On Hybridization of some Species of *Salix*. II.

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I. SUMMARY OF EARLIER RESULTS.

FIRST I will briefly recapitulate here what I have stated in my former paper concerning the same subject,¹ in so far as it is related to the present communication, because this will conduce much to clearness.

The hybridizations between two Japanese species of *Salix*, viz. *gracilistyla* and *multinervis*, were done in 1910 and 1911. The character which sharply distinguishes them is among others the hairy condition of their catkin: that of the former is characterized by being densely covered with greyish hairs, whilst that of the latter is very sparingly hairy (cf. Ikeno, l. c., Pl. I, Figs. 1–2). The cross *multinervis* × *gracilistyla* done in 1910 has given rise to fourteen F_1 hybrids. The latter were not uniform: eleven were provided with catkins densely hairy like *gracilistyla* (hence called *plants of G-type*), and three with catkins sparingly hairy like *multinervis* (hence called *plants of M-type*). It is to be noticed that each of the two parents was examined in respect to its genetical purity by means of fertilization *inter se*, and found to breed true to its respective type. The fertilization between male and female G-type has given rise in F_2 to many G-types and a few M-types, and that between male and female M-type to many M-types and a few G-types, while that between male G-type and female M-type has produced both types in almost equal number. I have tried to explain the formation of two types of progeny in F_1 , despite the apparent genetical purity of both parents, by the action of invisible factors.

¹ Journ. of Genetics, 1918, vol. viii, pp. 35–58.

The result of the same hybridization done in 1911 was quite different from that of 1910. It has given rise to almost fifty plants which were not hybrids, as was expected, but *multinervis*, i. e. identical with the mother-plant; all were found to be female.

II. FORMATION OF HYBRIDS.

1. General and F_1 Generation.

Though, as stated above, *multinervis* $\times$ *gracilistyla* has always given rise to a certain number of progeny, its reciprocal cross, i. e. *gracilistyla* $\times$ *multinervis*, was unsuccessful even by repeated trials (done in 1912 and 1918), and I stated in my former paper that this hybridization is wellnigh impossible.¹ This conclusion was, however, rather hasty. In 1919 I once more tried this operation, and was much astonished to see that this time I obtained success, though scanty. I have pollinated many ears, but only three have given some seedlings and I have obtained twenty-one hybrids in all. Of these four have died down, and I have now seventeen planted in my garden; they have not flowered yet, except two, each of which has borne G-type female catkins. So far as I am able to judge by their leaves, the remaining fifteen are certainly hybrids exactly similar to those ex *multinervis* $\times$ *gracilistyla*. The definite classification of all of them according to the nature of their catkins is thus still impossible, and they are consequently of not much use in my present experiment. I wish merely to point out the fact that *gracilistyla* $\times$ *multinervis* may be successful, though very rarely.

From *multinervis* $\times$ *gracilistyla* carried on in 1910 I got only fourteen hybrids. As this number is very small I repeated the same hybridization in 1918, and obtained many more offspring. Though seven of them have not yet produced flowers, those with flowers may be classified as follows:

TABLE I.

No. of Cross.	Progeny.					No. of Cross.	Progeny.				
	G-type.	M-type.	GM.	MM.	Totals.		G-type.	M-type.	GM.	MM.	Totals.
1	1	...	...	...	1	16	1	...	...	...	1
2	5	...	...	...	5	17	...	1	...	...	1
4	1	1	...	...	2	18	1	...	...	...	1
5	1	...	...	...	1	19	1	...	...	...	1
6	1	1	...	...	2	20	2	...	...	...	2
7	8	...	...	...	8	22	1	...	...	...	1
8	4	...	...	...	4	23	1	...	...	...	1
10	3	2	...	...	5	26	4	...	...	1	5
11	14	...	1	...	15	28	4	3	...	...	7
13	5	1	...	...	6	29	...	...	...	1	1
14	...	1	...	...	1	30	1	1	...	...	2
						Totals	59	11	1	2	73

¹ l. c., p. 36.

Each number in the above table includes plants derived from one catkin, and all catkins are taken from one tree. The table shows that very few plants are produced from each catkin—very often only one. This is first of all to be ascribed to the fact that each catkin is very poorly fertile, owing evidently to the great difficulty of the hybridization under discussion. Many more seeds were actually obtained than might be inferred from a simple inspection of the table; many of them, however, failed to germinate, or else many seedlings perished in very young stages. In the table Nos. 3, 9, 12, 21, 24, 25, and 27 are wanting, because no adult plants were available from these pedigree numbers, owing to one of the two causes stated above. In one plant from No. 11, ranked under the column GM, some branches bear G-type catkins, and others M-typed ones. Nos. 26 and 29 have each produced one female *multinervis* (placed under the column MM), to which I will refer later in this paper.

If we add together the F_1 hybrids from the crosses done in 1910 and 1918 we have:

TABLE II.

Year.	G-type.	M-type.	Multinervis.	GM.	Totals.
1910	11	3	...	...	14
1918	59	11	2	1	73
Totals	70	14	2	1	87
%	83.3	16.7	...	...	...

If we take simple G- and M-types into consideration, the three remaining plants being left out of account, we have eighty-four offspring in all, composed of 83.3 per cent. G-types and 16.7 per cent. M-types.

The important question in respect to the hybridization under discussion is: Why will it yield the two types G and M already in F_1 , despite the fact actually observed that each of the two parents breeds true respectively? Have we here to deal with a segregation? In my former paper I was led to the conclusion that we have in this case no segregation at all, and I tried to explain the appearance of two types in F_1 , as well as the behaviour of the F_2 progeny, by the interference of invisible factors X and Y .¹ The results of hybridizations between F_1 hybrids and either of the two parents (i. e. back-crossing) which were performed in 1917 and 1918 served for testing the validity of my hypothesis. They were by no means particularly favourable for it. Especially, the results from G-type $F_1 \times gracilistyla$ were contrary to my theoretical expectation: thus, if we denote by X the factor which is able to cause Gg to develop to G-type, as was done in my former paper,² we have G-type $F_1 = XXGg$, and *gracilistyla* = GG , and the cross $XXGg \times GG$ or $XXGg \times xxGG$ should give rise to $2(XxGG + XxGg)$, i. e. to G-type progeny exclusively, which was not

¹ l. c., p. 48 ff.² l. c., p. 50 ff.

actually the case, inasmuch as I have got by this crossing not only G-type, but a certain number of M-types (see Table V, Expt. 6, below). Such a fact, as well as certain other reasons which would not be needed here to be enumerated, have led me to abandon my hypothesis, at least in the form in which it was expressed in my former paper. How can we then explain the phenomenon? For the reasons which I will not repeat here I was formerly unwilling to accept the 'imperfection of dominance' for its explanation.¹ But if the view of 'invisible factors' is to be abandoned, I think that there will be no better means than to resort to that of the 'imperfection of dominance', for the latter will explain most simply what we observe in respect to *Salix* hybrids; though I am still of the opinion that the interference of certain other factors, either visible or invisible and variously combined, may be the ultimate cause of this phenomenon. What is discussed below is consequently founded on the view of the 'imperfection of dominance'.

We have seen in Table I that in F_1 of *multinervis* $\times$ *gracilistyla* we have 83.3 per cent. G-types and 16.7 per cent. M-types. We may regard the densely hairy condition of the catkin of *gracilistyla* to be *dominant* to the less hairy condition of that of *multinervis* as a rule, so that if we represent the former by *D* and the latter by *R*, all F_1 hybrids will agree in being *DR* genetically. The dominance here is not absolute; consequently only in the 83.3 per cent. is the dominant character observed, whilst in the remaining 16.7 per cent. it fails, and lets the recessive character appear externally. In short, the G-type as well as the M-type progeny in F_1 are *genotypically* equal, though *phaenotypically* different; we have evidently no segregation in this generation. Moreover, Table I shows us that many numbers therein, each of which corresponds to one catkin, contain both G- and M-types. Hence we may conclude that not only one plant individual, but also even one and the same catkin, possesses at the same time both gametes where usual dominance of *D* over *R* prevails and those where it fails.

2. F_2 Generation.

In order to obtain the F_2 progeny I have carried on the three following crosses between the G-type and M-type progeny.² The results are shown in the following table³:

¹ l. c., p. 48.

² Only in Expt. 3 is the male parent derived from F_2 (l. c., p. 42).

³ The number of progeny in Expts. 1 and 2 is the same as in my former paper (l. c., pp. 42-3), but that in Expt. 3 is a little larger, because I have added afterwards those plants which have borne flowers in 1919 and 1920. In calculating the percentage the new type was not taken into account.

TABLE III.

No. and Nature of Experiment.	Progeny.			Totals.
	G-type.	M-type.	New type.	
1. (G-type ♀ × G-type ♂)	187	32	...	219
2. (M-type ♀ × G-type ♂)	77	67	15	159
3. (M-type ♀ × M-type ♂)	4	19	8	31
Totals	268	118	23	409
%	69.4	30.6	...	...
Deviation from 75 % : 25 % expectation	-5.6	+5.6	...	...

Plants ranked under the column 'New type' in the above table have their catkins very similar to those of M-type, but distinguished from the latter by their perfect non-hairiness (l. c., p. 44). In my former paper I stated my supposition that they will perhaps breed true in later generations. In order to confirm it many seeds of this type produced by fertilization *inter se* were sown in 1918, and many germinated, but a large portion of the seedlings perished in the young stage, and I obtained only eleven adult plants. Of the latter two produced flowers in 1920, and proved themselves to be the male and female plant respectively, and are quite similar to their parents externally. The remaining nine do not bear flowers yet. My supposition was thus confirmed, at least partially. The problem, whether they were produced by the so-called 'loss-mutation' or by the new combination of certain factors, would not be definitely decided without further breeding experiments.

Since the crosses shown in Table III correspond to $DR \times DR$, we expect to get 75 per cent. D and 25 per cent. R , if we are dealing here with the 3 : 1 segregation. We have actually 69.4 per cent. G-types and 30.6 per cent. M-types, i. e. ± 5.6 per cent. deviation from this expectation. The deviation is *positive* on the side of M-type progeny, and this fact is evidently due to the imperfection of dominance: the F_2 progeny are to be represented genotypically by 25 per cent. DD + 50 per cent. DR + 25 per cent. RR ; in the case under discussion in 5.6 per cent. out of 50 per cent. DR the dominance failed, and the recessive character has made its appearance, or, in other words:

25 per cent. DD + 44.4 per cent. DR + 5.6 per cent. (DR) + 25 per cent. RR = (25 + 44.4) per cent. G-type + (5.6 + 25) per cent. M-type = 69.4 per cent. G-type + 30.6 per cent. M-type.

(DR) denotes the zygotes where the usual dominance fails.

In short, the segregation follows the 3 : 1 mode, and the deviation is due to the imperfection of dominance of the factor D over R .

3. Back-crosses.

The results of back-crossing of F_1 hybrids by either of the two parents, i. e. $DR \times R$ or $DR \times D$, have fully confirmed those obtained in the F_1 as well as the F_2 generation.

In respect to the back-cross $DR \times R$ we have to distinguish between two cases, viz. G-type $\times$ *multinervis* and M-type $\times$ *multinervis*. They were carried on in 1918, and the results as studied in 1920 and 1921 are as follows:

TABLE IV.

No. and Nature of Experiment.	Progeny.		
	G-type.	M-type.	Totals.
4. (G-type $\text{♀} \times$ <i>multinervis</i> ♂) . . .	125	88	213
5. (M-type $\text{♀} \times$ „ „) . . .	14	91	105
Totals	139	179	318
%	43.7	56.3	...
Deviation from 50 % : 50 % expectation	-6.3	+6.3	...

Since the segregation occurs according to 3:1 fashion, we expect to have in $DR \times R$ 50 per cent. G-types and 50 per cent. M-types. Actually we have 43.7 per cent. of the former and 56.3 per cent. of the latter. The deviation is therefore ± 6.3 , and is *positive* on the side of the M-type, just as was the case in F_2 (see Table III); this is evidently to be ascribed to the fact that in certain cases the usual dominance fails, i. e.—

$DR \times RR = (50 - 6.3)$ per cent. $DR + 6.3$ per cent. (DR) + 50 per cent. $RR = 43.7$ per cent. G-type + $(6.3 + 50)$ per cent. M-type = 43.7 per cent. G-type + 56.3 per cent. M-type.

(DR) denotes, as before, the zygotes where dominance fails.

In the back-cross $DR \times D$ we have to distinguish between G-type $\times$ *gracilistyla* and M-type $\times$ *gracilistyla*. These crosses were performed in 1919, and the results as studied in 1921 are as follows:

TABLE V.

No. and Nature of Experiment.	Progeny.		
	G-type.	M-type.	New type.
6. (G-type $\text{♀} \times$ <i>gracilistyla</i> ♂) . . .	184	8	...
7. (M-type $\text{♀} \times$ „ „) . . .	76	9	11
Totals	260	17	11
%	93.8	6.2	...
Deviation from 100 % : 0% expectation	-6.2	+6.2	...

In the above table the new type is not taken into consideration for counting the percentage.

Since the above crosses correspond to $DR \times D$ we expect to have 100 per cent. G-types and no M-types at all. Actually we have 6.2 per cent. of the latter, so that the deviation from the theoretical expectation is ± 6.2 , which is again *positive* on the side of M-type, i. e.—

$DR \times DD = 50$ per cent. $DD + (50 - 6.2)$ per cent. $DR + 6.2$ per cent. (DR) = 93.8 per cent. G-type + 6.2 per cent. M-type.

(DR) = zygotes where dominance fails.

If we compare the results shown in Tables III, IV, and V, we see that the deviations from the expectation are very similar to each other (5.6 per cent., 6.3 per cent., 6.2 per cent. respectively), which will indicate that the degree of failure of dominance is nearly the same in all three cases. In F_1 generation, however, where the theoretical expectation is 100 per cent. dominants and no recessives, the deviation, i. e. the failure of dominance, is 16.7 per cent. (see Table II), and consequently much larger than in the above three cases. To what cause may such a difference be ascribed? We may make several suppositions. Thus, for instance, in the crosses shown in Tables III–V one or both parents are heterozygous, i. e. DR , whilst in the cross in Table II both are homozygous; may not such a circumstance lead to the difference in the degree of failure of dominance? We may perhaps put forward another consideration: Does not that degree vary under the influence of external conditions? Such questions and all others which one may ask are naturally not to be definitely decided without further breeding experiments, and I must here be satisfied with simply indicating such problems.

A few words as to the dominance of the *gracilistyla* catkin. We have seen that the hairy condition of catkins of the latter is dominant to the less hairy condition of *multinervis* as a rule. I have observed that in *multinervis* $\times$ *gracilistyla* the non-hairiness of leaves is dominant to their hairiness (l. c., p. 38), which is consequently very different from what we see in the catkins in the same hybrid. Evidently the genetic factors concerning hairs covering leaves on one hand and those covering catkins on the other must be quite different.

4. Potency.

As we can easily see by comparing the results of the various crosses above enunciated, the degree of failure of dominance is not the same in G-type as in M-type. The question is, Do the G-type plants produce a larger proportion of G-type progeny than the M-type ones, and vice versa? Is the degree of 'potency' (to use the word and the expression adopted by Davenport¹) inherited?

Let us first see what other authors have observed in this respect. In their experiment on the crossing between extra toes and normal ones in poultry, where the former are dominant to the latter, Bateson and

¹ Carnegie Institution of Washington Publication, No. 121, 1909. The author says (p. 92), 'The potency of a character may be defined as the capacity of its germinal determiner to complete its entire ontogeny. If we think of every character as being represented in the germ by a determiner, then we must recognize the fact that this determiner may sometimes develop fully, sometimes imperfectly, and sometimes not at all. . . . Potency is variable. Even in a pure strain a determiner does not always develop fully. . . . But in a heterozygote potency is usually more or less reduced. When the reduction is slight, dominance is nearly complete; but when the reduction is great, dominance is more or less incomplete. . . .'

Punnett¹ have obtained, (a) from the heterozygote *DR* with usual dominance $\times$ recessive, 114 extra toes and 95 normal, i. e. 54.5 per cent. and 45.5 per cent. respectively, and (b) from the heterozygote *DR* where dominance fails $\times$ recessive, 128 extra toes and 151 normal, i. e. 45.7 per cent. and 54.1 per cent. respectively; consequently the class (a) contains comparatively a little more extra-toed progeny than the class (b). So that there may be a very slight inheritance of degree of potency, but this is quite insignificant. Much more marked inheritance has been found by Davenport² in respect to the crosses of Houdan fowls: he has calculated the correlation between total number of toes in the two parents and average number of toes in their progeny, and found the coefficient of correlation to be equal to $+0.683 \pm 0.092$.

Turning to our case, the simple inspection of the results of Expts. 1, 3, 5, 6 shown in Tables III, IV, V, will immediately indicate to us that the inheritance of the degree of potency is very significant in the present case. The results of our four experiments just enumerated were employed to make the following table of correlation:

TABLE VI.

Nature of Crosses.	Progeny.		Totals.
	G-type.	M-type.	
G-type $\times$ G-type and G-type $\times$ <i>gracilistyla</i> (Expts. 1 and 6)	187 + 184	32 + 8	411
M-type $\times$ M-type and M-type $\times$ <i>multinervis</i> (Expts. 3 and 5)	4 + 14	19 + 91	128
Totals	389	150	539

This gives $r = +0.724 \pm 0.021$. The coefficient or correlation is rather high, and this result confirms our supposition founded on mere inspection of data.

Since the appearance of the well-known work of Wichura³ it seems that botanists have generally believed in the constancy of *Salix* hybrids throughout later generations. In a short note Sirks⁴ has expressed his opinion that the work of Wichura by no means induces us to the belief that *Salix* hybrids are constant. In 1916⁵ and 1918⁶ I published the results of my investigations on the hybridization of some species of *Salix*, especially *S. gracilistyla* and *multinervis*: in these experiments I was able, contrary to the current belief of botanists, to observe the segregation of some few characters in *Salix* hybrids, but since the relative number

¹ Reports to the Evolution Committee of the Royal Society. Report II, 1905, p. 115.

² l. c., p. 23.

³ Die Bastardbefruchtung im Pflanzenreich erläutert an den Bastarden der Weiden. Breslau, 1865.

⁴ Zeits. f. induct. Abstamm.- u. Vererbungslehre, Bd. xv, 1915, pp. 164-5.

⁵ Bot. Mag., Tôkyô, vol. xxx, 1916, pp. 316-20.

⁶ Journ. of Genetics, vol. viii, 1918, pp. 35-58.

of segregates was somewhat different from what we might have expected in the case of usual Mendelian segregation, I could not then decide definitely whether we have here really to deal with such or not. In 1918 Heribert-Nilsson¹ published an elaborate memoir containing the results of his extensive experiments on *Salix* hybrids; he was able not only to prove the segregation of various characters, but also to discover the usual Mendelian ratio in many cases. My experiments concerning the catkin characters above mentioned have shown that their segregation occurs according to the simplest Mendelian ratio 3 : 1, though more or less obscured by the imperfection of dominance. Accordingly these experiments are a further contribution towards establishing the fact that the segregation of the various characters in *Salix* hybrids follows Mendel's law.

III. FORMATION OF PARENTAL FORM.

The hybridization *multinervis* × *gracilistyla* done in 1911 has given rise to almost fifty offspring, all of which were, contrary to our expectation, not hybrids, but female *multinervis*.²

I have myself had no doubt about the actual production of *multinervis* progeny in this case. The fact was, however, so unexpected that others may reasonably be in doubt about its reality. So I repeated the same hybridization in 1918 and 1919. The results of hybridization in 1918 are contained in Table I (see p. 176). Though, as is usually the case in this hybridization, each catkin has produced only few seeds—often none—yet I have obtained a comparatively large number of progeny—more than ninety plants in all; but the number of *multinervis* was small—in fact I had only two (Table I, Nos. 26 and 29). On the contrary, the hybridization carried on in 1919 has yielded very few seedlings, but almost all were *multinervis* progeny, thus:

TABLE VII.

No. of the Female Parent (<i>multinervis</i> ♀).	No. of Catkins.	No. of Progeny (<i>multinervis</i>).
I.	35	2
	36	1
	41	1
II.	4	1
	7	4
Total		<hr/> 9

Gracilistyla used as the pollen plant in the above experiments was the same in both cases, and identical with what we have employed in the experiments whose results are shown in Table I.

No progeny contained in the above table bear flowers yet, but that all of them are *multinervis* is quite unmistakable on account of their characteristic leaves, &c. Besides them, some plants which are not *multi-*

¹ Lunds Universitets Årsskrift, N.F., Afd. 2, Bd. xiv, No. 28, 1918.

² l. c., p. 51.

nervis, perhaps hybrids, have been produced, but since they perished before flowering they are not enumerated in the table.

From the results just enunciated we now can have no reason to doubt the production of *multinervis* progeny from *multinervis* mother without the action of *multinervis* pollen.

It is well known that sometimes in some *Salix* species a few male flowers are produced on the female catkin, or even a few flowers become hermaphrodite. One might perhaps think that the production of *multinervis* progeny in our case might be due to such abnormalities which have escaped my attention. I have looked for them in our *multinervis* trees carefully and repeatedly: it may be remarked in this respect that I have never met with even traces of such in our trees.

When my former paper was published the fact whether or no the *multinervis* progeny under discussion will breed true in later generations was not yet decided, though this seems to me to be highly probable. I pollinated in 1920 two such *multinervis* progeny (from Nos. 26 and 29 in Table I) by pollen taken from a *multinervis* male plant, and got a certain number of progeny. The latter were *multinervis* without exception, so that my supposition that they will breed true has been fully confirmed.

The next question will be, How are the *multinervis* progeny produced without the action of *multinervis* pollen? My conclusion in my former paper was that it may be ascribed neither to parthenogenesis nor to development of nucellar cells, but to so-called pseudogamous development of oospheres due to the stimulus of foreign pollen (l.c., pp. 51-4). Some doubt, however, arose in my mind in 1919 about this conclusion. Mr. S. Nohara, then assistant in my laboratory, covered a number of young catkins of a tree cultivated in our botanical garden, and very similar to *multinervis*,¹ with paper-bags for a certain purpose, and left them in this condition for a certain lapse of time, perhaps about two months. On opening them he was struck by the fact that some catkins had produced a few fruits. He notified me of the fact. I placed some seeds thus obtained upon a moist filter-paper within a Petri dish: some of them were so weak that they refused to germinate, but others germinated very well. Still others were sown in a seed-pan and came to germination.² From all these facts there will be no doubt about the occurrence of the 'apomictic' development without any pollination in this case, to use the word introduced by the German botanist Winkler.³

¹ It may be a variety of *multinervis*; it is chiefly distinguished from the latter by its smaller catkins.

² Plants derived from this germination are now cultivated in our garden. So far as we can judge by their leaves they are quite similar to their parent; one of them has already borne female catkins, and proved itself to be perfectly similar to its parent.

³ Parthenogenesis und Apogamie im Pflanzenreiche, Jena, 1908, pp. 8 ff.

In my former paper I stated the fact that female inflorescences of *multinervis* covered with paper-bags were never able to bear fruits.¹ This is quite true, but then only a few branches were used for the experiment. In view of the results obtained by Mr. Nohara, which are described above, the experiments were repeated in 1920 and 1921 on a much larger scale than formerly. In 1920 nine trees were chosen for the experiment: of these, three (Nos. 1-3 in Table VIII below) which are high and copiously branched are derived from the fertilization *inter se* of male and female trees used in my experiments done in 1910 and 1911; the remaining six are smaller, of which four (Nos. 4-7 in Table VIII) are the cuttings from a female tree used in the same experiments, and two are from Nos. 26 and 29 in Table I (i. e. plants produced without the action of *multinervis* pollen), designated in Table VIII as Nos. 8 and 9. The number of catkins in each tree was as follows:

TABLE VIII.

No. of Trees.	No. of Catkins.
1	1,105
2	326
3	818
4	20
5	29
6	19
7	36
8	55
9	95
Total	2,503

If we suppose that each catkin bears 100 flowers on average, and each flower contains three ovules, both of which are by no means high estimations, we should have a total of 750,900 ovules. All catkins enumerated in the above table were covered with paper-bags, and left in this condition for nearly two months. Though in certain catkins a few ovaries grew up somewhat more intensely than others, and consequently attracted our attention by their remarkable size, yet all of them, on opening, were found either to be quite empty or to contain a few seed-hairs only. All other catkins simply dried up and fell, with the exception of two catkins from No. 8 in the above table. Thus only from the latter did I get four seeds in all. Of these, two, being placed upon a moist filter-paper within a Petri dish, came to germination, whilst the remaining two did not.

Similar experiments were repeated in 1921 on the same nine trees used in 1920. The number of catkins produced in 1921 was somewhat smaller than in 1920; besides, either a certain number of branches covered with bags were broken down, or the latter were torn off by storms which

¹ l. c., p. 54.

raged several times in March and April. As catkins on such branches must necessarily have been left out of consideration, the number of those available for our experiment was considerably diminished. Nevertheless, we had 1,261 catkins which should contain no less than $1,261 \times 100 \times 3 = 378,300$ ovules in all, i.e. more than half the number of ovules experimented upon in 1920. All these catkins were covered with bags for more than two months. As in the former year I saw not unfrequently ovaries which grew somewhat more intensely than others, but generally they either simply shrivelled up gradually, or opened and proved themselves to contain no seeds at all. Only on some catkins of the tree designated as No. 1 in Table VIII did I get eight seeds. Of these, three came to germination, and one began to germinate but soon ceased to make any further growth. All remaining seeds refused to germinate.

In the two new experiments just mentioned I was thus able to get only very few seeds which have the power of germination, but nevertheless they have proven beyond all doubts that in our case the apomictic development of ovules without the application of any pollen is a matter of possibility, though it is of extraordinarily rare occurrence.

In consequence of the results of the new experiments just described my former view concerning the development of *multinervis* after the pollination with *gracilistyla*, as stated in my former paper, must necessarily change. To explain the result of our new experiments there are two possibilities, viz. the embryo formation from nucellar cells and the parthenogenesis, each of which is either autonomous or induced by the stimulus of foreign pollen. The definite conclusion whether the apomictic development in question will occur according to the first or the second mode just enunciated would naturally be possible only after a comparative cytological examination of the development of normal as well as apomictic ovules. Since, as above stated, ovules of the latter class are of extremely rare occurrence, it would be necessary for the purpose to examine innumerable specimens made by sectioning an enormous number of catkins, and even then there would be no great chance of meeting with such ovules. Thus we see that in the present case the cytological examination must be almost an impossibility. The embryo formation from nucellar cells is generally accompanied by polyembryony, as in *Funkia*, *Citrus*, *Opuntia*, &c., &c. Since I have never met with this in *Salix*, although I have observed the germination of many thousand seeds of various species of *Salix*, either pure or hybrid, including *S. multinervis*, I am rather inclined to the view that as the cause of apomictic development in our case parthenogenesis is much more probable than embryony. All the following discussion is accordingly founded on the supposition that we have here to deal with parthenogenesis, either autonomous or induced.

In *Thalictrum purpurascens*¹ as well as some species of *Hieracium* (*pilosella*, *excellens*, &c.)² it is well known that there are two kinds of ovules, viz. those which require fertilization for seed formation, and those which do not. It will naturally be quite the same in *Salix multinervis*. As we have here to deal in all probability with somatic parthenogenesis in the sense of Winkler,³ it follows that the former kind of ovules, which have undergone the reducing division of chromosomes during their development, become hybrids by pollination with foreign pollen, while the latter kind, which have undergone no such process, develop to *multinervis* without being fertilized. Moreover, that these two kinds of ovules are contained at the same time not only in one plant individual, but also in one and the same catkin, is clearly seen from the examination of No. 26 in Table I (see p. 176), inasmuch as in this number the progeny which are derived from one single catkin contain, besides one *multinervis*, four G-types (i. e. hybrids).

In my former paper I was led to the conclusion that in our case we have to deal with pseudogamy, i. e. parthenogenesis induced by the stimulus of foreign pollen, *gracilistyla* in the present case. This was the natural consequence deduced from the results of my former experiments, because then any catkin covered with a bag was found not to be able to bear a single seed. Since, however, our new experiments have shown us that in certain cases, though extremely rare, catkins wholly prevented from any pollination are able to produce a few seeds, my former conclusion is of course not quite right. The following remarks may, however, be made in consequence of the results of our new experiments. Although very rarely, the apomictic development without any pollination is possible, yet we have seen that the number of seeds then produced was so scanty that pseudogamy might also be not impossible in certain cases. For instance, in our experiment done in 1911, where the pollination with *gracilistyla* was several times carried on, I have got almost fifty *multinervis* individuals from a few catkins, while in the experiments done in 1920 and 1921, when no such pollination was practised, only five apomictic seeds which were able to germinate were obtained out of more than $700,000 + 370,000 = 1,070,000$ ovules!⁴ It is not improbable that although few ovules may develop parthenogenetically without the stimulus of foreign pollen (autonomous parthenogenesis), yet ovules may in many cases be induced to

¹ Overton: Bot. Gaz., vol. xxiii, 1902, pp. 363-75; Ber. d. Deutsch. Bot. Ges., Bd. xxii, 1904, pp. 274-83.

² Ostenfeld: Botanisk Tidsskrift, Bd. 27, 1906, pp. 225-48. Rosenberg: ibid., Bd. xxvii, 1907, pp. 143-70.

³ l. c., p. 17.

⁴ In the experiment carried on in 1919, when the pollination by *gracilistyla* was practised, I got only nine *multinervis* (see Table VII), but then only a very few catkins were used for experiment, and it is hardly doubtful that had I experimented upon a larger number of catkins I should have been able to obtain a much larger *multinervis* progeny.

development only by its stimulating action (pseudogamy). As before stated, I have observed frequently in catkins covered with bags ovaries which have grown up somewhat more intensely than others and yet remain quite sterile; may not such ovaries be able to produce some seeds by the stimulus of foreign pollen? This is, however, a mere supposition, and the fact ought to be studied in future more in detail, because it is quite possible that exceptionally a much larger number of ovules than I was able to observe in my experiments might develop to seeds without any pollination.

The results of experiments of Pellew and Durham with *Primula verticillata*, *floribunda*, and the hybrid between them, *P. Kewensis*,¹ agree in several respects with what I have seen in our *Salix*. It appears that they have also met with autonomous as well as induced parthenogenesis, for they say,² 'We incline to suppose that the ovules are such that while they can occasionally develop without fertilization they more commonly develop in consequence of that stimulus.'

Concerning the above experiments of Pellew and Durham, Winkler makes the following remarks, which are reproduced below in his own words: 'Vor Allem aber ist . . . zu bedenken, dass die apomiktische Samenbildung auch ohne jeden Bestäubungsreiz erfolgen kann, so dass ein solcher als Auslösung der vermuteten Parthenogenesis jedenfalls nicht unentbehrlich sein, sondern höchstens fördernd wirken kann. Dann kann man aber auch nicht von einer durch die Bestäubung induzierten Parthenogenesis sprechen . . . sondern nur davon, dass durch den Pollenschlauchreiz die an sich schon vorhandene Neigung zur apomiktischen Samenbildung gefördert wird. . . .'³ All that Winkler says may be true, and also applicable to our case of *Salix*. To decide, however, experimentally the fact whether pollination has induced parthenogenesis, or has simply accelerated ('fördern') the tendency for apomictic development, would not be easy.

Had we to deal in our case with parthenogenesis, either autonomous or induced, the following remark might not be without some interest. It is clear that parthenogenesis has been originally derived from normal fertilization in the course of phylogenetic evolution. In respect to the manner of its origin there may be two possible ways. Firstly, certain animal or plant forms which have reproduced themselves at first by normal fertilization acquire at once the power of complete parthenogenesis by mutation, so that all eggs or ovules reach suddenly a condition such that they need no fertilization for their further development. Secondly, the transition from fertilization to parthenogenesis is gradual, i. e. several stages intervene between the two extremes—as we see, for instance, in some

¹ Journ. of Genetics, vol. v, 1916, pp. 159–82.

² l. c., p. 160.

³ Ursache und Verbreitung der Parthenogenesis im Pflanzen- und Tierreich, Jena, 1920, p. 175.

animals, as *Rhabditis aberrans* (Nematoda) studied by Krueger.¹ *Thalictrum purpurascens* as well as certain species of *Hieracium*, where some ovules are parthenogenetic and others normal, may be considered to be in their way of this transition.² Our *Salix multinervis* is also to be ranked among such transitional forms, and in view of the extreme rarity of parthenogenetic ovules, as well as of the fact that parthenogenesis is not always autonomous but in many cases induced (considering my supposition above expressed to hold good), we may regard our *Salix* as being in the very beginning of such transition.

The opinion was expressed by some authors that parthenogenesis has taken its origin in consequence of the abortion of pollen and the decline of sexuality, while that of others, especially Winkler, is quite opposed to it.³ Now if such were really the case we should also observe such phenomena in our *Salix*, but we can there recognize neither abortion of pollen nor decline of sexuality. For instance, Nohara has examined the germinating power of pollen of our *Salix* by the use of 2–3 per cent. cane-sugar solution, and found it to be as high as 98 per cent.⁴ I asked him to examine the germinating power of seeds of this *Salix* species, and he found that out of 721 seeds, taken from four catkins artificially pollinated by a male plant of the same species, 655 came to germination, i. e. 92.2 per cent. The results of these observations are obviously decidedly in favour of the opinion of Winkler.

We come finally to the problem of the sex of our apomictic progeny. As before stated, nearly fifty *multinervis* individuals resulting from the experiment in 1911 were female without any single exception; so also were two *multinervis* obtained by the pollination done in 1918 (cf. Nos. 26 and 29 in Table I). The sex of nine *multinervis* derived from the pollination made in 1919 (Table VII) is yet unknown. In view of the small number of the *multinervis* progeny resulting from the experiment of 1918, we are not able to make any inference about them, but the fact that all *multinervis* offspring derived from the experiment of 1911, numbering almost fifty, are female, can hardly be without a certain significance. Before going farther, let us see first what we know about the sex of the progeny arising by parthenogenesis in animals and plants. In the former it is *arrhenotokous* (males produced exclusively), *thelytokous* (females produced exclusively), or *amphiterotokous* (both males and females produced) in different cases.⁵ Concerning a few dioecious plants where natural parthenogenesis has been discovered, the sex of the progeny arising by this process is known in

¹ Cf. Winkler, l. c., pp. 28 ff.

² Certain species of *Rubus* may also belong to such transitional form. (Cf. Lidforss, Zeits. f. indukt. Abstamm.- u. Vererbungslehre, Bd. xii, 1914, pp. 1–13).

³ Parthenogenesis und Apogamie im Pflanzenreich, pp. 133 ff.

⁴ Bot. Mag., Tôkyô, vol. xxvii, 1913, p. 185.

⁵ Cf. Winkler: Ursache und Verbreitung der Parthenogenesis, p. 15.

some cases. Thus, for instance, in *Bryonia dioica* Bitter has obtained nine parthenogenetic plants, all of which were male.¹ In *Thalictrum purpurascens* the sex of the progeny due to this process is not specially mentioned.² In *Antennaria alpina* males are very rare, and parthenogenesis seems to be chiefly thelytokous.³ In *Chara crinita*, known long since as the classical example of natural parthenogenesis in plants, Ernst⁴ has recently discovered that there are two strains: in one, which reproduces itself by normal fertilization, males and females are produced in almost equal number, whilst in the other, which reproduces itself by parthenogenesis, plants are exclusively female, i. e. parthenogenesis is thelytokous. Since in our *Salix* species our repeated observations have proved that even in the case of normal fertilization females are much superior in number to males, the fact above enunciated, that all fifty offspring are female, might be due simply to the latter circumstance, inasmuch as had we had more than fifty plants it would have been possible that some male offspring might have been obtained. If, however, parthenogenesis in our *Salix* should prove really to be thelytokous, the fact is easily comprehensible, whether the female is homozygous or heterozygous in respect to the sex-determining factors; because, since in our case egg-cells are supposed to develop without undergoing any reducing division of chromosomes, they will remain always in the state of the so-called female-producing ones, *XX* in the first case and *WZ* in the second according to the well-known nomenclature of Morgan.

IV. SUMMARY.

1. By pollination of *Salix multinervis* by *S. gracilistyla* we get hybrids, as well as *multinervis* exactly similar to the mother-plant.

2. Two kinds of hybrids appear in F_1 . The one characterized by its densely hairy catkin is called *G-type*; the other characterized by its less hairy catkin is called *M-type*, and is produced in much less number than the other (for instance, 17 per cent. against 83 per cent. in round numbers). Though the two differ externally they are genetically equivalent.

3. From the study of various crosses we are led to the conclusion that the hairy catkin of *gracilistyla* is dominant as a rule to the less hairy one of *multinervis*. If we represent them by *D* and *R* respectively the F_1 progeny agree in being *DR*.

4. That in F_1 some *DR* individuals are *G-type* and others *M-type* is due to the imperfection of dominance: in a few cases dominance fails,

¹ Abhandl. d. Naturwiss. Vereins zu Bremen, Bd. xviii, Heft 1, 1904. Original not seen.

² Overton, l. c.

³ Juel: Kongl. Svenska Vetenskaps Akademiens Handlingar, Bd. xxxiii, 1900, p. 11.

⁴ Bastardierung als Ursache der Apogamie im Pflanzenreich, Jena, 1918. Cf. especially chapter iii, pp. 49 ff.

and the recessive character appears externally, giving rise to M-types. The appearance of two types in F_1 is not to be regarded as Mendelian segregation.

5. The segregation occurs first in F_2 as usual. The F_2 generation resulting from the cross between the F_1 progeny is variously composed: G-type ♀ × G-type ♂ produces chiefly G-types and a few M-types, M-type ♀ × M-type ♂ chiefly M-types and a few G-types, M-type ♀ × G-type ♂ both types in almost equal number.

6. If we add the results of the above three crosses together we find that we have G-types and M-types in the approximate proportion of 3:1, with a certain *positive* deviation on the side of the latter type: this deviation is due to the imperfection of dominance of the factor D in relation to the factor R .

7. The above conclusion has been fully confirmed by the results of back-crosses, either $DR \times R$ or $DR \times D$.

8. The degree of potency is inherited: i.e. G-type plants produce a much larger proportion of G-type progeny than do M-type plants, and vice versa.

9. Rarely *multinervis* × *gracilistyla* gives rise to *multinervis* progeny which breed true in later generation. Their production from a *multinervis* mother without any pollination at all has been also observed, though very rarely. Whether the embryo formation from nucellar cells or parthenogenesis is the real cause of such 'apomictic' development is not definitely established.

10. We have here to deal with parthenogenesis, which is very occasionally autonomous; in many other cases, however, it seems possible that it is induced by the stimulating action of foreign pollen (pseudogamy).

11. If parthenogenesis is derived phylogenetically from normal fertilization, and if this transition is gradual, our *Salix multinervis* may perhaps be regarded as being in the way of such transition, and in its very beginning.

12. Neither abortion of pollen nor decline of sexuality is to be detected in our *Salix*, which contradicts the view often expressed that parthenogenesis sets in in consequence of such circumstances.

13. All plants of *multinervis* produced by apomixis are female, without any exception. The explanation for it is given.

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Ikeno, S. 1922. "On hybridization of some species of *Salix*. II." *Annals of botany* 36, 175–191. <https://doi.org/10.1093/oxfordjournals.aob.a089792>.

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