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Regeneration in Plants*

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The phenomena of regeneration in plants have been known much longer than those in animals. For cuttings of twigs and the separated leaves of many plants to become independent individuals, or to give rise to them, is an every-day occurrence. The anatomical changes which take place are, in general, well known. On the other hand, relatively few investigations have been made as to the factors which set up regeneration and determine the kind of organs and the manner of their formation in regeneration. It is evident without further discussion that a knowledge of these facts would be of the greatest importance for every theory of organic development and heredity, and in brief for all investigations which might be classed under "causal morphology." It is necessary to group the facts from some general point of view before a theory can be formulated. I have already attempted this some time since, and will give in the following a brief restatement of the conclusions already reached together with the results of some new experiments. It will be profitable to repeat some of my previously formulated propositions † in a somewhat modified form.

1. The phenomena of regeneration imply a development of dormant or latent rudiments. These rudiments (*Anlagen*) are present as vegetative points (embryonic tissue) and are set into

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† Goebel, K. Ueber Regeneration im Pflanzenreich. Biol. Centralb. **22**: 385–397, 417–438, 481–505. 1902. See also discussion and literature in Goebel, Organographie der Pflanzen, 35–43. 1898.

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activity by injuries, or they are outwardly invisible, there being simply a disposition or tendency toward the formation of new structures, as in adventitious buds, or adventitious roots. The two cases are not sharply distinguishable from each other, since in both the unfolding of a rudiment, or the awakening of a predisposition, is conditioned by the reciprocal connections of organs with one another, which are designated as "correlations."

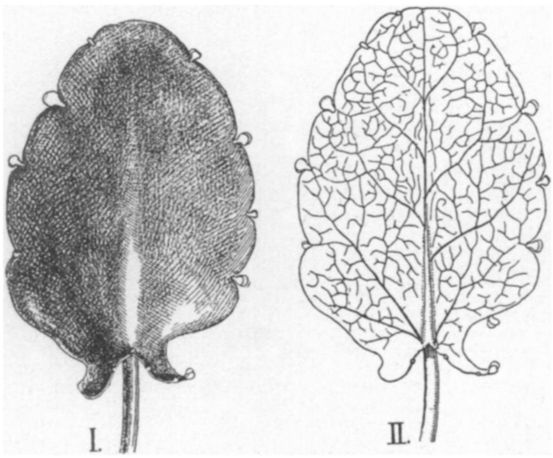

FIG I. *Bryophyllum crenatum*. Detached leaf, which has developed sprouts upon its edge, each with two small leaves. (In II made translucent so that the venation is visible.)

Some examples will make this point clearer. It is well known that every tree has hundreds of dormant buds which ordinarily do not awake, but which may be set into activity by cutting back, or by the destruction of the leaves during the vegetative season. My investigations were most closely concerned with the development of the buds which are normally present on the leaves of some Crassulaceae, as for example *Bryophyllum crenatum*, and which are laid down even in the embryonic condition of the leaves. Their presence implies that the leaves here serve the function of reproduction, since every leaf that is cut off, if laid in damp earth, produces numerous young plants in the notches of its edges.

The next question to be considered is by what means the development of the shoot-rudiments existing on the leaves is set in action. Investigation showed that this development may be induced by cutting the larger vascular bundles, and also the veins of the leaf. The leaf remains fresh and attached to the plant, but becomes covered with young sprouts through the growth of the rudiments on the edge of the leaf. We see by this that a disconnection, or interruption of the conducting system gives the stimulus for the further development of the resting vegetative point on the leaf; but why this interruption should act as a stimulus has not yet been explained. Further investigations may throw some light upon this point. The vegetative points were removed from a number of plants except those found on the margins of the leaves. The result of this operation was that the vegetative points which usually remain dormant became active. The conductive system was not broken but the goal of the system was removed in a manner. For the vegetative points are to be considered as centers of attraction for the constructive material which is carried thither to be used in the building up of new organs. We can say, therefore, that the presence of the vegetative points on the shoot prevents the development of those on the leaves. The former lay claim to the conducting channels that run through the leaf, and only when these are broken or the vegetative point of the shoot is removed do those on the leaves develop. A correlation is clearly apparent.

Whether we have to do in this case with a quantitative relation to constructive material, as was assumed above for the sake of simplicity, or with imperfectly understood specific reactions to stimuli along the conducting system must for the present remain unsettled.

It appeared of interest to me to determine the behavior of plants that have no vegetative points on the leaves, but possess only the tendency to construct adventitious shoots from cells which have passed over into permanent condition. The best known example of this is *Begonia Rex*. Florists propagate this plant by setting leaves cut from the stems in damp sand. At the base of the blade adventitious shoots appear; and one can easily induce them to appear on other places by cutting the larger veins of the leaf.

If the above proposition is correct, it would necessarily follow that we could induce the development of these adventitious shoots on leaves as well, which still retain their connection with the plant. If the attempt were to prove successful, we should have induced experimentally in *Begonia Rex* a phenomenon which is normally characteristic of two other species of this genus. Systematists state that in *B. sinuata* and *B. prolifera* both leafy and flowering shoots develop on the leaf-blade and even from its base.

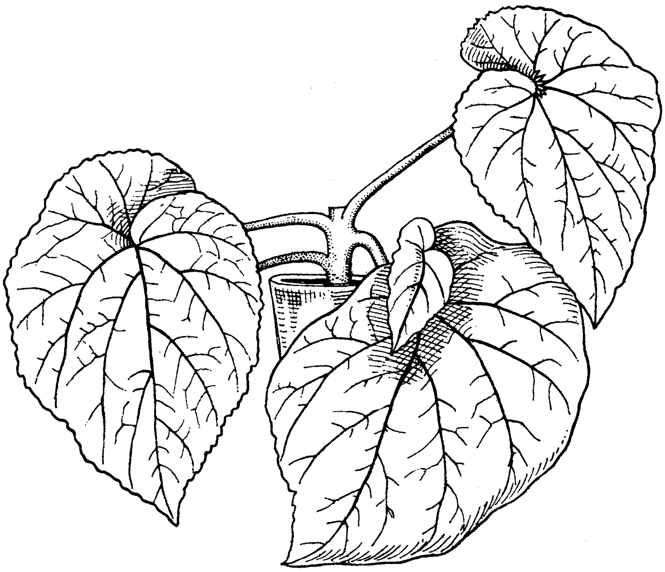

FIG. 2. *Begonia Rex.*

It was found possible to induce the production of shoots on the leaves, as shown in *f. 2*, although not, it is true, as quickly as in *Bryophyllum*. The method used was the same as in the previous instance. All vegetative points that could be found were removed. The plants soon began to develop dormant buds that had been hidden, and produced adventitious shoots in the axes: all these shoots were removed and the process was repeated. After about three months, there appeared on the leaves of the plants treated in this manner adventitious shoots which developed into plants. The shoots came from the bases of the leaf-blades, where the large veins join. Here then we have the same phenomenon as in *Bryophyllum*: that is the removal of the vegetative points of the shoots starts the development. In this case, however, we have to do, not with previously laid-down primordia, but with a disposition on the part of the tissues of the leaf; and we have actually brought about a form-relation present in other species but normally absent from *Begonia Rex*.

2. When a part of a plant is removed direct restoration occurs only when we have to do with embryonic tissue, such as that of the vegetative point. When parts of plants which have assumed their permanent character are concerned the rupture and wounding have the effect of inducing a portion of the cells to return to the embryonic condition and to produce structures which give rise to one or more new plants. Many seedlings have a remarkable power of regeneration.

There is no important and invariable difference in the phenomena of regeneration in plants and in animals, but the course of procedure that has been described is highly characteristic of plants. While in animals the parts that have been lost must be replaced directly, this is the exception in plants. A few illustrations will make this clearer.

(*a*) Embryonic tissue. It has long been known that vegetative points of roots and shoots, if wounded, easily regenerate what has been lost. Fern-fronds have, as is known, the peculiarity that the tip remains in an embryonic condition for a long time, while it soon passes into permanent form in most spermatophytes. I split young leaves on *Polypodium Heracleum* lengthwise into two similar or dissimilar halves. When the parts were alike the tips of each regenerated a perfect leaf; when the parts were unequal, the regeneration on the smaller was much less marked. Similar phenomena are to be observed in the tips of roots or shoots.

(*b*) On the other hand when the parts of a leaf which have assumed permanent form are removed, the restoration does not take place; a separated leaf of *Begonia* for example does not regenerate a new shoot-axis with roots which continue life as a part of the regenerated plant, but there develop on the leaf new plants which soon become entirely independent of the original leaf. The cells of the leaves of many plants are easily induced to develop new plants; they contain all the necessary germ-plasm, but are not in a position to coöperate with one another in such manner as to directly replace what was lost by newly constructed tissues.

The one exception to the above established by Hildebrandt, Winkler and others is the case of first leaves of the seedlings of *Cyclamen Persicum*. When one removes the leaf-blades in this plant there develop to the right and left of the petiole new blades. But in this case also, as I think I proved, there is only a development of latent rudiments. From the point of view of embryology, the leaf-stalk is a part of the leaf-primordium, in which the development of the leaf-blade is arrested. This condition of arrest is by no means so well established in the seedling as it is in later stages, and consequently it can be overcome. When the blades are removed from leaves in older plants these organs die without forming new blades.

Similar conclusions were arrived at in all cases in which a critical examination was made. The phenomena of regeneration in leaves, as described in the literature of the subject, will not bear careful examination. On the other hand, it can be shown that seedlings often possess a greater capacity for the formation of new organs than older plants. Not a few seedlings develop adventitious shoots on the hypocotyl even without being wounded. Such shoots have also been seen on the leaves of young plantlets of *Lycopodium inundatum*, but not on those of older plants.

3. The character of the organ constructed depends upon the condition of the plant at the time regeneration ensued. Illustrations of this fact are quite as apparent in the lower as in the higher plants. The fungi may be cited first. From investigations by Van Tieghem and Brefeld we know that when, for example, the pileus of certain agarics is cut off, there is a regeneration from the stalk, not of a new pileus, but of one or more complete fruiting bodies. We see here that a direct restoration of the part that has been lost does not take place, but the new organ is dependent upon the condition of the plant, in that as a result of the wound, the mycelium proceeds immediately to build up a new fruiting body, and does not go through a longer vegetative period.

It is to be remembered that the dependence of the formation of organs upon external conditions is much greater in fungi than in the higher plants. If the sporangium is removed from the fruiting hypha of *Phycomyces* there is developed a new fruiting hypha from the stalk of the old one. But when regeneration takes place on an entirely immersed fruiting hypha the external factors influential in the production of fruiting hyphae are lacking and a vegetative hyphal branch developes. It appears therefore that the above-mentioned relation generally holds good.

Formerly I believed that in certain cases in fungi a direct restoration of wounded fruiting bodies could take plaee. I cut

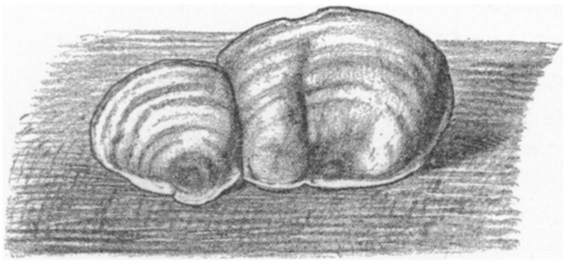

FIG. 3. *Stereum hirsutum*. Fruit-bodies from beneath.

pieces from the fruit of *Stereum hirsutum*, and found after some months, growth being slow in this instance, that the bodies had reassumed somewhat the shape of the fruit before wounding. Closer investigation showed that this was not the case however. The fruit-body exhibits a characteristic zonal formation (*f. 3*). The new formation does not in any way add to the zone formation of the old fruit-bodies but is entirely independent of it (*f. 4*).

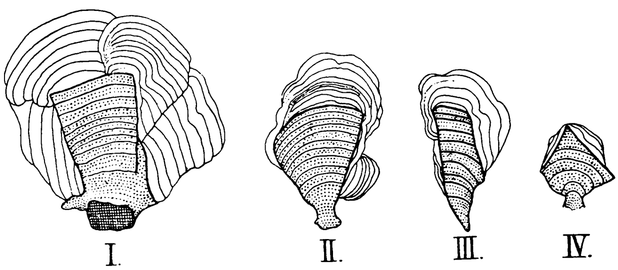

FIG. 4. *Stereum hirsutum*. Regeneration of the fruit-bodies. The old portions are dotted.

They are actually new fruit-bodies, but since they are scarcely individualized they soon grow together with the old fruit and so appear as regenerated parts of the latter. Injured fruit-bodies growing in close proximity often become concrescent and so show that the above conclusion is correct.

Among higher plants *Begonia* is a good example. When parts of leaves are taken from plants of *Begonia Rex* in a flowering condition, these proceed to the formation of flowers much more quickly than those parts taken from plants not in flower (Sachs), and in *Begonia discolor*, which forms bulbils in the autumn, we get leafy shoots as regenerated organs in the spring, but bulbils in the autumn (Wakker).

I have cited other examples of this relationship, which forms an important part of the theory of metamorphosis, in another place.

4. The character of the organs formed in regeneration is dependent to a very slight degree only on outside factors. It is conditioned by the structure of the parts of the plants concerned, particularly by the direction in which the constructive material moves; the wound-stimulus must also be taken into consideration.

Vöchting's well-known investigations have directed the attention of botanists to the polarity apparent in regeneration. New shoots are produced at the tips of old shoots, and roots from the bases. This process is reversed in root-cuttings. Leaves show no polarity in regeneration; the new organs appear at the base. The cause of these differences is the next point to determine. Vöchting held that above all *difference in growth* was the determining factor. Organs which have limited growth show regeneration at the base; those which have unlimited growth show polarity.

I can not agree with this conclusion. The relations described above are the ones which, in my belief, are to be considered most important. In a foliage leaf the constructive materials are in the process of movement toward the stem. When we cut off the leaf, the new organs are in accord with the above idea produced at the base. In shoots and roots we have a double movement: on the one hand toward the vegetative point of the shoot, and on the other toward the vegetative point of the root. This implies the existence of polarity in these instances.

Space does not admit the citation of all of the facts bearing upon this point, but a few examples may be mentioned. If Vöchting's theory were correct, *all* organs of limited growth would necessarily show similar relations. But this is not the case. The leaves of the foliaceous liverworts do not show any tendency to limit regeneration to the bases. This however is easily explainable from my point of view. These leaves are composed of only one or two layers of cells and so have at the time of separation neither a very large amount of constructive materials, nor definite conducting systems to carry them. They form adventitious shoots only when, after being cut off, they have been able to perform the photosynthetic processes for some time. No cause exists for the limitation of regeneration to the base of the leaf. The pieces from the flanks of the thallus of *Fegatella* behave similarly.

On the other hand the leaves of *Bryophyllum* produce, in general, no new structures of their own when cut off, all of the constructive material being used by the vegetative points already in existence in the indentations. The removal of these on a number of leaves caused the formation of roots at the base of the leaves, and in one instance of a bud also. These facts seem to lead to the conclusion that:

1. The vegetative points serve as centers of attraction for the constructive material necessary for the formation of new organs.

2. As long as the leaf remains attached to the stem the materials flow into the stem; when the leaf is detached, the materials are appropriated by the vegetative points of the leaf.

3. The removal of the vegetative points of the leaf is followed by the transfer of the place of origin of new structures to a point down the conducting tracts at the base of the leaf.

The above are the chief conclusions so far attained, but much more research will be necessary before the processes of regeneration are well understood.

MÜNICH, November 15, 1902.

[This paper was written in German; the English translation, which was read at Washington and is here printed, while authorized by Professor Goebel, has not been revised by him.—ED.]