

of an induced circuit wave of contraction the difference in the amounts of CO<sub>2</sub> produced is somewhat smaller than in the former experiment although the rate of pulsation in the activated half-disk is on the average 3.143 times as great as that of a half-disk pulsating under the control of its sense organs. It seems to be clearly established from this type of experiment that there is some other form of metabolic activity which is of greater importance as a source of CO<sub>2</sub> and which is more directly under the influence of the rhopalia than is the activity of the muscular system.

In the comparison of normally pulsating half-disks and those activated by a circuit wave of contraction a series of cuts, equal in extent to those used in forming the labyrinth in activated specimens, were made in the tissues of the half disks with sense organs in order to guard against any inaccuracy in the results due to inequality in the extent of the laceration of the tissues. The activity of the muscular tissue—rate of pulsation—was 3.143 times as great in the half-disk containing the circuit wave of contraction as in the normally contracting specimen, but after various periods of from 5 to 15 hours, when the titrations were made it was found that in every experiment except one the greater amount of CO<sub>2</sub> had been produced by the half disk on which the sense-organs remained. In the single experiment which proved an exception to the regular result the excess of CO<sub>2</sub> in favor of the activated specimen were very slight in comparison to the difference in rate of pulsation which for this specimen was 28 per minute for the half-disk with rhopalia and 118 per minute for the activated half-disk.

## HERITABLE VARIATIONS AND THE RESULTS OF SELECTION IN THE FISSION RATE OF *STYLONYCHIA PUSTULATA*

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In organisms in which an admixture of two parents occurs at reproduction, the problems of evolutionary change become most complex and difficult. Heritable changes appear abundantly, but most of these are shown to be the definite working out of the numerical rules of inheritance. Whether any of the heritable changes that occur are of a different character is in dispute. In reproduction from a single parent these difficulties disappear; if evolutionary changes occur independent of biparental admixture it should be possible to demonstrate this in uniparental reproduction. Yet most recent work agrees that in such uni-

parental reproduction inherited variations occur rarely or not at all, and that selection has no effect in altering racial characteristics.

Most investigators, following Johannsen ('03, '09, '11), have found that 'pure lines' or 'clones' are hereditarily constant under selection. Johannsen's results were obtained with self-fertilized lines of beans. Similar ineffectiveness of selection has been found by Hanel ('08) and Lashley ('15) as to the number of tentacles in Hydra multiplying by budding; by Jennings ('08, '09, '10) for size in infusoria; by Barber ('07) (in the main), and by Winslow and Walker ('09), in bacteria; by East ('10) in the vegetative reproduction of the potato; by Agar ('13 and '14) in Cladocera and aphids multiplying parthenogenetically; and by various other investigators on diverse organisms. Some discordant results have been recorded, but most of these are ill-defined or uncertain; it is mainly in bacteria, with their immense difficulties for precise technique in pedigree work, that heritable variations or modifications have been described. The preponderance of evidence has been that in uniparental reproduction heritable variations do not occur (save as rare mutations of marked character), and that selection of slight individual variations is without effect in altering the hereditary characteristics.

These results have given origin to the concept of the Genotype (Johannsen), as a designation for the permanent heritable constitution of the race. The present paper deals with the inheritance of variations and the effect of selection in the case of a most delicately poised and readily modifiable physiological character, the rate of fission of an Infusorian multiplying without conjugation.

The questions here raised are: Can we, with respect to the character examined, get from a single genotype by selection two genotypes that differ characteristically from each other under identical conditions; and that retain these differences from generation to generation? Is selection of small variations such as appear within the pure strain or clone an effective evolutionary procedure?

For one hundred and thirty days two halves of a single clone (or set of individuals derived from the fission of a single parent) of *Stylonychia pustulata* were subjected to selection in opposite directions; this produced a marked and steadily increasing difference in the average fission rate of the two halves. Expressing the excess of generations produced by the fast-selected lines as a percentage of the total number of generations produced by both sets, the difference was 6.9% for the first thirty days; 12.8% for the next twenty days; 19.3% for the next thirty days, and 21.2% for the last fifty days. The records also show that for the fast lines the number of generations produced per line during the one

hundred and thirty days ranges from 178 to 187, while for the slow lines the range is but from 116 to 128. The slowest fast-selected line produced 50 more generations than the fastest slow-selected line.

To determine whether the difference in fission rate thus produced is heritable, parts of the two sets were removed at intervals and subjected to culture without selection (by 'balanced selection,' in which unavoidable selections in one direction were always compensated by equal numbers of selections in the other direction). By long continued culture without selection, it was found that the difference was heritable. For example, after the two halves of the clone had been subjected to continuous opposite selection for 80 days it was found that the average difference per line per day had increased from 0.267 generation for the first thirty days to 0.415 generation for the last thirty days of that period. To test the permanence of this result these two sets of thirty lines were now subjected to ninety days of balanced selection, or ten days longer than the lines had been subjected to opposite selection. It was found that the average difference per line per day in favor of the progeny of the fast-selected set was for the three consecutive thirty-day periods of this experiment: 0.213, 0.256 and 0.284 generation.

Also, representatives of the two sets after 80 days of selection and 40 days of no selection ('balanced selection'), were subjected to mass culture for twelve days. Further balanced selection of these for fifty days showed that the inherited difference of fission rate still persisted. Thus the inherited difference produced by 80 days of selection had lasted for 102 days without selection.

Experiments with reversed selection showed that the inherited difference could be reversed in the same way that it is produced; the originally fast set was thus caused to become the slower one, and vice versa. Continuation of these two sets without selection showed again that the difference so produced was heritable.

Thus in this case the selection of small individual variations in fission rate has split the single clone (derived vegetatively from a single parent) into two heritably diverse divisions and the effect of selection was cumulative.

This experiment was now twice repeated. The first repetition was with the progeny of a single individual taken from one of the fast lines of the first set of experiments. Here again selection produced from the progeny of a single individual two sets differing hereditarily in average fission rate. Again, a new wild individual was obtained from a new mass culture. From this, two sets of thirty individuals each, all belong-

ing to the seventh filial generation, were obtained; and subjected, in the manner previously described, one to 'fast' the other to 'slow' selection for thirty days. The excess of generations produced by the fast-selected lines, expressed as a percentage of the total number of generations produced by both sets, was 1.99% for the first ten days; 4.36% for the second ten days, and 7.10% for the third ten days; thus showing a gradual increase and indicating a cumulative effect of selection.

To test the permanence of this result these two sets of lines were now subjected to balanced selection for 21 days. On every day but one the lines that had been subjected to fast-selection averaged higher than the others, and the percentage that the difference in favor of the fast set is of the total number of generations produced by both together was practically constant; it was 3.97% for the first ten days and 5.01% for the last eleven days. The results are thus the same as in our first set of experiments.

It was found that if conjugation occurred in the fast set, and likewise in the slow set, the difference produced by selection continued to exist after conjugation.

This third series of experiments has entirely corroborated the results of the first and second series. In a second clone, unrelated to that used for the first and second series of experiments, opposite selection for thirty days produced a heritable difference of average fission rate, a difference that gradually increased as selection progressed, indicating again that the effect of selection on this physiological character is cumulative. This average difference persisted through twenty-one days of balanced selection, twenty-nine days of mass culture followed by conjugation, and then fifteen days of further balanced selection.

All the experiments thus give concordant results; through selection of individual differences in fission rate it is possible to divide a clone into two divisions differing hereditarily in rate of multiplication. The effects of selection are cumulative; the hereditary differences between the two divisions become greater the longer selection continues. By reversing the direction of selection the hereditary differences between the sets are reversed. The hereditary differences between the sets persist through the ordeal of conjugation.

Hence, in reference to the physiological character studied, the selection of small individual variations such as appear within the pure strain or clone is an effective evolutionary procedure.

How are we to account for the difference between the results here set forth and those of older investigators? In *Stylonychia* we are dealing with an organism which is large enough to be easily handled and

followed individually, so that no question can arise as to the purity of the pedigrees (as sometimes occurs with reference to Bacteria). In this organism the facts as to the cumulative effects of selection are clear.

We are of course dealing with a delicate physiological characteristic, and this is perhaps more readily varied (even hereditarily) than the characters examined by most other investigators. Further, it is perhaps true that hereditary changes are more easily brought about in the Protozoa than in the more complex organisms, for in Protozoa the 'apparatus of heredity' is in close chemical contact with all the somatoplasm.

But a certain feature of the experimental procedure in the present case may have more importance than these conjectural considerations. It has been possible in my work to make a much greater number of actual selections (where plus and minus cases were both present to choose from), than in most of the work that has given negative results. And it has been found that a few selections give very slight results, and that a great number are required to give any marked differences between the sets. Thus, in my main experiment, on the average 39.86 plus selections were made in the fast-selected lines; 34.36 minus selections in the slow-selected lines. The difference between the two sets was thus the equivalent of some 74 selections extending through an average of 150 generations. This resulted in the production of a constant average difference per line of 0.42 of one fission per day.

Contrast with this great number of selections the *six* made by Johanssen in obtaining his negative results with beans, the three or four made by East with potatoes, the two made by Winslow and Walker with bacteria, and similar small numbers made by most other investigators along these lines; even indeed the selection through fifteen generations made by Agar, in Cladocera. It appears not at all inconceivable that in these organisms an equal number of selections, covering as great a number of generations, as were made in *Stylonychia*, would have given similar heritable effects. What all the work shows (and here my own is not in positive disagreement) is that heritable variations of considerable extent do not occur so frequently as was at one time supposed, so that a few selections are not sufficient for establishing a definite positive effect. But negative results from a few selections are not sufficient for disproving the occurrence of heritable small variations which may be gradually accumulated. This indeed has been admitted by many of those who have obtained negative results.

As a result of this work upon *Stylonychia* it is possible to give pre-

cise data as to the occurrence of heritable variations and their accumulation through selection, when sufficiently long continued. And this can hardly fail to have influence on the conception of the hereditary constitution or *genotype* as a fixed thing, changing only discontinuously by marked steps or mutations, that do not intergrade.

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## HEREDITARY ANCHYLOSIS OF THE PROXIMAL PHALAN- GEAL JOINTS (SYMPHALANGISM)

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There are many recognized forms of congenital malformations of the hands and feet. Walker in 1901 first described the type of deformity which is made the subject of this study, and showed that the condition had been transmitted through five generations, though the number of his recorded cases was too small to justify a definite conclusion on a Mendelian basis. Farabee in 1905, and Drinkwater in 1908, showed that another type of deformity of the hands, known as brachydactylism, was a dominant unit-character, transmitted in accordance with the Mendelian law.

The lesion in the condition under discussion consists of a congenital ankylosis, due apparently to the failure of formation of the joint between