ON THE RELATION BETWEEN NUMBER OF CHROMOSOMES AND NUMBER OF TYPES, IN *LATHYRUS* ESPECIALLY.

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(With Plate V.)

THE number of simultaneously and independently segregating pairs of factors[1] in the species investigated by genetic experts has, as we know, never yet been found so high as to exceed the haploid chromosome number of the species. Consequently, there is still nothing to subvert the theory that the genes have their morphological equivalent in the chromosomes and that these latter are—or can be—individually dissimilar in a given biotype as regards the genes included.

It is a question of very great theoretic importance, whether the simultaneously segregating pairs of factors, not mutually connected, can ever exceed the number of chromosomes in the species concerned, this being, so to speak, a decisive point as regards the value of the entire section of the study of chromosomes related to the science of genetics. If a biotype could be found to exhibit segregation of but a single pair of factors in excess of the number indicated by the haploid chromosome value, then, properly speaking, the theory as to the value of chromosomes as bearers of the genetic, segregating units would collapse at once. The nice agreement between reduction division and segregation would thus be irrevocably destroyed.

At a first glance, it might seem likely that we should be able, in highly varying species, to segregate without great difficulty a greater number of types than the chromosome number found for the species permits. We can, however, obtain a surprisingly large number of combinations even from a quite small number of chromosomes, and it must also be borne in mind that the theory of agreement between reduction division and Mendelian segregation would by no means be destroyed even if we did succeed in finding a greater number of independently mendling pairs of genes (or pairs of gene-complexes) within a Linnaean

[1] By this expression is meant pairs of factors, or groups of pairs, between which the phenomenon of linkage is not found.

species, than corresponds to the haploid chromosome number. Even a species with but a single chromosome in the haplophase, and two in the diplophase, might be allowed to contain an unlimited number of independently segregating genes, as the two chromosomes in the diplophase might very well be genotypically different between individuals within the species. The point is, that according to the theory, there must not be more independently mendling pairs of factors in a given *biotype* (individual or clone) than the chromosome number of the biotype indicates. As soon as two individuals (not to speak of more) not belonging to the same biotype are introduced into the experiment, the possibility of new combinations is considerably increased, unless the two original individuals are homozygotic.

An organism with only one chromosome in the haplophase ($x = 1$) will naturally only be able to have two different chromosomes at the outside in the diplophase. Let us call these A and a. Two different types of gamete can then arise, viz. A and a, and these can form three different types of zygote: AA, Aa, and aa, of which two are homozygotic.

With $x = 2$, a given biotype can be doubly heterozygotic; i.e. $AaBb$, and four kinds of gametes can be formed, viz. AB, Ab, aB and ab, of which in F_1 it will be possible to obtain nine diploid combinations, $AABB$, $AABb$, $AAbb$, $AaBB$, $AaBb$, $Aabb$, $aaBB$, $aaBb$, and $aabb$, of which four will be homozygotic in both characters.

Where $x = 3$, 8 different gametes can be formed, giving 27 different diploid combinations, of which 8 are homozygotic.

In a word: *With a given haploid chromosome number, x, we can by self-fertilisation and segregation of a single individual obtain, theoretically speaking, at the outside 2^x different gamete types and 3^x different diploid biotypes, of which 2^x will be homozygotic in all characters.*

If we are to entertain any hope of controverting the theory of identity between reduction division and segregation, then naturally it will be necessary to work with organisms having a low chromosome number, and capable of self-fertilisation. A plant with only eight chromosomes ($x = 8$) will on self-fertilisation be capable of forming 256 different gametes, and will in F_1 segregate 6561 different types.

If the segregation experiments be commenced with more than one biotype, which of course will as a rule be necessary when working with species not capable of self-fertilisation, the question becomes more complicated.

Two individuals with $x = 1$ can in the diplophase differ in both chromosomes, the one diplobiont having the formula $A\ A_1$, the other $a\ a_1$. On formation of the gametes, we can then obtain four different types, A—A_1—a—a_1; two from each. In F_1, we have $2 \times 2 = 4$ genotypically different diploid combinations, viz. Aa—Aa_1—A_1a—A_1a_1, and these will, on reduction division, again throw off the four gametes A—A_1—a and a_1. In F_2 and the following offspring generations, we can by free combination of these find 10 genotypically different combinations, viz. AA—AA_1—Aa—Aa_1—A_1A_1—A_1a—A_1a_1—aa—aa_1—a_1a_1, of which four are homozygotic; one for each gamete type.

On considering in the same way an organism with two chromosomes in the haplophase ($x = 2$), and presuming the segregation experiment to commence with the crossing of two individuals having different genotypic value for all the chromosomes of the diplophase (eight in all in the two individuals) then each individual will be able to form four gametes, or eight different gametes from both. On crossing these forms, we obtain 16 different biotypes in F_1; and in F_2, where the gametes (16 different) can combine altogether freely, there can arise 100 genotypically distinct diploid types, of which 16 will be homozygotic in all characters, i.e. one for each gamete type formed by F_1.

Where $x = 3$, we can by crossing two different individuals easily obtain, theoretically, eight gametes from each, i.e. 16 different in all. In F_1, 64 types will have arisen, able to form in all an equal number of gametes, and in F_2, 1000, of which 64 are homozygotic.

Briefly then, *if a segregation experiment be commenced by crossing two individuals of the species to be investigated, then we can in F_1 obtain 4^x and in F_2 and the following generations 10^x genotypically distinct forms, of which 4^x will be homozygotic in all characters, where x indicates the haploid chromosome number of the species.*

If, for instance, we commence by crossing two entirely different individuals, heterozygotic throughout, of a species with eight chromosomes in the haploid phase, then in F_1, there can arise 65536, and in F_2 a milliard different types.

On extending the analysis so as to include a further number of individuals, as to whose genotypic constitution nothing is previously known, then the number of possible combinations will of course be far higher even than this, as also when "crossing over" takes place. In this last case we cannot reckon the possibilities beforehand.

The advantage of working with self-fertilising organisms is thus entirely evident. It would be even better if we could make our genetic experiments with organisms where the haplophase was a richly developed and independently living individual, as for instance in the liverworts. This I have already pointed out in a previous work : "The Chromosomes. Their numbers and general importance" (*Comptes rendus des travaux du Laboratoire de Carlsberg*, Vol. XIII, Copenhagen, 1917). A species such as *Marchantia polymorpha*, with eight chromosomes, would thus only form, at the outside, 256 types on crossing between two individuals; and by taking an individual of a monoecious species, self-fertilised, then *eo ipso* we should obtain but one type—a pure line—if we may use this term also for cultures of haplobionts.

Lathyrus odoratus is one of the plants with which we may hope sooner or later to elucidate the important question as to agreement of reduction division with Mendelian segregation, as students of genetics have for a long time back been occupied with the genotypic features of this species.

Acting upon a suggestion from Prof. R. C. Punnett, of Cambridge, concerning the number of chromosomes in *Lathyrus odoratus*, I proceeded, in the summer of 1918, to fix and examine material of the species in question. In a written communication to me, Prof. Punnett stated that Mr R. P. Gregory had informed him that there were doubtless seven or eight chromosomes in the haplophase. Otherwise, as far as I am aware, nothing is stated in extant literature as to this; no mention is made as to any species of *Lathyrus* either by Tischler in his excellent "Chromosomenzahl, -Form und -Individualität im Pflanzenreiche" (*Progr. Rei Bot.*, Vol. V, 1915, p. 164) or by Ishikawa in his likewise very comprehensive work, "A list of the number of chromosomes" (*The Bot. Magazine*, Tokyo, Vol. XXX, 1916, p. 404).

On two occasions, in the early spring and early summer of 1918, Prof. Punnett sent me, from his cultures at Cambridge, freshly fixed material of young flowers of *L. odoratus*. On investigation, however, the former consignment was found to be at a too advanced stage, as the tetrad division had throughout been completed. The second batch of material, again, was too young, no stage beyond synapsis being discernible. In order not to delay the investigation further by correspondence under the present abnormal conditions prevailing in the postal service, I therefore fixed material myself, obtaining it from a garden at Marselisborg, near Aarhuus (Jutland) in the month of July. The material was fixed in Carnoy's liquid, and stained with Delafield's haematoxylin. Besides the above mentioned species, I also fixed, in September, by the same means, some young flowers of *Lathyrus latifolius*, cultivated in the experimental nursery of the Carlsberg Laboratory, Copenhagen.

A cytological investigation, carried out essentially with a view to ascertaining the chromosome number, gave the following result.

Lathyrus odoratus L.

The chromosomes are large and somewhat elongated in the heterotypic metaphase. They are present to the number of 7 ($=x$) (Figs. 1 and 2). There is no difference, on the whole, in the size of the different chromosomes. Splitting and transition to the anaphase proceed fairly regularly. One of the chromosome pairs can, however, at times be separated slightly earlier than the remainder (Fig. 3). The heterotypic telophase may often give an impression that the chromosome number is greater than seven, owing to the fact that the chromosomes at this stage are bent to an angle, and also exhibit splitting in the plane through which the subsequent homoeotypic division takes place. The chromosomes being bent to an angle (the angle pointing toward the pole of the nuclear spindle) will in a certain position each appear as two separate chromosomes (schematically shown in Fig. 4) and as the chromosome is further split in the plane of the angle, we find, extremely often, apparent groups of four chromosomes, to the number of seven, i.e. 28 altogether (Fig. 5). Fig. 6 shows the chromosomes in the metaphase of the homoeotypic division; here also we can with great certainty count seven chromosomes in each of the two nuclear plates of the spore mother cell. In the anaphase of the homoeotypic division also, we may find what would seem like more than seven chromosomes, the chromosomes here being likewise bent to an angle, though not split.

An investigation of the chromosomes in somatic cells showed entire agreement with the figure found in the haplophase, i.e. 14 (Fig. 7). As is usually the case, the chromosomes here were of slenderer form than during reduction division.

Lathyrus latifolius L.

This perennial species appeared in every respect as *L. odoratus*, and the cytological picture for the two species is so uniform that preparations of the one might be taken for those of the other. Not only is the chromosome number likewise seven, as the heterotypic metaphase in Figs. 8–9 shows, but the chromosomes themselves are also entirely alike in size and shape. Here, as in *L. odoratus*, twice the number could be counted with perfect certainty in somatic cells (Fig. 10).

These two species, then—the only species of *Lathyrus* hitherto investigated—present an instance of the ordered regularity with which in larger or smaller systematic groups in the animal and vegetable kingdoms, the chromosome members of closely related forms are found to be themselves related; i.e. either entirely alike, or multiples of a *cardinal number* characteristic of the group—as I have explained in my work above quoted.

The chromosome number seven is a comparatively low value to find among phanerogams, and should, *a priori*, count in favour of the employment of *Lathyrus* in genetic experiments. The theory as to agreement between reduction division and Mendelian segregation permits—commencing *either* with self-fertilisation of one heterozygotic individual *or* by crossing of two pure lines—that there be found at the outside seven independently segregating pairs of factors (or pairs of factor groups). A segregation experiment commenced in one or other of these two ways should not lead to the production of more than 128 different biotypes, all homozygotic throughout.

As to how far it may prove possible to find more than seven independently segregating pairs of factors or pairs of factor groups in a single biotype of the species, is a question which future investigations must decide. Personally I feel confident that they will *not* be found.

I have observed nothing in my material which might lead to the interpretation that chiasmatypy, in the sense of Janssens, is met with in *Lathyrus*. In my opinion, even in organisms in which breeding experiments have resulted in the view that parts of the chromosomes are exchanged during the reduction division, this process is not usually going on at so late a stage as shown by Janssens. More probably it occurs in synapsis where it cannot readily be demonstrated by microscopical methods.

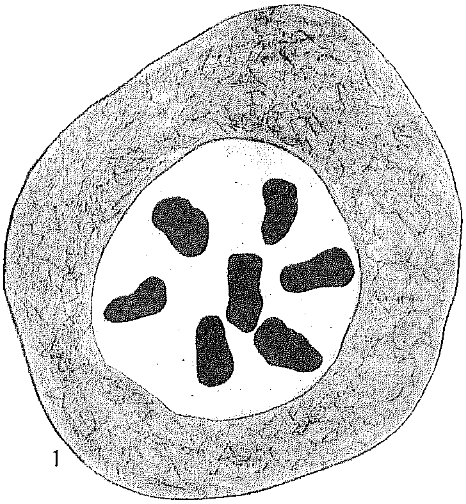
CARLSBERG LABORATORIUM, COPENHAGEN,
15 *October* 1918.

EXPLANATION OF PLATE V.

All figures are drawn with aid of Abbe's camera lucida, using Zeiss' homog. immers. 2 mm. and comp. oc. 18.

Figs. 1—7, *Lathyrus odoratus*. Figs. 8—10, *L. latifolius*.

Figs. 1—2. Pollen mother cell. Heterotypical metaphase (polar view).
Fig. 3. Pollen mother cell. Heterotypical meta-anaphase (side view).
Fig. 4. Two chromosomes from heterotypical anaphase (schematically).
Fig. 5. Pollen mother cell. Heterotypical anaphase (polar view). Apparently 7 groups of 4 chromosomes present.
Fig. 6. Pollen mother cell. Homotypical metaphase.
Fig. 7. Somatic cell with 14 chromosomes.
Fig. 8. Pollen mother cell. Heterotypical metaphase (polar view).
Fig. 9. Pollen mother cell. Heterotypical metaphase (side view).
Fig. 10. Somatic cell with 14 chromosomes.



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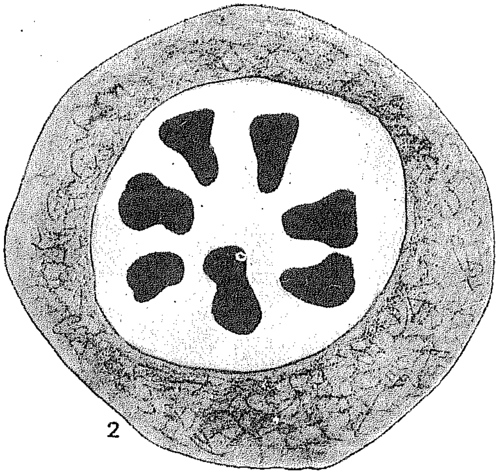

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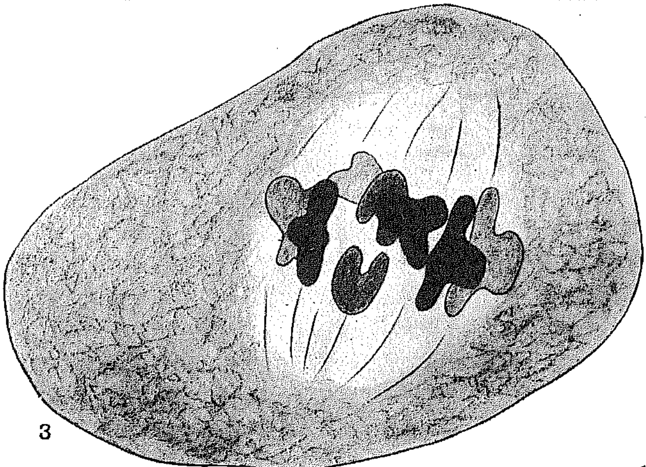

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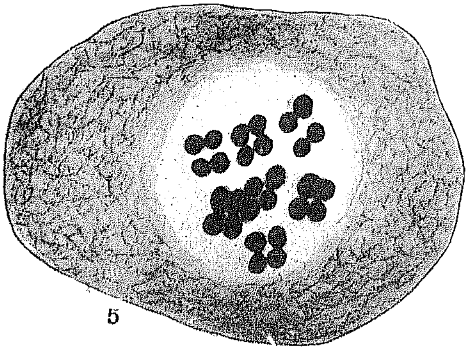

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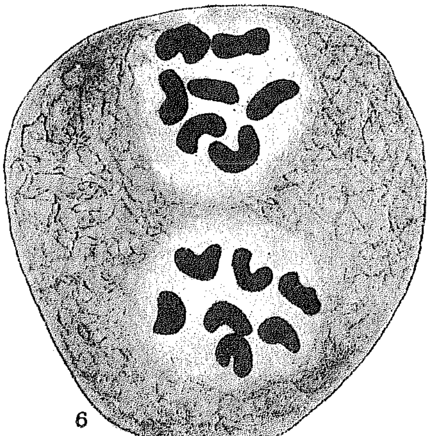

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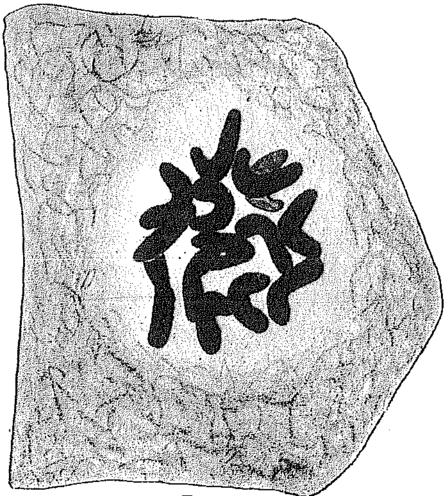
PLATE V



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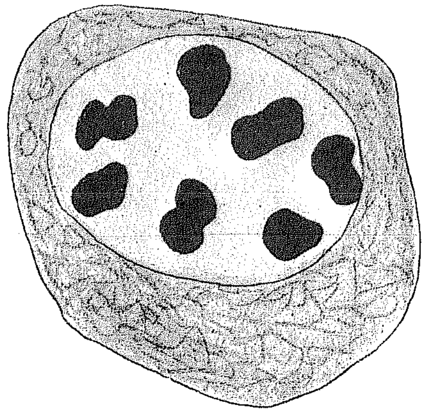

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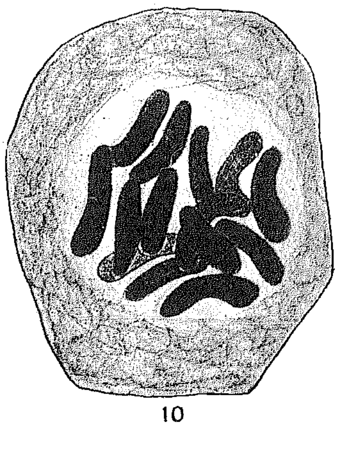

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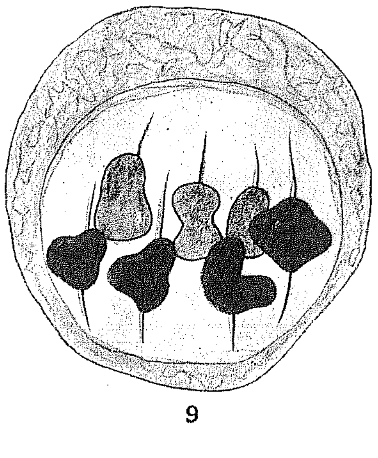

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