THE CONJUGATION OF THE CHROMOSOMES

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The conjugation of the chromosomes is a universal process in the whole of organic nature. It occurs not only among all multicellular organisms both in the animal and the vegetable kingdom but is also proved to exist in a great number of unicellular organisms. It is not only an indispensable condition for all bisexual reproduction but also of great importance for what we, in a vague term, call variability, and for all kinds of evolution, for in the meiosis the combination of different genes takes place, and thereby through various aberrations of the normal process of conjugation further favorable conditions for the production of mutations are created.

Meanwhile, our knowledge of conjugation and its real nature by no means stands in proportion to its importance and significance. REUTER in his profound and scholarly work says that the cause of conjugation lies in the manner of reproduction of the diploids. Yet neither he nor any one else can offer us an explanation of the power which sets free conjugation. We speak about the affinity of the homologous chromosomes without being able further to define what we mean by such an affinity, whether we think of it as physical or chemical, or as an unknown form of biological affinity. And why are the chromosomes in the somatic cells independent of this mystic affinity that does not set free the pairing of the homologous chromosomes until meiosis? These are the questions that are still awaiting a satisfactory solution.

That the affinity between the paternal and the maternal homologues has nothing to do with the sexuality is evident, although this assumption has been propounded by certain authors. For only the Y (the W) chromosome is bound to one sex, while all autosomes and the X (the Z) chromosomes sometimes happen to exist in individuals of one, sometimes in individuals of the other sex.

That the chromosomes at conjugation play an active and to a certain extent self existent part, independent of each other, seems to be fully proved by the fact that in certain cases the autosomes conjugate in the first maturation division, while the allosomes and also the microsomes do so only in the interkinesis. That the structure of the chromosomes must be of decisive importance in this case is evident, just as it plays a very important part in the conjugation. But on the other hand we have also clear evidence of the fact that the general physiological conditions in the cell may determine the process of conjugation. Critical experiments have further shown how conjugation depends on the surrounding influences. Finally, experimental research has during the last few years discovered special genes which determine the conjugation of the chromosomes. Thus we see that this important process mainly depends on the structure of the chromosomes but also on the genotype of the individual and, finally, on the environment in the cell itself and the surroundings in general.

As is the case when a complicated biological process is being investigated, it is not only from a careful examination of the normal course of the process that we can expect to arrive at an explanation of any problem. It is rather the deviations from the normal process which often give us a deeper insight into the real nature of the process. This is true in the case of the problem we are dealing with at present.

It is chiefly on four different principles that we try to solve the mystery which still obscures the conjugation of the chromosomes: (1) through a thorough study of the normal meiosis in diploid forms with large chromosomes which can be observed during all the different phases of the conjugation; (2) through observations on polyploid and heteroploid forms; (3) through systematic investigations of hybrids and the circumstances of conjugation of the different species chromosomes; (4) through research into the special genes, determining the conjugation and the outside factors influencing it.

Among the many scientists who have made the normal meiosis the subject of their special study, BELLING and DARLINGTON may be mentioned in the first place. They have added to our knowledge on the subject very essentially; our insight into the origin and importance of the chiasmata has made a step forward, and the phenomenon of crossing over has greatly gained in clarity while the synapsis or syndesis has, on the other hand, been pushed a little into the background.

DARLINGTON is of the opinion that the meiosis is an abnormal mitosis in which prophase contraction has anticipated the division of each chromosome into two threads. As at mitosis in this stage, the split halves of chromosomes are constantly associated side by side in pairs; this relationship is restored in meiosis by the association of whole, undivided chromosomes in pairs. DARLINGTON questions the existence of any kind of affinity between the chromosomes at metaphase. The four chromatids are kept together by chiasmas, and these prevent the separation of the chromosomes at diakinesis and metaphase, for, according to DARLINGTON, the repelling powers during these stages, especially during diakinesis, are stronger than the attracting powers.

I have no doubt that DARLINGTON is right, and I quite acknowledge his great merits; yet I cannot agree with the theory that the question of the mystic affinity is reduced to the question of whether chiasmas are produced. For a condition that the chiasmas should be produced is that the homologous chromosomes approach each other or conjugate, that is, that there exists an affinity between the homologues.

What powers are in action when the chromosomes approach each other is still a riddle. That the genic constitution of the chromosomes, or, to speak cytologically, of the specific chromomeres of the chromosomes, is in this case of primary consequence, can be considered sufficiently proved. Yet the structure of the chromosomes does not reign alone, and under certain circumstances other factors succeed in gaining supremacy over the chromosomes and prevent the conjugation, which can also be considered to be experimentally proved.

By the study of meiosis in polyploids scientists have succeeded in getting clear evidence of the fact that the linear structure of the chromosomes is of the greatest importance for the conjugation. In certain triploid forms, for instance, Canna, Tulipa, Primula, Hyacinthus and others, trivalent chromosomes have been found besides the bivalent ones while among tetraploid forms quadrivalent chromosomes occur, an evidence of the fact that the homologues attract each other regardless of their being either 2, 3, or 4 in number. A more detailed study of the manner of association of these multivalent chromosomes has given us evidence of the fact that the chromosomes are built up of small units, the chromomeres, which are arranged in a quite definite manner. Thus there never exists among the primaries of trisomics of Datura an association in a triangular form, because three congruent chromosomes can not unite in a triangle in such a way that two homologous ends meet.

In the secondaries of the trisomics among which an interchange of one half has taken place in one of the chromosomes, this changed chromosome can be inserted between the ends of the two unchanged chromosomes, and thus a triangle arises.

In this connection I also want to speak of the well-known cases in Drosophila in which an inversion of a section in one of the homologues has taken place. Such an inversion presumably prevents the normal synapsis, which makes it likely that an absolute homology between the chromomeres is necessary for a normal conjugation. Through the inversion the formation of chiasmata is undoubtedly prevented, which makes the pairing less stable.

The experiments of MULLER and PAINTER with translocations in certain chromosomes of Drosophila caused by radiation speak in favor of this theory. Only lately have DOBŽHANSKY and STURTEVANT, through a series of systematic crosses between normal individuals of Drosophila and those with translocations in the second and third chromosomes, clearly proved that chiasmata are caused between homologous chromomeres and fail between chromosomes of which one has a series of chromomeres disturbed by translocation.

The few haploid organisms of which the meiosis is known also prove the correctness of the theory that the structure of the chromosomes determines the affinity. Among haploids there is, as a rule, no synapsis, and where a tendency to a synapsis is sometimes observed it proves to be defective. Thus, where homologous chromosomes do not appear in the nucleus, there is not a normal tendency to conjugation.

We now pass on to the third group of experiments, comprising the species hybrids, and I shall, in connection with the second group, the polyploids spoken of above, begin my explanation by pointing out some extremely interesting observations concerning crossings of polyploid species, especially the autosyndesis appearing in such crossings.

Why chromosomes which never conjugate with each other among the parent species should suddenly be seized by a desire to enter into union with each other when they are brought together with another set of chromosomes, is more than mysterious; every autosyndesis was, to begin with, even considered impossible, and, when reported, was thought to be the result of erroneous observations. But the instances of autosyndesis are now so numerous, and behind the observations stand so many conscientious scientists, that we need not feel any doubt of their correctness. Attempts have been made to explain the fact in such a way, that if, for instance, among a certain species the two chromosome groups A_1 A_1 A_2 A_2, where A_1 and A_2 never conjugate with each other, are brought together through crossing with the groups B B C C of another species, and these chromosomes are not related to A_1 and A_2, the last-named would, of necessity, conjugate the one with the other. The said explanation would thus presuppose a tendency for conjugation, innate in the chromosomes, which must be satisfied at any price. Yet, apart from the fact that this explanation takes into account a real mystic affinity, we do not advance far with this theory. It builds exclusively on the relationship of the chromosomes, that is, on a more or less strongly characteristic homology, and considers it to be conclusive for the conjugation. In case this should be a correct supposition, conjugation ought to take place without disturbance among the autotetraploids. But this is in no way the case. Many tetraploids show a defective pairing. In this case recourse has been had to the expedient of declaring the forms to be allotetraploids rather than autotetraploids. Yet this expedient does not lessen the difficulty. For if it were correct, then, at least among forms which are proved to be amphidiploids, all the chromosomes ought to pair regularly, and they do not. On the contrary certain irregularities are the rule. For example, it is only recently that LEVITSKY and BENETZ KAJA ascertained that, among the wheat-rye amphidiploids with twice 21 wheat and twice 7 rye chromosomes, the number of bivalents in the same individual may vary from 25 to 28. Thus we have here 1 to 3 pairs of absolutely homologous chromosomes that do not conjugate with each other. The reason for this must be looked for in other circumstances than in the incompatibility between the chromosomes. Among many other amphidiploids similar irregularities seem to occur, although they have not hitherto been investigated very carefully.

The capricious character of the autosyndesis is perhaps most clearly illustrated by a comparison of the condition of the chromosomes at different crossings with the same species. In this respect the crossings made with different species of Digitalis are specially enlightening, as GERTRAUD HAASE-BESSEL rightly points out. At a crossing between the closely related Digitalis species, *lutea* with 24 chromosomes and *micrantha* with 48 (by some taxonomists taken to be forms), it became apparent that the hybrid had 36 bivalents in meiosis and that the hybridization set free autosyndesis in both *lutea* and *micrantha* chromosomes, but the hybrid is, in spite of the absolutely normal pairing of the chromosomes, absolutely sterile.

In the hybrid between *lutea* and *purpurea* there is no pairing at all between the chromosomes, and there are only univalents in the diakinesis. This hybrid consequently belongs to the Pygaera type according to the Täckholm division.

And finally, at the crossing of *lutea* with *lanata* the hybrid shows a variable number of bivalents and must thus be reckoned to belong to the Boreale type.

To summarize the above: Crossing with one species sets free the autosyndesis between the *lutea* chromosomes, in crossing with another species all the *lutea* chromosomes remain unpaired, and with a third species a variable number of them begin pairing with the chromosomes of this third species.

But also, in one and the same crossing, sister individuals can show quite different conjugation conditions. Thus HAASE-BESSEL describes two hybrids between *Digitalis canariensis* and *grandiflora,* of which one hybrid did not show any pairing whatever of chromosomes, while the other presented a variable number of bivalents.

The apparently great capriciousness in the matter of the chromosome conjugation has struck every one who has made the hybrid meiosis the subject of research. Hitherto, however, there have been comparatively few systematically carried out investigations of hybrids between different related species or between a number of individuals of the same crossing, and above all our knowledge restricts itself to the pollen development and spermatogenesis, while the oogenesis, technically so hard to study, has been thoroughly investigated only in extremely rare cases.

Having myself, during a number of years, been occupied with investigations of hybrids of Lepidoptera, I take the liberty of here recording some partly unpublished results. I will begin by referring to the table which illustrates the conditions of conjugation among a number of hybrids between closely related species of Sphingidae, all with the haploid chromosome number 29. From the table it appears that meiosis with the hybrid *Metopsilus porcellus*×*Chaerocampa elpenor* is practically normal, while the hybrid *Deilephila galii*×*D. euphorbiae* shows a meiosis with comparatively small deviations from the normal. The former of these hybrids is entirely fertile, and in the backcrossing with the parent species the resulting hybrids show a distinct segregation, both in the larval, pupal and imago stages. The latter hybrid is also fertile in a high degree. I regret to say that I have been able to state segregation only in the larval stage. Yet this reciprocal hybrid has been investigated by BYTINSKI-SALZ and has shown itself to be segregate in all stages.

The position is quite a different one with regard to the two hybrids *D. euphorbiae*×*Ch. elpenor* and *Ch. elpenor*×*D. galii*. For both we state a minimal affinity between the chromosomes of the parents, and we find that the spermatocytes are divided among a great number of different conjugation classes. It has not been in my power to make backcrossing between these hybrids and their parents. Only the males develop into imagoes, while the females, in consequence of a sub-lethal combination of the sex chromosomes, are not able to pass through the metamorphosis and therefore die as pupae. The hybrid males, on the other hand, develop so late in the autumn that there are no longer any females of the parent species left. But I believe that, without being too audacious, I dare assert that neither of these hybrid males is fertile.

The table meanwhile teaches us something else also: the conjugation of the chromosomes in the hybrids between the same parent species can turn out quite differently, and even the sibs in the same cross can show great dissimilarities in this respect. I refer only to the two investigated individuals of

TABLE 1

Number of chromosomes at metaphase I.

♀ × ♂ (Number of spermatocytes)	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	TOTAL
Metopsilus porcellus × *Chaerocampa elpenor*	..	..	47	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	49
Deilephila galii × *euphorbiae* Family 16	4	118	11	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	133
Deilephila galii × *euphorbiae* Family 16	..	7	74	12	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	95
Deilephila galii × *euphorbiae* Family 17	..	2	18	3	1	..	2	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	27
Deilephila galii × *euphorbiae* Family 17	..	1	14	2	3	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	20
Deilephila galii × *euphorbiae* Family 18	..	5	26	4	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	35
Deilephila euphorbiae × *Chaerocampa elpenor*	..	..	..	..	..	..	..	..	2	2	4	3	7	8	5	9	3	1	2	1	2	..	..	1	..	..	..	..	..	..	..	..	50
Deilephila euphorbiae × *Chaerocampa elpenor*	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	1	..	1	1	2	3	1	3	..	..	..	..	..	..	13
Chaerocampa elpenor × *Deilephila galii*	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	2	3	5	4	8	9	6	3	4	1	3	49

the family 16 of the cross *galii*×*euphorbiae,* of which one shows a maximum number of cells with number 29, characteristic of the parents, while the other has its maximum at 28. The number of examined cells is in both cases so great that there cannot be any doubt of the correctness of the observations. In the last named individual a secondary affinity has thus made two chromosomes associate into one. A similar case has been observed by me earlier at a crossing between the German and the Finnish form of *Dicranura vinula,* which both have the haploid number 21, while the hybrid has 20.

Such a very capricious conjugation of the chromosomes may be observed in almost all hybrids of which the greatest number of individuals is subjected to a careful investigation; this clearly proves that it is not only the chromosomal structure which determines the conjugation.

From the table one might perhaps be tempted to draw the conclusion that the conditions of conjugation of the hybrids might be used for a taxonomic purpose in order to fix the closer or remote relationship of the parent species. The greater the number of conjugating chromosomes, the closer the relationship. Yet this would be a hasty conclusion, I am sure. Of course, in the present case the supposition that *elpenor* is more closely related to *porcellus* than to *euphorbiae* and most remotely related to *galii* would agree with the opinion of the taxonomists. And it would also be justifiable to consider that *galii* and *euphorbiae* are more closely related to each other than to *elpenor.* Other instances also supporting this theory could be mentioned both from the botanical and zoological fields. But the conjugation is not at all a reliable taxonomic criterion. As an instance of its untrustworthiness I desire only to quote an example from the same family as the above named. The East Asiatic form Planus of the European *Smerinthus ocellata,* however, which are considered by all entomologists as being very closely related to each other, and which both possess 28 chromosomes, show, in the hybrid, a very low degree of affinity between the chromosomes.

From a table such as the one dealt with here, we can draw a conclusion concerning the fertility and the sterility of the hybrids. The fewer the different conjugation classes are which a hybrid comprises, the greater its fertility, and with a growing breadth of variation its sterility augments. It does not signify in this case whether the top of the curves corresponds with the haploid or the diploid number. The high and narrow curves are always to be found at these numbers; only in some backcrosses do they lie in their immediate neighborhood.

I should like to conclude my discourse on the conditions of conjugation of the species hybrids by giving a record of one case which will, perhaps better than anything else, show you how extremely difficult it is to solve the problem which confronts us. I have in view the altogether dissimilar conditions which in one case have proved themselves to exist between the male and female of the same hybrid. I mean the reciprocal hybrids between *Pygaera pigra*, $n=23$, and *curtula*, $n=29$, which in the male sex show no conjugation at all, or a conjugation between very few chromosomes, while in the female sex all the 23 *pigra* chromosomes conjugate normally with 23 *curtula* chromosomes, and of the six remaining *curtula* chromosomes the greatest number are eliminated. Here we have the most striking evidence in favor of the hypothesis that the conditions of conjugation cannot be used in the service of taxonomy, but we also arrive at the conviction that physiological conditions in the cell, and especially in the nucleus, are able in certain cases to assume sovereignty over the chromosomal structure. In the case in question the autosomes are absolutely the same in both sexes of the hybrid, and only the sex chromosomes are different in the reciprocal females. But as the hybrids in the reciprocal crosses act alike, one can hardly ascribe the effect of the sex chromosomes to the quite dissimilar conditions of conjugation in the two sexes. It only remains to assume that the cause why a seemingly normal conjugation is brought about under spermatogenesis, while it fails altogether in the oogenesis, lies in the dissimilar conditions during these processes. Yet it is true that they are chiefly regulated by the sex chromosomes.

Of course, the parallel with the dissimilarity in regard to crossing over in both sexes of Drosophila imposes itself. The reason why crossing over appears only in the female and not in the male has been taken to lie in the fact that chiasmata are formed only in the oogenesis, not in the spermatogenesis. It is natural to assume something similar also concerning the Pygaera hybrids. I regret to say that it is impossible here to examine chiasmata, because it is difficult to get sight of them in the Lepidoptera. Yet there are circumstances which speak for the opinion maintained by DARLINGTON that chiasmata at diakinesis and metaphase play a very important part. In certain individuals of the hybrid the existence of a manifestly short syndesis, also in spermatogenesis, can be discerned, which proves that a certain affinity yet exists, even if it is weak and of short duration, and that consequently it can not be traced at diakinesis and metaphase.

Altogether, many observations made by the way indicate that the meiosis can turn out quite differently in the pollen mother cells from that in the embryo sac mother cells and also differs in the spermatocytes from that in the oocytes, which fact ought to stimulate the cytologists to more careful investigations of the arduous but evidently satisfactory oogenesis.

There only remain to say some words regarding the last group of investigations concerning the outward and inward factors which have proved themselves to be regulators of conjugation.

After having spoken about moths, I should like here to state that during the normal spermatogenesis the metabolism, ruling in the testis, determines the conjugation. The chromosomes of those spermatocytes which first enter into the meiosis all pass through a regular conjugation, while the spermatocytes formed by the end of the spermatogenesis do not show any pairing of the chromosomes whatever but only univalents which divide irregularly. The spermatocytes give rise to the apyrene sperms which do not possess the power to fertilize the eggs. Through MACHIDAS' experiment to inject haemolymph from older larvae of Bombyx into younger ones and thereby to prove that the formation of apyrene sperms is accelerated, it is clearly shown that it is the quality of the haemolymph which decides whether or not the conjugation is brought about.

The influence of temperature on the conjugation of the moths has, on the other hand, been investigated by KOSMINSKY who has shown that in *Lymantria dispar* the bivalent chromosomes decrease with a rising temperature, so that in the highest temperatures which can be borne only diploid gametes are produced.

The influence of temperature on the meiosis has been very thoroughly studied by the botanists. Thus, SAKAMURA and STOW have, through influence of heat on bulbs of *Gagea lutea* during the time of the meiosis, succeeded in calling forth polyploid pollen cells and also pollen cells with aberrant chromosome numbers. These have shown themselves to possess the ability of germinating, and can thus give rise to new forms. BELLING has found that individuals of Uvularia that at night during winter were exposed to too much cold in a hothouse showed a number of abnormalities during the meiosis, in a great measure depending on abnormal conjugations.

The potato seems to be especially sensitive to high temperature. Conjugation takes place normally only at 20°C and leads to the formation of pollen cells with 24 chromosomes. In rising temperature the power of conjugation decreases and according to STOW is altogether suspended at 25 to 30°C, in consequence whereof diploid pollen cells with 48 chromosomes are formed. These are sterile. According to STOW the lower temperatures are more easily borne. At 8 to 12°C the conjugation proceeds normally, and 24 chromosomes can be counted but show a strong disposition to a secondary pairing so that, in certain cases, only 12 chromosomes are to be found.

HEILBORN has made certain kinds of apples grown in the north the subject of a careful investigation with regard to the influence of temperature on the process of conjugation and found that an already comparatively insignificant rise in the temperature above the optimum prevents conjugation, so that a larger or smaller number of univalent chromosomes can be observed. Different sorts show very different susceptibility. Among certain species as low a temperature as 20°C is injurious, while other kinds can bear without injury as much as 35°C.

That the age of the flower can influence conjugation is proved by ROSENBERG's observations of *Hieracium laevigatum* and *lacerum,* among which a formation of gemini does not generally occur. In old cells, however, ROSENBERG has succeeded in coming across a few gemini.

I should like further to state that even attacks of parasites can influence the normal process of meiosis. According to experiments by KOSTOFF and by KOSTOFF together with KENDALL in *Lycium halmifolium* and *Datura ferox,* many kinds of irregularities occur in the meioses because of attacks by mites.

And finally, some few words regarding specific genes which regulate the meiosis and specially influence the conjugation. BEADLE has described a couple of such genes in Zea. Among these, one gene called by BEADLE "asynapsis gene" is of interest in this connection. It is recessive, and its effect is characterized by partial or complete failure of synapsis during the prophase of the first division in the microsporocytes. This results in failure of reduction and production of diploid spores.

In Drosophila, GOWEN has discovered a gene which quite prevents crossing over and thus in some way or other must change the conjugation, probably through preventing the formation of chiasmata. This is also indicated by the development of all kinds of chromosomal mutations in such families. They must apparently be ascribed to the fact that synapsis failed partly or completely.

L. A. SAPEHIN has found in hybrids between *Triticum durum* and *vulgare* specific genes which determine normal meiosis; he calls them organisators, and their recessive alleles he calls disorganisators. These disorganisators cause all kinds of abnormalities in the chromosomes of the microsporocytes and SAPEHIN thinks he can declare that they segregate, although he has not been able to decide on the special type of segregation. Among these disorganisators there also exists one which prevents the segregation of the chromosomes both in the first and in the second maturation divisions and thus gives rise to diploid pollen cells. SAPEHIN seems to be convinced that there are a number of such gene organisators that regulate the whole meiosis, and he is sceptical about the influence of the plasma on the meiosis.

As appears from what I have said, I have not been able to offer any explanation of the phenomenon we call conjugation of the chromosomes, nor, I hope, have any of my hearers expected one. What I wish to convey to you is the extremely complicated nature of this universal process and its dependence on a number of inward and outward factors as yet too little subjected to research. And if my discourse succeeds in inciting scientists to take a deeper interest in the subject, and if it urges cytologists and geneticists together to undertake thorough investigations in this field of knowledge, I shall have gained my object.