuberosum. Of these, one was a diploid and the other a tetraploid, the chiasma frequencies being 1·22 and 1·27 respectively. Quadrivalents were observed in the tetraploid plant but unfortunately, owing to its hybrid nature the chiasma frequency cannot be regarded as representative of S. tuberosum itself. A chiasma frequency of 1·27 allows of six to seven two-chiasma bivalents and hence of three quadrivalents being formed per nucleus. If

the /

TABLE 34

The extent of quadrivalent formation in various autotetraploids.

Species	Numbers of cells with different numbers of quadrivalents													Xmta freq.	
	0	1	2	3	4	5	6	7	8	9	10	11	12		
Primula sinensis	-	-	-	-	-	-	-	-	-	1	11	9	-	1·7±	Darlington, 1931
Imp. Fertility Pear	-	-	-	1	2	2	-	4	4	3	1	2	1	-	Crane & Thomas, 1939
Solanum Lycopersicum	-	3	12	10	2	15	6	-	2	-	-	-	-	1·56	Upcott, 1935
Brassica oleracea	-	-	1	2	3	4	-	-	-	-	-	-	-	1·83	Howard, 1939
Kniphofia Nelsonii	-	-	2	4	4	-	1	-	-	-	-	-	-	1·64	Moffett, 1932

TABLE 35.

Numbers of cells with univalents in diploid and tetraploid Kniphofia (Moffett 1932).

		Chiasma frequency	Cells with different numbers of univalents			
			0	1	2	3
Kniphofia Nelsonii	2n	1·8		0	0	0
do.	4n	1·64	64	9	4	1

TABLE 36

The chiasma frequencies, extent of quadrivalent formation and chromosome length in various autotetraploids.

Species	Chiasma frequency	Percent. of potential quadrivs formed/cell	Number of quadrivs per cell	Chromosome length in	Authors
Primula sinensis	1·7±	90·0	10·4	1-3	Darlington, 1931
Kniphofia Nelsonii	1·64	57·5	3·5	7-11	Moffett, 1932
Tulipa turkestanica (2)	1·59	48·7	5·8	-	Upcott, 1939
Brassica oleracea	1·83	44·4	4·0	1·2 - 3·1	Howard, 1939
Solanum Lycopersicum	1·56	31·4	3·8	1·7 av.	Upcott, 1935
Allium Schoenoprasum	1·09	12·5-50·0	c.2·0(1·4)	3-7	Levan, 1936
Paeonia officinalis	1·5	11·9	-	-	Dark, 1936
Tulipa chrysantha	1·14	3·45	0·0	)	
T. Korolkowi	1·36	1·26	0·05	)	
T. turkestanica (1)	1·36	20·05	2·4	)	
T. turkestanica (2)	1·59	48·7	5·8	)	Upcott, 1939
T. stellata	1·45	7·14	0·7	)	
T. Whittalli	1·69	37·3	4·1*	)	
T. sylvestris	2·03	32·7	3·5	)	
T. Clusiana	1·66	-	1·8*	)	

* Including higher configurations.

the chiasma frequency of S. tuberosum L. itself proves to be in the neighbourhood of 1·27 the requirements of the breeding results will be fully met.

Two of the octovalents illustrated by Ellison (1936) (Figures 41a & b) require an average of 2·0 and 1·75 ½ chiasmata per chromosome for their formation; the septivalent (Figure 41c) and sexivalent (Figure 41d) both require 2·0 ½ chiasmata per chromosome. If the potato is really an octoploid the chiasma frequency must then be considerably higher than 1·27. But if this is so we might expect a higher average of quadrivalents per cell than the observations and figures in the literature would suggest. The alternative is the suggestion that these complex multivalents are the result of structural hybridity as in fact Meurman and Rancken (1932) have postulated. Structural hybridity within a tetraploid leading to the formation of complex rings of 6 and 7 up to 10 chromosomes was described by Meurman (1929b) in Aucuba japonica. Had the chromosomes not been morphologically differentiated it would have been an extremely difficult case to elucidate. Ellison (1936) figures a quadrivalent from the variety Arran Comrade whose constituent pairs are unequal in size which, he suggests, may be the result of segmental interchange having occurred. Propach's (1938a) observations of chromatin bridges in the hybrid S. acaule x S. Antipoviczii are perhaps of more significance in showing/

showing that structural hybridity does occur among potato species.

In the tetraploid Tulipa sylvestris, Upcott (1939a) found an association of eight at pachytene resulting from an interchange between two non-homologous chromosomes. But even with a frequency of 2·03 ½ chiasmata per chromosome chiasmata failed to form in the short paired segments and the average number of quadrivalents per cell at metaphase was only 3·5.

In species with short chromosomes and median or sub-median centromeres, such as Solanum Lycopersicum, S. tuberosum and Brassica oleracea, we should expect those types of quadrivalents which require the formation of several chiasmata in one arm to be rarely or never found. This is actually so, as the distributions in Table 37 show; types 19 and 20 (see Figure 26) are absent from S. Lycopersicum and types 12, 13, 19 and 20 from Brassica. Solanum tuberosum seems to fall into line here; examples of types 11, 16 and 17 can be identified in Ellison's (1936) figures and the quadrivalents observed by the writer were all of the chain and ring type (types 11 and 17). The predominance of symmetrical arrangements in S. Lycopersicum may account for the rarity of trivalents in the tetraploid, since these imply the existence of an asymmetrical/

TABLE 37.

The frequencies of certain types of quadrivalents in autotetraploids.

Species	∩∪	Y	X	◇	-O-	O--	⊂⊃	∞
Brassica oleracea	35·0%	-	-	55·0%	2·5%	2·5%	2·5%	2·5%
Sol. Lycopersicum	34·7%	6·7%	1·5%	51·3%	1·5%	2·1%	2·1%	-
Sol. tuberosum	?	-	-	?	-	?	-	-
Paeonia officinalis	16·2%	8·1%	2·7%	5·4%	-	8·1%	13·5%	5·4%

asymmetrical arrangement at prophase (Upcott 1935). If, as several workers have reported (Heyn 1930, Meurman and Rancken 1932, Ellison 1936) trivalents are common in the tetraploid potato the condition is an interesting one.

Ivanovskaja (1939) studied meiosis in a haploid seedling resulting from a cross S. tuberosum L. var. Aurora x S. Rybinii (2n = 24). In morphological features the haploid resembled the tetraploid more closely, showing none of the characters of the male parent, S. Rybinii, from which it seems legitimate to conclude that it arose by the parthenogenetic development of a reduced egg of S. tuberosum. The meiotic behaviour of the haploid is especially interesting in that 11-12 bivalents were formed, some with two chiasmata, and chromatin bridges were seen in about 7% of cells. Reduced (n = 12) and unreduced (n = 24) pollen grains were observed at maturity, and crosses of the haploid with other varieties of S. tuberosum showed that only these unreduced grains were functional.

From the sterility of the haploid gametes and the regular pairing at metaphase Ivanovskaja (1939) concludes that the potato must be an amphidiploid, the two original parental sets of chromosomes being sufficiently alike to pair regularly, as in Primula kewensis/

kewensis (Newton and Pellew 1929). But it is obvious that the behaviour of the haploid is equally in favour of autopolyploidy. Structural hybridity sufficient to produce 7% of bridges at anaphase might easily inhibit the production of viable haploid gametes. Morphologically the polyhaploid plant shows many of the quantitative differences which we are accustomed to associate with autopolyploidy. Thus, the leaves are thinner, less pubescent, differently dissected, the flowers smaller, and the plant less tall than the tetraploid. That allopolyploids may simulate autopolyploids in the matter of gigantism is illustrated by the wild allotetraploid watercress (Manton 1934, Manton and Howard 1940) so that gigantism in itself is not a reliable criterion of autopolyploidy.

Some very interesting results have emerged from Upcott's recent studies on polyploids (1937, 1939 a and b). The polyploid tulips show striking differences in the number of multivalents formed per cell. In the triploids the chromosomes are nearly all associated in threes, whilst in the tetraploids they are mostly in pairs (1939a). This is the result of a higher chiasma frequency in the triploids and a higher number of changes of partner at pachytene. Change of partner tends to reduce pairing and this factor will be of greater importance in triploids than in tetraploids.

Now, /

Now, a comparison of the chiasma frequencies of the triploid and tetraploid tulips shows that the average value of the tetraploids is lower than that of the diploids, whilst the value for the triploids is higher. If chiasmata are formed in proportion to the length of chromosome paired at pachytene, since this paired length is expected to be the same in diploids and triploids, the chiasma frequencies of the triploids are appreciably higher than those of the diploids. From which the inference is drawn that "the number of chiasmata formed in the triploids is proportional to the number of chromosomes and not to the amount of pachytene pairing" (Upcott 1939a). This important conclusion is supported by the behaviour of homozygous triploid Solanum Lycopersicum and Primula sinensis (Upcott 1935).

On comparing the chiasma frequencies of a number of tetraploids with those of the parental diploids Upcott (1939b) found the tetraploids had a consistently lower value. The reduction factor varied from 0·97-0·77. This reduction proved to be independent of the type of polyploidy, auto- or allo-, but did seem to be influenced by the chromosome size-nuclear volume relationship. Its significance is not clear.

Compared with their diploid relatives, autotetraploids generally show a higher incidence of sterility, varying/

varying in extent from one species to another. Thus, autotetraploid Solanum Lycopersicum has about 25% of bad pollen (Jørgensen 1928) and 20% of seeds formed compared with the diploid (Sansome 1933) whilst autotetraploid Brassica oleracea has only about 4% of bad pollen and 35% of the seed production of the diploid (Howard 1939).

As a rule autotriploids are more sterile than either diploids or autotetraploids, but Solanum nigrum and autotetraploid rape are anomalous in this respect (Table 38). The non-viable pollen produced by autopolyploids results principally from unequal segregation of multivalents giving both numerically and genetically unbalanced gametes. Hence, in a wild sexually reproducing tetraploid a low proportion of multivalents per cell and consequently a low chiasma frequency will be selected for. This we find strikingly illustrated by the polyploid tulips where the tetraploids are sexually reproducing and triploids asexual. In the tetraploid T. chrysantha with a frequency of 1·14 ½ chiasmata per chromosome no quadrivalents are formed and the only indications of its polyploid nature are the presence of occasional trivalents and pairs of univalents. In one strain of T. turkestanica however, about half the potential number of quadrivalents per cell is formed. The greater fertility of allopolyploids is usually regarded/

TABLE 38.

The fertility of certain experimental autotetraploids.

Species	Constitution	% good pollen	Seeds/fruit	No. of diploid seeds produced
Sol. Lycopersicum (Jørgensen 1928)	2n	95	80-100	
	3n	14	-	
	4n	75	10-40	33·3%
S. Lycopersicum (Upcott 1935)	4n	75	-	20·0%
S. nigrum (Jørgensen 1928)	2n	90	40-50	
	3n	37	5-8	14·7%
	4n	12	10-15	27·8%
Brassica oleracea (Howard 1939)	4n	96		c. 35·0%
B. napus (Morinaga & Kuriyama 1937)	2n	-	13·5	
	3n	-	3·9	28·9%
	4n	-	0·5	3·7%
Datura stramonium (Blakeslee, ~~Belling~~ & Cartledge 1926)	2n	99		
	3n	56		
	4n	95		

regarded as one means of distinguishing these from autopolyploids. But it is clear that the conditions of experimental production of autopolyploids are not those of the wild. Sterility is obviously a detrimental character and will be selected against, one means of escape being, as we have seen, reduction of the chiasma frequency. Relative fertility and the extent of metaphase pairing then become very little guide to the auto- or allopolyploid nature of a species.

An interkinesis of some length follows first division in the potato, during which a nuclear membrane and nucleoli may be organised (Heyn 1930). During this stage some of the chromosomes seem to remain connected with one another by fine chromatin threads; Ellison (1936) figures examples from the varieties St. Malo, Irish Queen and Katie Glover. These connections are also said to persist to second metaphase in the variety Sefton Wonder. They appear also in Müntzing's (1933) figures of second metaphases (Figures

The significance of the phenomenon is not at all clear. It was also found in the high polyploid (2n = c.180) Prunus laurocerasus by Meurman (1929a) where complex multivalents involving up to ten chromosomes were of frequent occurrence at first metaphase. These interkinetic figures were attributed to persistence of metaphase chiasmata following upon non-disjunction,

or/

or to secondary association. The presence of these connections is not a criterion of secondary association (see below) but this was, at that time, thought to be due to some type of chiasma formation, though in most cases the connections were too fine to be seen. The persistence of metaphase association of chromosomes to second division has been recorded in Aucuba by Meurman (1929b) and in Brassica oleracea by Howard (1939) but this explanation seems unlikely in the case of Prunus and the potato where terminalisation is complete at metaphase. I am inclined to think that the phenomenon is a fixational effect.

With the onset of second division, the chromosomes of the two interphase nuclei arrange themselves on the equators of two spindles whose axes are typically at right angles to one another but may take up any position between this and lying in the same plane with one another. If the metaphase plates are observed in polar view the chromosomes are seen to be associated in two's or larger groups (Figures

This phenomenon has already been mentioned in connection with first metaphase figures where it is only distinguishable with difficulty from true multivalent formation under certain conditions. To it the name secondary association was given by Darlington (1928) and most workers have regarded it as an expression of homology between/

between the chromosomes so associated. The present writer is not in entire agreement with this interpretation and, since the acceptance or rejection of its validity is of considerable importance in determining structural relationships of polyploids it will be discussed at a later stage. Tetrad formation occurs in the usual manner. Where micronuclei are present these also become rounded off and the pollen mother cells often contain three or four microcytes in addition to the tetrad of pollen grains.

This course of events is by no means the rule, since disturbances of various kinds intervene to reduce the potential fertility. Degeneration of the pollen mother cells may apparently take place at any stage though may not involve all the cells of an anther (Bleier 1931). In the variety Katie Glover Ellison (1936) describes cohesion of the prophase chromosomes followed by their dispersion through the cell at the ensuing anaphase and the thickening of the mother cell wall resulting in monad formation. Heyn (1930) describes similar abnormalities.

By far the most frequent abnormalities are those which result in the production of pollen grains with the somatic number of chromosomes (Figure 37). The principal causes are restitution nucleus formation and the fusion of second division spindles. Rosenberg (1927)/

(1927) coined the term restitution nucleus to describe certain types of diploid gamete formation in parthenogenetic Hieracia. Following a "semi-heterotypic" division, in which all homologous chromosomes failed to pair the irregular anaphase distribution was "interrupted by a premature homotypic division"; the entire spindle figure became surrounded by a nuclear membrane resulting in the production of a single large nucleus (the H. levigatum type). A second type was described from H. pseudo-illyricum where, at the commencement of I division a contraction phase intervened during which the chromosomes divided. Normal second division followed in both cases and the result was the production of gametes with the diploid number of chromosomes. Ellison (1936) describes a case of diad formation in the variety Arran Crest where a collapse of the spindle mechanism was responsible for restitution nucleus formation. Bleier (1931) correctly attributes the production of diads to this cause. Meurman and Rancken (1932) however, consider the fusion of second division spindles to be a more important mode of origin for giant pollen grains. The second division spindles may, as we have seen, lie at any angle with respect to one another, and where they both lie in the same plane inclusion of the two groups at one pole into one nucleus might easily follow. The/

The result will be the production of diads as before. Where the two spindles lie in the same plane but at an angle to one another triads may result from the inclusion of two of the anaphase groups into a single nucleus. Bleier (1931) describes and figures examples of both these types (cf. also Müntzing 1933). Larger grains with 96 chromosomes are described by Ellison (1935, 1936), and their origin is ascribed to the failure of wall formation to separate the four products of meiosis, as occurs also in Kniphofia (Moffett 1932). Again, following restitution nucleus formation the second division sometimes fails, the chromosomes, after division becoming irregularly grouped into several nuclei (cf. Prunus laurocerasus Meurman 1929).

The production of diads, following restitution nucleus formation has been described in Digitalis hybrids (Buxton and Newton 1928) Raphano-Brassica (Karpechenko 1927) Allium carinatum and A. oleraceum (Levan 1933) and Prunus spp. (Darlington 1930a), following spindle fusion also in Prunus avium vars. by Darlington (1930a). It now seems fairly certain that the frequency of diploid pollen grain production is determined by environmental influences. Buxton and Newton (loc. cit.) concluded as much from the distribution of fertile capsules on the spikes of their F_1 hybrids. Levan (loc. cit.), in a series of 25 fixations/

fixations from a single plant of the tetraploid Allium oleraceum made over a period of fourteen days, found the number of giant grains to vary from 0 to 51. Bleier (1931) found normal and giant grains within the same anthers of various potato varieties and concluded that their production was influenced by the environment. He fixed material from a series of plants of one variety kept for six hours at temperatures of -3°, +24°, +26° and +32°C. but was unable to detect any differences in the frequency of diad production. Kuwada's (1937) statement that the arresting of chromosome movement to the poles resulting in restitution nucleus formation is determined by an abnormal dehydration of the protoplasm perhaps suggest how these environmental influences act. In another connection Stow (1927) claimed that high temperatures influenced meiosis but his results were not confirmed by Heyn (1930) or Bleier (1931).

Most of the earlier workers on the potato (Stow 1927, Fukuda 1927, Heyn 1930, Longley and Clark 1930) assumed that diploid pollen grains resulted from the omission of one of the meiotic divisions. Smear preparations would be most liable to this misinterpretation since the previous history of the observed cells is unknown. Heyn (1930) thought that diploid grains might also arise from the collapse of the second division/

division spindle, while it is clear that Longley and Clark (1930) regard the presence of such grains as indicative of the omission of one meiotic division for which they suggest lack of harmony among the chromosomes to be responsible. Smith (1927) observing normal and diploid grains in different plants of one and the same variety decided that diploid and tetraploid (i.e. 2n = 96) races of cultivated varieties existed. This was easily disproved by root tip counts which always showed the somatic number to be 48.

Thus, at maturity most varieties will produce a very mixed output of pollen. Not only will there be microcytes and unbalanced grains with 23-25 chromosomes, but there will be a large proportion of diploid monads and diads together with normal haploid grains. Such differences in chromosome content are expressed by the variations found in pollen grain size. Heyn (loc. cit) found the diameters to vary from 17·57 to 25 . The curious fact about these diploid grains is that they are evidently non-functional, in marked contrast to those found in Brassica, Digitalis and Kniphofia. On numerous occasions such grains must have been used in pollination yet hexaploid or octoploid types have never arisen. The production of octoploids from tetraploid cultivated potatoes by treatment of the seeds with colchicine (Johnstone 1939) has shown that such plants are viable, if not fertile. Likewise,/

Likewise, in the absence of aneuploid plants (Ellison 1935) we are forced to conclude that selection against unbalanced gametes is equally severe.

It is clear that abnormal pollen-grain production is in a large measure responsible for reducing the pollen fertility of such varieties as reach this stage of development. The percentage of normally appearing tetrads was used by Meurman and Rancken (1932) as an index of the fertility of the varieties they investigated. The most fertile group produced 90% or more, the least fertile only 10%, the remainder consisting of atrophied grains, monads and diads. The classifications of other workers (Stow 1927, Fukuda 1927, Longley and Clark 1930) are essentially the same.

Again, some varieties, such as British Queen seem to produce abundant pollen, yet these are rarely successful as pollen parents. The pollen of British Queen on examination by Ellison (1936) was found to consist mostly of monads and diads, suggesting that only the haploid grains are functional. In addition to a number of continental varieties Bleier (1931) cites Great Scot and Arran Crest as producing diad grains only, which Ellison (1936) confirms in a list which includes the varieties Golden Wonder, Golden Marvel, St. Malo, Up to Date and Langworthy. Since the/

the potato appears to be particularly sensitive to changes in photoperiod it is suggested (see Appendix I) that these meiotic abnormalities may be in part an expression of the same causes which lead to poor production or complete supression of flowers.

There is less information about the incidence of female sterility, though there is clear evidence that it does exist (v. infra, Stout and Clark 1924). Degeneration may apparently occur at any stage during the development of the macrogametophyte (Rees-Leonard 1935), from the time of the meiotic division to the completion of embryo sac formation. Nothing in the nature of restitution or micro-nuclei have been observed but the above author and Ellison (1936) are agreed that there is a correlation between the extent of irregularities occurring in meiosis on the male and female sides.

An interesting consequence of the degeneration of the products of the first meiotic division is the development of the other into two macrospores, a single one usually developing (Rees-Leonard 1935). This may be one of the ways in which twin embryo sacs and, on fertilisation, twin seedlings arise. These are, however, usually associated with poly-embryony and such twin embryo sacs have been observed in the potato by several investigators (Young 1922, Lamm 1937a, 1938, Kausche 1937). Their importance lies in the possibility/

possibility of one member of such a twin developing into a haploid, following parthenogenetic development of one egg cell stimulated by the fertilisation of the other (Müntzing 1937). Several progenies from crossed and selfed named varieties have been germinated in a search for such haploid seedlings but so far without success. Lamm (1937) successfully raised two pairs of seedlings from up to Date; in one case both were tetraploid (2n = 48) but in the other one was tetraploid and the other diploid (2n = 24). Fuller details are given by him (1938) of the polyhaploid twin from the S. chaucha x tuberosum hybrid already discussed in another connection. Compared with the tetraploid the diploid plant was much more slender, the leaves were thinner and more tapering, and the pollen grains and stomata smaller, agreeing in most respects with most other diploid potatoes. A comparison of Lamm's measurements of leaf thickness, the length-breadth index of apical leaflets, size of stomata and diameter of pollen grains in the diploid and tetraploid shows that the differences are all highly significant. In the original only the difference between sizes of stomata is so indicated (Table 39). Ivanovskaja's (1939) haploid from the cultivated variety Aurora was evidently the result of parthenogenetic development too, stimulated by pollination with S. Rybinii pollen. Here also the plant was more branched and slender than the tetraploid/

TABLE 39.

Comparison of morphological features of a pair of twin seedlings from a tetraploid potato hybrid (Lamm, 1938).

	Somatic chromo. number	Number counted	M ± m	Coeff. of variability	$\frac{M_1-M_2}{E_1^2+E_2^2}$
Thickness of leaves in mm.	24	15	0·35±0·018	20·3	3·72
	48	15	0·45±0·020	17·6	
Length-breadth index	24	15	2·04±0·039	7·5	12·72
	48	15	1·39±0·033	9·1	
Size of stomata	24	50	14·86±0·386	18·4*	14·57
	48	50	22·65±0·370	11·6	
Diameter of pollen grains	24	50	21·91±0·246	7·9	8·02
	48	50	24·75±0·255	7·3	

* = difference significant.

tetraploid plant, the leaves thinner and more dissected, the tubers smaller and the petals more delicate. Now these are just the kinds of quantitative differences by which we distinguish autotetraploids from their parent diploids (Müntzing 1936). So far the cytological evidence is indecisive. The only indication that the cultivated potato is an allopolyploid is the presence of only a single pair of satellited chromosomes where two pairs might be expected if it were an autopolyploid. The pairing behaviour and the sterility of the polyhaploid supports an allo- or autopolyploid interpretation equally well. The genetic evidence demands that there shall be at least two sets of four chromosomes sufficiently homologous to form quadrivalents and hence that at least two chromosomes in the two complements of the allopolyploid be homologous.

In interpreting the genetical data it has been assumed that the potato is an autotetraploid. Ellison's (1936) suggestion that it is really an octoploid must be considered doubtful, both by reason of the probable low chiasma frequency and the absence of confirmatory evidence. That extensive homology exists among the four sets of S. tuberosum chromosomes must be concluded from the fact that quadrivalents do occur, sometimes as many as three or four per/

per cell. Several tetraploid tulip species have been proved to be autotetraploids from their frequencies of inversion crossing over yet from the low numbers or even absence of quadrivalents one might easily conclude that they were allopolyploids (Upcott 1939a). The reason being that the tetraploid tulips are sexually reproducing and consequently fertility has been attained at the expense of reduction of the chiasma frequency and consequent suppression of multivalent formation.

SECONDARY ASSOCIATION.

The theory of the octoploid nature of the potato rests on other grounds than the observations of high multivalents at metaphase I. Several workers have seen in the extent of the phenomenon of secondary association of chromosomes proof that the somatic complement of 48 is made up of more than four homologous sets of chromosomes. The principal criterion of secondary association of similar size and configuration at first and second metaphases of meiosis which, it is assumed indicates homology of the chromosomes so paired. This attractive hypothesis, extremely useful if it were reliable, on closer scrutiny has been found wanting, at least so far as the family Solanaceae is concerned.

The literature on secondary association is extensive and no attempt has been made to discuss it exhaustively. An endeavour has, however, been made to present a summary of the most important evidence and to indicate where the existing theories fail to explain satisfactorily the discrepancies mentioned above. I am aware that criticism may be justly levelled on the ground of unfamiliarity with the material, but, as a geneticist, I have attempted to assess the evidence for an important genetical proposition as impartially as possible.

The/

The close approximation of morphologically similar chromosomes in pairs was noticed so long ago as 1905 by Strasburger in embryonic nuclei of Galtonia and Funkia. Many other workers (v. Metz 1916) recorded similar observations but Kuwada (1910) and Ishikawa (1911) were the first to report similar pairing among chromosomes at both meiotic divisions in Oryza and Dahlia respectively. In his Prunus studies Darlington (1928, 1930a) again observed this phenomenon which he termed secondary association. This approximation of bivalents was recognised as not being "the development or continuation of a prophase relationship". Since in these first investigations secondary association was always observed in conjunction with multivalent formation at metaphase I difficulties of observation in the small chromosome Prunus and Pyrus species and incomplete knowledge of the prophase pairing conditions led to the assumption that it was not distinct from multivalent formation, the apparent absence of material connections being attributed to the extreme fineness of the connecting strands (Darlington 1928, 1930a, Darlington and Moffett 1930, Meurman 1929a).

Principally from his work on Dahlia species Lawrence (1931a,b,c) established the reality of secondary association as a separate phenomenon and developed the theory that this association was between homologous or/

or partly homologous chromosomes. Secondary association is characterised by the following features:-

(a) It is a post-synaptic phenomenon and does not affect segregation. It consists in a differential approximation of the bivalents in the equatorial plane, appearing at first and second meiotic metaphases.

(b) The bivalents or univalents so associated are similar in size and configuration, i.e. number of chiasmata per chromosome. They lie with varying degrees of closeness to one another but are not connected together as are the members of a multivalent association. The liability of confusion of secondary association with true multivalent formation in small chromosome species makes it imperative that only counts from second metaphases shall be regarded as critical evidence, except in such cases where multivalents are known not to occur, as in Brassica oleracea (Catcheside 1937).

(c) Secondary association is confined to polyploid species with small chromosomes.

The hypothesis is that "secondary association arises from the random approach of homologous chromosomes at pro-metaphase which then remain in juxtaposition until they are dispersed at the next repulsion phase. In many plants this second repulsion phase is interkinesis, but where this stage is short a certain/

certain proportion of association survives until the second division" (Lawrence 1931b).

Thus the frequency and size of secondarily associated groups should indicate the extent of reduplication of individual chromosomes in aneuploids and of whole sets of chromosomes in polyploids. By this means far reaching conclusions have been reached regarding the basic chromosome numbers of certain species and genera. These are of fundamental importance particularly in genera such as Solanum where species having the supposed basic number as their haploid complement no longer exist. Nevertheless a certain amount of evidence has accumulated which seems to disagree with Lawrence's interpretation (1931b) in this case inevitably leading to a questioning of the status of the theory as a whole.

Dealing first with the observations on wild and cultivated potatoes, any kind of association of chromosomes was regarded by earlier workers as a fixational effect but in recent years Müntzing (1933) Ellison (1936) and Propach (1937a) have made a special study of the phenomenon of secondary association.

Müntzing counted the number of secondarily associated chromosomes in second metaphase plates of diploid, triploid and tetraploid "Solanum tuberosum". It is not clear what is implied by the designation

S./

S. tuberosum here since diploid and triploid forms of S. tuberosum L. s. str. are unknown, nor have any members of the Eutuberosa been found in the Lake Titicaca region whence the material is stated to have come. Most probably the plants belonged to the Andigena group. Comparing these counts he found that association in groups of two was characteristic of the diploid, in groups of three of the triploid and in groups of four of the tetraploid. From which he concluded that the basic number of the genus is really 6. However, in every case his data show that associated groups of greater magnitude were observed which are not accounted for by this simple assumption but require the postulation of extensive structural hybridity within the complement if secondary association is governed by homology. Thus groups of 3, 4 and 5 chromosomes are recorded from the diploid, of 4 and 5 from the triploid and 5, 6, 7 and 8 from the tetraploid. In the tetraploid "in three cases even as many as 10, 11 and 15 respectively, were apparently connected". In spite of the affirmations that fixation was good several of the figures of triploid and tetraploid second metaphases show groups of chromosomes (up to 3 only in the former) joined apparently by chromatin threads (Figures 29b, 30a and b, 38 a and b, 40). Since "the occurrence of /

of persistent connections (from metaphase I) is definitely impossible with regard to the triploid Solanum tuberosum variety" it is difficult to see how these supposed threads can have arisen if they are not the result of fixation. A similar qualification must apply to the tetraploid plates showing connected groups of 10, 11 and 15 chromosomes since metaphase configurations of this magnitude have never been observed in the potato. Moreover it is clear that these connected groups have been counted as secondary associations and, consequently, I strongly doubt their validity as indicators of homology of the chromosomes concerned (on this point see also Meurman's figures in Prunus discussed above, and Figure 37).

Ellison (1936) came to the same conclusion as Müntzing after studying secondary association in the second metaphase plates of cultivated varieties. An analysis of 26 such plates showed the maximum association to be in groups of two whilst Müntzing found the highest percentage to be of single chromosomes in the tetraploid. Also in contrast to the latter author, the highest group observed was one of five chromosomes and this occurred only once. (Two discrepancies must be pointed out in Ellison's Table I (p. 243) in which the details of the counts are set out. If the total number of chromosomes is computed for each plate from the number of different associations recorded it is found/

found that this varies from 20 to 26; no mention is made of this fact. Secondly, the total number of groups of two for the 26 plates is 165 not 175 as shown, making the total number of chromosomes associated in groups of two 330 instead of 350. This slightly alters the percentage value for each chromosome group, but not significantly so.)

Groups of	Ellison (corrected values)	Müntzing
1	24%	32%
2	55%	20%
3	14%	15%
4	11%	13%
Higher	-	20%

In an analysis of 12 metaphase plates having 48 chromosomes, whether I or II metaphase is not stated, the percentage of chromosomes in groups of 3 and 4 was found to be higher than in the second division metaphases examined. Figures of second metaphase plates showing a number of chromosomes connected together by chromatin threads are given and Ellison accepts Meurman's interpretation (1929) that they are persistent I metaphase chiasmata. The size of these groups does not here exceed the expectations of this hypothesis, — the highest numbered group figured is one of four chromosomes which might conceivably arise from the disjunction of an octovalent. I am bound, however, to assume that the same objections apply here as were raised against Müntzing's findings. Again there is

no/

no means of knowing how often these connected groups have been counted as secondary associations.

Since both these authors use their data from secondary association counts as an argument for the basic number of Solanum being 6 and not 12 the evidence on this count can not be regarded as particularly convincing.

Secondary association was examined in several wild potato species by Propach (1937a). Particular care was taken in the matter of fixation and the counts were only made in the best fixed preparations. One hundred second metaphase plates were examined in each of the following:- S. chacoense Bitt. (2n = 24), S. acaule (2n = 48) x S. chacoense (2n = 24) (2n = 36), S. ajuscoense (2n = 48) and S. demissum (2n = 72); the number of groups of 2, 3, 4 etc. chromosomes counted, together with the percentages of the total chromosomes so associated being set out as in the accompanying table (Table 40). The group percentages in the diploid, triploid and tetraploid approximate very closely to Müntzing's corresponding figures for diploid, triploid and tetraploid "Solanum tuberosum". With the exception of the percentage of groups of three, however, Ellison's figures for true S. tuberosum are at variance with both sets of tetraploid figures.

Propach/

TABLE 40.

The extent of secondary association in certain tuber-bearing Solanum species and hybrids.

Species	No. of plates counted	Groups of chromosomes 1	2	3	4	5	6	7	8	Authors
S. chacoense 2n = 24	100	779	141	37	7					Propach, 1937.
	%-age*	80.89	14.61	3.83	0.72					
S."tuberosum" 2n = 24	46	303	106	8	2	1				Müntzing, 1933.
	%-age	72.14	25.24	1.90	0.48	0.24				
S. acaule x S. chacoense 2n = 36	100	724	281	91	33	12				Propach, 1937.
	%-age	63.45	24.63	7.98	2.98	1.05				
S."tuberosum" 2n = 36	40	338	93	49	5	1				Müntzing, 1933.
	%-age	69.55	19.14	10.82	1.29	0.21				
S. ajuscoense 2n = 48	100	819	280	125	74	35	18	6	3	Propach, 1937.
	%-age	60.22	20.59	9.19	5.44	2.57	1.32	0.44	0.22	
S. tuberosum 2n = 48	25	187	59	30	19	3	4	2	3	Müntzing, 1933.
	%-age	60.32	19.32	9.68	6.13	0.97	1.29	0.65	0.97	
S. tuberosum 2n = 48	26	143	330	63	68	5	-	-	-	Ellison, 1936.
	%-age	23.48	54.19	10.34	11.17	0.82	-	-	-	

* %-age = $\frac{\text{No. of groups}}{\text{Total groups}}$ x 100.

Propach sees in these figures no indication that the manner of distribution of chromosomes into groups of varying size is any other than would be determined by pure chance assortment — the more chromosomes per plate the greater the chances of high valency groups occurring. The curves relating group valencies with percentage frequency of occurrence for the diploid, triploid, tetraploid and hexaploid certainly bear out this statement (see Propach 1937a). Moreover the correspondence of the two sets of figures (Müntzing's and Propach's) over the same range of polyploidy for different material and presumably (though this is not certain) different species, seems significant since such a precise "distribution" of homology amongst the genomes of different species is scarcely to be expected. The lack of agreement of Ellison's figures with the general scheme would, of course, speak equally well for the contrary.

Assuming that secondary association is an indication of homology we might expect a degree of homology sufficient to cause the approximation of similar bivalents at metaphase I and of similar univalents at metaphase II to have important consequences in the polyhaploid (Katayama). No quadrivalents have been observed at meiosis in either of the haploid plants discussed above (Lamm 1938, Ivanovskaja 1939) presumably/

presumably because the homologous regions are too short and the chiasma frequency too low. Secondary association was observed and figured by Lamm (loc. cit) at metaphase II though Ivanovskaja makes no comment on this point and the rather poor figures do not give any helpful indications.

Lawrence inferred the existence of secondary association in a large number of genera and species from a survey of published figures of first and second metaphases. This practice is of course open to the serious objection that the quality of fixation of the material is unknown but the assumption was made that since the best fixed plates are usually the ones chosen for reproduction "it may be taken for granted that when association is seen in these plates, then it will most certainly be more pronounced in others not figured". Such data must, however, be regarded as of a somewhat doubtful nature. Since multivalents in polar view are liable to be confused with secondary associations only illustrations of second metaphases must be used for this purpose. From Longley and Clark's (1930) and Smith's (1927) figures of first metaphases of a number of diploid potato species Lawrence suggested thatthe basic number of the genus Solanum might be 6. On the other hand, association of chromosomes at first metaphase/

metaphase without chiasma formation in the 24-chromosomed haploid Nicotiana tabacum var. purpurea (Chipman and Goodspeed 1927) was interpreted as indicating that the basic number of the genus Nicotiana is 12. Two excellent microphotographs in Rybin's (1930) paper illustrate second metaphases in a European cultivated potato and in the diploid (2n = 24) S. chacoense . In the former appear three closely associated pairs of chromosomes and several others more loosely so, whilst one plate of the latter shows three closely associated groups of two chromosomes and three less so. The occurrence of such complete pairing in the diploid species is significant for the theory that the basic number here is 6.

The somewhat unsatisfactory nature of this evidence led me to question the accounts of secondary association in other genera and families and to a comparison of this phenomenon with the somatic pairing of Diptera with which it has been assumed to be homologous.

The pairing of chromosomes in the somatic cells of Drosophila was first observed by Stevens (1907, 1908) and its widespread occurrence among the Diptera by Metz (1916). Here it is difficult to escape the conclusion that this pairing "is not due to purely mechanical causes but is dependent in some way on the qualitative/

qualitative nature of the chromosomes". "Pairing is selective in the highest degree" and that "this association is not merely an assortment according to size is shown by the pairing of the unequal sex chromosomes in the male" (Metz loc. cit.). Drosophila melanogaster is anomalous in that there is no pachytene pairing or crossing over among the male autosomes. Presumably the intensified forces of somatic pairing are responsible for assembling the pairs of chromosomes on the metaphase plate and the repulsion of their respective centromeres for their regular disjunction at anaphase (Darlington 1934). The explanation put forward and which the relative positions of the four chromatids at metaphase appears to support is that "a common force of attraction exists between identical genes and that this attraction is almost or entirely saturated by the approximation (or association) of genes in pairs. Secondary attraction is then a residual attraction and its variation is merely the variation of degree of saturation by pairing" (Darlington loc. cit.).

The intensification of somatic synapsis following the uncoiling of the chromonemata in the development of polytene chromosomes and the sensitive manner in which structural re-arrangements and changes in homology are expressed by incompleteness or absence of/

of pairing in the segments concerned leaves no doubt that this pairing is determined by the homology of the chromosomes themselves. Metz (1922) in a study of tetraploid ovarian cells in the Dipteran Sarcophaga found that the four sets of six chromosomes were closely associated into tetravalent bodies, the association showing the same morphological selectivity as that in normal diploid cells.

How then does secondary association in polyploid plants compare with this situation?

An obvious difference is the variation in the valency of associated groups of chromosomes in polyploid plants. In some cases, at metaphase II, groups occur of higher valency or in greater numbers than are to be expected from the assumed basic number of the species, e.g. groups of 4 and 5 in the triploid Solanum and of 5, 6, 7 and 8 in the tetraploid; and an excess of groups of 2 and 3 in Brassica oleracea where none are expected. This has been easily explained by the postulation of extensive structural hybridity within the complement. The pairing of unequal bivalents in Brassica (Howard 1939) has been put forward as evidence in this connection. This explanation is particularly difficult to dispose of. On account of the usually small size of the chromosomes of species showing secondary association it is conceivable that structural re-arrangement could exist, which/

which owing to the low chiasma frequency would fail to be detected at meiosis by the formation of chromatin bridges and fragments. All the same structural hybridity must be assumed to be fairly extensive if all the aberrations are to be so accounted for. There is little information on the effect of structural hybridity on secondary pairing in Drosophila. However, Dobzhansky and Sturtevant (1930) found that the four chromosomes in an interchange heterozygote in Drosophila lay in a ring at mitosis. On the other hand Stern (1931) found that there was no attraction between the distal end of a normal X and the corresponding part of its homologue when this had been translocated to another chromosome. Presumably the attraction between the autosomes overcomes that between the X's. More frequently than "super-association" we find reduction or total absence of secondary pairing. Thus Lawrence (1931b) states, "the only proper criterion of association is the examination of a sufficient number of stages of diakinesis and metaphase in order to arrive at an estimation of the constancy or otherwise of the associations found".

A weakness of the homology theory is the manner in which the data are treated by different authors. Thus in Brassica oleracea (Catcheside 1937, Howard 1939), of the 11 types of arrangement of M I and M II plates only four of the observed types viz. 9(1)/

9(1), 1(2) + 7(1), 2(2) + 5(1) and 3(2) + 3(1), are considered to be "normal and characteristic and the remainder aberrant". The unimodal variation within this specially selected group is then taken as indicating that "there is a definite law of chance governing the occurrence of secondary pairing" according to the proximity of homologous bivalents on any given plate, (Catcheside 1937). Darlington and Moffett (1930) and Moffett (1931) on the other hand, in their work on apples deduce the basic number of the genus from the maximum degree of association observed at metaphase I, where in addition there is extensive multivalent formation. Lawrence observes the same procedure in Dahlia variabilis (1931a, b). This elasticity of treatment according to the hypothesis at stake is regrettable (cf. also Catcheside 1934, Riccharia 1937b and Sikka 1940 in this connection.)

Corresponding to the association in Diptera, there appears to be some degree of selectivity according to size and configuration, correspondence between pairs being most marked at anaphase separation. In small chromosome species where size differences assume critical importance it is clear that polar views of metaphase plates alone are not valid in this connection. The apparent size of bivalents will depend on the position of the spindle attachment and the number of chiasmata per bivalent which there is no reason to expect/

expect will remain absolutely constant.

In contrast to the Diptera, plants which show secondary association at meiosis do not show a corresponding degree of chromosome pairing in somatic cells. On this point Metz (1916) writes: "there seem to be various intermediate conditions between that of intimate pairing and that of very slight pairing", "the tendency to associate in pairs ..... being manifest in all the cells of some (organisms) but only in the maturing germ cells of others".

The most plausible explanation of this is certainly that mitotic chromosomes are usually more widely spaced and larger and consequently the attraction between homologues may be weaker, or if of the same strength as at meiosis physical conditions may oppose close approximation. However Skovsted (1933) and Davie (1934) have found secondary pairing in somatic cells and at meiosis in Gossypium.

Now if we accept the validity of data from secondary association in the potato we must necessarily assume that 6 is the basic number for the whole genus Solanum and for the family Solanaceae, unless it is extremely heterogeneous, which the uniformity of certain morphological characters within the family belies. Consequently we might expect to find some indication of secondary association in the published figures of metaphases/

metaphases of other Solanaceous species. Accordingly, I consulted as many of the references to work on the Solanaceae appearing in Gaiser's (1926-33) list of chromosome numbers as were easily accessible — and these included the majority. The figures were examined in as unbiassed a frame of mind as possible but with the possible exception of several tobacco species and hybrids in no case was secondary association so clearly manifested as to be beyond doubt. This discrepancy becomes clearer the nearer we approach to the Section Tuberarium. Thus S. luteum, S. nigrum and others belonging to the closely related section Morellae investigated by Jørgensen (1928) showed no suggestion of this phenomenon and there is no sign of it in the critical cytological investigations of Solanum Lycopersicum by Lesley and Lesley (1930) and Upcott (1935) nor in the extensive work on Datura. Afify (1933) found secondary pairing in the hybrid S. Lycopersicum v. racemigerum but in neither of the parents. Moreover neither in the haploid Datura (Blakeslee 1924) nor in the haploid tomato (Lindstrom and Koos 1931) is there any suggestion of pairing among the set of 12 chromosomes nor do the extensive genetical investigations on these plants suggest that there is a double set of chromosomes present. The Solanaceae are evidently not an isolated case. In the Cruciferae

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the Brassicas, Raphanus and Cardamine apparently exhibit secondary pairing whilst its existence is denied in Biscutella. Dr. Manton tells me that she has been able to produce varying degrees of association in water cress by altering the proportions of acetic acid in the fixative. Moffett (1934) found association in pears to be much less pronounced than in apples. Of 26 arctic grasses examined by Flovik (1938) only two showed secondary association and the only other two grass genera yielding extensive evidence were Oryza and Sorghum. No doubt other anomalies would be disclosed by a thorough search of the literature.

Where haploids are known these seem to follow the parent diploids in the matter of secondary pairing. Thus haploid tomato Solanum nigrum and Datura stramonium show neither primary nor secondary pairing at meiosis, in contrast to the haploid potato. Haploid rice shows some approximation of univalents at diakinesis (Morinaga 1934, Morinaga and Fukushima 1932).

Some explanation has therefore to be found for this limited distribution of the occurrence of secondary association in a family where it might reasonably be expected to be more widespread. Lawrence (1931a and b) has assumed that secondary pairing of chromosomes will be most pronounced in newly formed allopolyploids resulting from hybridisation between nearly related species. With the passage of time/

time the affinity between the genomes of the original parent species will tend to be decreased by structural re-arrangements and mutations. Consequently, the outward appearance of homology -- secondary association, will also tend to decrease in intensity, old allo-polyploids eventually functioning as diploids in this respect. Hence it could be claimed that the wide-spread nature of this phenomenon in the section Tuberarium is indicative of its youthful nature as compared with the rest of the Solanaceae. This must be pure speculation.

If we accept 6 as being the basic number of the genus then the development of the Solanaceae can be imagined as a progressive elaboration from an ancestral stock with n = 6 through Petunia (n = 7) and the aneuploid tobacco's (n = 8, 9, 10 and 11) to the remainder of the Solanaceous species (n = 12) and their polyploids (n = 24, 36, 72 etc.). In some Nicotiana species, some of the chromosomes are larger than others and it is conceivable that the haploid numbers 8, 9, 10 and 11 have in reality arisen from a basic set of 12 as the result of chromosome fusion, thus leaving Petunia as the only aberrant genus. Its inclusion in the family by virtue of parallel evolution from a separate source could be postulated but the speculation is unwarranted.

But/

But the difference between allo- and auto-polyploidy is merely one of degree, hence if secondary association occurs in allopolyploids it should surely be much more intense in autopolyploids where, in the case of artificially raised tetraploids the four sets of chromosomes are identical or nearly so. Yet we find here the same limitations as applied to diploids. Autotetraploid and triploid Datura, Tomato, and and Solanum nigrum show no secondary pairing, neither do autotriploid and tetraploid Primula sinensis nor autotetraploid Biscutella, all of which are comparatively small chromosome types. Whereas the probable auto-triploid Solanum Commersonii and autotetraploid Solanum tuberosum show a high degree of pairing. Primula Kewensis is probably the ideal allopolyploid, the parental sets of floribunda and verticillata chromosomes being sufficiently alike to pair regularly in the diploid hybrid and yet the tetraploid shows no secondary association (Newton and Pellew 1929, Upcott 1939b). No doubt such examples could be multiplied and they do present a serious obstacle.

At present there seems to be no satisfactory alternative explanation for this phenomenon than that proposed by Lawrence. One hesitates to dismiss it as a fixational artifact though this must be an important factor. Likewise the personal factor must complicate

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a situation where the only criterion is the degree of approximation — a problem which becomes more acute the more crowded the plate and the higher the valency of the groups expected. Propach's suggestion that association is simply the result of crowding on the metaphase plate does not cover all cases convincingly, particularly that of the haploid showing secondary pairing. Heilborn (1936, 1937) has put forward a mechanical theory based on evidence from his studies on Carices which may receive passing mention. The complements of most Carex species are made up of sets of chromosomes of different sizes, the members of each set being identical in size and "therefore supposed to be homologous, though this of course is a conjecture". Heilborn came to the conclusion that, as a result of the forces of nuclear division chromosomes of equal size are assorted irrespective of homology. Particular importance is attached to the case of Carex glauca having 4 pairs of large "A" chromosomes which, on occasions, form two quadrivalents, showing that the members of each set of four are homologous. Where one of the quadrivalents was replaced by bivalents it was found that at metaphase I one bivalent was paired as often with the other quadrivalent as with its homologous bivalent, demonstrating, according to Heilborn, that "association between the A chromosomes probably depends/

depends on size relations exclusively". The fact seems to have been overlooked however, that if the eight A chromosomes are not themselves homologues the basic number of this species will be 18, an abnormally high number for the group. As a parallel to his hypothesis Heilborn quotes Muller's (1910) figures of mitosis in Yucca aloifolia whose complement is made up of a number of very long chromosomes and a large number of very small ones. The small chromosomes pass to the pole first and this "centrifugal assortment" is visible in the telophase nucleus. Clearly this differentiation is one of time, the longer lengths of chromatin taking longer to separate than the shorter ones. Similarly in meiosis, where homologues are associated by chiasmata the speed of separation will be dependent on the chromosome length-chiasma frequency relationship and does not require the postulation of other external forces (cf. Darlington 1929a and O'Mara 1931). The argument for this hypothesis is therefore not a weighty one.

Secondary association seems to behave as an elusive quality with which some species are endowed and others not. Even the homology theory must be stretched to its limits to include all the variants and will certainly not bear the test of prediction. It is typical of most investigations dealing with the basic/

basic number of polyploid species that there are usually additional and more reliable indications to be obtained either from the range of chromosome numbers in the genus or from the extent of multivalent formation in the investigated species or its relatives. Thus Flovik (1938) concluded that 5 was the basic number in grasses from (a) the range in basic numbers in the Gramineae, (b) the occurrence of quadrivalents in diploids, bivalents in haploids and unexpected pairing in hybrids, (c) the range of chromosome morphology and (d) genetic evidence, in addition to the observations on secondary association. Even in Dahlia the clue to the basic number is given by the range of multivalent formation and the somatic numbers of the species themselves. The aneuploidy postulated in Dahlia Merckii and particularly that of Pyrus species still remain to be proved by genetical evidence. And this is the key to the situation. It is obvious that the proof of any hypothetical constitution deduced from secondary association must rest with genetic investigation — a difficult problem in most cases, impossible in others because polyploids form such unsuitable subjects for genetical analysis as a rule. The identification of sporadic duplicate factors cannot even be regarded as conclusive proof of duplicate sets of chromosomes for we know that such duplications/

duplications exist in normal diploids (e.g. the duplicate factors in diploid Brassica oleracea and Raphanus sativus quoted by Riccharia 1937a). The most complete piece of evidence available so far again comes from the grasses. In rice with n = 12 (5 + 5 + 2 according to Flovik) "there are according to Chao (1928) not less than five groups of polymeric factors known", and in maize, according to Huskins and Smith (1934) ten cases of duplicate and three of triplicate genes are known.

The information from secondary association modifies but little the conclusions reached from an examination of other aspects of meiosis regarding the cytological constitution of the potato.

The records of septi- and octovalents have been shown to be in need of confirmation and the secondary association data are of so conflicting a nature as to be of little help in deciding whether or not the true basic number of the genus Solanum is 6.

The phenomenon of secondary association is at least consistent in the fact that in those species where it is observed it appears in the haploid, diploid, triploid and all higher polyploids. If it is really an expression of latent homologies sufficient to cause approximation of bivalents in the diploid potato species such as S. chacoense (2n = 24) (Rybin 1930) it seems logical/

logical to suppose that some attempt at quadrivalent formation would be observed at meiosis in the polyhaploid of the cultivated potato. Pairing is solely by bivalents.

We have also seen that in a polyploid where secondary association is expected to result in the formation of multiple groups, two factors, the quality of fixation and the personal factor must complicate the situation. Müntzing's (1933) and Ellison's (1936) data for tetraploid potatoes include associations of higher valency than even an octoploid constitution would admit, without structural re-arrangements. Such postulated structural hybridity awaits definite proof and, from the statements of one of these authors and the figures of both of them it is difficult to escape the conclusion that some configurations are, in reality artifacts. If it is once admitted that fixation plays any part in the approximation of chromosomes it must be impossible to differentiate between what is and what is not an approximation dependent on homology unless the observer has preconceived ideas on the constitution of his subject. Moreover, on a crowded metaphase plate the limits of the associated groups of chromosomes may well be differently interpreted by different observers.

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The distribution of secondary association within and between genera almost suggests that it is a quality peculiar to certain types of chromosomes, but in those cases where it does appear it is impossible, at the present stage to affirm or deny its significance as an indication of latent homologies.

Four sets of tetrasomic genes, all alike in character, have been identified in this investigation and their inheritance does not conflict with the idea that the potato may, in reality, be an octoploid of similar constitution to Dahlia variabilis. If this is so it must be the result of previous hybridisation between two allotetraploids.

However, genetical and cytological data alike are more simply explained on the basis that the potato is an autotetraploid. Emme's (1936, 1937b) work with diploid potato species has given no indication of the duplicate factors which we might expect were these really tetraploids and the cytogenetic data from other Solanaceous species and from the polyploid potatoes presently to be discussed, all lend force to this conclusion.

POLYPLOIDY IN THE SECTION TUBERARIUM OF THE GENUS SOLANUM.

This investigation would hardly be completed without some discussion of the relations of S. tuberosum L. to the rest of the tuber-bearing Solanums. Since Vavilov's classical researches have shown what wealth of useful economic characters is to be found at the centres of origin of cultivated plants a somewhat romantic flavour attaches to the efforts of the numerous collecting expeditions which have visited South America — 'the home of the potato', within recent years. As a result of these, particularly of the Russian expeditions we now have a considerable amount of information about these S. American potatoes. It is not my purpose, nor am I in a position to discuss the detailed systematics of the group, but rather to indicate, from the available cytogenetic data, what processes have been most important in its evolution.

The Sect. Tuberarium Bitt. of the genus Solanum to which the potatoes belong is undoubtedly a difficult group in the taxonomic sense. The characteristic vegetative reproduction of the group ensures the perpetuation of variant and unbalanced cytological types which would not survive sexual reproduction. Variability is thereby increased to an extent which

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makes taxonomic differentiation of any but the largest units a difficult matter. In fact it has often been stated that the morphological range of the cultivated potato itself is as great as the collective range of several species of taxonomic rank (Juzepzuk and Bukasov 1929, Bukasov 1939). Though much has been written speculative and otherwise on the origin of the European cultivated potato we are still far from the solution of this problem. In their efforts to trace existing relatives of this species systematists have in the past associated it with types later proved to be unrelated morphologically and cytologically.

Bitter (1912, 1913) described a number of new species and divided up the section according to the articulation of the pedicels, the fruit shape, calyx form and distribution of pubescence. Since that time classification has been largely in the hands of the Russians and it has not been easy to follow its evolution, owing to the inaccessibility of the literature.

Three principal centres of concentration of species were found as a result of the Russian collections, viz. Mexico, the Peru-Bolivian Andes and Chile and, pending a revision of the taxonomy of the section, the species were divided up according to their source of origin (Rybin 1930).

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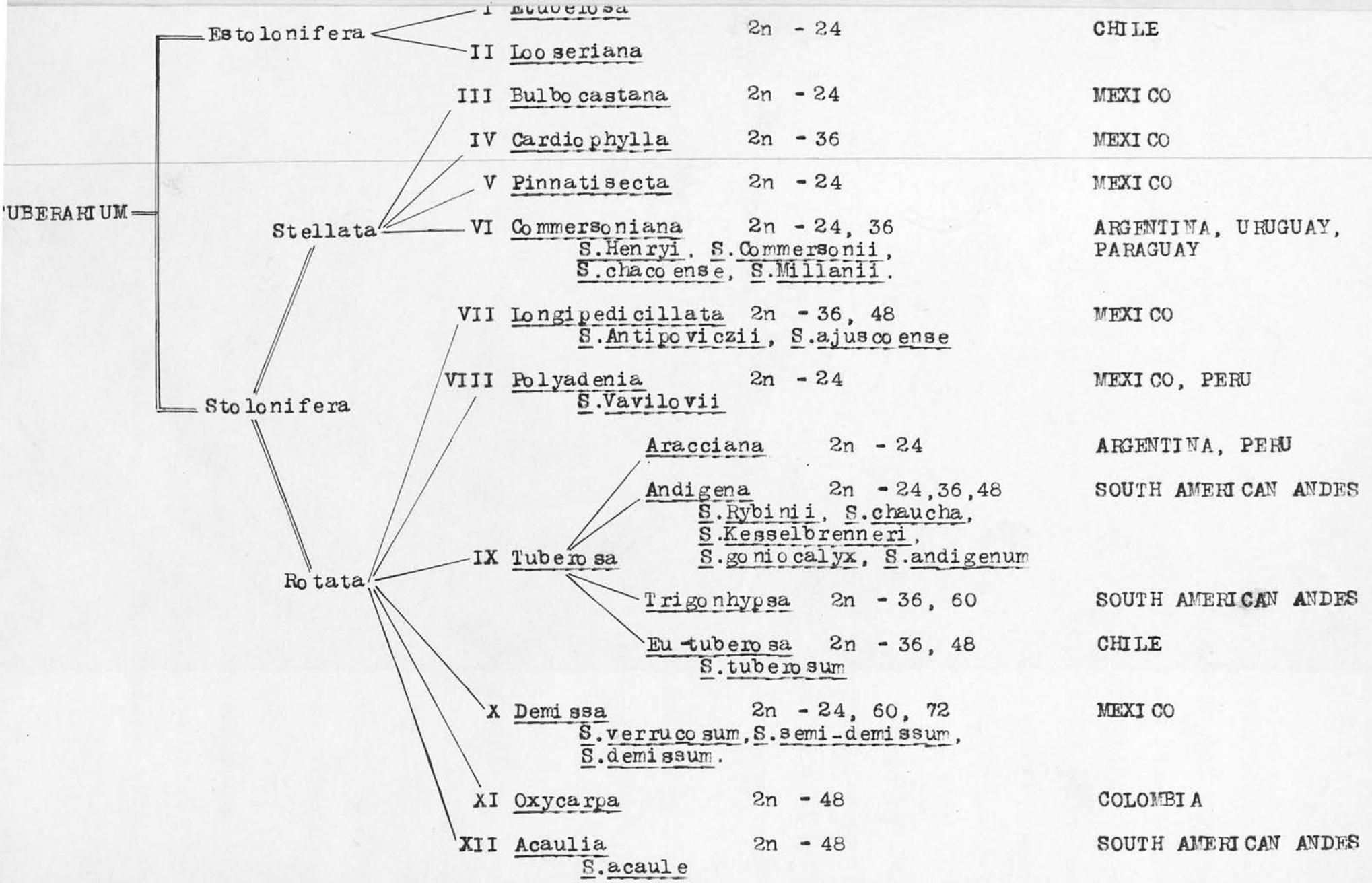

The systematic positions of those species mentioned in the text have been indicated.

The most important contributions to the cytology of the potatoes are those of Rybin (1930, 1933) working on this Russian material. His work shows the astonishing range of polyploid types to be found within the section Tuberarium and, in fact, leaves little doubt that polyploidy must have played a very large part in its evolution. In addition to describing tetraploids and hexaploids he reported in his 1930 paper the existence of triploids (2n = 36) for the first time.

In Bukasov's (1939) publication the systematy of the potatoes follows essentially the lines laid down by Bitter but the main divisions are here based on the stoloniferous or estoloniferous habit and possession of star or wheel shaped blooms. The Stellata and Rotata together include the majority of the species described. Further subdivision is into series characterised by one or a number of collective species. An abridged version of this classification is appended, together with details of the range of chromosome numbers within each series. Of the twelve series, Tuberosa is by far the largest, comprising diploids from Mexico (Polyadenia), a second group of diploids from Argentina and Peru (Aracciana), a large group from the Andes (Andigena) which includes diploids, triploids, tetraploids, and one pentaploid, and a smaller/

smaller group from Chile (Eutuberosa) including among other tetraploids S. Tuberosum L. sens. str. and two related triploids. The small series Demissa is confined to Mexico and is comprised of S. demissum, S. semidemissum and S. verrucosum, hexaploid, pentaploid and diploid respectively. The other important group is that of the Commersoniana (Stellata). The majority of the species in this series are diploids, there being only two triploids, S. Commersonii, the type species, and S. Millanii. The distribution of this series is confined to Argentina, Paraguay and Uraguay.

No descriptions of the taxonomic groups are given in Bukasov's account, nor have any published records been found, so that it is only possible to guess at their diagnostic characters. It is evident that several alterations from previous schemes are embodied in this arrangement which probably represents a more "natural" system than any previously attempted.

It is remarkable that there appear to be very few barriers to hybridisation between even the most remotely situated members of this system. S.tuberosum L. s. lat. crosses easily with species of the Demissa, Longipedicellata, and Commersoniana groups and with S. acaule (Acaulia) with some difficulty (Propach 1938b). Argentine lowland forms S. chacoense, S. Henryi cross

as/

as easily with S. acaule from the high Andes as with species from Mexico (S. demissum, S. ajuscoense). Diploids cross easily with tetraploids and hexaploids, triploids with difficulty (Propach 1938b, Olah 1938, Ivanov 1939). Self-sterility apparently exists in some species, particularly among the diploids e.g. S. Rybinii, S. chacoense (Bukasov 1939) Longley and Clark 1930) though there is little or no evidence of this in cultivated Solanum tuberosum (see below). Owing to the great sensitivity of potatoes to photoperiodic and climatic conditions it is difficult to get all species to flower satisfactorily. The blooms frequently drop before or after pollination and the number of berries set in proportion to the number of crosses made is very little guide to the compatibility or otherwise of the species.

The investigations of Propach and his coworkers at Müncheberg and of several Russian authors have thrown some light on the origin of this multiplicity of polyploid types. A timely word of warning is necessary, however, before interpreting these results since phylogenetic conclusions based on the pairing behaviour of hybrids between polyploids or between diploids and polyploids are open to a serious margin of error. Firstly, in hybrids where the length of homologous regions in similar chromosomes is short, competition/

competition in pairing will result in identical chromosomes being paired at pachytene at the expense of the partly homologous ones. Pairing at metaphase is then "determined by the formation of chiasmata in the pachytene thread proportionate in number to the length paired" (Darlington 1937). Secondly, the behaviour of hybrids depends on the frequency and distribution of chiasmata per unit paired length and comparisons cannot be made in the absence of any information about the chiasma frequencies of the parents. Further, reduction of metaphase pairing follows mere structural re-arrangement without genic differentiation. Taking these points into consideration then, the extent of metaphase pairing in a hybrid may give very little indication of the phylogenetic relationships of its parents.

Two interesting crosses between the diploid S. chacoense (2n = 24) (Commersoniana) and a European cultivated potato variety Pepo on the one hand (Propach 1938a) and with S. tuberosum L. s. str. from Chiloe on the other (Oppenheimer 1933) are available for comparison. Propach found that , with the exception of triploids, in all the wild species pairing took place by bivalents only. Rybin (1930) found this also in the hexaploid S. demissum and it was supposed that this indicated their allopolyploid nature. However,

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we have already seen in the Tulips how multivalent formation in an autopolyploid may be suppressed simply by the selection of a low chiasma frequency. Selection in this direction was shown to be most intense in sexually reproducing tetraploids.

	No. of flowers pollinated.	No. of berries	No. of seeds	Seeds/berry*	
S. chacoense (2n = 24) x var. Pepo (2n = 48)	64	4	260	65·0	Propach 1938
S. chacoense x S. tuberosum (Europe)	303	1	0	0	Oppenheimer 1933
S. chacoense x v. S. tuberosum s. str. (Chiloe)	103	24	2	0·8	Oppenheimer 1933

* Propach measures the compatibility of a cross by the number of seeds per cross made. In view of the great influence of external conditions on fruiting however, it is considered that the number of seeds per berry set gives a more reliable indication of this quality in view of the lack of exact information.

By rights the progeny of both these crosses should be triploid in constitution, as in fact Oppenheimer (1933) found. The morphological features of S. tuberosum predominated, as would be expected from the presence of two sets of tuberosum chromosomes, and/

and the hybrid is sterile. Reduction division was found to be irregular, and although some metaphases were observed with 12 bivalents and 12 univalents, pairing was variable. More significant, however, is the fact that three plants were found which proved to be tetraploid instead of triploid. Similarly Propach (1938a) raised seven plants from a cross S. chacoense x S. tuberosum var. Pepo, which were all tetraploids. These must have arisen through the functioning of a diploid egg cell of S. chacoense, as the greater similarity of the tetraploid to S. chacoense also suggests. In both sets of hybrids a small number of quadrivalents occurred (these are not mentioned by Oppenheimer specifically though evident in his figures but their existence has been deduced from his descriptions of metaphase pairing) but bivalents preponderated.

Hybrid	Numbers of cells with different numbers of quadrivalents				% quadrivalents
	0	1	2	3	
S. chacoense (2n = 24) x S. tuberosum (2n = 48) var. Pepo	63	28	8	1	3·9

On selfing his own 7 clones and one of Oppenheimer's Propach (1938a) found that five of these were self-sterile and three self-fertile and figures of the number of multivalents per cell are given to show that this sterility is probably not the result of excessive multivalent formation. In view of the self-sterility/

sterility of S. chacoense we may surmise that this factor has been carried over to the tetraploids also.

Multivalents/cell		Self-sterile	Self-fertile
50 cells	I	-	0.12±0.21
	II	23.24±0.51	22.82±0.63
	IV	0.38±0.23	0.56±0.33

Both authors attempt to draw conclusions from the extent of pairing as to the homologies of the four sets of chromosomes according to the "Drosera" scheme (Rosenberg 1909). The above figures show that a certain amount of autosyndesis must be taking place, but insufficient to justify any conclusions regarding the relationships between the two species or those of cultivated S. tuberosum with the Chilian species. The absence of trivalents in the triploid (Oppenheimer 1933) is perhaps more significant in this connection, particularly as we now know that S. tuberosum is probably autotetraploid. Cytological examination of a number of F_2 and back cross (to tuberosum) plants showed that viable gametes having 22-26 chromosomes were produced by the F_1's. This is of particular interest aneuploids have so far not been recorded from the wild (Oppenheimer 1933).

S. chacoense was used as the male parent in another hybrid cross, this time with S. acaule, a self-fertile/

self-fertile tetraploid species from the Andes (Propach 1937b). S. acaule is the type species of the somewhat isolated group Acaulia in Bukasov's scheme. The cross was evidently easily made although the parents differ in many morphological features.

	No. flowers pollinated	No. berries	No. seeds	Seeds/berry
S. acaule selfed.	20	18	2590	143.8
S. chacoense (self-sterile		-	-	-
S. acaule x S. chacoense	11	8	1348	168.5

An analysis of 50 metaphase plates of the hybrid showed the following distribution of multivalents.

Multivalents /cell. 50 cells	I 320	II 436	III 205	% trivalents 34.1
M±3m.	6.40±0.81	8.75±0.93	4.10±0.63	

The high frequency of trivalents probably indicates extensive homology between the three genomes here combined. Propach (1937b) comes to a similar conclusion by comparing these figures with Upcott's (1935) values for the related autotriploid Solanum Lycopersicum, a procedure which is clearly not justified in view of the statements set out above. The same procedure was/

was adopted in interpreting the degree of pairing in two other tetraploid hybrids, S. acaule x S. Antipoviczii (Propach 1938a) and S. demissum x S. verrucosum (Propach 1937b). S. Antipoviczii is a Mexican tetraploid belonging to the series Longipedicellata, whilst S. demissum and S. verrucosum, also Mexican and hexaploid and diploid respectively both belong to the series Demissa. The frequencies of multivalents found are given below. (Table 41)

Both sets of figures are very similar. Figures for the numbers of cells with different numbers of quadrivalents are only available for the second of these hybrids. The percentage of the possible number of quadrivalents formed is low in each case. The chiasma frequencies of all three polyploid parents must also be low since pairing in all of them is strictly by bivalents if Propach's (1937b) statements be correct. Hence we are probably justified in regarding the genomes of S. demissum and S. verrucosum as being extensively homologous with one another, as also those of S. acaule with S. Antipoviczii. Without more evidence however, it seems unwise to assume that the three polyploids are actually autopolyploids.

The cytology of S. Commersonii and of several hybrids between and taxonomically nearly related diploids/

TABLE 41.

Hybrid	Configs	I	II	III	IV	% quads.
S. demissum var. Klotschii x S. verrucosum	Total	124	882	46	98	
	M/cell	2.48±0.7	17.64±1.4	0.90±0.5	1.96±0.6	16.3
S. acaule x S. Antipoviczii	Total	96	948	44	69	
	M/cell	1.92±0.6	18.96±1.2	0.88±0.4	1.38±0.5	11.5

Hybrid	No. of cells with different numbers of quadrivalents.						% quads.
	0	1	2	3	4	5	
S. acaule x S. Antipoviczii	15	17	7	7	3	1	11.5

diploids has been dealt with by Olah (1938). S. Commersonii, itself a triploid, is the type species of the series Commersoniana which comprises a number of diploid species and one other triploid S. Millanii, all natives of the Argentine region. An analysis of 50 metaphase I plates of S. Commersonii gave the following figures.

Species	Configs.	I	II	III	% trivalents
S. Commersonii 50 cells	Total	208	383	288	
	M/cell	4.16±0.9	7.88±1.1	5.76±0.8	48.0

The very high number of trivalents per cell indicates the autopolyploid nature of this species. Additional evidence is afforded by the fact that only diploids are known in the region to which it is endemic, so that it is unlikely to be the product of hybridisation with a tetraploid. No doubt its origin may be traced to the fertilisation of an unreduced diploid egg. The same applies to the other triploid, S. Millani (Bukasov 1939). S. Commersonii is evidently not entirely male sterile since Olah (1938) reports two successful crosses with S. demissum made by Propach using S. Commersonii as the male parent. As would be expected anaphase separation in S. Commersonii itself is very irregular, a large part of the pollen consisting of diads, triads, pentads and hexads (Olah 1938). The hybrids/

TABLE 42.

	T	24	25	26	27	28	29	30	31	32	33	34	35	36	48
Tulipa (3n) x (2n)	50	-	-	7	5	11	10	3	7	3	2	2	-	-	-
Datura (3n) x (2n)	285	58	138	79	10	-	-	-	-	-	-	-	-	-	-
S. Commersonii (3n) x S. chacoense (2n)	18	-	-	-	-	-	-	-	-	6	9(8)	3(5)	-	-	-
† S. Commersonii (3n) x "papa chusa" (2n)	19	-	-	-	1	-	-	3	4	5	4	2	-	-	-
S. Commersonii (3n) x S. Henryi (2n)	14	3	3	5	1(2)	-	-	-	-	1	-	-	-	-	1*

* The exceptional individual with 48 chromosomes must be the result of fertilisation of an unreduced egg of S. Commersonii.

† A diploid cultivated S. American variety closely related to S. Commersonii.

greater on both male and female sides in Datura. We have no detailed information on the distribution of chromosomes at gametogenesis in the potatoes, but it seems most probable that, apart from any selection against unbalance in the gametes themselves selection is most rigorous against (numerically) unbalanced zygotes. For example, no aneuploid types have so far been found among wild material. Similarly, although it is known that numerically unbalanced gametes are produced in the cultivated potato the absence of unbalanced types forces the conclusion that they are in fact inviable. This situation is in marked contrast to other unstable tetraploids such as Biscutella laevigata (Manton 1934, 1937) and Silene ciliata (Blackburn 1933), all the more remarkable in view of the predominant vegetative mode of reproduction of the potato. This is certainly a point in need of investigation.

I have collected a number of scraps of information regarding the relationships of other species within the section Tuberarium (Bukasov 1939, Emme 1934, 1935, 1937). In most instances however, the facts are merely stated in summarised form without the detailed evidence from which one might judge the validity of the conclusions and they have therefore not been included. This applies particularly to the groups/

groups Acaulia, Oxycarpa and Eutuberosa. In the first two instances only tetraploids are known and in the third, triploids and tetraploids but no diploids in the same region, from which they might have arisen. In the absence of any definite evidence the gaps can only be bridged by speculation.

However, if the evidence so far obtained is typical also of species in the wild there seems to be ample opportunity for such a polyploid system to arise. Thus Propach (1938) and Oppenheimer (1933) have shown that the diploid S. chacoense may produce unreduced gametes. Fertilisation of unreduced eggs by normal haploid gametes would give rise to triploids. By the fusion of two such unreduced gametes, or the meeting of a gamete from a tetraploid with an unreduced diploid gamete a tetraploid might arise. Again, the production of viable unreduced gametes by the wild tetraploids S. Antipoviczii (Ivanov 1939), Rybin (quoted by Bukasov 1939), S. acaule (Dremlyug 1937) and S. ajuscoense (Nikolaeva, quoted by Ivanov 1939) has given rise to fertile hexaploids on crossing with a tetraploid species. Hexaploids and tetraploids might also arise from the functioning of unreduced triploid gametes, the occasional formation of which we have seen in S. Commersonii (Olah 1938). It requires little imagination to see how, from these beginnings an array of polyploids such as is to be found/

found among the potatoes could arise.

In addition, the capacity for regeneration and the production of sectors of polyploid tissue seems to be a characteristic of the Solanaceae. The ease with which polyploid shoots have been produced by Jørgensen (1928) and others simply by decapitation in Solanum Lycopersicum, S. nigrum, and S. luteum bears witness to this fact. So far polyploid shoots have not been obtained by this means in the potato (Jørgensen 1928, Johnstone 1939) but several workers have found polyploid chimaeras in different tuber-bearing species. Rybin (1930) found two cases where the whole root tip was composed of tetraploid cells (2n = 96) and others where a portion of the tissue was tetraploid. Similarly, Dremlyug (quoted by Bukasov 1939) found chimaeras in hexaploid S. acaule hybrids where the cells had 144 chromosomes. All of which conditions would seem to favour the origin of polyploid types by somatic chromosome doubling although this has not as yet been observed (v. also Biscutella, Manton 1937).

The predominance of vegetative reproduction within the section Tuberarium introduces another important factor in that it permits the survival of unbalanced cytological types, such as triploids which would otherwise be eliminated if perpetuation were by seed alone./

alone.

Supposing that a population turned from being completely sexually reproducing to being completely asexual, we should expect its initial range of variation to remain static -- each individual in fact forming a separate clone. This initial variability, dependent on the heterozygosity of the individuals composing the population will only be increased by the occurrence of dominant mutations occurring at random in different individuals. Clearly there can be no spread of these mutations through the population and they will be preserved providing they do not radically upset the relations between the individual and the environment. One might therefore expect vegetative reproduction to carry with it an inability of the organism concerned to pursue any definite evolutionary course — an inability to conform to radically changed conditions. Asexual reproduction moreover, acts as a shelter behind which chromosomal re-arrangements, inversions, translocations, may develop without impairing the survival of the individual since they always remain heterozygous in the absence of sexual reproduction. It is when such individuals do reproduce sexually after a period of vegetative increase that this accumulation of structural changes produces its effects in the form of hybrid sterility./

sterility. In fact, the process is a cumulative one, inversions which reduce fertility leading to the prolongation of the asexual condition, accumulation of more inversions and eventually complete failure of sexual reproduction, comdemning the individual to a clonal existence (Upcott 1937b).

If, in a less extreme case, the effect of vegetative reproduction is merely to lengthen the periods between successive seed productions, crossing over will prevent the accumulation of inversions to any great extent and will so safeguard fertility. Comparing the cultivated varieties and species of Tulipa Upcott (1937) has found that the comparatively young diploid garden varieties, propagated as clones are less structurally hybrid than the older diploid species. The triploid varieties are more hybrid than the species and, at the present stage it can only be surmised that this is partly the result of greater initial hybridity. In the tetraploid species (there are no tetraploid garden varieties) the situation is different; they occasionally set seed and show a low percentage of inversions. In the potatoes we have very little information on structural hybridity or on the balance between vegetative and sexual reproduction in the diploid and even numbered polyploids. The triploids must inevitably behave as clones but the only case

where/

where inversions have been detected is in the hybrid S. acaule x S. Antipoviczii where Propach (1938a) found about 5% of pollen mother cells with chromatin bridges at anaphase I. It may be that inversions escape detection by reason of the low chiasma frequency. Nevertheless it seems plausible to suppose that this type of differentiation must have played some part in species evolution in this group.

In the foregoing account certain species from their genetical or cytological behaviour have been described as autopolyploids. The terms auto- and allo-polyploidy have now lost much of their original significance when first defined (Kihara and Ono 1927) since it is clear that the difference between them is one of degree only. They are not distinguishable on the basis of genic or structural differences as Müntzing's (1936) definition might suggest and there is, in fact no boundary line between them. At best they give us some idea of the relative "distance apart" or degree of differentiation of the two parental genomes combined in a polyploid. More and more evidence is coming to light to show that few of the various criteria of auto- or allopolyploidy hold in all cases. Particularly striking in this connection is the tetraploid watercress (Manton 1935) whose autotetraploid nature seemed almost beyond doubt.

Compared/

Compared with the diploid and triploid, the tetraploid showed most of those morphological features we are accustomed to associate with autotetraploids and its taxonomic isolation seemed to render any possibility of allopolyploidy unlikely. Yet comparison of the cytological behaviour of the wild tetraploid with the autotetraploid artificially raised by colchicine treatment of diploid seeds shows that the former must indeed be an allopolyploid, probably from hybridisation with the nearly related genus Cardamine (Manton and Howard 1940). In artificially raised autotetraploids, fertility is considerably reduced compared with the related diploids (see above), for which multivalent formation and subsequent irregular segregation seems largely to account. Fertility is obviously an attribute of the greatest importance both in intra- and inter-specific competition and one means of achieving this in an autotetraploid will be the suppression of multivalent formation by the reduction of the chiasma frequency. This is what actually seems to have occurred in Tulipa (Upcott 1939a) and species which appear to be allopolyploid from their behaviour at metaphase show in other directions that they are really autopolyploid. In Allium Schoenoprasum (Levan 1936) again, the range of variability within one species is sufficiently great to permit of the/

of the origin of auto- and allo-tetraploid races. These few instances demonstrate with what care investigations of wild polyploids must be undertaken. The detailed study of polyploid behaviour is opening up new fields and as yet it is too early to generalise either on the relative proportions of these two forms of polyploids in nature or their relative importance from the point of view of evolution.

An interesting fact has emerged from the study of the inheritance of single dominant factors in autotetraploids, namely that the variance of the tetraploid is less than that of the diploid (Lindstrom 1935). Haldane (1930) has shown, that, whereas a small amount of random mating in a normally self-fertilising tetraploid population will have little effect on the proportion of recessives, a similar small amount of self-fertilisation in a normally random mating population will have a very considerable effect. The slight alterations in the proportions of recessives and dominants resulting from crossing over in quadrivalents will have a negligible effect in the wild. However, characters determined by single genes are the exception rather than the rule and we should expect polyploidy, in multiplying the numbers of genes determining particular characters to result in greatly increased variability of the population./

population. The bulk of the individuals composing such a population will be heterozygotes, the range of recombination will be greater and homozygotic segregants rarer than in the diploid populations. Admittedly the visible mutation rate will be less than that of diploids (Haldane 1931, Stadler 1929) but I do not agree with Stebbins' (1940) contention that evolution in polyploids will be slow. In fact the evidence seems to point to the greater adaptability and rate of spread of polyploids compared with diploids. In the first place, the greater range of variation and other physiological qualities connected with polyploidy will enable them greatly to extend their range. Secondly, providing, in the case of autopolyploids, generational sterility is not too great, increased plant size will in general mean increased seed production. In competition with diploids then, polyploids should increase their population size by the greater number of seeds produced alone.

In connection with the first of these points, Manton (1934) remarks upon the much greater variability of tetraploid Biscutella laevigata compared with the diploid which, she says, has frequently been commented upon by systematists. "There will always be a risk that infraspecific subdivisions will represent artificial categories of merely local or distinctive segregants"/

segregants" — referring to the tetraploid. So far I have not been able to find any other comparisons between the variability of auto- and allo-tetraploid with diploid populations. It seems, however, to be a characteristic of polyploid races (of diploid species) that they occupy continuous areas of distribution in contrast to the diploids which, although possibly more widely spread remain as comparatively small and uniform populations at various points within the distribution area. Again Biscutella provides a striking illustration. The diploid B. laevigata is only found at a number of widely separated localities each of which "is characterised by its own assemblage of morphological types to which subspecific and varietal rank has been given" (Manton 1934). By contrast the tetraploid subspecies occupy the whole of the region formerly covered by the Alpine Ice Sheet. Other examples are easily come by. Thus the diploid Tradescantias are limited in their distribution in N. America to small unglaciated areas in the south, the polyploid species being spread over the glaciated northern regions (Anderson and Sax 1936, Anderson and Woodson 1935). The diploid Campanula rotundifolia (Böcher 1936) and Vaccinium uliginosum (Hagerup 1933) are arctic in their distribution whilst the tetraploids have a larger and more southerly range. Diploid

Empetrum/

Empetrum nigrum is a temperate species, whilst its tetraploid E. hermaphroditum has an arctic range (Hagerup 1927). Similarly we find species like Biscutella laevigata with the tetraploids confined to the Alpine portion of its C. European range and others such as Phleum alpinum (Gregor and Sansome 1930, Müntzing 1936) where the alpines are diploid and the northern forms tetraploid. Plantago maritima represents yet another type, the northern forms being diploid and the Alpine populations mixtures of diploids and tetraploids (Gregor 1939). These anomalies of distribution seem explicable if the greater variability conferred by polyploidy means that a species is better able to adapt itself to a wide range of habitats. Probably the diploid species at one time likewise had a continuous distribution but environmental and other changes split up the area and reduced the size of the potentially interbreeding population. Decreased population size eventually leads to decreased variability and loss of ability to respond to environmental changes. This seems to be the only means of accounting for the markedly specialised ecological habitats occupied by some of the diploid subspecies of Biscutella and other relict species. Müntzing (1936) comparing the proportions of polyploid species in floras of different latitudes finds that 55% of the species/

allopolyploids will have a greater range of variation than autopolyploids since they presumably combine the variability of both their parents. In support of this he cites the Crepis complex where, apparently, the autopolyploids are almost as restricted in their distribution as the diploids, the allopolyploids being far the most widespread. What part apomixis plays in this scheme is hard to say. It would be of great interest in this connection to know what were the relative areas occupied by the auto- and allotetraploid races of Allium Schoenoprasum.

Regarding the second point raised above, namely the possibility of greater output of seed by the polyploid, there is also little information to be had. There seems no reason why the gigantism of autopolyploids should not lead to increase in the number of inflorescences per plant and in the number of flowers and seeds per inflorescence. This is marked in the allotetraploid watercress and Levan (1936) states that seed production in the autotetraploid race of Allium Schoenoprasum is higher than in the diploid forms examined. It has already been emphasised that the generational sterility of autopolyploidy will be selected against, the successful forms being those with a low chiasma frequency and high pollen and ovule fertility. If gigantism extends to the seed size this again may give seedlings of polyploids an initial advantage./

advantage.

In many cases one receives the impression that the origin of a polyploid race has been a single event in time and space. Yet it is clear that in those species where polyploid derivatives are frequent the same conditions — production of diploid gametes, polyploid chimaeras, which have once produced a polyploid will do so again if not many times. Since the polyploids, in most cases tetraploids, are genetically isolated from the parent diploids they must cross among themselves and the development of a new, highly variable population can be visualised straight away. And herein seems to lie the evolutionary importance of polyploidy. It is an isolating mechanism which splits up the diploid species into races incompatible with it. These new races "although not new species in themselves .... no doubt provide material from which such may differentiate" as Jørgensen (1928) appreciated from his work with artificially produced polyploids. If not actually an evolutionary advance in the sense of providing material from which other fundamentally new genera may develop, polyploidy is an advance in the sense of producing variations on and extending the potentialities of the original diploid species though perhaps no essentially new characters are evolved.

SUMMARY.

1. Evidence has been presented to show that the top necrotic reactions induced in certain potato varieties by graft infection with viruses A, X, B and C are separately determined by four genes designated N^A, N^X, N^B, and N^C respectively.

2. These four genes are alike in showing tetrasomic inheritance and being present and dominant in a single does in most of the cultivated varieties dealt with. One variety has been found to be duplex for the N^X gene.

3. The N^A and N^X genes are apparently situated in the same chromosome. The location of N^B is at present uncertain, this gene showing some indication of linkage with N^A and N^X, but N^C is evidently situated in a separate chromosome.

4. A critical examination of the cytological evidence has borne out the conclusion from the genetical data that the potato is an autotetraploid.

5. In conclusion, some indications have been given of the bearing of this work upon theoretical aspects of cytological and genetical science beyond the somewhat restricted compass of the paper.

ACKNOWLEDGMENT.

Very special thanks are due to Dr. Cockerham for allowing access to much unpublished information, for permission to use the data from a number of progenies tested during the years 1935-1938 and for the numerous photographs used to illustrate the virus section of this paper; also to Dr. Mather of the John Innes Horticultural Institution for advice on the statistical treatment of the material.

APPENDIX I.

Sterility in the Potato.

The contents of this section have been anticipated in the cytological review but as the insertion at that point of a lengthy discussion of the sterility and non-blossoming habit of the potato would have broken the continuity of the argument it seemed best to include it as an appendix.

The limitations to organised breeding programmes imposed by male sterility and non-blooming are very great and most workers have attempted a solution of the puzzle. The cytologists have seen an explanation for pollen sterility in the meiotic abnormalities already described but it is obvious that some more fundamental complex of genic and environmental factors is responsible for the great variability in flowering and fruiting which potatoes show. All gradations may be found between the non-blossoming condition and complete blooming, such as abscission of all buds before the flowers open, the opening of a few flowers which immediately fall and the persistence of a few flowers for several days.

For a long time it was widely believed that failure of sexual reproduction in vegetatively reproducing subjects was the result of diversion of the plant's/

plant's energies to food storing. Linked with this was the Lamarckian idea that improvement of tuber formation has resulted in the degeneration of sexual functions; other plants cited to support this view were Lilium, Hemerocallis, sweet potato and sugar cane. This apparently logical idea has been disproved by experiment. East (1908) removed the tubers as they were formed from several different varieties and found that there was no resulting increase in persistence of flowers in those varieties where the buds dropped before opening, nor was there any increase in the amount of viable pollen in the pollen producing varieties. Far more significant were the early observations that the abundance of flowering varied with the season and the locality, which are supported by information from a number of experiments.

Stout and Clark (1924) compared the behaviour of two identical sets of 15 potato varieties raised from cut halves of the same tubers, one set of which was planted at Presque Isle, Maine, and the other at the New York Botanic Garden. Of the 13 commercial varieties included, 2 bloomed well in both localities but more abundantly at Presque Isle, 2 produced few flowers at New York but bloomed abundantly at Presque Isle; the remainder bloomed freely at Presque Isle while not a flower opened at New York. In the same connection/

connection Stevenson and Clark (1933) state that "during the season of 1930 naturally fertilised seed was produced by 18 seedling varieties of potatoes at Estes Park, Colorado, at an elevation of 7,500 feet but only 3 of the same varieties produced seed at Presque Isle". Again, "potato seed cannot be produced on a large number of varieties and strains at University Farm, St. Paul, but many of the same varieties will produce seed at the N. E. Branch Experiment Station at Duluth, and even better seed setting is obtained at Castle Danger, 50 miles N.E. of Duluth" (see also KRANTZ et alii. 1938). Differences in environmental conditions between this station (Corstorphine) and the Ainville sub-station (Kirknewton, Midlothian) were sufficient in 1939 to induce at the latter bud dropping and abortion of flowers in several varieties which bloomed abundantly at Corstorphine, though no exact figures are available for comparison. Comparing different cytological accounts it was often seen that the same varieties studied in various parts of Europe frequently behaved differently.

In their classical researches on the influence of photoperiod on plant behaviour Garner and Allard (1920) were able to show that distinctions in time of flowering and fruiting of early and late maturing/

maturing and sterile or non-flowering varieties of plants were traceable primarily to responses to different day lengths. "Sexual reproduction can be attained by the plant only when it is exposed to a specifically favourable length of day; the requirements in this particular varying widely with the species and the variety" (Garner and Allard loc. cit.). It is obvious that vegetatively reproducing plants will be able to survive under conditions which are unfavourable for sexual reproduction.

Some rather inconclusive experiments by Stevenson and Clark (1933) indicate that length of photoperiod does bear some relation to the blossoming behaviour of the potato. Ten seedlings which produced an abundance of naturally fertilised seed in the field, together with 10 seedlings from the same progenies which produced no seed under the same conditions were divided into three groups, "each group containing one or more of each of the seedlings and varieties grown". One of these was exposed to natural day length, and the second and third to natural day length plus artificial illumination with a 1000 watt lamp and a 500 watt lamp respectively, from 5.30 to 11.30 p.m. each day from Feb. 11th to April 12th 1932. of the first set of seedlings 73% came into full bloom under the 1000 watt lamp, 68% under the 500 watt lamp but/

but only 50% under natural conditions. Of the second set 20% flowered under both the 1000 watt and the 500 watt illumination and none under natural conditions. It is difficult to make out from Stevenson and Clark's report whether there was any corresponding variation in pollen fertility.

Rather more information was obtained by Clark and Lombard (1939) from an extensive series of experiments. In one set of these carried out in 1937, 13 varieties were exposed to the natural day length, which varied during the experiment from 10-13½ hours, and to the natural day length supplemented by artificial illumination with 60 watt bulbs, giving a light intensity of 20-30 foot candles at bench level. In a second more accurate series of experiments performed in 1938, eight varieties were exposed to photoperiods of 9, 11, 13 and 16 hours. Seven of the varieties used (164-6, 164-7, 1010-68, 1019-69, 1019-106, Earlaine, Katahdin) set seed naturally under field conditions. The remaining six were pollinated in the experiments with one of the three self-fertile sorts, 164-7, Earlaine and Katahdin. In 1937 three of the naturally self-fertile seedlings produced a considerable number of berries under natural illumination but the number was substantially increased under the artificially lengthened photoperiod. This increase was statistically significant between the ordinary day length and the/

the other three photoperiods but there was no significant difference between these latter. Different varieties varied considerably in their behaviour. The more detailed 1938 experiments showed that the differences in numbers of flowers produced between varieties and between light treatments were highly significant, the number increasing as the photoperiods became longer. The percentage of flowers which set seed was approximately equal under all four photoperiods (9, 11, 13 and 16 hours), showing that it is the greater production of flowers under the long photoperiods which is responsible for the increased set of berries. This, in the case of the selfed varieties, Katahdin and Earlaine, may presumably be taken as indicating that there is little or no alteration in the proportion of fertile pollen with changes in photoperiod. In fact, differences in varietal behaviour were still marked, 80% of the flowers setting berries in Earlaine and only 50% in Katahdin. For all varieties the 16 hour photoperiod gave the best results.

Fresh light has been thrown on the mechanism of photoperiodic response in connection with flowering through the recent work of Moskov, Čajlachjan, Melchers and others. It was found that flowering could be initiated in short day plants held under long day conditions/

conditions by grafting these with scions from flowering, related, long day species, and vice versa, (Čajlachjan 1936, Čajlachjan and Yarkova 1937, Moskov 1937). To explain these results Čajlachjan postulated the existence of a hormone in the flowering plants which he termed "florigen" and which, diffusing out of the scion, is responsible for the initiation of flower primordia in the non-flowering stock. The florigen produced by different plants is apparently of the same nature (Čajlachjan and Yarkova 1937). From their extensive work with the short day plant Xanthium pennsylvanicum, Hamner and Bonner (1938) were able to show that the mature leaves of this plant alone were capable of manufacturing the hormone. It seems, in fact, that the initial photoperiodic effect is actually received by the leaves. Some long day plants may be brought into flower under short day conditions if they are first subjected to a period of long day conditions, and vice versa; this, termed induction. Using this technique with Xanthium, and subjecting one portion of the plant to different photoperiodic conditions from the other Hamner and Bonner demonstrated that the hormone could pass up or down the plant. All plants do not react similarly in this respect, and it is clear that localisation or diffusion of the response varies with the vegetative condition of the plant.

The/

The duration of the dark period was found to be the crucial factor in the production of florigen in Xanthium, since flower primordia were only formed in those plants subjected to photoperiodic cycles where the dark periods were longer than 5-8 hours. Temperature during light periods had no influence on the process, but hormone production was seriously hindered by low temperatures during the dark periods.

Melchers (1936, 1937, 1938, 1939) has carried the analysis of flowering behaviour a stage further by his work with annual and biennial Hyoscyamus plants and the short day Maryland Mammoth tobacco. The Hyoscyamus strains differ by a single gene but both flower only under long day conditions. Biennial plants flower only in the first year if their growing points have been subjected to low temperatures. Grafts from Maryland Mammoth tobaccos grown under long day conditions, and therefore presumably free of florigen since they are non-flowering, when transferred to biennial Hyoscyamus plants caused these to flower in their first year. The tobaccos must therefore contain a hormone which determined the first year flowering of the Hyoscyamus plants but is not present in sufficient quantities to initiate the flowering process in the tobaccos themselves. Melchers has termed this hormone "vernalin". Non-flowering Maryland/

Maryland Mammoth tobaccos under long day conditions were induced to flower by grafting to them leaves of flowering annual Hyoscyamus plants containing florigen. Leaves from non-flowering biennial Hyoscyamus did not induce such flower production. From these results it is concluded that florigen is only formed if vernalin is already present. The relationships of these hormones are by no means clear and more information is needed about the behaviour of other plants before any useful generalisations can be made. But the relating of differences in photoperiodic behaviour to genic differences is at least significant.

We are now in a somewhat better position to review the conditions existing in the potato, In the accompanying table (43)/162 varieties have been graded into four arbitrary classes (a), (b), (c) and (d) according to the number of flowers which open and are capable of pollination; those varieties in class (a) regularly produce an abundance of flowers, class (b) varieties produce fewer flowers, class(c) very few and class (d) no flowers at all. Secondly, the varieties in classes (a) and (b) have been graded according to the amount of fertile pollen they produce, the index (1) representing the highest of these classes. A query mark signifies that the pollen fertility of those varieties is unknown, 0 that no pollen is produced/

TABLE 43.

Maturity	Total	Distribution of degree of flowering within groups (a)	(b)	(c)	(d)	Distribution of pollen fertility within classes (a) and (b) $(a)_1$	$(a)_2$	$(a)_3$	$(a)_0$	$(a)_?$	$(b)_1$	$(b)_2$	$(b)_3$	$(b)_0$	$(b)_?$
1st Early	25	5	8	11	1	2	2	-	1	-	2	2	1	3	-
%-age	100.0	20.0	32.0	44.0	4.0		16.0					20.0			
2nd Early	36	15	7	6	8	7	1	0	3	4	1	1	0	4	1
%-age	100.0	58.3	19.4	16.7	22.2		22.2					5.6			
Early Maincrop	41	18	8	12	3	12	2	1	1	2	-	1	3	1	3
%-age	100.0	43.9	19.5	29.3	7.3		36.6					9.8			
Maincrop	28	19	6	3	-	11	3	-	2	3	-	1	-	2	2
%-age	100.0	67.9	21.4	10.7	0.0		50.0					3.6			
Late Maincrop	32	23	2	6	1	6	2	1	6	8	-	-	-	-	2
%-age	100.0	71.9	6.3	18.8	3.1		28.1					0.0			

For correlation between degree of flowering and time of maturity,
= +0.4587

For correlation between degree of pollen fertility and time of maturity
= +0.6391

produced at all. It must, of course, be kept in mind that the data have been assembled from various sources and that both the flower and pollen fertility gradings are largely arbitrary, the behaviour of individual varieties being liable to vary in different years, though this variation is likely to be least among the most abundant bloomers and pollen producers. If, now, we compare the percentage of class (a) varieties in each of the maturity groups, with the exception of the early main crops, there is seen to be a regular increase of this figure coincident with the lengthening of the life cycle, the late main crops having the highest proportion of free flowering varieties. The correlation between lateness of maturity and free blooming is positive and strong, though not actually significant. Again, if we take that proportion of class (a) varieties in each maturity group which produces any pollen at all and compare the figures there is again a correlation, this time between lateness of maturity and pollen fertility with the main crops showing the highest proportion of pollen fertile varieties.

Ellison (1936) has also remarked on the fact that the first early varieties he investigated all showed a higher incidence of pollen sterility than the later maturing ones. In flowering times the varieties follow/

follow the same sequence as their times of maturing that is to say the first earlies flower first, followed by the second earlies and main crops and this order is not greatly upset by different orders of planting. The main crops are usually in flower by mid-July, at which time the day length is 16-17 hours. I have therefore interpreted these figures as indicating that the main crop varieties are the most floriferous and pollen fertile because conditions in this country represent the optimum for the expression of their photoperiodic genotype. The sterility of the early maturing varieties is attributed to their being, so to say, out of step with their environment. It is conceivable that individuals may arise of a sufficiently balanced type to permit of their flowering earlier in the season, thereby accounting for the occasional early maturing varieties which do produce an abundance of flowers and fertile pollen. It is noteworthy that a day length of 16 hours was found by Clark and Lombard (1939) to induce the greatest production of flowers.

A preliminary experiment with eight varieties was set up to see whether freely flowering varieties or tomatoes produced any hormone which would induce the production of flowers in habitually non-flowering sorts. Jones and Borthwick (1938) working with one variety, Sebago/

Sebago, found that flower primordia were differentiated in complete darkness. This has also been noticed by the present writer in the case of seedlings stored in the dark, and if true also of habitually non-flowering varieties means that environmental conditions must intervene between the time the sprouts appear above ground and the normal flowering time to cause the abscission or degeneration of these primordia. In the varieties chosen 1st-Early, 2nd-Early, Early Maincrop and Late Maincrop maturities were represented and of nine plants of each, one was kept ungrafted, three were grafted with scions of seedling tomatoes, var. Essex Wonder, three were grafted with shoots from a known freely flowering potato variety and two were grafted with shoots from the same plant as a control on the act of grafting. Two shoots on each plant were grafted. The varieties were further divided into two sets, those necrotic to both the A and X viruses and those necrotic to neither and the flowering varieties from which scions were taken chosen so that their virus reactions would be compatible with those of the stocks to which they were to be grafted. The early maincrop Waverley and the second early Flourball respectively were used for these two groups. In addition two scions from each of the varieties tested were grafted to tomato stocks, also of the variety Essex/

Essex Wonder. All the plants were raised from tubers planted in six inch pots placed outside in mid-May 1940, taken into the greenhouse to be grafted, and again set outside after the grafts had united. These grafted plants, including the tomatoes, were set out in a field plot between the 15th and 18th of June. In nearly every case the graft unions were excellent but a very dry spell after planting out caused severe wilting and it was some considerable time before growth was resumed. The potato grafts on tomato stocks grew strongly as did the tomato scions on the other potato varieties, flowering and setting fruit. None of the Flourball or Waverley scions flowered nor did the stock plants of these varieties flower from which the scions were taken. The results of the experiment were entirely negative. No flower buds were observed on the habitually non-flowering Arran Crest, Harbinger, Herd Laddie, Duke of Perth or Arran Luxury and there was no indication of increased persistence of flowers in King Edward, Duke of York or Great Scot, whether grafted with potato or tomato scions or grafted to tomato stocks. No conclusion can be drawn in view of the lack of uniformity of the environmental conditions to which the plants were subject and a more careful investigation is needed. It seems significant, however, that Stout and Clark (1924) also found no increase in fruitfulness resulting from grafting 16 varieties/

varieties to stocks of Solanum ciliatum, S. sysimbrifolium, S. miniatum and S. nigrum, which presumably would also be flowering under the conditions of the experiment.

We have already seen that, outside the mechanical sterility consequent on bud dropping, pollen sterility is accompanied by a greatly increased production of diad and monad pollen grains which, apparently are non-functional (Meurman and Rancken 1932, Heyn 1930, Ellison 1936). The male fertility of a plant may therefore be judged by the proportion of apparently normal tetrads in the pollen. The reason for the production of the abnormal grains seems to lie with environmental influences and it is not unreasonable to suppose, that, in spite of Clarke and Lombard's (1939) results, abnormal photoperiodic conditions may also play a part. If the plant is out of step with the environment this may well be reflected in the meiotic behaviour. Upcott (1937) has described a case of male sterility apparently due to unbalance in the timing of the meiotic processes in Lathyrus odoratus. Although the anthers of the normal and male sterile plants grew at the same rate there was a lag in meiosis of the sterile one, such that the latter only reached the first meiotic division at the time when the normal plant showed binucleate pollen. Degeneration often followed first anaphase.

Various classifications of potato varieties have been put forward on the basis of the percentage of normal tetrads observed, and all agree in that even the best pollen producers show a certain amount of inviable pollen (Clark 1927, Stout and Clark 1924, Meurman and Rancken 1932). Female sterility on the other hand has been largely overlooked and it has been assumed that varieties which at least open their flowers can act as seed parents. Stout and Clark (1924) detected variation in female fertility by crossing all the varieties to be tested with pollen from the most fertile male sorts. From the results of such crosses the varieties were assembled into four groups:-

A. Varieties highly productive of berries when pollen of the most male fertile varieties is used.

B. Varieties with a feeble production of berries.

C. Varieties which produced no berries with seed to any pollination.

D. Varieties which tended to produce parthenocarpic fruits.

Some varieties were found to fruit poorly even with the best pollen, but as with potato flowers, female fertility, as judged by the set of berries, was extraordinarily liable to variation with environmental conditions. From the figures for a large number of varietal/

varietal crosses, for which I am indebted to Dr. Black, it has been possible to demonstrate similar differences in female fertility among European varieties. The figures only admitted of assessing the fertility of ten varieties from the number of crosses made with male parents known to be of the $(a)_1$ grade of fertility (see Table 43), since no one variety has been used sufficiently often to make the results reliable. Also, the figures are only for the number of berries set per number of crosses made and not per number of flowers pollinated. The ten female parents are set out in Table 44 in the order of their fertility. In this case there seems to be little indication of a correlation between the time of maturity and degree of fertility.

Table 45 has been drawn up from the results of some crosses made by the present writer in the summer of 1939. Comparing the results of crossing seven different varieties with four other male fertile sorts and using the number of seeds per berry as an index of the compatibility of the union it will be seen that this figure does not show a correlation with the percentage of pollinated flowers which set fruit. Moreover, there are marked differences in performance of the same female parent pollinated with different males and between different females pollinated with the same males, as judged by the average number of seeds/

TABLE 44.

Maturity	Variety	No. berries / No. crosses	%-age crosses set
2nd Early	British Queen	8/9	88
Late Maincrop	Golden Wonder	5/6	83
Late Maincrop	Kerr's Pink	5/7	71
Early Maincrop	Craigs Defiance	2/3	67
2nd Early	Epicure	6/11	54
Maincrop	Up to Date	3/7	42
1st Early	Arran Pilot	3/9)	33
2nd Early	Catriona	3/9)	
Maincrop	967(c) 38	14/44	31
Early Maincrop	Red King	0/8	0

seeds per berry. As before, these differences are indicative of varying output of viable gametes rather than of any intervarietal compatibility.

Turning now to the question of the inheritance of pollen sterility, the tendency has been to treat sterility as a clear cut character. For example, Salaman (1910) and Salaman and Lesley (1922) attempted to explain the inheritance of this character as being dependent on a single factor difference segregating in disomic ratios. As the polyploid nature of the potato is well established and the numbers of plants dealt with very small this work can scarcely be considered seriously. Recently, Krantz, Becker and Fineman (1938) claim to have shown that, regarding the potato as a tetraploid, pollen sterility is determined by the segregation of four factors independently, the double recessive pollen being non-viable. The discussion of the results is preceded by an admirable account of the various conditions upsetting fruiting of the potato. These workers investigated the incidence of pollen sterility in the F_1-F_4 generations of a cross between the variety Lookout Mountain and an unknown rogue "found as a mixture in a commercial field of Irish Cobbler". Both parents had a fair amount of pollen. The percentage of pollen stainable in aceto-carmine was taken as an index of the amount of viable pollen produced by/

by an individual.

By this means it was claimed that 16 F_1 plants were classifiable into 3 distinct groups; 5 with 0-17% of stainable pollen, 6 with 30-46%, and 5 with 70-77%. Of these 16 F_1's only 7 produced berries and the progenies derived from these 7 produced only 14, 10, 8, 15, 6, 10 and 10 seedlings respectively. From observations on the amount of stainable pollen in each of these plants three types of segregation are detected among the F_2 plants. Four progenies ranged from high to medium percentage of stainable pollen; two for high medium and low, and two for medium and low.

The F_3 and F_4 progenies also contain low plant numbers ranging from 17 to 3 but from similar determinations to the above it was considered that the 38 families involved showed three distinct breaks in the frequencies of the means as follows: between 20·6 and 27·8, 37·8 and 41·2, and 50·6 and 57·7. It is concluded that the segregation observed suggests that this may be a natural grouping and "that the parents of the 38 families may have been distributed among four different genotypes with respect to sterility".

Throughout this investigation no attention is paid to the possible coexistence of female sterility. But even assuming that male and female sterility in any plant correspond roughly in their extent, in the present/

present writer's opinion the size of the progenies raised is not large enough to justify the conclusions drawn. In every case it is quite conceivable that increase in the number of plants would have caused sufficient overlapping in the pollen classes as to render any clear cut classification impossible.

Further the results are interpreted as indicating "that pollen sterility may be inherited as a simple mendelian character determined by a pollen lethal gene, designated V, the vv pollen being fully lethal and the Vv partly so". Although the existence of such factors is highly probable — and some such possibility has already been envisaged in the breeding results discussed above, there are so many other conditions influencing non-fruiting that the scanty nature of the data scarcely appears to justify such a simple factorial hypothesis. Such strictly alternative fertility factors are likely to be most easily detected among varieties which normally show high degrees of male and female fertility.

At present there seems to be no straightforward solution to the problem. Investigations are always liable to be hampered by environmental conditions and the fact that usually only a small proportion of any progeny flowers in the same year. It would seem that since sterility is so closely connected with the/

the photoperiodic reaction this relation would repay further investigation and perhaps lead to results of practical value.

APPENDIX II.

Gametic segregation in auto-tetraploids.

The proportions of gametic types are derived as follows:-

Simplex case, Aa_3

In an individual simplex for a factor A whose locus is such that crossing over may occur between it and the centromere, then separation at this locus will be

		AA	Aa	aa
reductional	in (1 - e) of cases	-	2	2
equational	in e (1 - a) of cases, paired chromosomes passing to opposite poles giving	-	2	2
	in ea of cases, paired chromosomes passing to the same pole, giving	($\frac{1}{4}$ -)	2	2
		($\frac{1}{4}$ 1)	-	3
		($\frac{1}{4}$ -)	2	2
		($\frac{1}{4}$ 1)	-	3
		2ae	8-4ae	8+2ae

if α = ae, these proportions become α $4-2\alpha$ $4+\alpha$

and if the heterozygotes are indistinguishable from the homozygous dominants $4-\alpha$ $4+\alpha$

The gametic proportions expected from a triplex individual A_3a, may be easily written down once those of the simplex are known, viz. $A, 8-\alpha, a, \alpha$.

Duplex/

Duplex case, A_2a_2

The situation is complicated by the fact that in one third of the cases like chromosomes may be paired with like whilst in the remaining two thirds like and unlike chromosomes are paired. In half of these latter cases a given chromosome will be paired with like and unlike chromosomes whilst in the other half it will be paired with two unlike chromosomes. Where like chromosomes are paired with like there will be no equational separation but where unlike chromosomes are paired the possibility of independent double reduction of the two pairs of loci must be considered. Thus, where like chromosomes are paired separation will be

		AA	Aa	aa
reductional	at both pairs of loci in $4(1 - e)(1 - e)$ of cases giving	-	4	-
	at both pairs of loci in $4e^2(1 - a)$ of cases, paired chromosomes passing to opposite poles giving	-	4	-
	at both pairs of loci in $4ae^2$ of cases, paired chromosomes passing to the same poles, giving	2	-	2
equational	at one pair of loci and reductional at the other in $8e(1 - e)(1 - a)$ of cases, paired chromosomes passing to opposite poles giving,	-	4	-
	at one pair of loci and reductional at the other in $8e(1 - e)a$ of cases, paired chromosomes passing to opposite poles, giving	2	-	2

Following/

Following a similar procedure where like and unlike chromosomes are paired,

in $2(1-e)(1-e)$ of cases	the result is		2	4	2
" $2e^2(1-a)$	"	($\frac{1}{4}$	2	-	2
		($\frac{1}{4}$	-	4	-
		($\frac{1}{4}$	1	2	1
		($\frac{1}{4}$	1	2	1
" $2ae^2$	"	($\frac{1}{4}$	-	4	-
		($\frac{1}{4}$	1	2	1
		($\frac{1}{4}$	1	2	1
		($\frac{1}{4}$	2	-	2
" $4e(1-e)(1-a)$	"	($\frac{1}{2}$	1	2	1
		($\frac{1}{2}$	1	2	1
" $4e(1-e)a$	"	($\frac{1}{2}$	1	2	1
		($\frac{1}{2}$	-	4	1
Summing these proportions we get			$8+8ae$	$32-16ae$	$8+8ae$
simplifying and substituting for the product ae, these become			$1+\alpha$	$4-2\alpha$	$1+\alpha$
and if the heterozygotes are indistinguishable from the homozygous dominants			$3-\alpha$		$1+\alpha$

ANALYSIS OF SINGLE FACTOR SEGREGATIONS

In the present case it is of greater importance to determine whether the different families are in agreement with one another in their behaviour rather that to detect their deviations from any fixed ratios. Accordingly the progenies have been divided up into groups of like parentage and tested for the existence of heterogeneity within and between groups. This has been done by using Brandt and Snedecor's method (see Mather 1938). Values of P, the probability of obtaining a similar result by chance, of 5% or less have been taken as indicating significant deviations from the

ANALYSES OF SEGREGATIONS CONCERNING THE GENES N^X, N^A, N^B and N^C

N^X

N^X x n^x	a_1	a_2	n	a_2^2/n		
Epicure x Shamrock	41	51	92	28.27	$\frac{(n_t)^2}{a_1 t a_2 t}$	4.050
" x Alness	33	35	68	18.01		
" x Argyle Fav.	14	22	36	13.44		
" x Alannah (1)	11	21	32	13.78	Difference	0.63
" x " (2)	29	31	60	16.02	χ_4^2	2.552
	128	160	288	89.52		
				88.89	P	0.70-0.50
Epicure x Pepo	10	12	22	6.55	$\frac{(n_t)^2}{a_1 t a_2 t}$	4.010
White City x Pepo	51	54	105	27.77		
Liddes. Lads x Pepo	29	29	58	14.50	Difference	0.08
Maud Meg x Pepo	72	84	156	45.23	χ_4^2	0.3208
Southesk x Pepo	62	69	131	36.34		
	224	248	472	130.39		
				130.31	P	0.99-0.98
Benest x Alness	30	41	71	23.68	$\frac{(n_t)^2}{a_1 t a_2 t}$	4.000
Craigs Def.x Flourball (1)	91	77	168	35.30	Difference	0.74
Craigs Def. x Flourball (2)	60	60	120	30.00	χ_2^2	2.96
	181	178	359	88.98		
				88.24	P	0.30-0.20
Epicure x Alannah (1)	11	21	32	13.78	$\frac{(n_t)^2}{a_1 t a_2 t}$	4.069
" x " (2)	29	31	60	16.02		
	40	52	92	29.80	Difference	0.41
				29.39	χ_1^2	1.668
					P	0.20-0.10
Craigs Defiance x Flourball (1)	91	77	168	35.30	$\frac{(n_t)^2}{a_1 t a_2 t}$	4.01
Craigs Defiance x Flourball (2)	60	60	120	30.00	Difference	0.14
	151	137	288	65.30	χ_1^2	0.5613
				65.16	P	0.50-0.30

The indices of χ^2 indicate the number of degrees of freedom.

N^X x n^X	a_1	a_2	n	a_2^2/n		
Epicure x Pepo	10	12	22	6.55	$\frac{(nt)^2}{a_1^t a_2^t}$	
Epicure x Others	128	160	288	88.89		4.05
	138	172	310	95.44	Difference	0.03
				95.41	χ_1^2	0.1215
					P	0.80-0.70
Epicure x Others	128	160	288	88.89	$\frac{(n_t)^2}{a_1^t a_2^t}$	
Others x Pepo	224	248	472	130.31		4.022
	352	408	760	219.20	Difference	0.17
				219.03	χ_1^2	0.6837
					P	0.50-0.30
Epicure x Others	128	160	288	88.89	$\frac{(n_t)^2}{a_1^t a_2^t}$	
Benest x Alness)						4.007
Craigs Defiance)					Difference	0.33
x Flourball)	181	178	359	88.24	χ_1^2	1.322
(1) and (2))	309	338	647	177.13		
				176.80	P	0.30-0.20
Others x Pepo	224	248	472	130.31	$\frac{(nt)^2}{a_1^t a_2^t}$	
Benest x Alness)						4.002
Craigs Defiance)					Difference	0.15
x Flourball)	181	178	359	88.24	χ_1^2	0.6003
(1) and (2))	405	426	831	218.55		
				218.40	P	0.50-0.30
F_2 N^X selfed						
Edgecote Purple	15	8	23	2.783	$\frac{(n_t)^2}{a_1^t a_2^t}$	
Isis	107	33	140	7.779		5.312
	122	41	163	10.562	Difference	0.249
				10.313	χ_1^2	1.323
					P	0.30-0.20

Crosses concerning Kepplestone Kidney

n^x x N^x	a_1	a_2	n	a_2^2/n		
President x K.K.	50	50	100	25.00		
Up to Datex "	78	22	100	4.84	$\frac{(n_t)^2}{a_1{}^t a_2{}^t}$	4.141
Scot x "	92	47	139	15.89		
Claymore x "	93	64	157	26.09		
Peachbloom x "	58	62	120	32.03	Difference	6.88
Kerr's Pink x"	73	53	126	22.29		
British Queeen x K.K.	45	27	72	10.13	χ_9^2	28.49
Pepo x K.K.	41	25	66	9.47		
Ar. Victory x K.K.	84	67	151	29.73	P	very small
Golden Wonder x K.K.	98	73	171	31.16		
	712	490	1202	206.63		
				199.75		
Claymore x K.K.	93	64	157	26.09		
Kerr's Pink x "	73	53	126	22.29	$\frac{(n_t)^2}{a_1{}^t a_2{}^t}$	4.116
British Q. x "	45	27	72	10.13		
Pepo x "	41	25	66	9.47		
Ar. Victory x "	84	67	151	29.73	Difference	0.37
Gold. Wonder x "	98	73	171	31.16		
	434	309	743	128.87	χ_5^2	1.523
				128.50	P	0.30-0.20
President x K.K.	50	50	100	25.00	$\frac{(n_t)^2}{a_1{}^t a_2{}^t}$	
Up to Date x "	78	22	100	4.84		4.187
Scot x "	92	47	139	15.89	Difference	6.37
Peachbloom x "	58	62	120	32.03	χ_3^2	27.29
	278	181	459	77.76	P	very small
				71.39		
K.K. x 70(13)	12	12	24	6.00	$\frac{(n_t)^2}{a_1{}^t a_2{}^t}$	
" x Alness	7	13	20	8.45		4.229
" x Shamrock	37	65	102	41.42	Difference	0.39
	56	90	146	55.87	χ_2^2	1.649
				55.48	P	0.50-0.30

Backcross	a_1	a_2	n	a_2^2/n		
Kep. Kid. as ♀	56	90	146	55.48	$\frac{(n_t)^2}{a_1{}^t a_2{}^t}$	4.376
Kep. Kid. as ♂	712	490	1202	199.75	Difference	5.68
	778	580	1348	255.23	χ_1^2	24.86
				249.55	P	very small
Kep. Kid. x Shamrock	37	65	102	41.42	$\frac{(n_t)^2}{a_1{}^t a_2{}^t}$	4.129
Epicure x "	33	35	68	18.01	Difference	0.61
	70	100	170	59.43	χ_1^2	2.519
				58.82	P	0.20-0.10
Kep.Kid. x Alness	7	13	20	8.45	$\frac{(n_t)^2}{a_1{}^t a_2{}^t}$	4.058
Epicure) x "	63	76	139	41.55	Difference	0.18
Benest)	70	89	159	50.00	χ_1^2	0.7304
				49.82	P	0.50-0.30
Pepo x Kep. Kid.	41	25	66	9.47	$\frac{(n_t)^2}{a_1{}^t a_2{}^t}$	4.001
Others x Pepo	224	248	472	130.31	Difference	1.25
	265	273	538	139.78	χ_1^2	5.001
				138.53	P	0.05-0.02
Kep.Kid. x a_4	56	90	146	55.48	$\frac{(n_t)^2}{a_1{}^t a_2{}^t}$	4.022
Rest x a_4	530	589	1119	310.03	Difference	1.05
	586	679	1265	365.51	χ_1^2	4.223
				364.46	P	0.05-0.02
F_2 N^X selfed						
Kep.Kid. s. 1	13	6	19	1.895	$\frac{(n_t)^2}{a_1{}^t a_2{}^t}$	
2	27	7	34	1.441		5.079
3	11	4	15	1.067	Difference	0.203
4	52	21	73	6.041	χ_3^2	1.031
	103	38	141	10.444		
				10.241 P		0.80-0.70

F_2	a_1	a_2	n	a_2^2/n		
Kep. Kidney selfed	103	38	141	10.24	$\frac{(n_t)^2}{a_1 t a_2 t}$	5.214
Rest selfed	122	41	163	10.31	Difference	0.02
	225	79	304	20.55	χ_1^2	0.1043
				20.53	P	0.80-0.70

DUPLEX N_2^X

n^X x N_2^X

Golden Wonder x Cardinal	74	12	86	1.674	$\frac{(n_t)^2}{a_1 t a_2 t}$	6.053
Abundance x Cardinal	70	26	96	7.042	Difference	0.782
	144	38	182	8.716	χ_1^2	4.733
				7.934	P	0.05-0.02

F_2 N_2^X selfed

Cardinal selfed 1	44	3	47	0.1915	$\frac{(n_t)^2}{a_1 t a_2 t}$	12.77
2	27	2	29	0.1379	Difference	0.1097
3	25	4	29	0.5517	χ_2^2	1.400
	96	9	105	0.8811	P	0.50-0.30
				0.7714		

SIMPLEX N^A

N^A x n^a

Liddesd. Lads x Pepo	21	25	46	13.59	$\frac{(n_t)^2}{a_1 t a_2 t}$	4.051
Maud Meg x Pepo	57	92	149	11.39	Difference	1.73
Southesk x Pepo	37	27	64	56.81	χ_2^2	7.008
	115	144	259	81.79	P	0.05-0.02
				80.06		

Craigs Defiance x Flourball (1)	57	44	101	19.17	$\frac{(n_t)^2}{a_1 t a_2 t}$	4.047
do. (2)	46	39	85	17.89	Difference	0.02
	103	83	186	37.06	χ_1^2	0.08094
				37.04	P	0.80-0.70

N^A x n^a	a_1	a_2	n	a_2^2/n		
Others x Pepo	115	144	259	80.06	$\frac{(n_t)^2}{a_1 t\ a_2 t}$	4.002
Rest x a_4	103	83	186	37.04	Difference	1.30
	218	227	445	117.10	χ_1^2	5.203
				115.80	P	0.05-0.02
Arran Victory x Kep. Kidney	80	63	143	27.76	$\frac{(n_t)^2}{a_1 t a_2 t}$	4.001
Others x a_4	218	227	445	115.80	Difference	0.53
	289	290	588	143.56	χ_1^2	2.121
				143.03	P	0.20-0.10

SIMPLEX N^B

Backcross

n^b x N^B						
Liddesd. Lads x Pepo	21	23	44	12.02	$\frac{(n_t)^2}{a_1 t a_2 t}$	4.032
N^B x n^b					Difference	0.13
Craigs Defiance x Flourball (1)	62	70	132	37.12	χ_2^2	0.5242
do. (2)	39	53	92	30.53	P	0.80-0.70
	122	146	268	79.67		
				79.54		
N^B x n^b						
Ar. Victory x Kep. Kid.	70	64	134	30.57	$\frac{(n_t)^2}{a_1 t a_2 t}$	4.008
Others x b_4	122	146	268	79.54	Difference	0.41
	192	210	402	110.11	χ_1^2	1.643
				109.70	P	0.20-0.10

SIMPLEX N^C

N^C x n^c						
Cr.Defiance x Flourball (1)	83	78	161	37.79	$\frac{(n_t)^2}{a_1 t a_2 t}$	4.0027
do. (2)	55	53	108	26.01	Difference	0.00
	138	131	269	63.80	χ_1^2	0.00
				63.80	P	1.00

F_2 N^C x N^C

Epicure x Alannah	(1)	21	7	28	1.7500	$\frac{(n_t)^2}{a_1 t a_2 t}$	4.6886
do. x do.	(2)	34	18	52	6.2308	Difference	0.1683
		55	25	80	7.8125	χ_1^2	0.7891
						P	0.50-0.30

Estimation of the index of separation of the N^X gene.

Estimates of α and β, the simplex and duplex indices of separation respectively, for a given gene may only be arrived at if data from backcross and selfed F_2 matings are available.

Part of the data from the N^X gene were obtained by backcrossing varieties simplex for this gene, and by selfing similar varieties. The frequencies of dominants and recessives in each case were

Backcross	533 N^X,	586 n^X
Self	122 N^X,	41 n^X

The expectations from the gametic segregations given above are

Backcross	$\frac{1}{8}(4-\alpha)N^X$,	$\frac{1}{8}(4+\alpha)n^X$
Self	$\frac{1}{64}(48-8\alpha-\alpha^2)N^X$,	$\frac{1}{64}(16+8\alpha+\alpha^2)n^X$

The likelihoods of obtaining such segregations separately are

Backcross	C_1	$\left[\frac{1}{8}(4-\alpha)\right]^{533}$	$\left[\frac{1}{8}(4+\alpha)\right]^{586}$
Self	C_2	$\left[\frac{1}{64}(48-8\alpha-\alpha^2)\right]^{122}$	$\left[\frac{1}{64}(16+8\alpha+\alpha^2)\right]^{41}$

The likelihood of obtaining these two segregations (see Mather 1938) jointly is the product of the two individual likelihoods. The method of maximum likelihood depends on the maximisation of this product with respect to α, and is most easily done if the various terms are converted to their logarithms. Then the logarithm of the joint likelihood will be given by the sum of the individual logarithm likelihood expressions.

L /

$$L = 533 \log (4-\alpha)+586 \log (4+\alpha)+122 \log (48-8\alpha-2\alpha^2)+ 41 \log (416+8\alpha-\alpha^2)$$

Differentiating with respect to α and equating to zero leads to the equation of estimatinn

$$\frac{dL}{d\alpha} = -\frac{533}{4-\alpha} + \frac{586}{4+\alpha} - \frac{244(4+\alpha)}{48-8\alpha-\alpha^2} + \frac{82(4+\alpha)}{16+8\alpha+\alpha^2} + \frac{82(4+\alpha)}{16+8\alpha+\alpha^2} = 0$$

Whence $1445\alpha^2 + 15824\alpha - 2576 = 0$

$\therefore \alpha = +0.1604$

The variance of α is obtained by taking the second differential of the original expression, substituting the expected values for the observed and equating to $-\frac{1}{V_\alpha}$. The less the variance of the estimate the greater the amount of information concerning α in the data so that $\frac{1}{V_\alpha}$ may be written I_α.

Then $$\frac{d^2\alpha}{d\alpha^2} = \frac{1119}{8}\left(\frac{1}{4-\alpha} + \frac{1}{4+\alpha}\right) + \frac{163}{64}\left[\frac{(8+2\alpha)^2}{48-8\alpha-\alpha^2} + \frac{(8+2\alpha)^2}{16+8\alpha+\alpha^2}\right] = I_\alpha.$$

Substituting for α

$$I_\alpha = 139.875\ (.2604+.2404)+2.5469\ (1.4828+4.0000)$$

$$= 70.0494 + 13.9641 = 84.0135$$

$\therefore V_\alpha = 0.01190$ and $S_\alpha = \sqrt{0.01190} = \pm 0.1091$

$\therefore \alpha = 0.1604 \pm 0.1091$

An exactly similar procedure is followed in the calculations of the duplex index of separation, β, for the N^X gene. Here, the frequencies of dominants and recessives in the two types of matings were

Backcross	144 N^X,	38 n^x
Self	96 N^X,	9 n^x

The/

The corresponding combined logarithm expression is

$$L = 144 \log (5-\beta) + 38 \log (1+\beta) + 96 \log (35-2\beta-\beta^2) + 9 \log (1+2\beta+\beta^2)$$

$$\therefore \frac{dL}{d\beta} = \frac{-144}{5-\beta} + \frac{38}{1+\beta} - \frac{192(1+\beta)}{35-3\beta-\beta^2} + \frac{18(1+\beta)}{1+2\beta+\beta^2} = 0$$

$$\therefore 392\beta^2 + 1648\beta - 760 = 0$$

$$\therefore \beta = 0.4193$$

$$I_\beta = \frac{182}{6}\left(\frac{1}{5-\beta} + \frac{1}{1+\beta}\right) + \frac{105}{36}\left[\frac{(2+2\beta)^2}{1+2\beta+\beta^2} + \frac{(2+2\beta)^2}{35-2\beta-\beta^2}\right]$$

Substituting for β

$$I_\beta = 30.3(0.9230 + 0.7047) + 2.917(4.003 + 0.2371)$$

$$= 27.97 + 12.368 = 40.338$$

$$\therefore V_\beta = 0.02479 \text{ and } s_\beta = \sqrt{0.02479} = \pm 0.1575$$

$$\therefore \beta = 0.4193 \pm 0.1575$$

To determine whether β is significantly greater than α the t test is employed. The formula being

$$t = \frac{\beta - \alpha}{\sqrt{\frac{V_\alpha}{n_\alpha+1} + \frac{V_\beta}{n_\beta+1}}}$$

where n_α is the number of degrees of freedom for α, i.e. 2 and n_β the same for β, i.e. 1 (Mather 1936)

$$\therefore t = \frac{0.2589}{\sqrt{0.01636}} = \frac{0.2589}{0.1279} = 2.024$$

Testing the heterogeneity of the simplex and duplex backcross and selfed data concerning the N^A gene

A method for doing this test has been developed by Mather (1937b) (see Mather 1938) which is based on joint estimation by the method of maximum likelihood. For calculating χ^2 we require to know the amount of information, I, contributed by each set of data, backcross and

F_2/

F_2, separately and D, the deviations of the backcross and F_2 expressions of the maximum likelihood expression from zero. Then $\chi_1^2 = S\left(\frac{D^2}{I}\right)$

Now, the values of I Backcross and I self have already been calculated in determining the standard error of

so that, $I_B = 70.0494$ and $I_S = 13.9641$

Then $D_B = \frac{-244(4+\alpha)}{48-8\alpha-\alpha^2} + \frac{82(4+\alpha)}{16+8\alpha+\alpha^2}$

and $D_S = \frac{-533}{4-\alpha} + \frac{586}{4+\alpha}$ Substituting for α

$D_B = +2.1$

$D_S = -2.03$

Then $\chi_1^2 = \frac{D_B^2}{I_B} + \frac{D_S^2}{I_S} = \frac{2.03^2}{70.049} + \frac{2.1^2}{13.96}$

$= 0.37473$ and P = 0.7-0.5

$\therefore$ the two sets of data are in agreement with one another.

The procedure is precisely the same when testing the heterogeneity of the duplex backcross and self data.

Here, $I_B = 27.97$ and $I_S = 12.368$

and $D_B = \frac{-144}{5-\beta} + \frac{38}{1+\beta}$

$D_S = \frac{-192(1+\beta)}{35-3\beta-\beta^2} + \frac{18(1+\beta)}{1+2\beta+\beta^2}$

Substituting for β (= 0.4193)

$D_B = -4.66$ and $D_S = +4.675$

$\therefore \chi_1^2 = \frac{4.66^2}{27.97} + \frac{4.675^2}{12.368} = 2.5434$

and P = 0.20-0.10

The two sets of data are therefore showing relatively good agreement with one another.

Estimation of the index of separation of the N^C gene

Of the remaining genes studied, data of the two types, backcross and self, are only available for the N^C gene.

Maximisation of the expression

$$L = 138 \log (4-\alpha)+131 \log (4+\alpha)+55 \log (48-8\alpha-\alpha^2) +25 \log (16+8\alpha+\alpha^2)$$

gives the value of α as 0.0666. The standard error, calculated as above is ±0.2060. The unsatisfactory nature of this estimate of α must be attributed to the inadequacy of the data.

Testing the heterogeneity of the backcross and self N^C data.

Performing the same test as was done on the N^X data and using the above value of α (0.0666) $\chi_1^2 = 1.7261$ and P = 0.20-0.10, so that the two sets of data concerning the N^C gene are in agreement with one another.

Linkage Determinations.

For the detection of linkage the data have been grouped according to the genes concerned and the frequencies of the various classes set out as in the accompanying tables. Since all the families are backcrosses the/

the assumption is made that the two genes in each case are segregating in accordance with a 1:1 ratio and independently of one another. A χ^2 for the joint deviation of all the observed frequencies from their expectations may be calculated by using the formula $\chi^2 = S\left(\frac{a^2}{mn}\right) - n$ where a is the observed and mn the expected frequency in each case, n the total number of individuals and S the sum over all classes. This χ^2 has three degrees of freedom, two concerned with the deviations of the individual gene segregations from the expected 1:1 ratio and the third with detecting association of the two factors in segregation. Then if the two gene pairs be A,a and B,b

$$\chi_A^2 = \frac{(a_1+a_2-a_3-a_4)}{n} \qquad \chi_B^2 = \frac{(a_1-a_2+a_3-a_4)}{n}$$

and, by application of the principal of orthogonality (see Mather 1938) $\chi^2_{\text{Linkage}} = \frac{(a_1-a_2-a_3+a_4)}{n}$ where a_1 a_2 a_2 a_4 are the observed frequencies of the four classes and n the total number of individuals. This is done for each family separately and for each total of the combined families. The analysis into deviation and heterogeneity portions is carried out as in the analysis of the single factor segregations. In each case the deviation has one degree of freedom. The number of degrees of freedom of the heterogeneity χ^2 is one less than the number of families combined. As before, the 5% level of P has been taken as indicating significant deviation.

TABLE 46.

Detection of Linkage between N^A, N^X, N^B, N^C.

Parentage	Frequencies of combinations				Totals	Components of		χ^2 L	χ^2
	AX	Ax	aX	ax		A,a	X,x		
Ar. Victory x Kep. Kid.	75	5	6	57	143	2.20	2.525	102.4	D.F. 3
Lid. Lads x Pepo	21	0	2	23	46	0.03477	0.000	38.33	A,a = 1.96587
Cr. Def. x Flourball (1)	49	7	1	42	99	1.707	0.0101	69.60	P = 0.70-0.50
do. x do. (2)	38	6	5	34	83	0.3011	0.0482	44.82	X,x = 1.1573
	183	18	14	156	371	4.55587	2.5833	255.15	P = 0.70-0.50
χ_3^2 = 258.106 P = v. small					Joint deviation	2.590	1.426	254.00	L = 1.150 P = 0.80-0.70
	AB	Ab	aB	ab		A,a	B,b	L.	
Ar. Victory x Kep. Kid.	43	30	29	30	132	1.485	1.091	1.485	D.F. 3
Lid. Lads x Pepo	13	6	7	17	43	0.5813	0.2092	6.719	A,a = 2.2454
Cr. Def. x Flourball (1)	33	22	16	26	84	2.011	0.0000	5.249	P = 0.50-0.30
do. x do. (2)	20	25	16	23	97	0.3711	1.485	0.0412	B,b = 2.7740
	109	83	68	96	356	4.4484	2.7852	13.4942	P = 0.50-0.30
χ_3^2 = 10.36 P = 0.02-0.01					Joint deviation	2.203	0.0112	8.193	L = 5.3012 P = 0.20-0.10
	XB	Xb	xB	xb		X,x	B,b	L.	
Ar. Victory x Kep. Kid.	42	32	29	31	134	1.462	0.3657	1.075	D.F. 3
Lid. Lads x Pepo	14	8	6	16	44	0.000	0.3637	5.817	X,x = 1.2669
Cr. Def. x Flourball (1)	35	31	24	37	127	0.1968	0.6378	2.016	P = 0.80-0.70
do. x do. (2)	16	27	22	25	90	0.1778	2.178	0.7112	B,b = 2.6332
	107	98	81	109	395	1.8366	3.5452	9.6192	P = 0.50-0.30
χ_3^2 = 5.0 P = 0.20-0.10					Joint deviation	0.5697	0.9120	3.465	L = 6.1542 P = 0.20-0.10
	AC	Ac	aC	ac		A,a	C,c	L	
Cr. Def. x Flourball (1)	27	30	29	15	101	1.673	1.198	2.273	D.F. 1
do. x do. (2)	28	17	17	22	84	0.4285	0.4285	3.047	A,a = 0.1495
	55	47	46	37	185	2.1015	1.6255	5.320	P = 0.70-0.50
χ_3^2 = 3.54 P = 0.50-0.30					Joint deviation	1.952	1.562	0.0054	C,c = 0.0645 P = 0.80-0.70 L = 5.3146 P = 0.05-0.02
	BC	Bc	bC	bc		B,b	C,c	L.	
Cr. Def. x Flourball (1)	35	27	35	34	131	0.3741	0.6183	0.3741	D.F. 1
do. x do. (2)	16	23	33	19	91	1.857	0.5386	4.846	B,b = 0.4301
	51	50	68	53	222	2.2311	1.1569	5.2201	P = 0.70-0.50
χ_3^2 = 3.86 P = 0.30-0.20					Joint deviation	1.801	1.153	0.6486	C,c = 0.0039 P = 0.98-0.95 L = 4.5715 P = 0.05-0.02
	XC	Xc	xC	xc		X,x	C,c	L	
Cr. Def. x Flourball (1)	37	45	45	30	157	0.3121	0.3121	3.369	D.F. 1
do. x do. (2)	29	23	25	27	94	0.0000	0.1702	0.6794	X,x = 0.1245
	66	68	70	57	261	0.3121	0.4823	4.0484	P = 0.80-0.70
χ_3^2 = 1.54 P = 0.70-0.50					Joint deviation	0.1877	0.4636	0.8622	C,c = 0.0187 P = 0.90-0.80 L = 3.1862 P = 0.10-0.05

Only in the case of the $N^X + N^A$ genes is linkage sufficiently close to warrant an estimation of the cross over value, h. The frequencies of the different genotypes are $N^A N^X$ 183, $N^A n^x$ 18, $n^a N^X$ 14, $n^a n^x$ 156. Since the genes are in single coupling the gametic series expected is

$N^A N^X$	$4 - ac - 2h + ah + \frac{1}{2}ach_2$
$N^A n^x$	$2h - ah - \frac{1}{2}ach_2$
$n^a N^X$	$2h - 2ah + ach_2$
$n^a n^x$	$4 + ac - 2h + 2ah - ach_2$

Neglecting the terms involving ach_2, a, c and h may be determined by maximising the expression

$$183 \log (4 - \alpha - 2\gamma + \delta) + 18 \log (2\gamma - \delta) + 14 \log (2\gamma - 2\delta) + 156 \log (4 + \alpha - 2\gamma + 2\delta)$$

for all three variables, where α stands for ac, γ for h, and δ for ah. This gives the three simultaneous equations

(1) $$\frac{156}{4 + \alpha - 2\gamma + 2\delta} = \frac{183}{4 - \alpha - 2\gamma + \delta}$$

(2) $$\frac{18}{2\gamma - \delta} = \frac{14}{2\gamma - 2\delta}$$

(3) $$\frac{156}{4 + \alpha - 2\gamma + 2\delta} = \frac{18}{2\gamma - \delta}$$

From (2) $\delta = 0.3636\,\gamma$. Substituting in (1)

$$156 (4 - \alpha - 2\gamma + 0.3636\,\gamma) = 183 (4 + \alpha - 2\gamma + 0.7273\,\gamma)$$

$$\therefore \alpha = -\left(\frac{108 + 22.374\,\gamma}{339}\right) = -(0.3185 + 0.066\,\gamma)$$

Substituting for α and δ in (3)

$$156(2\gamma - 0.3636\gamma) = 18(4 - 0.3185 - 0.066\gamma - 2\gamma + 0.7273\gamma)$$

$$\therefore 255.278\gamma = 66.267 - 24.097\gamma$$

$$\therefore \gamma = \frac{66.267}{279.375} = 0.2372 = h$$

$$\therefore \delta = 0.3636 \times 0.2372 = 0.08624 = ah$$

$$\therefore \alpha = -(0.3185 + 0.066 \times 0.2372) = -0.33415 = ae$$

$\therefore h = 0.2372 = 11.860\%$ of crossing over.

$a = 0.3636$

$e = -0.9189$

The standard error of h may be calculated by use of the formula

$$V(h) = S\left\{nh\left(\frac{dh}{da}\right)^2\right\} - n\left(\frac{dh}{dn}\right)^2 \text{ (Mather 1936)}$$

$h = \frac{2b(c - \frac{1}{2}b)}{n}$ or in this case $\frac{28(c - \frac{1}{2}b)}{n}$

where a, b, c & d are the frequencies of the various classes, n the total number of individuals and h the recombination value.

$$\therefore \frac{dh}{dc} = \frac{28}{n}, \quad \frac{dh}{db} = -\frac{14}{n}, \quad \frac{dh}{dn} = -\frac{h}{n} +$$

$$\frac{dh}{da} = \frac{dh}{dn} = 0$$

$$\therefore \frac{1}{n}V(h) = \frac{c}{n}\left(\frac{28}{n}\right)^2 + \frac{b}{n}\left(\frac{14}{n}\right)^2 - \frac{h^2}{n}$$

$$V(h) = \frac{28^2 c}{n^2} + \frac{14^2 b}{n^2} - \frac{h^2}{n}$$

Substituting the observed values for b, c, n & h

$$V(h) = \frac{28^2 \times 18}{371^2} + \frac{14^2 \times 14}{371^2} - \frac{0.2372^2}{371}$$

$$= 0.1025 + 0.01993 - 0.0001517$$

$$\therefore V(h) = 0.1222783$$

$$\therefore S(h) = 0.3497 \text{ & } S_{\frac{1}{2}h} = 0.17485$$

$$S_{\frac{1}{2}h} = 17.485\%$$

$$\therefore h = 11.86 \pm 17.485\%$$

On De Winton & Haldane's (1931) formula

$$h = \frac{32}{371} \times 100 = 8.624\%$$

Key to abbreviations in references.

A.N. = American Naturalist.

A.P.J. = American Potato Journal.

C. = Cytologia.

G. = Genetics.

H. = Hereditas.

J.G. = Journal of Genetics.

P. = Phytopathology.

P.N.A.S. = Proceedings of the National Academy of Sciences (U.S.A.).

P.R.S.B. = Proceedings of the Royal Society (London) Series B.

S.P.R.D.S. = Scientific Proceedings of the Royal Dublin Society.

Z.i.A.V. = Zeitschrift für induktiv Abstammungs- und Vererbungslehre.

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