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Bacteriology. — *"On different forms of hereditary variation of microbes"*. By Prof. M. W. BEIJERINCK.

The interesting lecture of Prof. HUGO DE VRIES in the last meeting of the Academy on the origin of new forms in higher plants, induces me to draw attention to some observations regarding the same subject in microbes.

Though the culture of microbes, compared to that of higher plants and animals is subject to many difficulties, it cannot be denied that, these once mastered, microbes are an extremely useful material for the investigation of the laws of heredity and variability. The starting from the single individual, which of course is required here, is commonly almost as simple as for the higher organisms, and it is want of practice only which makes it appear so troublesome. The generations succeed each other quickly; huudreds, nay thousands of individuals can be very easily surveyed in their posterity; far remote classes of the natural system are represented by microbes; in many the variability is great [1]). Even the difficulty of determining the species and varieties, which is frequently only possible by means of biochemical investigation, can become an advantage, for the very reason that biochemical methods of distinction are very accurate, can be extended in various directions and compared by measurement. Thus the species and varieties of lactic-acid ferments are distinguished by titration, alcohol ferments by means of the saccharometer, while different carbohydrates can be selected as the base of lactic-acid and alcohol fermentation. To all this is added the circumstance that it is easy to perform with microbes experiments of competion, which is difficult or impossible with higher plants and animals, and it is well-known how delicate the distinctions are which are thereby revealed.

In comparing the results obtained with microbes to the rules found in higher organisms, account should be kept, first, with the want of sexuality, by which the variation of microbes becomes comparable to the bud-variation of the higher plants, and, second, with the unicellularity of the microbes. As to the first point the experiences with the bud-variants of higher plants seem to prove that an essential difference between bud-variation and seed-variation does not exist. As to the uni-cellularity of the microbes, it is my opinion that by it the phenomena of variation are rendered clearer but are not changed, when

[1]) Compare RODET, De la variabilité dans les microbes, Paris 1894 Bibliography wants in this book und not all data are trustworthy.

compared to the multi-cellular organisms. According to the point of view, the individual microbe can be compared to the whole individual of the higher organism, or to a single tissue-cell of it, — both comparisons are correct [1]).

1. *Degeneration.*

In bacteriological laboratories it is well-know that by prolonged culture many microbes undergo slow, but great changes, even in so much, that certain long continued cultures do not agree any more with the descriptions given of them by the discoverers, short after their first isolation from nature. In some cases the way in which the change takes place can be rather minutely traced; three forms of variability are therein more salient: degeneration, transformation and common variation.

A species is isolated from nature and it is found that at the culture during the first series of inoculations, in which hundreds or thousands of cell-generations succeed each other, it develops well, so that in the beginning the impression is obtained of a thorough knowledge of the nutrition and other conditions of life. But by and by it becomes more difficult to make the new inoculations thrive and at last the culture-material grows troublesome and uninteresting and would be quite unrecognisable if not the various phases of the degeneration-process had been exactly observed. Prolonged cultivation above the optimum temperature of the growth, and a too strong concentration of the nutriment are in some cases the cause of degeneration. In some microaerophilae, for instance the bactery of the "lange-wei" *(Streptococcus hollandiae)* [1]), the irrational regulation of the oxygen tension causes a rapid, in few days complete vanishing of the slimeformation, while after a much longer time, by the same cause, the vegetative power of the bactery completely disappears. In other cases, for instance with a phosphorescent bactery, very common in the sea *(Photobacter degenerans* FISCHER*)*, the degeneration is accomplished without known cause, and in a very short time, so that, within a few weeks the cultures may cease to exist. The degeneration goes not by leaps but continuously and affects all the

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[1]) An interesting view herewith connected is found in WHITMAN, The inadequacy of the cell-theory of development. Biological Lectures at the Wood's Holl Laboratory, 1893, pag. 105, Boston 1894.

[2]) Used in Holland for cheese-making.

individuals in culture equally, so that it cannot be checked by colony-selection.

2. *Transformation.*

At the transformation all the individuals brought in culture lose a characteristic, while either another comes in its place, or a new characteristic arises, or, lastly, the characteristic disappears without a distinct substitute. Thus the cultures of *Photobacter luminosum* grow dark in the course of some months by a slow process of transformation, whereby they change into a more rapidly growing form, which acts more strongly on the nutriment than the normal form. Here, thus increase of vegetative power has supplied the decrease of phosphorescence.

It is remarkable that the transformation in this phosphorescent bactery sometimes suddenly ceases and is replaced by a process of variation where, beside a completely dark form (the variant), the phosphorescent form with the full primitive phosphorescent power again springs up. This is not the same as common atavism, where the stock which throws off the atavist does not change further, but it is probably comparable to the splitting of a bastard into the two components. Very slow cell-partition, caused for instance by culture at a low temperature, furthers this phenomenon. On the other hand, the cause of the transformation may be a too rapid process of cell-partition in which the photoplasm, which seems to grow more slowly than the rest of the protoplasm, remains behind in its development.

In another phosphorescent bactery of the sea, common on our coast, *Ph. hollandiae*, I hitherto only saw transformation, so that this species quickly disappears from the cultures as a phosphorescent bactery.

In a pigment bactery (*Bacillus viridis*) I saw, apparently without any other change, the at first very strong power of liquefying gelatin, by and by get lost in all the individuals.

On the other hand, I have seen in some vibrions, in a corresponding way, from non-gelatin-liquefying individuals come forth liquefying ones.

The new forms, thus called into life give, at superficial examination, quite the impression of new constant species. They cannot, however, be valued as such as they differ only by one or very few characteristics from the mother forms. This is the cause why they must be classified as variants, quite like those of the following case.

3. *Common variation.*

The third and most frequent form of variability is *common variation.* Here the normal form continues unchanged, but now and then throws off individuals, the variants, which, from the beginning, are likewise constant and remain so, but which every now and then again throw off other variants, among which the normal form may occur as an atavist. These variants probably correspond with many well-known so-called varieties or races of culture plants and domestic animals, and likewise, I should think, with the interesting new forms obtained by Prof DE VRIES from *Oenothera lamarckiana* [1]). They remind us in some respects also of the Pleomorphy in the Fungi, which especially in the Ustilaginae, can easily be observed in the laboratories and about which, in particular BREFELD, has made many researches [2]).

The names variant and sub-variant I have chosen, because in the here discussed products of hereditary variation, which differ apparently very much, but in fact only little from the normal form, I think to see the lowest degrees of the natural system following above the individual, and to them are given those names according to the rules of botanic nomenclature [3]).

Regarding the divisions above the species, DE CANDOLLE does not think it necessary to give definitions, in which I quite agree with him. But singularly enough he does try to do it for the ranks beneath the species, where he takes the greater or lesser constancy at sowing as a criterion for the differences. This is not logical, here too, definitions are unnecessary.

Probably various causes give rise to the production of variants. Lengthened growth at insufficient nutrition, and the prolonged action of the own secretion products of the microbes may, with some probability, be considered as such causes.

The variant seems seldom, perhaps never, by one single cell-partition to result from the mother-form, but only after some intermediary partitions, rapidly accomplished. With these latter partitions correspond the sub-variants, with a disposition for atavism or further variation, and only keepable by colony-selection.

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[1]) These Proceedings. Meeting of 29 Sept. 1900 pag. 246. Comptes rendus. T. 131 pag. 124 en 561, 1900.

[2]) Botanische Untersuchungen uber Hefenpilze. Heft 5, 1883.

[3]) A. DE CANDOLLE, Lois de la nomenclature botanique, 2e Ed. pag. 15, 1867, and Nouvelles remarques, pag. 48 and 63, 1883.

I will now describe some instances of common variation; first a few cases of the originating of hereditary-constant variants, which seem unable to return to the stock, then the more complicated case of constant and variable variants, among which some with a great disposition for atavism, which case I have nearer investigated in the West-Indian phosphorescent bactery and its relatives.

I might augment these instances with many more, for most of the microbes with which I occupied myself for a length of time, produced in my cultures more or less hereditary-constant variants. Extremely variable are the mycelia of the Fungi, for which I refer to the complicated relations of the aethyl acetate-yeast, which I described and demonstrated in 1895 [1]), and where transformation and common variation both occur.

4. *Variation in Schizosaccharomyces octosporus* [2]).

This curious maltose-yeast I detected in 1893 on dried orient-fruits as currants, dates, raisins, and figs. I found a good method to separate this species from the other microbes, by which it is possible as often as desired to bring it from nature into culture. It proved to be a generally spread organism, which is found in Greece, Turkey, Italy, Asia Minor and Java in one and the same variety. After many isolations I found in 1897 [3]) a new variety on dates from N.-Africa. The culture is effected in the like way as of beer-yeast on wort-gelatin. Maltose, like glucose and levulose, undergoes a vigorous alcoholic fermentation, cane-sugar not at all.

As well the usual form as the new variety produce 8-spored spoangia, the spores of which colour intensely blue with iodium. During the growing a small quantity of diastase is secreted. The vegetative condition which precedes the spore-formation, as also the vegetative variant, which produces no more spores, of which more below, multiply by partition (not as in other yeasts by budding) and colour yellow by iodium; glycogene wants completely in it. Accordingly it is possible, by treating a culture with iodium, from thousands of colonies, instantly to recognise those containing spores, and from the intensity of the blue colouring with some certainty to make out

1) Handelingen van het 5e Natuur- en Geneesk. Congres te Amsterdam pag. 301, 1895.

2) Centralblatt fur Bacteriologie Bd. 16 pag. 49, 1894 and Ibid. Abth. 2. Bd. 3 pag. 449, 1897. In 1897 I put the variant on a level with a "vegetative race", but as I now think, in doing so I rated its systematic value too high.

3) Together with a new quite different species of *Schizosaccharomyces.*

the number of spores present. The variety of dates differs from the main form by the sporangia of the latter being ellipsoidal and thickest in the middle, while in the variety, on the other hand, they are just in the middle constringed, and moreover by several other little salient characteristics, which only become discernible by practice.

Both, main form as well as variety produce, as the cultures grow older, a variant so much deviating from the normal forms, that, if these variants were met with in nature, they would certainly be proclaimed a new species if no new genus. The cells are globular, and not as in the normal form elongated, but the multiplication is here also exclusively effected by partition. Spores are not at all formed.

This variant springs, so far as I have been able to find out till now, at once form the normal form, which for the rest propagates unchanged, and can constantly anew throw off the variant. The first variants are found in cultures which have continued growing a few weeks without re-inoculating, and they go on some time multiplying on the nearly exhausted culture medium, after the normal form does no more do so. This points to a gain of vegetative power, at least in the conditions that prevail in the old culture-medium, but in new nutriment I could observe nothing of this difference.

The variant after repeated re-inoculation, at present already during more than three years and consequently after thousands of cell-generations, has remained perfectly constant; never could even a single sporangium be found, which, with the help of the iodium reaction, can be seen at a glance in the microscopic preparation. Whether in the variant the faculty of forming spores continues latent is possible, even probable, but not proved.

In the variety, isolated from dates, also occur sub-variants, that is intermediary forms between normal form and variant, while in that of currants I have found no sub-variants. The sub-variants still produce some sporangia, mostly 8-spored. Without much trouble I could isolate from a thousand colonies three sub-variants, belonging to two types; both types proved at re-inoculation to be constant, but growing older they throw off, in the habitual way, the asporgene variant, so that, in order to be preserved, they must be propagated from the spores. This can be done by pasteurising the sowing material at 55° C., by which the vegetative cells die and the spores alone survive.

In continuing this manipulation I have obtained new sub-variants. One of these produces 4- or 8-spored globular sporangia and is at

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first sight a new species. Cells and sporangia remind of the vegetative variant which should have regained the power of producing spores. But all the characteristics are limited between those of the normal form and the asporogene variant. So that, although this form too is hereditary-constant, I cannot see a new variety in it, but only a new variant.

It is noteworthy in this case, that the variants of the same generation, that is those which result from the same sowing, always differ by distinct breaks in the tint of the iodium reaction, and form no flowing series between main form and main variant. But I think this to be the consequence of the limited number of colonies which can be overseen at each experiment, and amount to no more than one or two thousand, and that it will be possible to fill up the gaps with sub-variants from other cultures, which perhaps grow rarer as the leaps are smaller. The question why sub-variants are so much rarer than main variants, I cannot as yet fully answer, but the existence of sub-variants proves that the great and sudden leaps, observed in the variability everywhere in the vegetable and animal kingdoms, are no necessary attribute of variability. Furthermore these sub-variants prove that even slight deviations may be in high degree hereditary-constant [1]).

5. *Variation in Bacillus prodigiosus.*

This well-known red pigment-bactery is cultured by me in three distinct natural varieties. One of them does not liquefy the the culture-gelatin [2]), of the two others which do, one [3]) has the power of causing various carbon-hydrates to ferment under production of hydrogen, the other not [4]). All three produce, in older cultures, a variant which is completely colourless, but in all other respects possesses the properties of the normal form whence it has taken birth, so that there are non-liquefying and fermenting, and liquefying non-fermenting colourless variants. All these variants have remained hereditary-constant in my experiments and produce no

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1) For the more complicated phenomena of variation in some species of *Saccharomyces*, I refer to my paper "Sur la régénération des spores chez les levûres etc., Archives Néerlandaises, Sér 2, T. 2 pag. 269, 1899.

2) Isolated from potatoes grown hollow in the soil and given me by Prof. RITZEMA BOS.

3) Isolated from tubercles of red clover.

4) Isolated from bones kept at the open air on the bone-hill of the gelatin- and glue-factory at Delft.

atavists like to the mother form, i. e. red-coloured colonies. There is no doubt but, if these variants were met with in nature, not accompanied by the normal form from which they arise, they would be taken for as many new species. Still it would be an error to admit them as species into the system, as a more minute investigation shows that, except in the power of forming pigment, they correspond in all other respects with the normal forms, and one single point of difference determines only a variant.

I doubt by no means that *B. prodigiosus* can also vary in other directions; this follows already from the fact that I could find three very different natural varieties, which all produce red pigment [1]). But I have not taken pains to trace other variations.

Sub-variants between the normal forms and the said colourless variants are, or at least seem rarer than the main variants. They are rose-coloured and at colony-selection almost as constant as the normal form. They also produce like the latter the constant colourless main-variant, and moreover show a propensity for atavism. In each natural variety I have found only one or two rose-coloured sub-variants.

6. *Variation in Photobacter indicum.*

This phosphorescent bactery was isolated by Prof. FISCHER of Kiel, from seawater in the vicinity of the isle of Santa Cruz, one of the Antillies, on January 10, 1886. I received material of it in May 1887 and have without interruption cultured it till now. Already in 1887 I perceived, that with the growing older of the cultures, two main variants arise and even in so great a number that the normal form can be supplanted by them for the greater part, though not quite. One of these is either completely or almost completely dark, the other grows much more slowly than the normal form and is almost motionless, while the normal form is extremely motile. I will call these variants *Ph. indicum* vnt. *obscurum* and *Ph. indicum* vnt. *parvum*. Later I found some more variants which are less common. There are besides sub-variants of which I have examined those standing between the normal form and *obscurum*; they produce now and then atavists, and vary also towards *obscurum*, but can be kept constant by colony-selection.

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[1]) The red pigment of *B. prodigiosus* is a product of excretion found between the living and partly accumulated in dead bacteria. It is in my opinion the product of specific chromoplasm, which forms a small part of the protoplasm in general.

Notwithstanding this great variability it has been possible, likewise by means of colony-selection, during the more than 13 years continued laboratory culture, to keep up the stock unchanged, which is remarkable, when thinking of the place where it was first found.

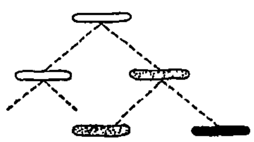
The variants and sub-variants always spring from the stock in the same way. They may be reduced to two types: variable and unvariable. All phosphorescent variants are more or less variable.

The variant *parvum* shows an extreme disposition for atavism, so that already after its first re-inoculation on a new culture-medium, various normal forms spring from it.

The *obscurum*-variants are more constant They are either perfectly constant, so that, as it seems, phosphorescent forms never again arise from them, or imperfectly, so that after going through a few cell-partitions, answering to as many sub-variants, the normal form returns with the full phosphorescent power. Dark variants, in this way producing luminous cultures, prove that progressive variability [1]) also occurs in the laboratory cultures

The variant is not the product of a single heterogene cell-partition, but of the passing through some preparatory cell-partitions, answering to as many sub-variants. I was able without difficulty to distinguish two of these leaps or sub-variants, but it is possible that there are more, too slightly differing for my observation. It is also probable that by the conditions of culture, these preparatory cell-partitions, and with them the sub-variants, existing between the normal form and the main variant, will grow more or less numerous.

Fig. 1.



Probable course of development of the dark variant by direct heterogene cell-partition or evolution. The first partition produces from the single luminous bacillus one of the same, and another of lessened luminosity. The second partition produces from the latter again one of the same luminosity, and another quite-dark.

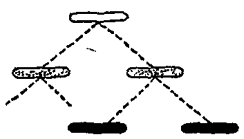
The *obscurum*-variant is probably produced in accordance with the scheme of Fig. 1.

For the sake of simplicity here is only figured one intermediary stadium (sub-variant) by the dotted rod; the dark main variant is drawn black, the normal form white. This scheme answers to what may be called the development of the cell-variant by heterogene cell-partition or evolution.

Less probable is the development of the variant by transformation or epigenesis represented in Fig. 2.

[1]) Distinction can be made between: — retrograding or analytic variability, in which a characteristic disappears entirely or partly, — replacing variability, in which a characteristic is wholly or partly supplanted by another, — and progressive or synthetic variability, in which a new characteristic is added to those already existing.

Fig. 2.

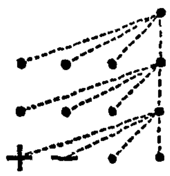


Less probable course of development of variant by indirect heterogene cell-partition or epigenesis.

The preparatory cell-partitions at the atavism of the luminous normal form from the dark or feebly luminous sub-variants still liable to retrogression, probably answer likewise to the schema of Fig. 1.

If the normal form is indicated by •, the *obscurum*-variant by —, and the *parvum*-variant by +, unreckoned the sub-variants, the pedigree of the normal form of *Ph. indicum* can be represented by Fig. 3, which means, that at the two first re-inoculations only the normal form is produced, and that at the third likewise *obscurum*- and *parvum*-variants have orginated, but from cells which were subjected to particular conditions. The numbers 2 and 3 for the generations are chosen arbitrarily, for the number of generations, after which the variation occurs, can be regulated at will, for by early re-inoculating the young cultures on fish-broth-agar (not on fish-broth-gelatin) the variation can be kept back for a long time [1].

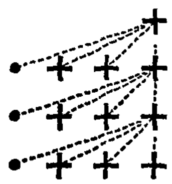
Fig. 3.



Pedigree of *Ph. indicum*, normal form.

For the variant *parvum* the pedigree becomes somewhat different from that of the normal form, there being much atavism (Fig. 4).

Fig. 4.



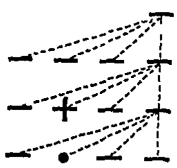
Pedigree of *parvum*.

The pedigree of *obscurum* can, as to the constant form, be represented by a single mark.

The variable, *obscurum*, again produces atavists, but less than *parvum*, and besides *parvum*-variants (Fig. 5).

In these three last schemes are, as said, the sub variants left out.

Fig. 5.



Pedigree of variable *obscurum*.

I have not succeeded from *Ph. indicum* to obtain a perfectly constant luminous form, that is, one which produces no variants, though I have tried for years to do this by selection. It is evident that the conditions of culture inavoidably give cause to the rise of variants. That for the rest the faculty of varying in a very determined way, is deeply rooted in the nature of the cell, is proved by the following observations.

A few years ago Mr. FISCHER at Kiel again sent me some material of *Ph. indicum*, which had thus during many years been cultiv-

[1] To these relations I hope to refer at another occasion.

ated in his laboratory. There was a considerable difference, compared to my stock, but the normal form and the two variants *obscurum* and *parvum*, I could still obtain from it as constant forms by means of colony-selection.

At the examination of numerous samples of seawater in all the seasons, taken near Scheveningen, Bergen op Zoom and den Helder, partly far from the coast [1]), I have never found *Ph. indicum* itself, but, even three times, forms which, with a broad conception of the species, might be considered as varieties of it, and else as very closely allied species. I call them *Ph. splendidum* and *Ph. splendor maris.* Short after the isolation already they produced variants, one of which is quite dark and multiplies in such a number that in cultures which are negligently re-inoculated the normal form, and with it the photogenic power, wholly disappear.

Thus, a culture at 22° C. of *splendor maris*, going out from a single phosphorescent colony, after being in 12 days six times re-inoculated on fish-broth-gelatin, produced 1800 dark variants on 22 colonies of the normal form. The culture re-inoculated six times in the same space of time on fish-agar did not yet contain any variants, whilst at the 12th re-inoculation on agar their number was also very great. The first not further re-inoculated cultures on gelatin, which accordingly had only had little opportunity to grow, after 12 days did not contain variants, in accordance with the rule that at eessation of growth no variability is manifested.

The *parvum*-variant also is in *Ph. splendidum* and *Ph. splendor maris* as distinctly recognisable as in *Ph. indicum* itself, and here too, frequently produces the primitive forms as atavists.

Basing on these experiences I think it probable, that the cause which calls forth the variants is not exclusively active in our artificial cultures, but can also be active in the sea itself, so that in this case there is a chance that dark forms, isolated from the sea will at first be taken for particular species, but after more minute observation, will prove to be variants of known phosphorescent bacteria.

By observing certain general conditions the production of dark variants can, as said, be greatly slackened, but not wholly prevented.

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[1]) Many of these samples I owe to the kindness of Dr. HOEK. Various species of luminous bacteria have been found in them to the amount of 0.1 to 5, even 7 pCt., of all present bacteria. Especially *Ph. luminosum*, and a species difficult to distinguish from it, but still quite different, *Ph. hollandiae*, occur very often. *Ph. degenerans* also is frequent.

Among these are: strong nutrition and vigorous growth a little below the optimum temperature, free access of oxygen, such as can be attained in cultures on agar-agar, and total exclusion of the influence exerted by the secretion-products of the bacteria themselves, which is attainable by re-inoculating the young cultures very often on a new medium.

7. *Conclusion.*

I will begin with pointing to the fact that hereditary variability is a function of growth, in particular of slackened growth, but that at cessation of growth no change takes place. And furthermore that variability attacks only one independent characteristic at a time. In the sub-variant one characteristic of the normal form is partly, in the main-variant it is wholly changed. In new varieties and species more characteristics are varied.

Furthermore resuming the above given statements I come to the following conclusions. The here discussed forms of hereditary variability belong to three types: At *degeneration* all individuals, by a slow process of variability, lose their vegetative power, so that the species may cease to exist. At *transformation*, which seems to appear more seldom, all individuals lose a specific characteristic and acquire either or not another instead. At the common hereditary variability or *variation*, the normal form, probably by heterogene cell-partition, throws off some individuals, the variants, mostly differing from it by a strongly salient characteristic. The normal form itself propagates beside it quite unchanged. The variants are constant in a way corresponding with independent species; sometimes this constancy is perfect, in other cases atavists are produced, like to the normal form. Subvariants i. e. intermediate forms between normal form and variant, are less *found* than the variants themselves, but they are perhaps never wanting, and are in the same way constant as the normal forms. Whether the sub-variants are also originally *formed* in smaller number than the main variants is uncertain; what is seen is that they rapidly disappear from the cultures and are supplanted by the normal form and main variants if they are not fixed by colony-selection. Besides, each well-defined degree of variation, however slight, seems to be fixable.

The rare occurrence of the sub-variants throws some light, *First*, (by the comparison of the individual microbes with the individuals of the higher organisms) on the marked distinctness by which in higher plants and animals most varieties and species are separated, —

for they originate by repeated variation processes, relative to different characteristics- and the chance that the common and distinctly discernable variants will partake therein and not the rare sub-variants, more difficult to distinguish, is accordingly greatest [1]).

Second, (by the comparison of the individual microbes with the tissue-cells of the higher organisms) on the no less marked confines between the tissues and the organs of one and the same individual, — for these are constituted of as many cell-variants of the embryonal cells, cell-variants, which will supplant the cell-sub-variants.

That many so-called new species will prove only variants of other species and no "good species", is not improbable. Especially in the microbes, where the want of crossing must strongly favour the prolonged continuing of the once formed variants, it is to be foreseen that in nature will often be found variants, which will long maintain themselves at their habitat. If they are isolated, the discoverer will at first be almost sure to see new species in them, and only after an accurate investigation recognise them as variants of another species.

The sub-variants of the microbes prove, that the characteristics which in the main variants are quite wanting disappear by little leaps from the normal forms. In other cases, however, the main variants seem to appear suddenly, whence it would follow, that a characteristic can also vanish at a single leap at the cell-partition; but here the sub-variants may have escaped from observation.

The variants of the microbes, regarded as cell-variants, prove that out of a cell daughter-cells may spring unlike to the mother-cell. Though the way in which this is effected is still insufficiently known, it proves the existence of heterogene cell-formation, whether by direct heterogene cell-partition (fig. 1), or, by the less probable transformation (fig. 2).

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[1]) I perfectly agree with Professor DE VRIES, that the origin of species should often be sought in the almost suddenly produced variants, or mutants, as he calls them. This is also the conclusion to which GALTON has come regarding the races, and to which he referred repeatedly since 1892, the last time, so far as I know, in Nature T. 58, pag. 247, 1898 in these words: "I have frequently insisted that these sports or "aberrances" (if I may coin the word) are notable factors in the evolution of races. Certainly the successive improvements of breeds of domestic animals generally, as in those of horses in particular, usually make fresh starts from decided sports or aberrances and are by no means always developed slowly through the accumulation of minute and favourable variations during a long succession of generations." Along quite distinct ways GALTON, DE VRIES and myself have thus arrived at the same conclusion regarding the probable origin of many races and species. But the great difficulty which lies in the explanation of the adaptions, has not been removed, neither by GALTON's "aberrants", DE VRIES' »mutants", nor my "variants".

In order to show how decidedly heterogene cell-formation is still considered as impossible, so that it is not superfluous to afford a new evidence for its existence, I refer to the well-known book of O. HERTWIG "Die Zelle und die Gewebe", p. 64 Bd. 2, Ed. 1898, where we read as follows: „Die Theorie der heterogenen Zeugung, wo sie aufgestellt wurde, ist als grober Irthum bald beseitigt worden. So gilt als ein allgemeines Grundgesetz in der Biologie der Ausspruch „Gleiches erzeugt nur Gleiches" oder besser „Art erzeugt stets seine Art" Bei allen einzelligen Lebewesen ist erbgleiche Theilung ihres Zellenorganismus die einzige die vorkommt und vorkommen kann. Auf ihr beruht die Constanz der Art. Wenn es möglich wäre, dass bei irgend einem einzelligen Organismus die Erbmasse (Idioplasma) durch Theilung in zwei ungleiche Componenten zerlegt und auf die Tochterzellen ungleich übertragen werden könnte, dann hätten wir den Fall einer heterogenen Zeugung, den Fall der Entstehung zweier neuer Arten aus einer Art. Wie indessen alle Beobachtungen lehren, werden auch bei den Einzelligen die Arteigenschaften so streng und bis ins Kleinste überliefert, dass einzellige Pilze, Algen, Infusoriën auch noch im millionsten Gliede, ihren weit entfernten Vorfahren genau gleichen. Der Theilungsprocess als solcher erscheint daher auch bei den einzelligen Organismen nie und nirgends als Mittel um neue Arten ins Leben zu rufen."

The preceding pages prove that this view is erroneous, so that the far reaching conclusions, drawn from it in relation to ontogeny vanish at the same time.

So far there is thus no reason in contradiction with observation, which forbids admitting, that the ontogeny of the higher organisms consists in a regular course of variation processes, and that full-grown plants and animals are built up of as many cell-variants of the embryonal cells, as they contain different tissues composed of identic cells.

Botanics. -- "*On the development of Buds and Bud-variations in Cytisus adami*". By Prof. M. W. BEIJERINCK.

Cytisus adami is a hybrid between the common laburnum, *Cytisus laburnum*, and a little shrub from Styria, *Cytisus purpureus*, with purple flowers. Now and then are found on *Cytisus adami* buds