

A LAMARCKIAN EXPERIMENT INVOLVING A HUNDRED GENERATIONS WITH NEGATIVE RESULTS

BY W. E. AGAR.

Professor of Zoology, the University of Melbourne.

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(With Two Text-figures.)

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INTRODUCTION.

THE present state of the controversy over the problem of Lamarckian inheritance, or the inheritance of acquired characters, to use the misleading phrase sanctioned by recognised usage, is certainly a reproach to biology, if only because of the extremely uncritical use so often made of evidence from direct experiment or indirect indications.

If we review the experiments cited in evidence by those biologists who believe in the reality of Lamarckian inheritance, we find that it consists almost entirely of cases where the altered environment is said to have produced a pronounced and inheritable effect in a single generation. Now it seems certain that if Lamarckian inheritance is a general feature of organisms, it can only, as a rule, be brought about by gradual action through many generations. It is a matter of common experience both in everyday life and in genetic experiment that animals do not ordinarily inherit their parents' acquirements. Positive evidence in the same direction, which seems not to have attracted the attention it deserves, is the lack of inheritance of the somatic differences existing between members of an asexually produced population of Metazoa, or of a pure line, as shown both by the inefficacy of selection, and by the characteristic absence of a significant coefficient of correlation between parent and offspring in such population (*e.g.* Agar, 1914).

Hence biologists who accept Lamarckian inheritance, although they may quote Brown-Séguard or Kammerer as evidence, must envisage the process as normally taking place much more gradually, perceptible results only appearing after application of the stimulus through many generations. This is specially true in the case of those who hold that inheritance and memory are fundamentally of the same nature, for repetition is the essence of habit formation, or of all but the highest types of learning.

If, however, we turn to the few experiments where the same stimulus has been applied over a succession of generations, we find scarcely any support for the Lamarckian view until we come to the quite recent—and indeed still unfinished—experiment of McDougall (1930) on the inheritance of training in rats. While past experience of apparently successful Lamarckian experiments inclines to caution, it is certainly difficult at present to suggest any plausible explanation of the results of this experiment other than the Lamarckian one. Thus it becomes important to extend this type of experiment, which deals with reactions of a definitely vital nature, repeated over a long series of generations, as in the experiment about to be described.

THE NATURE OF THE EXPERIMENT.

The reactions of organisms differ in respect to the degree of their vital character. Psychological reactions, such as employed by McDougall, undoubtedly exhibit this quality in its most developed form. At the other end of the scale a difference in size, or shape, induced by change of temperature or external conditions of development may be a physico-chemical reaction only, not concerning the living organism as such. A very typical vital reaction is the restitution of a lost part, and the reaction finally chosen was the regeneration of the dorsal branch of the biramous second antenna of *Simocephalus gibbosus* and *Daphnia carinata*.

When this branch of the antenna is amputated, a new one is formed, but this is very imperfect; moreover, the amount of regeneration is very variable, so there is plenty of opportunity for "improvement with practice." The problem therefore was: If these animals are made to regenerate an antenna for a series of generations, will the regeneration become more perfect or alter in any way? Or, failing that, will the normal growth of the antenna be affected?

Simocephalus and *Daphnia* present many advantages for an experiment of this sort.

Firstly, their parthenogenetic mode of reproduction greatly simplifies matters.

Secondly, their life cycle is short, and therefore many generations, that is to say, many repetitions of the reaction, can be obtained in a short time.

Thirdly, and most important of all, the general features of inheritance in these animals in relation both to their parthenogenetic and sexual modes of reproduction have been studied by the author, Banta (1921), and others, and we know that, with the exception of rare mutations, all members of a clone (descended by parthenogenesis from a single ancestor) are genetically identical. It is not necessary to emphasise the enormous advantage of being able to exclude the chances of Mendelian segregation from the possible causes of differences between the experimental animals and

the controls, and thus avoid a suspicion which has greatly reduced the evidential value of many Lamarckian experiments.

While this work was conceived purely as an experiment in Lamarckian inheritance, it was found desirable, as it proceeded, to investigate several matters concerned with the process of regeneration and the factors influencing it. The results of this have been embodied in a separate paper (Agar 1930) to which the reader is referred for details.

The standard culture method adopted throughout these experiments was to rear each animal separately in a cylindrical glass phial containing 50 c.c. of culture medium. This amount was actually measured when similarity of condition was essential (as in the test experiments), but judged by the eye, when this was not so relevant.

Details of the culture medium used, and also of the life cycle, growth, ecdyses, etc., are given in my other paper.

THE STRUCTURE OF THE ANTENNA, AND GENERAL FEATURES OF THE REGENERATION.

The operation consisted in the amputation of the dorsal branch of the second antenna. To understand the course of the regeneration it is necessary to know something of the structure of the antenna (Fig. 1).

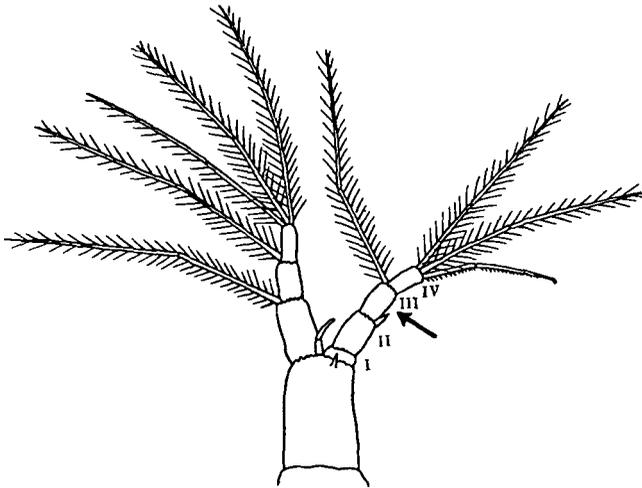


Fig. 1. Second antenna of *Simocephalus*. The arrow shows the level of amputation. In *Daphnia* the amputation was performed through segment II.

In both genera the antenna consists of a basal segment carrying two branches. The ventral branch consists of three segments and five setae, the dorsal branch has four segments and four setae.

Each seta is jointed near the middle into two segments, and is thickly covered with very fine hairs, which are longer in *Daphnia* than in *Simocephalus*. In the latter,

the most dorsally situated of the terminal setae of the dorsal branch differs from all the others in that the hairs are replaced by a fine comb, and the tip of the seta is sharply recurved to form a minute hook, by which the animal suspends itself from solid objects in its characteristic fashion.

The antenna contains bundles of muscles which run up into both branches, and give off a twig to each seta. The latter can lie flat against the axis of the antenna, with the point of the seta directed towards the apex of the antenna, or they can stand out at right angles to it. Observation of muscular twitches in freshly amputated and still living antennae show that these changes in the position of the setae are produced by muscles inserted into them.

The setae can bend at the joint in the middle, but again only through one right angle.

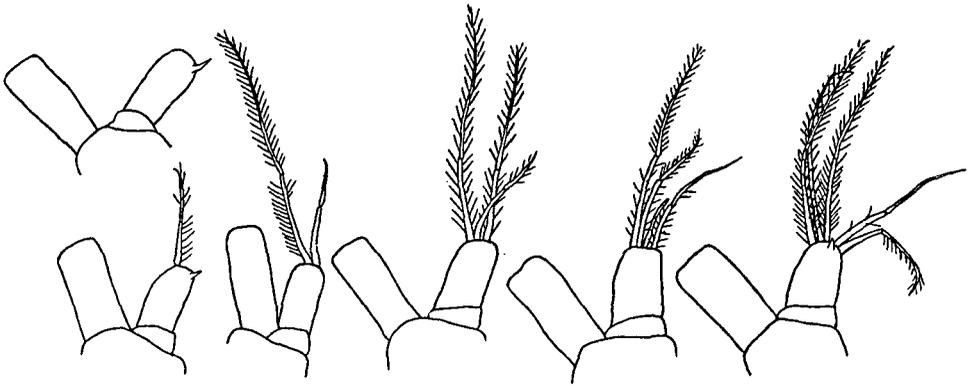


Fig. 2. Examples of regenerated antennae in *Simocephalus*, taken at the first adult instar. The number of setae regenerated in these examples varies from 0 to 5. The basal joint of the ventral branch of the antenna is shown in each case.

Thus the setae are fairly complicated structures. They are provided with muscles (inserted into their bases at least), they have definite joints, one at the base and one near the middle, and they are set with hairs.

The operation consisted in the amputation by a fine needle of the dorsal branch through the middle of segment III in *Simocephalus* and of segment II in *Daphnia*. The operation was carried out in the first instar, and the degree of regeneration was measured in the first adult instar (*i.e.* when the animal was carrying its first batch of eggs in its brood pouch), except where otherwise stated.

The operation in *Simocephalus* removes one and a half segments together with four setae (Fig. 1); in *Daphnia*, two and a half segments and the four setae are removed. Regeneration consists essentially of the formation of new setae, the missing segments of the axis never being reproduced. There may be a little growth of the stump of the amputated segment, but the missing joints are not formed. The regenerated antenna—or rather, the dorsal branch of it—consists of a variable number of setae growing out in a branch from the end of the truncated stump. Fig. 2 illustrates the variation in regeneration in *Simocephalus*. It is to be noted that

the special hooked dorsal seta apparently is never regenerated—the new setae are always of the more generalised type, even when four or more are regenerated.

The average number and length of setae regenerated, and the thickness of their hairy covering, is greater in *Daphnia* than in *Simocephalus*.

The regenerated antennae were examined and recorded in the first adult instar, except in the first tests of the lines designated *DR* and *DB*, when the second adult instar was used. Only very rarely are additional setae formed after maturity in *Simocephalus*, but this not uncommonly happens in *Daphnia*. This matter is dealt with fully in my other paper.

The regenerated setae are usually very good replicas of the normal ones, being provided with the proximal and median joints, furnished with muscles which move them on the antenna stump, and they are supplied with the usual hairy covering. The latter, however, is seldom as dense as in the normal seta. The regenerated setae rarely or never attain the length of the normal setae, and are occasionally devoid of hairs, unjointed, or misshapen.

There is often a considerable degree of necrosis of the regenerated setae in later life.

MEASURING AND RECORDING THE AMOUNT OF REGENERATION.

Two principal measures of regeneration were used:

(1) The number of setae regenerated. This varies in my experiments from 0 to 6 in *Simocephalus*, and from 0 to 9 in *Daphnia*.

(2) The length of the setae regenerated. This was expressed as the summed lengths of all setae regenerated (total length of setae).

While these are primarily quantitative measures, the latter is indirectly qualitative also, for long setae are nearly always better formed and more thickly set with hairs than short setae.

The setae were measured on camera lucida drawings of the antennae, dissected off, and mounted in dilute glycerine. All measurements quoted in this paper have been converted into actual, unmagnified dimensions in μ .

Experiments made to test the point showed that no change takes place in the number or length of the setae during one instar. Immediately after ecdysis the rudiments of any setae which have been forming grow out, and those already formed expand, and then no further measurable change takes place till after the next ecdysis. Consequently all measurements of different animals made in the same instar (the first adult in this case) are strictly comparable.

As will be seen from the tables, most of the test experiments involved between 50 and 100 individuals. These were provided by a number of families, averaging between 17 and 18 to each test. Hence there were often several individuals belonging to the same family. In sexual reproduction there exists of course a correlation between members of a family from genetic causes, and therefore in such cases it would not be permissible to base the probable error of the mean of a population composed of a number of fraternities upon the total number of individuals. In monoclonal populations such as ours, no genetic source of fraternal correlation

exists. It is, however, conceivable that such a correlation might be brought about by other causes (such as persistent effects of intra-ovarian conditions in the mother), and this had to be tested to see if the basing of the probable error of the mean on the total number of individuals was justifiable.

The fraternal correlation for degree of antenna regeneration was found in two populations each of *Simocephalus* and *Daphnia*. The four populations contained 110, 82, 72 and 57 individuals respectively, provided by 31, 22, 20 and 11 families. The coefficients of fraternal correlation were found for number of setae regenerated, and for total length of setae. Some of the coefficients were positive, some negative. Most of them were less than their probable errors, and none of them reached twice its probable error. Thus there is no significant fraternal correlation, and it is permissible to base the probable error of the mean for the whole population on the total number of individuals composing it.

VARIATION IN AMOUNT OF REGENERATION AND ITS CAUSES.

The subject of this brief section formed the main part of my other paper, to which the reader is referred for fuller details and analysis.

The distribution of the number of setae regenerated per animal in the combined test experiments is given in Table I.

Table I. *Distribution of number of setae regenerated per antenna in all the test experiments combined.*

Number of setae	0	1	2	3	4	5	6	7	8	9	Total
<i>Simocephalus</i>	96	135	147	104	50	5	—	—	—	—	537
<i>Daphnia</i>	24	27	90	168	173	38	6	2	1	1	530

The immediate cause of the variation in the number of setae regenerated is to be looked for in numerous factors localised in the regeneration blastem. The combined action of all these factors determines the "regeneration potential."

The value of this varies approximately according to the normal probability distribution, and expresses itself primarily in the number of setae produced. The left-hand end of the distribution of number of setae resulting from the distribution of potential value is telescoped owing to the fact that the potential must rise above a certain threshold value before any setae can be produced.

The conclusion that the factors which combine to produce the regeneration potential are mainly localised in the regeneration blastem, and that this is little influenced by differences in general internal conditions of the animals (within the limits through which they varied in these experiments) is mainly based on the following fact. In populations of *Daphnia* and *Simocephalus* in which both antennae have been operated upon, although there is very great variation in the number and length of setae regenerated on the antennae, there is no significant coefficient of correlation between the right and left antennae of the same animal as regards either number of setae or total length of setae.

Nevertheless, the degree of regeneration is affected by the general complex of external conditions, as shown by the difference in the mean degree of regeneration in populations reared under intentionally different conditions, or at different periods. This factor is avoided in the present experiment by comparing only animals reared contemporaneously, and by insuring that external conditions were practically identical for them all—or rather, that any unknown differences of conditions were distributed at random among the individuals of the two groups under comparison.

One more finding remains to be mentioned. No “improvement with practice” in regeneration within the lifetime of a single individual could be discovered. Naturally, the negative evidence is not worth much. It is impossible to make an animal regenerate the same antenna many times in succession, and even if it were, it would be impossible to exclude the possibility that any alteration in later regenerations as compared with earlier ones might be due to other causes than facilitation due to practice. However, as described more fully in my other paper, an experiment was carried out which showed that animals which had already regenerated one antenna and were subsequently made to regenerate the other, did this second regeneration no better, and possibly worse, than animals without previous experience of regeneration. This negative result was to be expected; if for no other reason, from the fact already mentioned, that the factors determining regeneration are mainly localised in the regeneration blastem itself.

THE LINES USED FOR THE EXPERIMENT.

(1) *Simocephalus*.

As we have seen, every individual of this form used was descended by parthenogenesis from a single female, from which the three experimental lines and the control line were derived.

Two principal lines were used for the Lamarckian experiment, and one for an experiment in which the possible Lamarckian factor was combined with selection.

One line, designated SR_1 was carried on as far as possible from the first broods of each generation, thus shortening the life cycle and giving the maximum number of generations in a given time. The total duration of the 102 generations of this line was 32 months.

The usual procedure was to operate on four first instar young ones in each generation. These were kept isolated, each in its own phial, and labelled 1 to 4 at random. No. 1 was destined to be the parent of the next generation, the other three being kept as reserves in case of death or accident. Within 24 hours of No. 1 producing its first brood, four of these were operated upon, and so the line was maintained. The parents were also retained as reserves until their offspring had themselves produced young, which proved useful chiefly on those occasions when the operated individuals turned out to be males. I found it extremely difficult to discriminate between females and the much less numerous males, in the young of this species of *Simocephalus*. This difficulty does not arise in the case of *Daphnia*.

The other line in the Lamarckian experiment, designated SR_3 , was bred from the third broods of each generation (fourth in a few cases). The animal is nearly twice as old when it lays its third batch of eggs as when it lays its first, and the process of regeneration—at any rate, the growth of the regenerated setae—continues all this time. It seemed possible, therefore, that the later produced eggs might be more influenced by the regeneration process than the earlier ones.

The line SS , used for the combination of selection and the Lamarckian factor, was bred mainly from first broods.

In all these lines of *Simocephalus* the right antenna alone was operated upon.

In addition to these three lines a control line of unoperated animals was maintained.

(2) *Daphnia*.

Again, every individual traces its descent by parthenogenesis back to the same original female.

Two lines were used for the Lamarckian experiment, DR in which only the right antenna was operated upon and DB in which the dorsal branches of both antennae were amputated. Both these lines were bred mainly from second broods. The duration of the 80 generations of the DR line was 31 months. The line DS , bred from first broods, was used for selection combined with the Lamarckian factor. A control line of unoperated animals was also maintained.

METHOD OF CARRYING OUT THE TESTS.

Owing to the variability of the regeneration, and the impossibility of keeping external conditions constant over a large number of generations, it is useless to measure the amount of regeneration in the four operated individuals of each generation, with a view to finding whether this undergoes any progressive change. The method adopted therefore was to make periodical large-scale tests, in which a number of individuals of the experimental line (with ancestral experience of regeneration) were compared as to their powers of regeneration with a number of animals from the control line, regenerating their antennae for the first time in the history of their line.

Experiments directed to this purpose showed that no significant difference in the amount of regeneration as measured in the first adult instar resulted from variation in the age at which the animal was operated upon, so long as this was in the first instar (the duration of which is about 24 hours at the temperature employed). Nevertheless, to guard against any possibility of this source of error, at the time when the parents (generally about 20 in number) of the animals which were to be used for a test were about to give birth to young, an hourly examination was carried out, and all operations (with insignificant exceptions) were performed 2 to 2½ hours after the birth was recorded.

Immediately after operation each animal was placed in a separate phial, special care being taken to keep conditions as uniform as possible.

In preparation for a test a large quantity of culture medium was made up, and keeping it well stirred the while, 50 c.c. of it was measured out into each phial into which an animal was to be placed. All the phials, each containing a single experimental or control animal, were mingled together and stood in a water bath maintained at 22.5° C. by a thermostat. (In some of the earlier tests a lower temperature was used, but of course the same for all animals in any one test.)

RESULTS OF THE LAMARCKIAN EXPERIMENT.

These are summarised in Tables II-IV, which set out the results of each test separately. It is necessary to compare the experimental and control animals of the same test only; for reasons already indicated, it is useless to compare one test with another.

Table II. *Inheritance tests in the Simocephalus line SR₁.*

Total length of setae regenerated per animal is given in μ

Test number	1	2	3	4	5
<i>SR line₁</i>					
No. of generations of operated ancestry	20	33	48	73	101
No. of individuals	27	25	35	50	36
No. which failed to regenerate	3	1	10	6	12
Mean number of setae regenerated	1.59 ± 0.12	2.56 ± 0.15	1.46 ± 0.13	1.72 ± 0.10	1.17 ± 0.11
Mean total length of setae regenerated	413 ± 27	558 ± 30	505 ± 34	530 ± 23	390 ± 29
<i>Controls</i>					
No. of individuals	25	36	33	34	47
No. which failed to regenerate	3	4	11	7	13
Mean number of setae regenerated	1.76 ± 0.14	2.56 ± 0.16	1.39 ± 0.14	1.85 ± 0.15	1.23 ± 0.10
Mean total length of setae regenerated	494 ± 32	631 ± 30	511 ± 34	622 ± 37	399 ± 24
<i>Difference in favour of operated line</i>					
No. of setae regenerated	-0.17 ± 0.18	0 ± 0.22	+0.07 ± 0.19	-0.13 ± 0.18	-0.06 ± 0.15
Total length of setae	-81 ± 42	-73 ± 42	-6 ± 48	-92 ± 44	-9 ± 38

In reading these tables, the following points must be borne in mind:

(1) In connection with the "number of generations of operated ancestry" it must be remembered that a certain percentage of operated animals fail to regenerate any setae at all (see Table I). The amount of regeneration of the single individual

Table III. *Inheritance tests in the Simocephalus line SR₃.*

Test number	1	2
<i>SR line₃</i>		
No. of generations of operated ancestry	21	33
No. of individuals	29	26
No. which failed to regenerate	4	7
Mean number of setae regenerated	2.07 ± 0.15	1.23 ± 0.13
Mean total length of setae regenerated	501 ± 27	427 ± 36
<i>Controls</i>		
No. of individuals	36	26
No. which failed to regenerate	4	4
Mean number of setae regenerated	2.56 ± 0.16	1.58 ± 0.14
Mean total length of setae regenerated	631 ± 30	467 ± 31
<i>Difference in favour of operated line</i>		
No. of setae regenerated	-0.49 ± 0.22	-0.35 ± 0.19
Total length of setae	-130 ± 40	-40 ± 48

Table IV. *Inheritance tests in the Daphnia lines DR and DB. In line DB, in which both antennae were operated upon, the number of individuals refers to antennae, not animals.*

Test number	<i>DR line</i>		<i>DB line</i>	
	1	2	1	2
<i>Operated line</i>				
No. of generations of operated ancestry	9	79	15	57
No. of individuals	29	48	44	94
No. which failed to regenerate	6	0	7	2
Mean number of setae regenerated	3.21 ± 0.30	3.46 ± 0.09	3.48 ± 0.24	3.16 ± 0.08
Mean total length of setae regenerated	1365 ± 105	1443 ± 51	1737 ± 76	1337 ± 39
<i>Controls</i>				
No. of individuals	28	50	44	94
No. which failed to regenerate	3	2	4	3
Mean number of setae regenerated	3.39 ± 0.28	3.20 ± 0.11	3.41 ± 0.23	3.26 ± 0.08
Mean total length of setae regenerated	1329 ± 106	1544 ± 59	1581 ± 85	1590 ± 39
<i>Difference in favour of operated line</i>				
No. of setae regenerated	-0.18 ± 0.41	+0.26 ± 0.14	+0.07 ± 0.33	-0.10 ± 0.11
Total length of setae	+36 ± 149	-101 ± 78	+156 ± 114	-253 ± 55

used as parent in each generation was not recorded in every case, but the following are the approximate number of them which failed to regenerate out of the total number of parents shown in the last test of each line:

SR_1 line, 15; SR_3 , 3; DR , 16; DB , 3.

(2) The mean number of setae regenerated includes those antennae which did not regenerate at all (the number of which is shown in the tables), but the mean total length of setae is exclusive of these.

(3) The number and total length of setae are not independent measurements, but highly correlated.

Little need be added to the information contained in the tables. It is seen at once that on the whole the animals with ancestral experience of regeneration regenerate no better than those which are doing it for the first time. The bottom sections of the tables show the inevitable small differences that exist between the mean degree of regeneration of the experimental and control groups. Out of a total of 22 comparisons, 5 are in favour of the experimental animals, 16 in favour of the controls, and the remaining 1 is zero. Only 2 (both in favour of the controls) exceed three times their probable errors. Indeed, there appears to be some indication that the animals with ancestral experience of regeneration regenerate worse than the controls, though it is very improbable that the evidence on the whole is significant. If it is so, the effect must be due to immediate parental influence only, for the disadvantage is not cumulative.

Even though continued ancestral regeneration does not affect the powers of regeneration of the descendants it is possible that it might influence the normal growth of the antenna, as Kammerer believed resulted from a single amputation and regeneration of the siphons in *Ciona*. Or possibly, antibodies produced to toxins formed at the wound surface might act as Guyer has described as consequent upon injury to the eye in rabbits. To test this, the following experiment was carried out. Six operated individuals of the 15th generation of the *DB* line were taken as parents. From each of their second broods 8 unoperated young were isolated for examination of the normal growth of the antenna. As controls, 8 young of each of 7 control parents were taken. All of the 112 individuals matured, but accidents when dissecting off the antenna for measurement reduced the number to 47 experimental animals and 50 controls. The proximal segment of the seta which springs from the joint between the third and fourth segments of the dorsal branch of the antenna (one of the setae which is removed in operated animals) was measured. To compensate for variations in general body size, this is expressed as the ratio, body length divided by seta. The mean ratio for the experimental animals was 8.83 ± 0.03 and for the controls 8.91 ± 0.04 .

A similar experiment was carried out in the 86th generation of the same line (*i.e.* with 85 generations of operated ancestry). The mean ratio for 40 experimental animals was 8.48 ± 0.03 , and for 45 controls 8.49 ± 0.03 .

It is clear therefore that 85 generations of amputation and regeneration does not affect the normal growth of the seta.

THE LAMARCKIAN FACTOR COMBINED WITH SELECTION.

An experiment to test this was carried out with both species. In each generation 10 new-born young of the selected individual were operated on in the usual way. After operation they were put together in a jar containing about 500 c.c. of culture medium. They were examined again when carrying their first batch of eggs, and the amount of regeneration recorded. The animal showing most regeneration was isolated, and 10 of its young were treated in the same way.

Table V. *Inheritance tests in lines SS and DS.*

Test number	<i>Simocephalus</i> , line SS			<i>Daphnia</i> , line DS		
	1	2	3	1	2	3
<i>Operated and selected line</i>						
No. of generations of operated ancestry	5	21	33	23	34	42
No. of individuals	30	35	48	43	40	42
No. which failed to regenerate	3	1	16	1	2	2
Mean number of setae regenerated	2.57 ± 0.15	1.86 ± 0.12	1.52 ± 0.14	3.70 ± 0.17	2.83 ± 0.13	2.83 ± 0.12
Mean total length of setae regenerated	586 ± 29	462 ± 32	479 ± 36	1540 ± 55	1473 ± 48	1457 ± 59
<i>Controls</i>						
No. of individuals	36	33	41	42	33	44
No. which failed to regenerate	4	11	8	6	5	1
Mean number of setae regenerated	2.56 ± 0.16	1.39 ± 0.14	1.66 ± 0.13	2.64 ± 0.17	2.52 ± 0.16	3.32 ± 0.13
Mean total length of setae regenerated	631 ± 30	511 ± 34	439 ± 33	1264 ± 67	1463 ± 90	1676 ± 58
<i>Difference in favour of operated line</i>						
No. of setae regenerated	+0.01 ± 0.22	+0.47 ± 0.18	-0.14 ± 0.19	+1.06 ± 0.24	+0.31 ± 0.21	-0.49 ± 0.18
Total length of setae	-45 ± 42	-49 ± 47	+40 ± 49	+276 ± 87	+10 ± 102	-219 ± 83

In estimating the amount of regeneration for selection purposes, the total length of seta regenerated was the criterion used. In the great majority of cases this means the animal with the largest number of setae. Where two or more had the same number, the one in which the setae were longest and most perfect was chosen. In a very few cases an animal with fewer large setae had a greater total seta length (as judged by the eye on the living animal) than one of its sisters with more but shorter setae, and in such a case the animal with the fewer setae was chosen.

Owing to mortality, and to the selected animal occasionally having a brood of less than 10, the number of animals from which the selection was made, though

usually 10, was frequently somewhat less. The mean number in each generation from which the selection was made was 8.9 in *Simocephalus* and 8.7 in *Daphnia*. The mean number of setae regenerated by the selected parents happens to be the same in the two genera, namely 3.7. They include none which failed to regenerate at all.

The problem of whether any progressive change in power of regeneration was produced was tested in the same way as in the main Lamarckian experiment, by making periodical large-scale tests in precisely the same way as in that experiment. The results of these tests (three in each species) are set out in Table V. A glance at the bottom section of the table, where experimental and control animals are compared, shows that the results are negative. No change in power of regeneration was produced.

Besides confirming the lack of any Lamarckian effect, these experiments are in accord with the great majority of experiments on intracloal selection in Metazoa, which agree as to its inadequacy to produce results, save such occasional ones as can reasonably be explained by the rare occurrence of mutation.

SUMMARY.

The experiment was devised to find out whether regeneration of the dorsal branch of the second antenna of *Simocephalus* and *Daphnia*, repeated for many generations, would result in an improvement in the regeneration (which is very imperfect) or in any other alteration in the process. Neither the character nor extent of the regeneration was influenced in any measurable degree in any of the experimental lines, even after 100 generations, nor was the normal growth of the antenna affected.

A similar experiment, except that selection was practised in addition, likewise produced negative results, confirming both the lack of the Lamarckian effect and the inefficacy of intracloal selection.

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